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PRINCIPAL INVESTIGATOR: W. Dalton Dietrich, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
Miami, Florida 33136

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The First Joint Symposium of the National and International Neurotrauma Societies 2002

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Fort Detrick, Maryland 21702-5012

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The First Joint Symposium of the National and International Neurotrauma Societies was held October 27 through November 1, 2002, at the Saddlebrook Resort, Tampa, FL USA. Over 750 scientists/physicians participated in this five-day meeting, which represented a unique partnership between the International and National Neurotrauma Communities. Various research topics targeting CNS injury and repair were discussed by some of the leading authorities in the world. The success of the meeting was greatly dependent upon support from various funding agencies, including the Department of the Army. These monies were used to support conference/symposium activities. A copy of the program, including the scientific committee, programs, and abstract/poster presentation is included with this report.
THE FIRST JOINT SYMPOSIUM OF THE NATIONAL AND INTERNATIONAL NEUROTRAUMA SOCIETIES

The Twentieth Annual National Neurotrauma Society Symposium & The Sixth International Neurotrauma Symposium

October 27 - November 1, 2002
Saddlebrook Resort
Tampa, FL USA
THE FIRST JOINT SYMPOSIUM OF THE NATIONAL AND INTERNATIONAL NEUROTRAUMA SOCIETIES

October, 2002

Dear NINTS 2002 Attendees:

Welcome to the first joint meeting of the National and International Neurotrauma Societies (N-INTS) at the Saddlebrook Resort.

This five day meeting represents a unique partnership between both the international and national neurotrauma communities, both of whom have been historically committed to excellence in both research and the medical management of the brain and spinal cord injured patient. It is our hope that the joining of these two historically successful societies will provide a unique intellectual environment in which to promote the exchange of contemporary scientific communication on the basic mechanisms and treatments of acute and chronic CNS injury.

We express our sincere gratitude to the outstanding group of basic and clinical scientists who helped make this meeting possible by serving on the program committee and participating as faculty in the years' symposium. It is through their efforts that we can offer you such an extraordinary program.

Thank you for your participation in this year's symposium. We hope that you will find it both challenging and enjoyable.

Warmest regards,

Douglas K. Anderson
W. Dalton Dietrich
Linda J. Noble
THE FIRST JOINT SYMPOSIUM OF THE
NATIONAL AND INTERNATIONAL
NEUROTRAUMA SOCIETIES

October, 2002

Dear N-INTS Attendee:

On behalf of the International Neurotrauma Society (INTS), it is my pleasure to welcome all participants and guests to the Sixth International Neurotrauma Symposium and the first to be held in conjunction with another neurotrauma organization, the National Neurotrauma Society.

The purpose of the INTS is to foster the worldwide dissemination of neurotrauma research and to supervise international neurotrauma symposia throughout the world. The intention continues to be to alternate the venue of the symposium meetings between Australasia, Europe and The Western Hemisphere every two or three years. To do so, the INTS authorizes a local host for each meeting and assists the local host’s organizing committee thorough the International Scientific Advisory Board of the INTS.

Professors Douglas Anderson and Dalton Dietrich were chosen from scientists at several distinguished North American universities to organize and host this Sixth INTS meeting. They have had a most difficult task to achieve scientific and social comparability to that of the Fifth International Neurotrauma Symposium in Germany and have been given the additionally difficult task of integrating the INTS meeting with the meeting of the National Neurotrauma Society. This represents the first attempt of either society to present a combined meeting.

The INTS has been exceptionally impressed by the organizational capabilities of Professors Anderson and Dietrich and their teams from the University of Miami and the University of Florida. The cooperation of Professor Linda Noble and her colleagues in the National Neurotrauma Society has been superb and, thus, we are confident that we are about to experience a very special scientific and social program that will advance the science of neurotraumatology and will draw scientists from all nations much closer together. In this regard, I wish you a most productive symposium and pleasant camaraderie.

Thomas A. Genarelli, MD
President, International Neurotrauma Society
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COMMITTEES

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In compliance with the Accreditation council for Continuing Medical Education (ACCME) Standards for Commercial Support of CME, the Office of Continuing Medical Education discloses all current relationships that program faculty report with companies whose products they may discuss during their presentations.

The following invited speakers have disclosed relationships with commercial supporters:

- Doug Anderson, Ron Hayes and Paul Reier are faculty members at the McKnight Brain Institute.
- Arlene Chiu and Mary Ellen Michel are employed by the National Institute of Neurological Disorders and Stroke.
- W. Dalton Dietrich and Martin Oudega are faculty members at the Miami Project to Cure Paralysis.
- Ron Hart is a faculty member at the W.M. Keck Center for Collaborative Neuroscience.
- David Hovda is the Director of the UCLA Brain Injury Research Center.
- Claire Hulsebosch has received funding in the past from the Kent Waldrep National Paralysis Foundation, Pfizer and the Paralyzed Veterans of America.
- Robin Roof is employed by Pfizer.
- Marion Murray receives funding from EPVA.
- Andrew Maas is the Chairman of the Pharmos steering committee for International Multicenter Study.
- Graham Teasdale has received financial support from Pharmos through Glasgow University.

We have been unable to obtain information regarding relationships with commercial supporters from the following speakers: Ross Bullock, Susan Harkema, Jun Chen, Paul Reier.

The following session chairs have disclosed relationships with commercial supporters:

- Mary Bunge is a faculty member at the Miami Project to Cure Paralysis. She is also a member of the Ameritec board which determines the recipient of the Ameritec Prize for Paralysis Research and is on the Scientific Advisory Board for Acorda Therapeutics.
- Mary Eaton is a faculty member at the Miami Project to Cure Paralysis and has been a past recipient of grants from the Paralyzed Veterans of America.
- Edward Hall is a former employee of Pharmacia and is currently employed by Pfizer.
- Dena Howland is a faculty member at the McKnight Brain Institute.
- Bruce Lyeth and Paul Vespa receive funding from a research grant from the UCLA Brain Injury Research Center.

The following oral abstract presenters have disclosed relationships with commercial supporters:

- Paul Vespa (P333), Grace Griesbach (P115) and Cheri Osteen / Christopher Giza (P545) are all members of and/or receive support from the UCLA Brain Injury Research Center.
- Stephen Larner (P105) and Eric Johnson are both members of the McKnight Brain Institute.

All other oral abstract presenters report having no relationships with the commercial supporters of CME.
ACKNOWLEDGEMENTS OF COMMERCIAL SUPPORT

(Supporters listed alphabetically)

The National and International Neurotrauma Societies and the School of Medicine at Virginia Commonwealth University gratefully acknowledge the following companies for their generous support of N-INTS 2002:

Acorda Therapeutics
Ameritec Foundation
Aventis Pharmaceuticals
DePuy AcroMed
Eastern Paralyzed Veterans Association
Evelyn F. & William L. McKnight Brain Institute at the University of Florida
Geron Corporation
Kent Waldrep National Paralysis Foundation
Miami Project to Cure Paralysis
The National Institutes of Health
The National Institute for Child Health & Human Development
The National Institute of Neurological Disorders and Stroke
Paralyzed Veterans of America
Pfizer
Pharmacia
Pharmos Corporation
United States Department of the Army
United States Department of the Navy
United States Department of Veterans Affairs
UCLA Brain Injury Research Center
University of Miami Department of Veterans Administration
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Aventis Pharmaceuticals

Custom Design & Fabrication

Geron Corporation

Integra Neurosciences

INTS 2004 - Australia

Laboratoires Fournier

Lippincott Williams & Wilkins

Moor Instruments, Inc.

Oxford Optronix

United States Department of Veterans Affairs

University of California at Irvine

W.M. Keck Center for Collaborative Neuroscience

Women in Neurotrauma (WINTR)
GENERAL INFORMATION

NINTS CREDENTIALS DESK AND BASIC INFORMATION

INFO DESK PHONE: EXTENSION 4322

Located in the Royal Palm Foyer, the Credentials Desk will be open daily from at 7am until 6pm. Conference staff will be on hand to assist participants in every possible way, so as to ensure your NINTS 2002 experience is both enjoyable and rewarding.

WWW.NEUROTRAUMA2002.ORG

The NINTS Conference website will be updated with Conference highlights, awards and winners of the Poster Competition following the completion of the conference.

FUTURE CONFERENCES

The Organizing Committee of the 7th International Neurotrauma Symposium invites all members of the international neurotrauma community to join their colleagues on September 12-16, 2004 in Adelaide, South Australia. For details, please contact events@plevin.com.au.

The 21st Annual Meeting of the National Neurotrauma Society will be held on Nov. 6-7, 2003. The meeting will be a satellite to the annual Neuroscience meeting in New Orleans, Louisiana. Student travel awards, student poster competition and an excellent scientific program are planned. Details will be available soon at www.neurotrauma.org.

OFFICIAL NINTS 2002 T-SHIRTS

Don’t leave NINTS without an Official Conference T-Shirt! Available in various sizes for only $15.00. Don’t forget to pick up a few extra for your colleagues back in the lab... The T-Shirt sales desk is located next to the NINTS Central Information Desk in the Royal Palm Foyer. Supplies are limited!

NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your tickets with you for admittance to ticketed events.

RESPONSIBILITY

By registering and participating in NINTS 2002, the attendee shall hold harmless the National and International Neurotrauma Societies, Society Administration and elected officials, Conference Co-Chairs and Conference Organizers in the case of any damage or personal injury claims. In addition non of the party’s shall be liable for non-performance including but not limited to, strikes or labor unrest, delay in transportation, delay in delivery by suppliers, fire, wars, acts of governments, unavailability of power or other utilities, or acts of nature.

CME ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) by the School of Medicine, Virginia Commonwealth University, Medical College of Virginia Campus (VCU) and the National Neurotrauma Society. VCU is accredited by the ACCME to provide continuing medical education for physicians.

Physicians may claim up to 33.5 hours in Type 1 or Type 2 CME on the Virginia Board of Medicine Continued Competency and Assessment Form required for renewal of an active medical license.

VCU designates this educational activity for a maximum of 33.5 hours in category 1 credits toward the AMA Physician’s Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the activity.

This continuing education activity meets the criteria of Virginia Commonwealth University and the Southern Association of Colleges and Schools. 3.0 CEUs will be awarded and recorded with the University.
GENERAL INFORMATION

SOCIAL EVENTS INCLUDED WITH REGISTRATION

Continental Breakfast will be held in the Royal Palm Ballroom each morning for all registered delegates and registered accompanying guests.

Sunday, October 27th

Welcome Reception
6:30 pm - 8:00 pm

Attendance is included for all registered delegates and registered accompanying guests.

Monday, October 28th

Delegate Luncheon (Provided by Pharmacia)
11:45 am - 1:00 pm, Pegasus Ballroom

Attendance is included for all registered delegates.

Thursday, October 31st

Masquerade Gala Dinner
7:30 pm - 10:30 pm, Grand Pavilion

Join us for a traditional American Halloween celebration at Saddlebrook Resort!

We invite you to attend and, if you like, Dress in costume or wear a mask for our festive celebration on All Hollow's Eve.

Attendance is included for all registered delegates and registered accompanying guests.
GENERAL INFORMATION

OPTIONAL TICKETED SOCIAL EVENTS

To purchase tickets for these events, please visit the Credentials Desk unless otherwise noted.

Monday, October 28th

"An Evening in the Tropics" Dinner  
7:00 pm, $58.00 pp

All participants are cordially invited to attend an evening celebrating the local flavors and rich cultures of tropical Florida.

Tuesday, October 29th

Women in Neurotrauma Luncheon Ticket  
11:45 am-1:00 pm, $14.00 pp

Women in Neurotrauma Reception  
5:30 pm-6:30 pm, $12.00 pp

Wednesday, October 30th

National Neurotrauma Society Business Meeting  
11:45 pm-1:00 pm, $22.00 pp

All National Neurotrauma Society members are invited to attend. Lunch tickets may be purchased in advance.

Thursday, October 31st

Lunch at Saddlebrook  
11:45 am-1:00 pm, $22.00 pp

Avoid the rush at the restaurants and network with your colleagues during a casual buffet lunch.
GENERAL INFORMATION

INSTRUCTIONS FOR AUTHORS

PUBLICATION
All accepted abstracts will be published in the Journal of Neurotrauma which is included with the attendees’ credential kits.

POSTERS

All posters will be displayed in the Royal Palm Ballroom. For your convenience, push pins will be provided for your use in displaying your poster. Be sure to set up and remove your posters during the times indicated below. Posters left remaining after their session will be discarded. Please be sure you are stationed at your poster at the scheduled session time to present your abstract:

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<td>Tuesday, Oct 29</td>
<td>12:00-12:30 pm</td>
<td>3:00-4:30 pm</td>
<td>After 4:30 pm</td>
</tr>
<tr>
<td>437-512</td>
<td>5</td>
<td>Wednesday, Oct 30</td>
<td>7:30-10:15 am</td>
<td>11:45-1:15 pm</td>
<td>1:15-1:30 pm</td>
</tr>
<tr>
<td>513-588</td>
<td>6</td>
<td>Thursday, Oct 31</td>
<td>7:00-8:00 am</td>
<td>10:15-11:45 am</td>
<td>11:45-12:00 pm</td>
</tr>
</tbody>
</table>

*Posters 101-132 will be reviewed by the Abstract Judging Committee on Monday and Tuesday during Poster Sessions 1-3. Please be sure you are present at all three judging sessions to answer any questions the judges may have. All posters being reviewed by the committee will be displayed for the entire week.

ORAL PRESENTATIONS

Each abstract selected for oral presentation will be allotted 10 minutes for their presentation, followed by 5 minutes for discussion. Please be sure to arrive at Royal Palm Ballroom East (1/2/3) at least 15 minutes prior to your presentation with your presentation on a PC formatted disk in order to coordinate with the A/V technician. Please refer to the schedule below for the date & time of your presentation:

<table>
<thead>
<tr>
<th>Free Communications Session</th>
<th>Date</th>
<th>Session Time</th>
<th>Abstract Number (in order of presentation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Monday, Oct 28</td>
<td>4:30-6:00 pm</td>
<td>107, 108, 113, 123, 128, 318</td>
</tr>
<tr>
<td>2</td>
<td>Tuesday, Oct 29</td>
<td>4:30-6:00 pm</td>
<td>105, 115, 116, 120, 126, 313</td>
</tr>
<tr>
<td>3</td>
<td>Wednesday, Oct 30</td>
<td>10:15-11:45 am</td>
<td>104, 110, 114, 122, 127, 519</td>
</tr>
<tr>
<td>4</td>
<td>Thursday, Oct 31</td>
<td>3:00-4:30 pm</td>
<td>106, 125, 131, 333, 375, 545</td>
</tr>
</tbody>
</table>

TRAVEL GRANTS

Thanks to generous support in the form of a grant from NIH, NINTS is able to offer $10,000 in travel grants encouraging students to attend the conference. The travel grants are awarded based on financial need and merit. We are pleased to announce that this year's awardees are: María Briones-Galang, Oleg Butovsky, Szu-Fu Chen, Jyoti Chuckowree, Pauline Dergham, Julie Friedland, Maria Jimenez Harman, Caitlin Hill, Nicole Klapka, Iris Kulbatski, Paul Lea, Jie Liu, Yan Long, Andrew Luciano, Andreas Menke, Otani Naoki, Eugene Park, Nicholas Phan, Neggy Rismanchi, Tomoko Sengoku.
GENERAL INFORMATION

LOCAL TRANSPORTATION

TO TAMPA INTERNATIONAL AIRPORT

Saddlebrook Resort is providing a shuttle service to Tampa International Airport (TPA) for $19.00 per person each way. For reservations, please call extension 4455. Please plan to depart 2 hours or more prior to your flight departure time. Shuttles to the airport depart on the hour except for the first shuttle, which is 4:30am. Transportation charges will be billed to your room account at Saddlebrook. Children 17 & under traveling with an adult are free.

Cabs are also available to the airport, however the average one way fare is approx. $75.00 USD.

TO ORLANDO

Bus departs at 1:30 p.m. from the Royal Palm Mall area. Advance reservations required.

For delegates who will be attending the 31st Annual Meeting of the Society for Neuroscience, held on Nov. 2-7 in Orlando, we are providing One Way bus transportation to Orlando for $25.00 pp. Even if you will not be attending the Orlando conference, you may wish to take advantage of this opportunity to spend a day at Walt Disney World or one of the many other attractions in the area before returning home.

RENTAL CARS

We have negotiated special discounted rates with Enterprise Rent-a-Car for NINTS 2002 attendees, including:

- Sunset Special 50% off rentals beginning between 4:00-5:30 pm and ending by 8:00 am the following day.
- $15.99 a Day Weekend Rate Applies to a compact car rented from Friday through Monday for a 3 day total of $47.97 with 300 free miles included.
- Complimentary Pick Up or Delivery available at Tampa International Airport and Saddlebrook Resort

If you have a need for a rental car while in Tampa, please make your reservations by calling Enterprise at 813-949-7458 (local) or 813-282-1680 (Tampa airport). If you need further assistance, please contact Marta Apostulu at 727-539-0702 x. 211. To receive your discounted rate, please refer to Account #686876 when calling to make a reservation.

PARKING FACILITIES

Valet parking charges are currently $5.00 for day parking and $10.00 for overnight parking. Self parking is also available on a complimentary basis.
# GENERAL INFORMATION

## LOCAL AREA BUSINESSES

### BANKS

1.1 miles  **Sun Trust Bank**  
5310 County Road 581  
Wesley Chapel, FL  
813-907-1335

1.2 miles  **Southtrust Bank**  
5227 County Road 581  
Wesley Chapel, FL  
813-973-2265

6.0 miles  **Community Bank**  
19910 Bruce B Downs Blvd  
Tampa, FL  
813-991-9206

6.5 miles  **First Union National Bank**  
8902 Regents Park Dr  
Tampa, FL  
813-276-4449

### MOVIE THEATERS

7.7 miles  **Movio Theaters**  
18002 Highwoods Preserve Pkwy  
Tampa, FL  
813-558-9755

8.9 miles  **Zephyrhills Cinema 6**  
6848 Gall Blvd  
Zephyrhills, FL  
813-782-2222

### GROCERY STORES

1.1 miles  **Publix Supermarkets Inc**  
5400 County Road 581  
Wesley Chapel, FL  
813-907-1699

1.1 miles  **Winn-Dixie**  
5351 Village Mart  
Wesley Chapel, FL  
813-973-3000

### PHARMACIES

1.0 miles  **Walgreens**  
28115 State Road 54  
Zephyrhills, FL  
813-973-2095

6.7 miles  **Eckerd Drug**  
8809 New Tampa Blvd  
Tampa, FL  
813-632-8989

### SHOPPING CENTERS

1.0 miles  **Bealls Outlet**  
5417 Village Market St  
Zephyrhills, FL  
813-994-3550

6.0 miles  **Wal-Mart Supercenter**  
19910 Bruce B Downs Blvd  
Tampa, FL  
813-994-6543

7.3 miles  **Dollar General**  
36524 State Road 54  
Zephyrhills, FL  
813-780-6808

7.3 miles  **K Mart**  
22920 State Road 54  
Lutz, FL  
813-949-6303

### PLACES OF WORSHIP

0.5 miles  **Atonement Lutheran Church**  
29617 State Road 54  
Wesley Chapel, FL  
813-973-2211

0.5 miles  **First Baptist Church of Wesley Chapel**  
29716 State Road 54  
Wesley Chapel, FL  
813-973-7185

1.8 miles  **Faith Baptist Church**  
6300 Oakley Blvd  
Wesley Chapel, FL  
813-907-9462

4.5 miles  **Wesley Chapel Seventh Day**  
33420 State Road 54  
Wesley Chapel, FL  
813-788-1550

4.5 miles  **Trinity United Methodist Church**  
33425 State Road 54  
Wesley Chapel, FL  
813-788-2898

7.1 miles  **Our Lady of the Rosary Church**  
2348 Collier Pkwy  
Land O Lakes, FL  
813-949-4565
# PROGRAM AT A GLANCE

## SUNDAY, OCTOBER 27<sup>TH</sup>

<table>
<thead>
<tr>
<th>Time</th>
<th>Royal Palm Foyer</th>
<th>Grand Pavilion</th>
<th>Executive Boardroom</th>
<th>Boardroom Two</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00</td>
<td>Guest Check-In</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td>INTS Executive Committee &amp; Scientific Advisory Board Meeting</td>
<td></td>
</tr>
<tr>
<td>1:00</td>
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<td></td>
<td></td>
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<tr>
<td>1:30</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2:00</td>
<td></td>
<td></td>
<td></td>
<td>NNS Officer Meeting</td>
</tr>
<tr>
<td>2:30</td>
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<td></td>
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<tr>
<td>3:00</td>
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<tr>
<td>3:30</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4:00</td>
<td></td>
<td></td>
<td>Opening Ceremony</td>
<td></td>
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<tr>
<td>4:30</td>
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<tr>
<td>5:00</td>
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<tr>
<td>5:30</td>
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</tr>
<tr>
<td>6:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:30</td>
<td>Welcome Reception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:00</td>
<td>(poolside)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:30</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

## NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your ticket with you for admittance to ticketed events.
## PROGRAM AT A GLANCE

### MONDAY, OCTOBER 28TH

<table>
<thead>
<tr>
<th>Time</th>
<th>Royal Palm Ballroom &amp; Foyer</th>
<th>Grand Pavilion</th>
<th>Pegasus Ballroom</th>
<th>Royal Palm East 1-2-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Credentials Desk Opens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:30</td>
<td>Continental Breakfast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:30</td>
<td></td>
<td>General Session 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:00</td>
<td></td>
<td>Bedside to the Laboratory</td>
<td></td>
<td></td>
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<tr>
<td>9:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td>Coffee Break</td>
<td>Poster Session 1</td>
<td>Pharmacia</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Provided by Pharmos</em></td>
<td>&amp; Visit Exhibits</td>
<td>Luncheon</td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11:00</td>
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<td>11:30</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td>General Session 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td>Cell Death &amp; Survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:00</td>
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<td>1:30</td>
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<td>2:00</td>
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<td></td>
</tr>
<tr>
<td>2:30</td>
<td>Coffee Break</td>
<td>Poster Session 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Provided by Pharmos</em></td>
<td>&amp; Visit Exhibits</td>
<td></td>
<td></td>
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<tr>
<td>3:00</td>
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<tr>
<td>3:30</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>4:00</td>
<td></td>
<td>Breakout Session 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4:30</td>
<td></td>
<td>Bedside to the Laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5:00</td>
<td></td>
<td>Breakout Session 2</td>
<td></td>
<td>Free Communications</td>
</tr>
<tr>
<td>5:30</td>
<td></td>
<td>Cell Death &amp; Survival</td>
<td>Session 1</td>
<td></td>
</tr>
<tr>
<td>6:00</td>
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<td></td>
<td></td>
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<tr>
<td>6:30</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7:00</td>
<td>to 10:00</td>
<td>Total Banquet: An Evening in the Tropics Dinner, Poolside</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your ticket with you for admittance to ticketed events.
# PROGRAM AT A GLANCE

## TUESDAY, OCTOBER 29TH

<table>
<thead>
<tr>
<th>Time</th>
<th>Royal Palm Ballroom &amp; Foyer</th>
<th>Grand Pavilion</th>
<th>Pegasus Ballroom</th>
<th>Royal Palm East 1-2-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Credentials Desk Opens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:30</td>
<td>Continental Breakfast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Provided by Aventis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:00</td>
<td></td>
<td>General Session 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:30</td>
<td></td>
<td>Guidelines and Management in CNS Injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:00</td>
<td></td>
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<td>9:30</td>
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<tr>
<td>10:00</td>
<td>Coffee Break</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Provided by Aventis</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>Poster Session 3 &amp; Visit Exhibits</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11:30</td>
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<td>12:30</td>
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<td></td>
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<tr>
<td>1:00</td>
<td></td>
<td>General Session 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:30</td>
<td>Age &amp; Gender Differences After CNS Injury</td>
<td></td>
<td>Ticketed Event: Women in Neurotrauma Luncheon</td>
<td></td>
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<tr>
<td>2:00</td>
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<td></td>
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<tr>
<td>2:30</td>
<td>Coffee Break</td>
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<tr>
<td></td>
<td>Provided by Aventis</td>
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<tr>
<td>3:00</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3:30</td>
<td>Poster Session 4 &amp; Visit Exhibits</td>
<td></td>
<td></td>
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<tr>
<td>4:00</td>
<td></td>
<td>Breakout Session 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guidelines and Management in CNS Injury</td>
<td></td>
<td>Breakout Session 4</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Age &amp; Gender Differences After CNS Injury</td>
<td></td>
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<tr>
<td>4:30</td>
<td></td>
<td></td>
<td>Free Communications Session 2</td>
<td></td>
</tr>
<tr>
<td>5:00</td>
<td>Ticketed Event: Women in Neurotrauma Reception, Little Club Patio</td>
<td></td>
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</tr>
<tr>
<td>5:30</td>
<td>Ticketed Event: Ybor City / Columbia Dinner</td>
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</tr>
</tbody>
</table>

## NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your ticket with you for admittance to ticketed events.
## PROGRAM AT A GLANCE

### WEDNESDAY, OCTOBER 30TH

<table>
<thead>
<tr>
<th>Time</th>
<th>Royal Palm Ballroom &amp; Foyer</th>
<th>Grand Pavilion</th>
<th>Pegasus Ballroom</th>
<th>Pegasus South</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Credentials Desk Opens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:30</td>
<td>Continental Breakfast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:00</td>
<td></td>
<td>General Session 5 Functional Recovery After CNS Injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:30</td>
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<td>9:00</td>
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<td>9:30</td>
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<td></td>
</tr>
<tr>
<td>10:00</td>
<td>Coffee Break</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td></td>
<td>Breakout Session 5 Functional Recovery After CNS Injury</td>
<td>Free Communications Session 3</td>
<td></td>
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<tr>
<td>11:00</td>
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<tr>
<td>11:30</td>
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</tr>
<tr>
<td>12:00</td>
<td>Poster Session 5 &amp; Visit Exhibits</td>
<td></td>
<td></td>
<td>Ticketed Event. NNS Business Luncheon Meeting</td>
</tr>
<tr>
<td>12:30</td>
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<tr>
<td>1:00</td>
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<td></td>
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<tr>
<td>1:30</td>
<td></td>
<td></td>
<td>Ticketed Event. Busch Gardens Excursion</td>
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<tr>
<td>to 6:30</td>
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<tr>
<td>6:30</td>
<td></td>
<td></td>
<td>Ticketed Event. Florida Aquarium / Starship Dining Yacht</td>
<td></td>
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<tr>
<td>to 10:30</td>
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<td></td>
<td></td>
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</tbody>
</table>

## NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your ticket with you for admittance to ticketed events.
### PROGRAM AT A GLANCE

#### THURSDAY, OCTOBER 31ST

<table>
<thead>
<tr>
<th>Time</th>
<th>Royal Palm Ballroom &amp; Foyer</th>
<th>Grand Pavilion</th>
<th>Pegasus Ballroom</th>
<th>Royal Palm East 1-2-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Credentials Desk Open</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7:30</td>
<td>Continental Breakfast</td>
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<tr>
<td>8:00</td>
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<td></td>
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</tr>
<tr>
<td>8:30</td>
<td></td>
<td>General Session 6</td>
<td>Stem Cells &amp; Neurotransplantation</td>
<td></td>
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<tr>
<td>9:00</td>
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<tr>
<td>9:30</td>
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</tr>
<tr>
<td>10:00</td>
<td>Coffee Break</td>
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<td>11:00</td>
<td>Poster Session 6 &amp; Visit Exhibits</td>
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<td>Optional Ticketed Lunch</td>
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<td>1:00</td>
<td>General Session 7 Plasticity &amp; Regeneration</td>
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<td>3:00</td>
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<td>Breakout Session 6 Stem Cells &amp; Neurotransplantation</td>
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<td>4:30</td>
<td>Abstract Awards Presentation</td>
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<tr>
<td>7:30 to 10:30</td>
<td>Masquerade Gala Reception and Dinner (costumes optional)</td>
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### NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your ticket with you for admittance to ticketed events.
# PROGRAM AT A GLANCE

## FRIDAY, NOVEMBER 1<sup>ST</sup>

<table>
<thead>
<tr>
<th>Time</th>
<th>Royal Palm Ballroom &amp; Foyer</th>
<th>Grand Pavilion</th>
<th>Pegasus Ballroom</th>
<th>Royal Palm East 1-2-3</th>
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<tbody>
<tr>
<td>7:00</td>
<td>Credentials Desk Opens</td>
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<td>General Session 8</td>
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<td>Inflammation/Immune</td>
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<td>Response to CNS Injury</td>
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<td>Coffee Break</td>
<td>General Session 9</td>
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<td>10:30</td>
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<td>Genomics &amp; Proteomics: Where Do We Go From Here?</td>
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<td>1:00</td>
<td><strong>Ticketed Event: Bus to Orlando Departs, Royal Palm Mall</strong></td>
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## NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your ticket with you for admittance to ticketed events.
# N-INTS 2002 Scientific Program

## Sunday, October 27th

### All day
- **Guest Check-In**
  - Location: Royal Palm Foyer

### 12:00 PM
- **Credentials Claiming Opens**
  - Location: Royal Palm Foyer

### 2:00-5:00 PM
- **INTS Executive Committee & Scientific Advisory Board Meeting**
  - Location: Executive Boardroom

### 3:00-5:00 PM
- **NNS Officer Meeting**
  - Location: Boardroom Two

### 5:30-6:30 PM
- **Opening Ceremony**
  - Dr. Thomas Genarelli, President, INTS
  - Dr. Linda Noble, President, NNS
  - Program Chairs: Doug Anderson, W. Dalton Dietrich and Linda Noble
  - Keynote Speakers: Marilyn Anderson, Alexander Rabchevsky, Senator Rod Smith
  - Location: Grand Pavilion

- **Presentation of the Ameritec Prize for Paralysis Research**
  - 2002 Award Recipient: Dr. Stephen Strittmatter, Yale University

### 6:30 PM
- **Welcome Reception**
  - Location: Poolside

## Monday, October 28th - Day 1

### 7:00 AM
- **Credentials Claiming Opens**
  - Location: Royal Palm Foyer

### 7:30-8:00 AM
- **Continental Breakfast**
  - Location: Royal Palm Ballroom

### 8:00-10:00 AM
- **General Session 1: Bedside to the Laboratory**
  - Co-Chairs: Russ Nockels, Alexander Rabchevsky
  - TBI: History and Challenges for the Future - Graham Teasdale
  - History and Challenges for the Future in SCI Research: Closing
  - The Gap Between Basic Science and Clinical Practice - Anders Holtz
  - Novel Approaches to the Clinical Investigation of CNS Injury - Susan Horne
  - Location: Grand Pavilion

### 10:00-10:15 AM
- **Refreshment Break – Provided by Pharmos Corporation**
  - Location: Royal Palm Ballroom

### 10:15-11:45 AM
- **Poster Session 1 (P133-P208) & Visit Exhibits**
  - Location: Royal Palm Ballroom

### 11:45-1:00 AM
- **Delegate Luncheon – Provided by Pharmacia**
  - Location: Pegasus Ballroom

### 1:00-2:45 PM
- **General Session 2: Cell Death and Survival After CNS Injury**
  - Co-Chairs: Jackie Bresnahan, Robert Clark
  - Overview of Cell Death Mechanisms After CNS Injury - Tracy McIntosh
  - Mitochondrial and Nuclear DNA Damage - Ella Englander
  - Molecular Pathways to CNS Neuronal Apoptosis Involving Nitrosative and Oxidative Stress - Stuart Lipton
  - Location: Grand Pavilion

### 2:45:3:00 PM
- **Refreshment Break – Provided by Pharmos Corporation**
  - Location: Royal Palm Ballroom

### 3:00-4:30 PM
- **Poster Session 2 (P209-P284) & Visit Exhibits**
  - Location: Royal Palm Ballroom
### MONDAY, OCTOBER 28th - DAY 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Location</th>
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</table>
| 4:30-6:00| **BREAKOUT SESSION 1: BEDSIDE TO THE LABORATORY**  
Co-Chairs: John Povlishock, Ed Wirth  
Chronic Complications of SCI - Paul Muizelaar  
Mild Traumatic Brain Injury - David Hovda  
Conference Report of TBI Clinical Trials - Mary Ellen Michel | Grand Pavilion |
| 4:30-6:00| **BREAKOUT SESSION 2: CELL DEATH AND SURVIVAL AFTER CNS INJURY**  
Co-Chairs: Ed Hall, Kathy Saatman  
DNA Damage & Repair – Jun Chen  
Oxidative Stress - Joe Beckman  
Mitochondrial Dysfunction - Pak Chan | Pegasus Ballroom |
| 4:30-6:00| **FREE COMMUNICATIONS SESSION 1**  
Co-Chairs: Ed Dixon, Lynne Weaver | Royal Palm East (1-2-3) |

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<thead>
<tr>
<th>Time</th>
<th>Presentation Title</th>
<th>Authors/Organizers</th>
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<tbody>
<tr>
<td>4:30-4:45</td>
<td>P107. Local Treatment With Phosphocreatine Improves Injury-Induced Metabolic And Electrophysiologic Changes After TBI</td>
<td>Oscar L. Alves, Thomas M. Reeves, Ross Bullock</td>
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<tr>
<td>5:00-5:15</td>
<td>P113. Activated EGFR Signaling And Transplanted Neural Stem Cell Motility</td>
<td>John A. Bockvar, Joost Schouten, Saori Shimuzzo, Rachel C. Hoover, Donald M. O'Rourke, Tracy K. McIntosh.</td>
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<tr>
<td>5:15-5:30</td>
<td>P123. Quantitative Diffusion Weighted Imaging Analysis Of Cell-Permeant Calcium Buffer Induced Neuroprotection After Cortical Devascularization In Rats</td>
<td>Brenda Bartnik, Igor Spigelman and André Obenaus</td>
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<tr>
<td>5:45-6:00</td>
<td>P318. Age Related Effects Of Acute Nmda Blockade On Functional Outcome After Controlled Cortical Impact In Immature Rats</td>
<td>PD Adelson*, CE Dixon, DS Davis, DJ Santone, AS Gordon, LW Jenkins, PM Kochanek.</td>
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### TUESDAY, OCTOBER 29th - DAY 2

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<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00 AM</td>
<td>Credentials Claiming Opens</td>
<td>Royal Palm Foyer</td>
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</table>
| 7:30-8:00| Continental Breakfast  
Provided by an unrestricted grant from Aventis Pharmaceuticals | Royal Palm Ballroom |
| 8:00-10:00| **GENERAL SESSION 3: GUIDELINES IN MANAGEMENT OF CNS INJURY**  
Co-Chairs: Tom Genarelli, Paul Vespa  
Management Controversies in Traumatic Injury - Ross Bullock  
Critical Care in TBI - Elisabeth Ronne-Engstrom  
Critical Care in SCI - Michael Fehlings | Grand Pavilion |
| 10:00-10:15| Refreshment Break  
Provided by an unrestricted grant from Aventis Pharmaceuticals | Royal Palm Ballroom |
| 10:15-11:45| **POSTER SESSION 3 (P285-P360) & VISIT EXHIBITS** | Royal Palm Ballroom |
TUESDAY, OCTOBER 29th - DAY 2

11:45-1:00  SPECIAL WOMEN IN NEUROTRAUMA LUNCHEON
            (Optional Ticketed Lunch)
            Challenges We Have Met - Elaine Aparicida Del Bel, Lisa McKerracher,
            Lisa Schnell, Esther Shohami

1:00-2:45  GENERAL SESSION 4: AGE AND GENDER DIFFERENCES
            AFTER CNS INJURY-CLINICAL CONSIDERATIONS
            Co-Chairs: Helen Bramlett, Ann-Christine Duhaime
            Gender - Patricia Hurm
            Pediatric - Donna Ferreiro
            Age and Outcome - Andrew Maas

2:45:30    Refreshment Break
            Provided by an unrestricted grant from Aventis Pharmaceuticals

3:00-4:30  POSTER SESSION 4 (P361-P436) & VISIT EXHIBITS

4:30-6:00  BREAKOUT SESSION 3: GUIDELINES IN MANAGEMENT
            OF CNS INJURY
            Co-Chairs: David Adelson, David Graham
            Management of Acute Brain Injury: New Directions - Geoff Manley
            Clinical Care in Severe TBI - Claudia Robertson
            Population Based Study on Risk Factors and Quality of Management
            in TBI - Alex Baethmann

4:30-6:00  BREAKOUT SESSION 4: AGE & GENDER DIFFERENCES
            AFTER CNS INJURY
            Co-Chairs: Sean Grady, Stuart Hoffman
            Metabolic changes in the Developing Brain after TBI - Mayumi Prins
            Gender and TBI - Robin Roof
            Oxidative Injury and Gender Differences After TBI - Takuji Igarashi

4:30-6:00  FREE COMMUNICATIONS SESSION 2
            Co-Chairs: Nariyuki Hayashi, Mary Eaton

4:30-4:45  P105. Effects Of Injury Severity On Regional And Temporal Caspase-12
            mRNA and Protein Expression Levels After Traumatic Brain Injury In Rats

4:45-5:00  P115. Voluntary Exercise Therapy After TBI: A Critical Window Of
            Opportunity
            G.S. Griesbach*, R. Molteni, F. Gomez-Pinilla & D.A. Hovda

5:00-5:15  P116. UP-Regulation Of The Cell Cycle/ Inhibitor Of Apoptosis Protein
            Survivin In Astrocytes And Neurons After TBI In Rats.

5:15-5:30  P120. Co-Accumulation Of Amyloid-Beta, Beta-Secretase, And Presenilin-1
            In Cultured Axons Following Stretch Injury

5:30-5:45  P126. Rapid Functional Recovery After Thoracic Spinal Cord Injury In
            Young Rats
            K.M. Brown*, B.B. Wolfe. and J.R. Wrathall

5:45-6:00  P313. Treatment Of Cold Injury-Induced Brain Edema With A Nonspecific
            Matrix Metalloproteinase Inhibitor MMI270 In Rats
            Nobuyuki Kawai*, Masanobu Okauchi, Seigo Nagao
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<td>7:30-8:00</td>
<td>Continental Breakfast</td>
<td>Royal Palm Ballroom</td>
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<td>8:00-10:00</td>
<td><strong>GENERAL SESSION 5: FUNCTIONAL RECOVERY AFTER CNS INJURY</strong></td>
<td>Grand Pavilion</td>
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<td><strong>Co-Chairs: Michele Basso, Kyoung-Suk Cho</strong></td>
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<td><strong>Outcome Measures and Human TBI - Jennie Ponsford</strong></td>
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<td><strong>Recovery After Traumatic Brain Injury: Animal Studies - Tim Schallert</strong></td>
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<td><strong>Recovery After Spinal Cord Injury: Human &amp; Animal Studies - Susan Harkema</strong></td>
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<td>10:00-10:15</td>
<td>Refreshment Break</td>
<td>Royal Palm Ballroom</td>
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<td>10:15-11:45</td>
<td><strong>BREAKOUT SESSION 5: FUNCTIONAL RECOVERY AFTER CNS INJURY</strong></td>
<td>Grand Pavilion</td>
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<td><strong>Co-Chairs: Angelika Mautes, Roi Ann Wallis</strong></td>
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<td><strong>Bladder Function After SCI - Jean Wrathall</strong></td>
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<td><strong>Brain Injury-Induced Epileptogenesis: Animal Studies - Doug Coulter</strong></td>
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<td><strong>Inflammation After TBI - Cristina Morganti-Kossmann</strong></td>
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<td>10:15-11:45</td>
<td><strong>FREE COMMUNICATIONS SESSION 3</strong></td>
<td>Pegasus Ballroom</td>
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<td><strong>Co-Chairs: Peter Blumbergs, Linda Phillips</strong></td>
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<td>10:15-10:30</td>
<td>P104. Quantitative Analysis of Neurofilament Compaction and Axonal Transport Following Diffuse Traumatic Brain Injury</td>
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<td></td>
<td>C. R. Marmarou, S.A Walker, J.R. Stone, E. Suehiro, Y. Ueda, R.H. Singleton and J. T. Povlishock</td>
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<td>10:30-10:45</td>
<td>P110. Temporal and Spatial Profile of Phosphorylated Mitogen-Activated Protein Kinase Pathways Following Lateral Fluid Perfusion Brain Injury in Rats</td>
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<td>Nakoji Otani, Hiroshi Nawaishiro, Katsuji Shima</td>
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<td>10:45-11:00</td>
<td>P114. Inhibition of NOGO-A Improves Recovery of Neuromotor and Cognitive Function Following Experimental Traumatic Brain Injury in Rats</td>
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<td>PM Lenzlinger, FM Bareyre, M Motta, S Shimizu, A Luginbuhl, RC Hoover, H Thompson, A Clause, KE Saatman, ME Schwab, and TK McIntosh</td>
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<td>11:00-11:15</td>
<td>P122. Relationship Of 40kD, 10kD, AND 3kD Fluorescent Indicators Of Altered Axolemmal Permeability To Impaired Axoplasmic Transport In Traumatic Axonal Injury.</td>
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<td>11:30-11:45</td>
<td>P519. Caspase Inhibition Attenuates Mitochondrial Release of Cytochrome C and Apoptosis-Inducing Factor After Traumatic Brain Injury in Rats</td>
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<td>11:45-1:15</td>
<td><strong>POSTER SESSION 5 (P437-P512) &amp; VISIT EXHIBITS</strong></td>
<td>Royal Palm Ballroom</td>
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<tr>
<td>11:45-1:15</td>
<td><strong>NATIONAL NEUROTRAUMA SOCIETY BUSINESS MEETING</strong></td>
<td>Pegasus South</td>
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<td>(All NNS Members are invited to attend. Ticketed Lunch optional)</td>
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<tr>
<td>1:15 pm</td>
<td>Free Afternoon</td>
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### Thursday, October 31st - Day 4

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<tr>
<td>7:00 AM</td>
<td>Credentials Claiming Opens</td>
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<td>7:30-8:00</td>
<td>Continental Breakfast</td>
<td>Royal Palm Ballroom</td>
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<td>8:00-10:00</td>
<td><strong>GENERAL SESSION 6: STEM CELLS AND NEUROTRANSPLANTATION</strong></td>
<td>Grand Pavilion</td>
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<td></td>
<td>Co-Chairs: Katsuji Shima, Bjorn Shefler</td>
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<td>History of Transplantation - Paul Reier</td>
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<td>Endogenous Stem Cells as a Transplant Source - Alain Privat</td>
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<td>Adult Stem Cells, In Vitro and In Vivo - Ariene Chiu</td>
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<tr>
<td>10:00-10:15</td>
<td>Refreshment Break</td>
<td>Royal Palm Ballroom</td>
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<tr>
<td>10:15-11:45</td>
<td><strong>POSTER SESSION 6 (P513-P587) &amp; VISIT EXHIBITS</strong></td>
<td>Royal Palm Ballroom</td>
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<tr>
<td>11:45-1:00</td>
<td>Lunch (on your own)</td>
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<td></td>
<td>Lunch at Saddlebrook (Ticketed Event)</td>
<td>Pegasus West</td>
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<td>1:00-2:45</td>
<td><strong>GENERAL SESSION 7: PLASTICITY AND REGENERATION</strong></td>
<td>Grand Pavilion</td>
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<td>Co-Chairs: Mary Bunge, Dana McTigue</td>
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<td>CNS Plasticity - Thomas Woolsey</td>
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<td>Factors that Influence Axonal Outgrowth - Lisa Schnell</td>
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<td>Spinal Transplants: What are the Limitations on Repair? - Marion Murray</td>
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<td>2:45:3:00</td>
<td>Refreshment Break</td>
<td>Royal Palm Ballroom</td>
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<td>3:00-4:30</td>
<td><strong>BREAKOUT SESSION 6: STEM CELLS AND NEUROTRANSPLANTATION</strong></td>
<td>Grand Pavilion</td>
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<td>Co-Chairs: Bruce Lyeth, Dena Howland</td>
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<td>Fate of Transplanted Stem Cells - Scott Whittemore</td>
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<td>Regulation of Neuronal Differentiation in Multipotent Human</td>
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<td>Neural Stem Cells - Angelo Vescovi</td>
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<td>Olfactory Ensheathing Glia and CNS Injury - Osamu Honmou</td>
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<td>3:00-4:30</td>
<td><strong>BREAKOUT SESSION 7: PLASTICITY AND REGENERATION</strong></td>
<td>Pegasus Ballroom</td>
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<td></td>
<td>Co-Chairs: Harry Goshgarian, Hans Kierstead</td>
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<td>Genetic Models - Phil Horner</td>
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<td>Functional and Dysfunctional Plasticity After CNS Neurotrauma</td>
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<td>- Claire Hulsebosch</td>
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<td>Use of Transplantation Approaches to Enhance Regeneration</td>
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<td>- Martin Oudega</td>
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<td>3:00-4:30</td>
<td><strong>FREE COMMUNICATIONS SESSION 4</strong></td>
<td>Royal Palm West (1,2,3)</td>
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<td>3:00-3:15</td>
<td>P106. 5,6-Epoxeyicosatrienoic Acid - Mediated Ca2+ Signaling Is</td>
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<td>Enhanced In Microglia Activated By Exposure To Soluble Factors From</td>
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<td>Traumatically Injured Astrocytes.</td>
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<td>3:15-3:30</td>
<td>P125. Differential Gene Expression Profiling In The Embryonic And Adult-Injured Spinal Cords</td>
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<td>Paul Gris* and Arthur Brown.</td>
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THURSDAY, OCTOBER 31st - DAY 4

3:00-4:30  FREE COMMUNICATIONS SESSION 4 (continued)  Royal Palm West 1-2-3


3:45-4:00  P333. Mild Fluid Percussion Injury Lowers The Threshold To Kainic Acid-Induced Seizures Which In Turn Elicit Recurrent Increases In Glutamate And Energy Demand In Vulnerable Tissue  P Vespa, E Roncati Zanier, E Shieh, D Hovda.

4:00-4:15  P375. The Potential Role Of The Chemokines MCP-1 AND IL-8 As Well As ICAM-1 In Traumatic Brain Injury  Mario Rancan*, Thomas Kossmann, Maria Cristina Morganti-Kossmann.

4:15-4:30  P545. Injury-Induced Changes In NMDA Receptor Subunit Composition Contribute To Prolonged Calcium-45 Accumulation In Intact Cortex  C.L. Osteen*, C.C. Giza, and D.A. Hovda.

4:40-6:00  ABSTRACT AWARDS PRESENTATION  Grand Pavilion
Top Abstract Student Poster Competition Awards
Women in Neurotrauma Award
Outstanding Young Investigator (ICCP) Awards

7:30-10:30  MASQUERADE GALA RECEPTION AND DINNER  Grand Pavilion
(Costumes optional)

FRIDAY, NOVEMBER 1ST - DAY 5

7:00 AM  Credentials Claiming Opens  Royal Palm Foyer

7:30-8:00  Continental Breakfast  Royal Palm Ballroom

8:00-10:00  GENERAL SESSION 8: THE INFLAMMATORY/IMMUNE RESPONSE TO CNS INJURY: THERAPEUTIC OPPORTUNITIES  Grand Pavilion
Co-Chairs: John Bethea, Patrick Kochanek
Inflammatory Responses to CNS Injury - Phillip Popovich
Protective Autoimmunity in CNS Injury - Michal Schwartz
Cytokines: Pro and/or Anti-Inflammatory Mediators - Esther Shohami

10:00-10:15  Refreshment Break  Royal Palm Ballroom

10:15-12:15  GENERAL SESSION 9: GENOMICS & PROTEOMICS - WHERE DO WE GO FROM HERE?  Grand Pavilion
Co-Chairs: Alan Faden, Ramesh Raghupathi
The Use of Multiple Proteomic Approaches in the Study of TBI - Larry Jenkins
Applications of Proteomics to the Study of Traumatic Brain Injury: Opportunities and Challenges - Ron Hayes

FINAL SUMMARY & ADJOURN
P101. REGIONAL HYPERGLYCOLYSIS IS CHARACTERIZED BY DECREASED GLUCOSE TRANSPORT AND PRESERVED HEXOKINASE ACTIVITY FOLLOWING TRAUMATIC HEAD INJURY.
N.Hatton1, SC.Huang2, HM.Wu3, WH Liao3, TC Glenn2, PM.Vespa4, M.Phelps4, DA. Hovda1, 2. M.Bergsneider2. (Dept. of Molecular and Medical Pharmacology, UCLA Brain Injury Research Center. David Geffen School of Medicine at UCLA. Los Angeles, CA).

P102. TOPICAL L-ARGININE, BUT NOT NITRIC OXIDE DONOR, RESTORES CEREBROVASCULAR PRESSURE AUTOREGULATION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS: POSSIBLE ROLE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE.

P103. TUMOR NECROSIS FACTOR RECEPTOR FAMILY MEMBERS MEDIATE POSTTRAUMATIC CELL DEATH AFTER CONTROLLED CORTICAL IMPACT IN MICE
Michael J. Whalen*, Jianhua Qiu, Deirdra McCarthy, and Michael A. Moskowitz. (Massachusetts General Hospital, Boston, MA US).

P104. QUANTITATIVE ANALYSIS OF NEUROFILAMENT COMPACTION AND AXONAL TRANSPORT FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY

P105. EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL CASPASE-12 mRNA AND PROTEIN EXPRESSION LEVELS AFTER TRAUMATIC BRAIN INJURY IN RATS

P106. 5,6-EPOXYEICOSATRIENIOIC ACID - MEDIATED Ca2+ SIGNALING IS ENHANCED IN MICROGLIA ACTIVATED BY EXPOSURE TO SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.

P107. LOCAL TREATMENT WITH PHOSPHOCREATINE IMPROVES INJURY-INDUCED METABOLIC AND ELECTROPHYSIOLOGICAL CHANGES AFTER TBI.
Oscar L. Alves, Thomas M. Reeves, Ross Bullock (Medical College of Virginia, Virginia Commonwealth University, Richmond, VA USA).

P108. HEME OXYGENASE-2 PREVENTS LIPID PEROXIDATION-MEDIATED CELL LOSS AND PROMOTES FUNCTIONAL RECOVERY AFTER TRAUMATIC BRAIN INJURY

P109. GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ASSESSMENT OF F2–ISOPROSTANE LEVELS IN CSF AFTER TRAUMATIC BRAIN INJURY IN RATS

P110. TEMPORAL AND SPATIAL PROFILE OF PHOSPHORYLATED MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS

P111. TRANSPLANTATION OF NGF-EXPRESSION NT2N NEURONS ATTENUATES A LEARNING DEFICIT FOLLOWING CONTROLLED CORTICAL IMPACT BRAIN INJURY IN MICE
Deborah J. Watson*, Luca Longhi*, Scott Fujimoto2,4, Adam Longibou*, Carl T Fulp*, Nicolas Royo*, Chen Zhang*, Kathryn E. Saatman*, John H. Wolfe*, Tracy K. McIntosh*. (Neurology, Children's Hospital of Philadelphia, PA. 2Departments of Neurosurgery, University of Pennsylvania. 3Anesthesia and Critical Care Medicine, Ospedale Maggiore Policlinico, IRCCS. Milan, Italy. 4Veteran Administration Medical Center, University of Pennsylvania.).

P112. NEURAL PROGENITOR CELL TRANSPLANTS SHOW LONG-TERM SURVIVAL AND ENHANCE BEHAVIORAL RECOVERY IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY
Deborah A. Shear1, Matthew C. Tate2, David R. Archer2, Stuart W. Hoffman3, Verne D. Hulce4, Michelle C. LaPlaca5, and Donald G. Stein1, 2. (Dept of Psychology. 3 Neurology. 4 Emergency Medicine. 5 Pediatrics. Emory University; Dept of Biomedical Engineering, Georgia Tech/Emory. Atlanta. GA. 2Field Neurosciences Institute. Saginaw. MI).

P113. ACTIVATED EGFR SIGNALING AND TRANSPLANTED NEURAL STEM CELL MOTILITY
John A. Boockvar, M.D., Joost Schouten, M.D., Saori Shimizu, M.D., Ph.D., Rachel C. Hoover, B.S., Donald M. O'Rourke, M.D., Tracy K. McIntosh, M.D. (University of Pennsylvania, Philadelphia, PA US).
P114. INHIBITION OF NOGO-A IMPROVES RECOVERY OF NEUROMOTOR AND COGNITIVE FUNCTION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS
PM Lenzlinger1,2, FM Bareyro3, M Motta1, S Shimizu4, A Luginbuhl5, RC Hoover3, H Thompson1, A Clause4, KE Saatman1, ME Schwab5, and TK McIntosh1. (1Dept. of Neurosurgery, University of Pennsylvania, Philadelphia PA, USA; 2Div. of Trauma Surgery, University Hospital, Zurich, Switzerland. 3Brain Research Institute, University and Swiss Federal Institute of Technology, Zurich, Switzerland).

P115. VOLUNTARY EXERCISE THERAPY AFTER TBI: A CRITICAL WINDOW OF OPPORTUNITY.

P116. UP-REGULATION OF THE CELL CYCLE/ INHIBITOR OF APOPTOSIS PROTEIN SURVIVIN IN ASTROCYTES AND NEURONS AFTER TBI IN RATS.
E.A. Johnson1, B.R. Pike. P. Tolentino3, R.L. Hayes & J. Pineda1,2. (Center for Traumatic Brain Injury Studies, U of FL McKnight Brain Institute Gainesville. FL USA 1Dept. of Neuroscience. 2Division of Pediatric Critical Care Medicine. 3Dept. of Neurosurgery).

P117. A SUBPOPULATION OF MITOTICALLY-ACTIVE CELLS MIGRATE ECTOPICALLY FROM THE ANTERIOR SUBVENTRICULAR ZONE FOLLOWING EXPERIMENTAL BRAIN INJURY
C.T. Fulp4, S. Shimizu3, J.E. Davis1, T.K. McIntosh1. (1The Head Injury Center and Dept. of Neurosurgery, Univ. of Penn. School of Medicine. 2Veterans Administration Medical Center. Philadelphia PA).

P118. GENDER DIFFERENCES IN COGNITIVE RECOVERY AFTER INTERVENTION WITH ENVIRONMENTAL ENRICHMENT FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY

P119. EXTRACELLULAR SIGNAL-RELATED KINASE/MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IS CRITICAL FOR ASTROCYTE PROLIFERATION AND MIGRATION IN THE SETTING OF BRAIN INJURY
W. Shawn Carbonell* and James W. Mandell. (University of Virginia, Charlottesville, VA US).

P120. CO-ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE, AND PRESENLIN-1 IN CULTURED AXONS FOLLOWING STRETCH INJURY

P121. IDENTIFICATION OF MULTIPLE DISTINCT PATHOLOGIC NEURONAL PHENOTYPES WITHIN DIFFUSELY INJURED BRAIN
Richard H. Singleton* and John T. Povlishock. (Medical College of Virginia/VCU, Richmond, VA US).

P122. RELATIONSHIP OF 40KD, 10KD, AND 3KD FLUORESCENT INDICATORS OF ALTERED AXOLEMMAL PERMEABILITY TO IMPAIRED AXOPLASMIC TRANSPORT IN TRAUMATIC AXONAL INJURY.

P123. QUANTITATIVE DIFFUSION WEIGHTED IMAGING ANALYSIS OF CELL-PERMEANT CALCIUM BUFFER INDUCED NEUROPROTECTION AFTER CORTICAL DEvascularization IN RATS
Brenda Bartnik1,2, Igor Spigelman* and Andre Obenaus1,2. (1Department of Anatomy & Cell Biology, University of Saskatchewan, Saskatoon, SK. Canada. 2Department of Radiation Medicine, Loma Linda University. Loma Linda. CA. USA, 3Division of Oral Biology & Medicine, UCLA School of Dentistry, Los Angeles. CA. USA).

P124. SPINAL CORD OLIGODENDROGLIA EXPRESS ACTIVATED CASPASE-3 FOLLOWING K+ INDUCED DEPOLARIZATION AND NMDA EXPOSURE
S.A. Nottingham* and J.E. Springer. (Anatomy and Neurobiology, Spinal Cord and Brain Injury Research Center, University of Kentucky Medical Center. Lexington. KY USA).

P125. DIFFERENTIAL GENE EXPRESSION PROFILING IN THE EMBRYONIC AND ADULT-INJURED SPINAL CORDS
Paul Gris* and Arthur Brown. (The John P. Roberts Research Institute, University of Western Ontario, London, Ontario CA).

P126. RAPID FUNCTIONAL RECOVERY AFTER THORACIC SPINAL CORD INJURY IN YOUNG RATS

P127. BENEFICIAL EFFECT OF AN EARLY ANTI-INFLAMMATORY STRATEGY AFTER ACUTE SPINAL CORD INJURY: COMPARISON TO THE EFFICACY OF METHYLPREDNISOLONE
P128. OLFATORY ENSHEATHING CELLS PROMOTE ROBUST AXON GROWTH FOLLOWING COMPRESSIVE SPINAL CORD INJURY
Boyd, JG, Lee, J, Skilhar, V, Doucette R, and Kawaja, MD. (Queen's University, Kingston, ON CA).

P129. CYCLIC AMP INDUCES FUNCTIONAL REGENERATION-ASSOCIATED GENES AND REPRESSES GAP-43
Jason B. Carmel\(^1\), Marie A. Handler\(^2\), Zixuan Cao\(^2\), Wilfredo Mellado\(^2\), Patricia Soteropoulos\(^3\), Peter Tolias\(^3\), Wise Young\(^4\), Marie T. Fillip\(^5\), Ronald P. Hart\(^1\). (\(^1\)W. M. Keck Center for Collaborative Neuroscience, Rutgers University, Piscataway, NJ; \(^2\)Department of Biological Sciences, Hunter College, CUNY, New York, NY; \(^3\)Center for Applied Genomics, PHRI, Newark, NJ USA).

P130. NOVEL SYNTHETIC Grafts THAT ARE BIOCOMPATIBLE AND PROMOTE AXONAL REGENERATION AFTER SPINAL CORD INJURY
Eve C. Tsai\(*\), Paul D. Dalton, Molly S. Shoichet, Charles H. Tator. (University of Toronto, Toronto, Ontario CA).

P131. NOGO-66 RECEPTOR ANTAGONIST PEPTIDE PROMOTES AXONAL REGENERATION AND FUNCTIONAL RECOVERY AFTER SPINAL CORD
Shuxin Li\(*\), Tadzia GrandPré and Stephen M. Strittmatter. (Yale University, New Haven, CT US).

P132. TRANSPLANTATION OF RODENT SKIN-DERIVED PRECURSOR CELLS ONTO RAT HIPPOCAMPAL SLICE CULTURE
Nao R. Kobayashi\(*\), Karl J.L. Fernandes, Amelie Rioux-Tache and Freda Miller. (Montreal Neurological Institute, McGill University, Montreal, QC. Canada).
P133. PROGNOSTIC VALUE OF SPECT IN PATIENTS WITH POSTTRAUMATIC TRANSENTORIAL HERNIATION
Martin Smrčka, M.D., Karel Máca, M.D., Viliém Juráš, M.D., Milan Vidišák, M.D., Vladimir Smrčka, M.D., Jiří Prášek, M.D., Roman Gál, M.D. (Neurosurgery, University Hospital Brno, Brno, CZ).

P134. LEFT-RIGHT ASYMMETRY OF THE ESTIMATION OF CEREBRAL PERFUSION PRESSURE USING TRANSCRANIAL DOPPLER ULTRASONOGRAPHY IN HEAD INJURY: A PRELIMINARY REPORT.
Schmidt EA*, Czonsnyka M*, Matta BF, Balestrieri M*, Piechnik SK, Steiner LA1,2, Pickard JD1, (1Academic Neurosurgery, 2Department of Anaesthesiology Addenbrooke's Hospital, Cambridge, UK).

P135. PERFUSION WEIGHTED MAGNETIC RESONANCE IMAGING (MRI) IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY.
Paul Mullins*, Xiao Di, Allan Faden. (University of New Mexico, Albuquerque, NM US).

P136. EFFECTS OF EARLY AND LATE INFUSION OF NOREPIINEPHRINE ON CEREBRAL BLOOD FLOW, BRAIN TISSUE OXYGENATION, AND BRAIN EDEMA FORMATION IN BRAIN-INJURED RATS

P137. EFFECTS OF HYPERAEMIA ON POSTTRAUMATIC CEREBRAL PERFUSION AND EDEMA FORMATION AFTER CONTROLLED CORTICAL IMPACT INJURY IN RATS.

P138. MAPPING FLOW-METABOLISM AND EVOLVING AXONAL INJURY AFTER EXPERIMENTAL BRAIN TRAUMA
Szu-Fu Chen, Hugh K. Richards, Piotr Smielewski, Peter Johnström, John D. Pickard, Neil G. Harris*. (1Academic Neurosurgery Centre for Brain Repair, 2Wolffson Brain Imaging Centre, & 3Clinical Pharmacology, University of Cambridge, UK).

P139. INTERPRETATION OF CEREBRAL LACTATE REDUCTION IN SEVERE HEAD INJURY: A MICRODIALYSIS STUDY.
*S Magnoni1, V Valeriani1, E Roncati Zanier1, S Ross1, A Prat1, F Prada1, N Stocchetti. (1Department of Anesthesia and Intensive Care and 2Department of Neurosurgery, Ospedale Maggiore Policlinico IRCCS, Milano).

P140. AMYLOID BETA 1-42 AND TAU IN CEREBROSPINAL FLUID AFTER SEVERE HUMAN TRAUMATIC BRAIN INJURY
G. Franz*, R. Beer, A. Kampfl. K. Engelhardt, E. Schmutzhard, H. Ulmer, and F. Deisenhammer. (Departments of Neurology and Biostatistics, University Hospital Innsbruck, Austria).

P141. TEMPORAL AND SPATIAL PROFILE OF BID CLEAVAGE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
K. Engelhardt*, R. Beer, G. Franz, S. Krajewsawi, J.C. Reed, B.R. Pike, R.L. Hayes, K.K. Wang, E. Schmutzhard, and A. Kampfl. (Department of Neurology, University Hospital Innsbruck, Austria; The Burnham Institute, La Jolla, California; McKnight Brain Institute of the University of Florida, Gainesville, Florida; Department of Neuroscience Therapeutics, Pfizer Inc., Ann Arbor, Michigan, U.S.A).

P142. TEMPORAL AND SPATIAL PROFILE OF CASPASE-6 EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
R. Beer1, G. Franz1, K. Engelhardt1, S. Krajewsawi1, J.C. Reed1, A. Buki2, T. Doczi1, N. Letfner1, E. Schmutzhard1, and A. Kampfl. (1Department of Neurology, University Hospital Innsbruck, Austria; 2The Burnham Institute, La Jolla, California, U.S.A.; 3Department of Neurosurgery, Pécs University, Hungary).

P143. MULTIDIMENSIONAL IMPAIRMENTS OF ATTENTION FOLLOWING PEDIATRIC TRAUMATIC BRAIN INJURY
Shelley C. Heaton1,2, Danielle A. Becker2, Eileen B. Fennell1,2, Olivia Puyan2, David Gribbins2 (1Center for Traumatic Brain Injury Studies, Evelyn F & William L McKnight Brain Institute of the University of Florida; 2Dept of Clinical & Health Psychology, University of Florida, Gainesville, FL).

P144. TEMPORAL PROFILE OF alpha-II-SPECTRIN BREAKDOWN PRODUCTS AFTER TRAUMATIC BRAIN INJURY IN IMMATURE RATS.
Jose.A. Pineda*, Jada M. Aikman1, Erik A. Johnson1, Brian R. Pike1, Barbara E. Osteen1, Tao Fan1 and Ronald L. Hayes1. (1Center for Traumatic Brain Injury Studies, Evelyn F & William L McKnight Brain Institute of the University of Florida. 2Division of Pediatric Critical Care Medicine, University of Florida Dept of Pediatrics).

P145. ZINC CHELATION ALTERS THE MOLECULAR PROFILE OF STRESS SIGNALING PATHWAYS IN TRAUMATIC BRAIN INJURY
H.L. Hellmich*, C. Frederickson, D.S. DeWitt, R. Saban, M. Parsley, R. Stephenson, D.S. Prough. (University of Texas Medical Branch, Galveston, Texas US).
P146. EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL mRNA EXPRESSION LEVELS OF CALPAINS AND CASPASES AFTER TRAUMATIC BRAIN INJURY IN RATS

P147. THE PREDICTIVE VALUE OF PROCALCITONIN AND S 100 B IN TRAUMATIC BRAIN INJURY
Linda E. Pelinka*, MD, Albert Kroepfi, MD, PhD and Heinz Redl, PhD. (Ludwig Boltzmann Institute of Experimental and Clinical Traumatology, A-1200 Vienna, Austria).

P148. CONTEXTUAL FEAR CONDITIONING TO ASSESS COGNITIVE DYSFUNCTION IN BRAIN INJURED MICE

P149. A 4-AXES MODEL OF THE STRESS RESPONSE
Iliadis Charalampis and Alexandra Kunz*. (Harvard University, Boston, MA US).

P150. INDUCTION OF HIGH PURITY OLIGODENDROCYTE CULTURES FROM HUMAN EMBRYONIC STEM CELLS
Gabriel I. Nistor, Minadora O. Totoiu and Hans S. Keirstead*. (Reeve-Irvine Research Center, Placentia, CA USA).

P151. QUANTIFICATION OF DIFFUSION TENSOR IMAGING PREDICTS DIFFUSE AXONAL INJURY FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

P152. RESPONSE OF NEURONS CULTURED IN TWO- AND THREE-DIMENSIONS TO DYNAMIC SHEAR DEFORMATION
D. Kasy Cullen and Michelle C. LaPlaca. (Department of Biomedical Engineering, Georgia Tech, Atlanta, GA USA).

P153. ADAPTATION OF SENSORIMOTOR AND COGNITIVE TASKS FOR USE WITH MICE: EFFECTS OF CONTROLLED CORTICAL IMPACT INJURY AT VARIOUS INSULT LOCATIONS
Yelena K. Baskin*, Annmarie J. Bramwell, W. Dalton Dietrich and Edward J. Green. (Departments of Psychology and Neurological Surgery, University of Miami, Miami, FL USA).

P154. PROGESTERONE IMPROVES BEHAVIORAL AND MORPHOLOGIC OUTCOMES AFTER TRAUMATIC BRAIN INJURY IN MALE C57BL/6 MICE
Douglas W. Lowery*, Joshua E. Logan, Deborah A. Shear, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).

P155. THE NEUROTECTIVE EFFECTS OF PROGESTERONE ARE ASSOCIATED WITH MODIFIED GENE EXPRESSION IN RAT CORTICAL IMPACT MODEL
Edward H. Pettus*, David W. Wright, Stuart W. Hoffman, Donald G. Stein. (Emergency Medicine, Emory University, Atlanta, GA US).

P156. ANESTHESIA AFFECTS GENDER-RELATED FUNCTIONAL OUTCOME FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY IN RAT
Christine O'Connor, Iboja Cernak and *Robert Vink. (*Department of Pathology, University of Adelaide, Adelaide SA, Australia; and Department of Neuroscience, Georgetown University, Washington DC, USA).

P157. A PARALLEL RANDOMIZED DOUBLE-BLIND MULTICENTRE CLINICAL TRIAL FOR THE EFFICACY AND SAFETY OF NALOXONE IN ACUTE TRAUMATIC BRAIN INJURY
Yuanli Zhao MD1, Jiyaio Jiang MD2, Li Li MD3, et al. on behalf of the National Naloxone Study Group. (Beijing Neurosurgical Institute. Shanghai Neurosurgical Institute).

P158. DOWNREGULATION OF AMYLOID PRECURSOR PROTEIN (APP) mRNA EXPRESSION FOLLOWING POST-TRAUMATIC CYCLOPSORIN-A ADMINISTRATION
*James J. Donkin1, Corinna Van Den Heuvel1, John W. Finnie2, Peter C. Blumbergs3, Tim Kuchel4, Barbara Koszyca5, Jim Manavis6. *Nigel R. Jones1, Peter L. Reilly2 and Robert Vink1. (Departments of 1Pathology and 2Neurosurgery, University of Adelaide, and 3Department of Neuropathology and the 4Veterinary Division, Institute of Medical and Veterinary Science, Adelaide, Australia).

P159. THE NEUROTECTIVE EFFECTS OF PROGESTERONE AND ALLOPREGNANOLONE AFTER CONTROLLED CORTICAL IMPACT IN RATS
Myriam J. Djebali*, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).

P160. PREGNENOLONE FACILITATES RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY
Melissa A. Arellano*, Robert M. Simkins, IV, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).
P161. STEROIDS IN SEVERE TBI: A META-ANALYSIS
Anne-Marie Guerguerian, Alexander Agthe, Sean Berenholtz, Elizabeth Bradley, Christopher Conners and Suzan Gerhardt. (Johns Hopkins Medical Institutions, Baltimore, MD US).

P162. DELIBERATE MILD HYPOTHERMIA FOR TREATMENT OF SEVERE BRAIN INJURY
Roman Gal1, Ivan Cundrte, Martin Smrcka2 (1Department of Anaesthesia and Intensive Care, 2Department of Neurological Surgery, University Hospital Brno, Brno, Czech Republic).

P163. REGULATION OF HYDROGEN PEROXIDE PRODUCTION BY BRAIN MITOCHONDRIA BY CALCIUM AND A BH3 DEATH DOMAIN PEPTIDE
Gary Fiskum3 and Anatoly Starkov. (University of Maryland School of Medicine, Baltimore, Maryland US).

P164. EFFECT OF DEXTROMETHORPHAN - A NON-COMPETITIVE NMDA ANTAGONIST - ON THE SECONDARY GROWTH OF A CORTICAL NECROSIS FROM FOCAL COLD INJURY

P165. NEURAL STEM CELL TRANSPLANTS FOLLOWING BRAIN INJURY IN RATS: CELLULAR SURVIVAL AND BEHAVIORAL CONSEQUENCES
H.M. Bramlett1, D.A. Castellanos, W.D Deitchler, E.Green, J. Sagen. (Miami Project to Cure Paralysis, U of Miami School of Medicine, Miami, FL, USA).

P166. COMPARISON OF TWO PROTOCOLS TO DIFFERENTIATE BONE MARROW STROMAL CELLS INTO NEURONS OR GLIA

P167. THE EFFECT OF RUTHENIUM RED, AIDA AND MK-801 ON MITOCHONDRIAL MEMBRANE POTENTIAL (MMP) IN STRAIN-INJURED ASTROCYTES
Karen A. Willoughby, Anthony Pellicane, Jennifer Shea and Earl F. Ellis1. (Department of Pharmacology and Toxicology, Medical College of Virginia Campus of Virginia Commonwealth University, Richmond, VA USA).

P168. GLIAL NEURONAL SIGNALING IN NEUROTUMA, STUDIED IN PRIMARY CULTURES

P169. SIGNALING FROM ATP RECEPTORS TO ERK IN AN IN VITRO MODEL OF TRAUMATIC BRAIN INJURY
Joseph T. Neary,1 Yuan Kang1, Minh Tran1, Karen A. Willoughby1, Earl F. Ellis2. (1Research Service, VA Med. Ctr., Dept. Pathol. and Biochem. & Molec. Biol. and Neuroci. Program, University of Miami School of Medicine, Miami, FL and 2Dept. Pharmacol. & Toxicology, School of Medicine, Virginia Commonwealth University, Richmond, VA).

P170. DELAYED TREATMENT WITH DEHYDROEPIANDROSTERONE SULFATE ENHANCES GENE EXPRESSION RELATED TO NEUROPLASTICITY
Stuart W. Hoffman1, Edward H. Pettus, Robert M. Simkins, IV, Donald G. Stein. (Emory University, Atlanta, Georgia US).

P171. TIMING OF PHYSICAL EXERCISE FOLLOWING MILD TRAUMATIC BRAIN INJURY: IMPACT ON STROOP TASK PERFORMANCE

P172. THE DELAYED ADMINISTRATION OF DEHYDROEPIANDROSTERONE SULFATE PROMOTES RECOVERY OF FUNCTION AFTER CORTICAL IMPACT INJURY
Sharad Virmani, Robert M. Simkins, IV, Donald G. Stein, Stuart W. Hoffman. (Emory University, Atlanta, Georgia US).

P173. TRAUMATIC PERIMESENCEPHALIC SUBARACHNOID HEMORRHAGE: A SIGN OF BRAINSTEM INJURY
Won-kyong Bae1, Kyung-suk Lee2 (Departments of Radiology1 and Neurosurgery2, Chonan Hospital, Soonchunhyang University, Chonan, Chungnam, Korea).

P174. LOSS IN CORRELATION BETWEEN ADMISSION GCS AND OUTCOME IN PATIENTS WITH MULTIMODAL BEDSIDE MONITORING
Balestren1, Steiner LA1,2, Chatfield DA1, Schmidt EA1, Czosnyka M1, Pickard JD1. (1Academic Neurosurgery and 2University Department of Anaesthesia, Addenbrooke’s Hospital, Cambridge, UK).

P175. DIFFUSION WEIGHTED MAGNETIC RESONANCE IMAGING OF EDEMA FOLLOWING TRAUMATIC BRAIN INJURY IN RATS
P176. VASCULAR TUNNEL CREATION AND FURTHER SPACE WINNING METHODS IN THE TREATMENT OF AGGRESSIVE BRAIN SWELLING.
Andras Csokay*, Laszlo Nagy, Gergely Pataki. (National Institute of Traumatology, Budapest, HU).

P177. CONCEPT OF 'TRUE ICP' IN MONITORING AND PROGNOSTICATION IN HEAD TRAUMA
Marek Czosnyka*, Luzius Steiner, Marcella Balestrii, Eric Schmidt, John D.Pickard. (Academic Neurosurgical Unit, Addenbrooke's Hospital, Cambridge, UK).

P178. ACCUMULATION OF CALPAIN AND CASPASE-3 CLEAVED ALL-SPECTRIN BREAKDOWN PRODUCTS IN CSF OF PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY.

P179. MODELLING INTRACRANIAL PRESSURE INSULTS IN HEAD-INJURED PATIENTS USING ARTIFICIAL NEURAL NETWORKS.
Peter D. Hay*, Hannah K. Bayes, Ian R. Piper, Laurence T. Dunn. Centre for Mathematical and Computational Science in Medicine, University of Glasgow, UK; Department of Neurosurgery, Institute of Neurological Sciences, University of Glasgow, UK.

P180. THE EFFECT OF MICROGlia ABLATION FOLLOWING TRAUMATIC BRAIN INJURY IN MICE
Mieko Eda. (Chiba University Hospital, Chiba JP).

P181. OVEREXPRESSION OF X-LINKED INHIBITOR OF APOPTOSIS PROTEIN IMPROVES FUNCTIONAL RECOVERY AFTER CONTROLLED CORTICAL IMPACT INJURY IN THE MOUSE.
Susan M. Knoblauch*, Xiao D., Peter Liston, Alan I. Faden. (Department of Neuroscience, Georgetown University; Children's Hospital of Eastern Ontario, Ottawa, Ontario).

P182. INFORMATION PROCESSING DEFICITS AND THEIR RELATIONSHIP TO NEUROIMAGING FOLLOWING MODERATE AND SEVERE TRAUMATIC BRAIN INJURY

P183. "KEY HOLE" APPROACH FOR THE MANAGEMENT OF NEURO-TRAUMA
I. Melamed*, G. Zucker, V. Merkin, E. Reichenthal. (Soroka Medical Center, Beer-Sheva, IL).

P184. DETRIMENTAL ROLE OF BRADYKININ B2 RECEPTORS FOLLOWING CLOSED HEAD INJURY IN MICE
Helfant F., Prunet D., Croci N., Palmier B., Potkine M. and Marchand-Verrechia C. (UPRES, Université René Descartes, Paris, France; Laboratoires Fournier, Dax, France).

P185. SECONDARY GROWTH OF A CORTICAL NECROSIS FROM COLD INJURY IN WILD-TYPE AND INOS-DEFICIENT MICE

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P433. SRC FAMILY KINASE INHIBITOR PP1 IMPROVES MOTOR FUNCTION AFTER SPINAL CORD CONTUSION IN RATS.
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P435. S-100BETA LEVELS AND MYELOPEROXIDASE ACTIVITY AFTER SPINAL CORD INJURY IN THE RAT.
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P439. THE EFFECTS OF DELAYED BUT PROLONGED HYPOTHERMIA ON THE PIAL VASCULAR RESPONSE AFTER TRAUMATIC BRAIN INJURY IN RATS
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P450. TRANSCRIPTIONALLY PROFILING THE EFFECTS OF CHRONIC METHYLPHENIDATE TREATMENT IN RATS AFTER TRAUMATIC BRAIN INJURY
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P454. MILD OR MODERATE TRAUMATIC BRAIN INJURY: BEHAVIORAL AND HISTOPATHOLOGICAL OUTCOMES IN MICE
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P457. TRAUMATIC AXONAL INJURY DIFFERENTIALLY IMPAIRS FAST- VS. SLOW-CONDUCTING CORPUS CALLOSUM FIBERS.
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P459. NON-INVASIVE ASSESSMENT OF ICP FROM CEREBRAL BLOOD FLOW VELOCITY AND ARTERIAL BLOOD PRESSURE USING A FUZZY PATTERN CLASSIFICATION METHOD
B. Schmidt1, S.F. Bockisch1, M. Päßler1, M. Czosnyka1, J.J. Schwarz2, J. Klingelhöfer1. (1Dept. of Neurology, Chemnitz Medical Centre, Chemnitz, Germany; 2Dept. of Systems Theory, Technical University, Chemnitz, Germany; 3Academic Neurosurgical Unit, Addenbrooke's Hospital, Cambridge, UK).

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P464. CASPASE INHIBITION AFTER TRAUMATIC BRAIN INJURY ALTERS AMYLOID PRECURSOR PROTEIN AND AMYLOID-BETA PRODUCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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P466. INCREASED EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN RAT BRAIN AFTER TRAUMATIC BRAIN INJURY
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P467. EXPLORATORY STUDY OF ACUPUNCTURE TREATMENT ON TRAUMATIC BRAIN INJURY (TBI) IN RATS
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P468. CHRONIC IMPAIRMENT OF EXTRACELLULAR K+ HOMEOSTASIS FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT.
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P469. THE mGluR1 ANTAGONIST AIDA REDUCES POST-TRAUMATIC EMPTYING OF CALCIUM STORES IN NEURONS AND ASTROCYTES
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S. Belé*, A.Brawanski (Departement of Neurosurgery, University of Regensburg, Regensburg, Germany).

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P479. DOWN REGULATION OF AQUAPORIN-4 IN AREA ADJACENT TO BRAIN INJURY IN A TRAUMATIC RAT BRAIN MODEL.
*Ming-Chieh Sun*, M.B., Christopher R. Honey*, D.Phil†, Ming-Chieh Sun†‡, Christopher R. Honey§, Norman L. M. Wong†, Joseph K.C. Tsui*. (Cardinal Tien Hospital and Fu-Jen Catholic University, Taipei, Taiwan; †Divisions of Neurosurgery, ‡Nephrology, and §Neurology, University of British Columbia, Vancouver, British Columbia).

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In your opinion, was information presented by each speaker presented fairly and without commercial or promotional bias? 
__________ Yes  __________ No  If no, please explain: ____________________________

Please rate the overall program by circling the appropriate letter in both columns. (A=Excellent, B=Good, C=Fair, D=Poor)

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Please rank the deciding factors in attending: ___Topics ___Location ___Faculty ___Other

What did you find most interesting?

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Suggestions for next course?
COURSE OBJECTIVES

Please rate how well N-INTS 2002 met the following educational objectives.

Upon the completion of The Symposium, participants will be able to:

- Discuss the recent, innovative techniques in CNS injury that include transgenic and gene knockout mouse models, advanced MR methods, stem cell biology, biopolymers and developmental molecules in cord lesions, the role of proteases in neuronal injury and nogo and axonal regeneration in the CNS.

- Outline molecular mechanisms of cell death in cerebral ischemia, the role for caspases, cyclooxygenases and excitotoxin in death due to stroke, and describe the significance of late-breaking news in CNS injury research.

- Discuss the characteristics of successful clinical trials in CNS injury and their limitations.

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___ INTS 2004 in Australia  ___ 2003 Society for Neuroscience  ___ Other: ____________________________

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On behalf of the International Neurotrauma Society (INTS), the Organising Committee of the 7th International Neurotrauma Symposium invites all members of the international neurotrauma community to join colleagues in Adelaide, South Australia 12-16 September 2004.

The Conference will be held at the Adelaide Convention Centre, in the centre of the city and adjacent to Adelaide's theatre and restaurant districts.

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Abstracts

The First Joint Symposium of the National and International Neurotrauma Societies

The 20th Annual National Neurotrauma Society Symposium and

The Sixth International Neurotrauma Symposium

October 27–November 1, 2002 Tampa, Florida
P101.
REGIONAL HYPERGLYCEMIA IS CHARACTERIZED BY DECREased GLucose TRANSPORT AND PRESERVED HEXOKINASE ACTIVITY FOLLOWING TRAUMATIC HEAD INJURY.


Koranic analysis of dynamic F-18 fluoro-deoxyglucose (FDG) positive emission tomography (PET) permits in-vivo assessment of glucose transporter and hexokinase activities regionally in human brain. The purpose of this study was to investigate changes in glucose delivery and phosphorylation regionally in patients with focal head injury with relation to hyperglycemia. Methods: Twelve patients (4 women, 38 ± 12 years old, range 17 to 58 years) with focal head injury underwent three-dimensional dynamic PET with FDG. Median initial GCS was 14 (range: 3-14) and PET scans were performed 3.4 ± 2.7 days after injury. Dynamic tissue activities were obtained from the regions-of-interest (ROIs) on the reconstructed PET images. Activities of glucose transporter (K1) and hexokinase (K3) were estimated using a 2-compartment kinetic model of FDG and a non-linear least-square fitting. ROIs of minimal FDG uptake, while FDG uptake was relatively preserved in remote cortex (remote). All patients had at least one Peri-Contusional area with decreased FDG uptake (Peri-Low), while 7/12 also had pericortical areas showing increased FDG uptake (Peri-High) compared to remote cortex. Peri-High showed slightly higher metabolic rate of glucose (MGR) than Remote (4.8 ± 1.3 vs. 4.0 ± 0.7, mg/100g/min, p < 0.05). Kinetic analyses showed preserved K1 in Peri-High (0.056 ± 0.020 min) compared to Remote (0.055 ± 0.016 min, p = n.s.), which was higher than K3 in Peri-Low (0.049 ± 0.012 min, p < 0.05) and Cupton (0.042 ± 0.018 min, p < 0.05). On the other hand, K1 in PC-High (0.068 ± 0.015 ml/min) was lower than Remote (0.088 ± 0.015 ml/min, p < 0.05), similar to PC-Low (0.059 ± 0.026 ml/min, p = n.s.), and higher than Contusion (0.024 ± 0.019 ml/min, p < 0.001). Conclusions: Regional FDG uptake generally reflects activity of glucose transporter. However, in the pericontusional region with high FDG uptake, the glucose transporter and hexokinase activity is uncoupled, showing preserved hexokinase activity despite of reduced glucose transporters. (NS30308; UCLA Brain Injury Research Center).

P102.
TOPICAL L-ARGININE, BUT NOT NITRIC OXIDE DONOR, RESTORES CEREBROVASCULAR PRESSURE AUTOREGULATION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS: POSSIBLE ROLE OF ENDOTHELIAL NITRIC OXIDE SYNTHESE.


Cerebral blood flow (CBF) is maintained constant over a range of systemic blood pressure by autoregulation. Impaired pressure autoregulation has been reported in both clinical brain injured patients and animal models of traumatic brain injury (TBI). Nitric oxide (NO) plays a significant role in maintaining pressure autoregulation. We sought to determine 1) whether NO donor or NO synthase (NOS) substrate can improve the post-traumatic pressure autoregulation, 2) if so, which isofoms of NOS participate in the autoregulation. Halothane anesthetized rats underwent controlled cortical impact (CCI) injury. Controlled hemorrhage was performed to create a stepwise fall in arterial pressure. Cortical CBF was monitored by laser Doppler flowmetry on an open cranial window. Static CBF pressure autoregulation curve in the hypertensive phase was plotted. Moderate CCI resulted in disruption of autoregulation during hemorrhagic hypotension. The slopes of the autoregulation curves in sham-injured group (n = 7) are significantly lower than that of the CCI group (n = 10) when mean arterial pressure (MAP) dropped from 90mmHg to 70mmHg (p < 0.05, t-test). Topical superfusion of S-nitroso-N-acetylpenicillamine (SNAP) does not affect the impaired autoregulation curve in injured rats (n = 10; p > 0.05 from sham-injured, ANOVA). In contrast, superfusion of L-arginine fully restores the autoregulation curve during the above MAP range (n = 10; p > 0.05 from sham-injured, ANOVA). Further, this restoration effect by L-arginine was not attenuated by intraperitoneal injection of 7-nitroindazole, a selective neuronal NOS inhibitor, (n = 10; p > 0.05 from sham-injured, ANOVA). We demonstrated that there is a loss of static CBF pressure autoregulation after moderate CCI. NOS substrate, but not exogenous NO, can restore the autoregulation following TBI. Our data suggests that regulatory NOS activity is required for CBF pressure autoregulation. Furthermore, the fact that 7-nitroindazole does not attenuate the restoring effect rendered by L-arginine indicates that endothelial NOS, but not the neuronal NOS, may be responsible for mediating CBF pressure autoregulation.

P103.
TUMOR NECROSIS FACTOR RECEPTOR FAMILY MEMBERS MEDIATE POSTTRAUMATIC CELL DEATH AFTER CONTROLLED CORTICAL IMPACT IN MICE

Michael J. Witten*, SC Hoang, RH Ueda, David McCarthy, and Michael A. Moskowitz. (Massachusetts General Hospital, Boston, MA, US).

We previously reported upregulation of Fas death inducing signaling complexes (DSC) associated with activation of caspasas in brain after experimental and human traumatic brain injury (TBI) Neurosurgery 2002, 22:330-331). To test the hypothesis that Fas mediates cell death after TBI, we performed controlled cortical impact (0.9 mm depth, 6 m/s) in Fas knockout vs. wild type mice. Fas knockout mice did not differ from wild type in the number of cortical TUNEL positive cells at 6 or 24 h, number of caspase 3 p20 immunoreactive cells at 48 h, or in caspase volume at 21 d, suggesting that other death receptor(s) might compensate the deletion of Fas. In support of this hypothesis, we detected upregulation of tumor necrosis factor receptor factor 1 (TNFR1) and TNFR1 DSC assembly in brain homogenates early after CCI using immunoprecipitation and Western blot. In addition, fluorescence immunohistochemistry revealed laminar demarcation of cortical necrosis, foci of caspase activation, and colocalization of TNFR1 with its adapter proteins TRADD and FADD in neurons and with TUNEL positive neurons early after CCI. However, posttraumatic contusion volume did not differ between TNFR1 knockout and wild type mice. To test the hypothesis that Fas or TNFR1 may compensate antagonism of the other death receptor, we administered blocking anti-TNF antibodies (2 µg i.c.v./2 mg kg i.p.) to wild type (n = 4) or Fas knockout mice (n = 3) and then performed CCI. Contusion volume in treated Fas knockout mice (Mean + SD; 3.3 ± 0.7 mm3) was reduced by over 2.5 fold compared to untreated wild type mice (8.5 ± 0.9 mm3) (p < 0.001). These results suggest that both Fas and TNFR1 contribute to cell death after TBI, and that strategies targeting both death receptors simultaneously, or their biochemical convergence points, are required to inhibit posttraumatic cell death. Support: NINDS K08 NS41969-01 (MWF) and 5 R01 NS37141-05 (MM).
P105.
EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL CASPASE-12 mRNA AND PROTEIN EXPRESSION LEVELS AFTER TRAUMATIC BRAIN INJURY IN RATS.

A novel apoptotic pathway involving the endoplasmic reticulum (ER) and ER stress has emerged with caspase-12’s discovery that is independent of the previously described intrinsic and extrinsic pathways. We examined regional and temporal caspase-12 mRNA and protein expression and its potential downstream protein target caspase-3 after traumatic brain injury (TBI). mRNA expression levels of caspase-9 were also examined. The mRNA transcript levels were determined in ipsilateral cortex and hippocampus after cortical impact TBI by quantitative RT-PCR.

Caspase-12 cortical mRNA expression increased significantly to 1.376%, 1.374%, and 3.315% of naïve by 120 hours while hippocampal mRNA levels reached 677%, 571%, and 695% within six hours post-TBI for 1.6mm, 1.2mm, and 1.6mm injury magnitudes, respectively.

In addition, we have shown that caspase-3 RNA levels increased by 400% in the cortex and by 200% in the hippocampus samples compared to naïve. In contrast, cortical and hippocampal mRNA levels for caspase-9 either narrowly or never exceeded naïve.

Western blots showed caspase-12 protein upregulation and activation within 24 hours, peaking within three days, while activated caspase-3 days.

The over 1300% increase in cortical caspase-12 mRNA expression and near 600% for the hippocampus plus significant increase in active form of caspase-12 protein suggests that caspase-12 may play an important role in cellular apoptosis following TBI and indicates an ER-mediated pathway to caspase-3 activation. The results also suggest that caspase-3 may be the downstream target of caspase-12. (Supported by DAMD 17-99-1-9365 and NIH R01 NS 39091)

P106.
5,6-EPoxyEicosatrienonic Acid - MEDIATED Ca2+ SIGNALING IS ENHANCED IN MICROGLIA ACTIVATED BY EXPOSURE TO SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.
S. Merchant*, S. Farde*, R. Fry*, B. Balster*, C. Liang*, J.R. Fultz*, and B. A. Rozgański*, (University of Central Florida, Dep of Molecular Biology & Microbiology, Orlando, FL, University of Texas, Southwestern Medical Center, Dep of Biochemistry & Pharmacology, Dallas, TX USA).

Traumatic brain injury (TBI) induces a state of microglial activation, including upregulation of macrophage characteristics and activation of an inflammatory response. Activated microglia (MÎ) are reported to have both neuroprotective and neurodegenerative effects, depending on the degree of activation and type of injury. However, the microglial signaling pathways leading to activation in response to TBI are not clear. Using an in vitro model for TBI (cell strain or stretch), we have previously shown that MÎ are not directly activated by strain injury. Indirect activation of MÎ was induced by exposure of unjured MG to medium conditioned by traumatally injured astrocytes. We now report that indirect activation of MÎ increases activity of intracellular store-operated Ca2+ channels (SOC). The arachidonic acid epoxide 5,6-epoxyeicosatrienonic acid (5,6-EET), is a putative "Ca2+ Infux Factor", which activates SOC-mediated Ca2+ influx. Exposure of resting MG to 5,6-EET elicited only a weak influx of extracellular Ca2+. Exposure of indirectly activated MÎ to 5,6-EET induced a dose-dependent influx of Ca2+, suggesting increased SOC channel activity. The glutamate- and 5,6-EET-stimulated Ca2+ influx activated MG, was blocked by the imidazole antymotic econazol, the SOC inhibitor SKF90063, and MS-POPH, a specific inhibitor of the P450 isozyme that produces 5,6-EET. These results suggest that injured astrocytes release soluble factors that upregulate SOC activity in MG, possibly via increased microglial cytotoxicity P450 activity and production of 5,6-EET. Supported by NS40490.

P107.
LOCAL TREATMENT WITH PHOSPHOCREATINE IMPROVES INJURY-INDUCED METABOLIC AND ELECTROPHYSIOLOGICAL CHANGES AFTER TBI.
Oscar L. Alves, Thomas M. Renes, M. Ross Bullock (Division of Neurosurgery, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA USA).

Introduction: Energy failure is a ubiquitous complication after traumatic brain injury (TBI). We have studied whether phosphocreatine (PCr), a high energy phosphate, delivered through a microdialysis probe, would ameliorate TBI-induced metabolic and electrophysiological changes.

Material and Methods: Adult Sprague-Dawley rats were submitted to 2.1 ± 0.5 atm fluid percussion injury. A custom probe (4 mm microdialysis membrane with attached recording electrodes) was inserted in the injured hemisphere. After 1 hour of baseline recording, using 0.9% NaCl perfusion, 10 mg/ml CrP was added to the perfusion solution, and measurements continued for 3 hours. Brain biopsies under frozen conditions were obtained for tissue adenosine triphosphate (ATP) measurements.

Results: Local treatment with CrP resulted in 73 ± 5% increases in diastolic glucose (p < 0.05), 60 ± 4% decreases in lactate (p < 0.001), and 70 ± 16% decreases in glutamate (p < 0.001). Additionally, multifocal neuronal firing rate was increased, as well as brain ATP levels.

Conclusions: Local treatment with CrP seems to ameliorate brain energy metabolism by bolstering intracellular “energy” substrates, and possibly through a stabilizing effect of creatine on mitochondria permeability transition pore.

Supported by NS 12587 and FCT no SFHR/B/D/342/2000.

P108.
HEME OXYGENASE-2 PREVENTS LIPID PeroxIDATION-MEDIATED CELL LOSS AND PROMOTES FUNCTIONAL RECOVERY AFTER TRAUMATIC BRAIN INJURY.
EE Chang*, T Igarashi, RJ Wong, HJ Vreman, DK Stevenson, LJ Noble. (University of California, San Francisco, San Francisco, California USA).

After traumatic brain injury, extracellular heme, derived from both hemorrhage and cell injury causes oxidative stress to neuronal tissue. Oxidative stress, induced by free radical reactions and lipid peroxidation, has been implicated as a key mediator of secondary traumatic brain injury. Heme oxygenase (HO) has been proposed as a critical mediator of the detoxification of heme, because it metabolizes the pro-oxidant heme to the potent antioxidant, bilirubin. It is currently unclear what role HO-2, the predominant and constitutively expressed isozyme in neurons, plays in traumatic brain injury. We used HO-2 knockout mice to determine the extent and mechanism of damage following controlled cortical impact (CCI) injury. Based on NeuN (neuronal nuclei antibody) immunohistochemical cell counting, regional cell loss was more severe in knockout than in wildtype animals, especially in the periinjurious cortex and thalamus. In addition, HO-2 knockout mice demonstrated significantly limited recovery on rotarod and inclined beam walking tasks, suggesting compromised motor function and behavior. Brain sonicates of knockout mice revealed significantly less total HO activity than wildtype littermates. Knockout mice also demonstrated decreased ability to reduce oxidative stress, as measured with an Fe2+/ascorbic acid-mediated CO generation assay for lipid peroxidation. Finally, Western blots showed that the low HO-1 expression did not change in injured HO-2 knockout animals. These findings suggest that HO-2 activity protects neurons in the setting of traumatic brain injury by reducing lipid peroxidation, possibly through its catalysis of heme.

Supported by NS 14543 and the UCLA Neurotrauma Initiative.
P109.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ASSESSMENT OF F2-ISOPROSTANE LEVELS IN CSF AFTER TRAUMATIC BRAIN INJURY IN RATS

Traumatic brain injury (TBI) causes 50,000 deaths/year. Although recent studies indicate that oxidant injury may be deleterious, progress in this area has been hampered by lack of adequate biomarkers for oxidant injury. F2-isoprostane is a stable peroxidation product of cell membrane phospholipids that increases dramatically during oxidant injury. The objective of our study was to provide a quantitative and sensitive assessment of F2-isoprostane levels in CSF after TBI.

A cortical impact injury device was used to produce TBI in rodents (n = 30). Sprague-Dawley rats (230-300 g) were anesthetized with isoflurane and mounted in a stereotactic frame. A craniotomy was performed, and TBI was produced by impacting the cortex with a 5 mm diameter impactor tip at a velocity of 3.5 m/s with a 1.6 mm compression and 120 ms dwell time. Sham-injured animals (n = 20) underwent identical surgical procedures without receiving an impact injury.

Methods: CSF was collected at −1 to time points after sham-injury or TBI. F2-isoprostane levels were measured with a stable isotope dilution assay using capillary gas chromatography / negative ion chemical ionization mass spectrometry. Investigators performing measurements were blinded to the experimental condition the animals. Group means were compared by one-way analysis of variance where p < 0.05 was considered statistically significant.

Results: Mean (±SD) F2-isoprostane CSF levels for sham-injured vs. TBI animals were 11 ± 10.36 ± 18 ± 10 ± 30 min post-injury (p = 0.003), 1 ± 2 vs. 91 ± 60 ± 2 hours post-injury (p = 0.05), and 1 ± 4 x 2 ± 4 ± 3 ± 13 ± 6 hours post-TBI (p = 0.0003), respectively.

Conclusion: Compared to the sham-injured group, CSF F2-isoprostane levels were significantly higher in the TBI group at 30 minutes, 1 and 6 hours after TBI. These results indicate that oxidant injury occurs rapidly after TBI, and that assessment of F2-isoprostane levels in CSF can provide a quantitative and sensitive measure of oxidant damage. (Supported by DAMD17-99-1-9565, DAMD17-01-07655, NIH ROI NS39091, and NIH ROI NS0182)

P110.

TEMPORAL AND SPATIAL PROFILE OF PHOSPHORYLATED MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS
Naoki Ota, Hiroshi Nakashiro, Katsuki Shima, National Defense Medical College, Tokorozawa, Saitama, JP.

(Introduction) Mitogen-activated protein kinases (MAPK), which play a crucial role in signal transduction, are activated by phosphorylation in response to a variety of mitogenic signals. The MAPK cascades are composed of extracellular signal-regulated protein kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 pathways. The aim of this study was to investigate the temporal and topographic expression of the activated MAPK pathways after traumatic brain injury (TBI) in rats. (Material & Methods) Adult male Sprague-Dawley rats (300–400 g) were subjected to lateral fluid percussion injury of moderate severity (3.5–4.0 atm) using the Dragonfly device model (No. HPD-1700). The phosphorylated- or total-MAPKs protein level 3, 15, 30 min, 1, 6, 24, 72 hrs after TBI was quantified using Western blot analysis. Each of the cortical or hippocampal tissue. Topographic distribution of immunoreactivity for p-MAPKs was examined using immunohistochemistry at the same time course. (Results) TBI significantly increased the p-ERK and p-JNK levels, but not the p-p38 protein levels. The immunoreactivity of the p-JNK was uniformly induced regardless of any regional selective vulnerability to TBI. In contrast, the immunoreactivity for p-ERK was confirmed up to 30 min after TBI in the superficial neuronal layers, and was not detected in the CA1 neurons, but was localized in the dentate hilar and the damaged CA3 neurons after 30 min of TBI. Double immunostaining using a glial-specific marker demonstrated that p-ERK was prominent in astrocytes 6 hrs after TBI. (Conclusion) The current results suggest that the ERK and JNK pathways, but not the p38 MAPK pathways are involved in signal transduction after TBI. Strong immunoreactivity for p-ERK was observed in the dentate hilus and the CA3 pyramidal neurons, selective hippocampal vulnerable lesions to TBI. These findings suggest that a distinct MAPKs cascade might therefore participate in the selective vulnerability after TBI.

P111.

TRANSPLANTATION OF NGF-EXPRESSING NT2 NEURONS ATTENUATES A LEARNING DEFICIT FOLLOWING CONTRA-LATERAL CORTICAL IMPACT BRAIN INJURY IN MICE
Deborah J. Watson*, Lucas Long*, Scott Fujimoto, Adam Langhille, Carl T. Fulp, Nicolas Royer, Chen Zhang, Kathryn E. Sautman, John H. Wolfe, Tracy K. McIntosh, (Neurology, Children's Hospital of Philadelphia, PA. 1Department of Neurosurgery, University of Pennsylvania. 2Anesthesiology and Critical Care Medicine, Galileo Maggiore Policlinico IRCCS, Milan, Italy. 3Veteran Administration Medical Center, University of Pennsylvania.)

In this study, we tested the hypothesis that nerve growth factor (NGF)-expressing NT2 neurons transplanted into the basal forebrain of brain-injured mice can attenuate long-term cognitive dysfunction by protecting the NGF-responsive cholinergic neurons of the septo-hippocampal pathway. Undifferentiated NT2 cells were transplanted with a lentiviral vector to release NGF (0.2 ng/hr/10(4) cells), differentiated into NT2/N neurons by exposure to retinoic acid and transplanted (20,000 cells in 2 ul) into the medial septum of mice 24 hours following contralateral cortical impact (CCI) brain injury or sham surgery in anesthetized mice. Mice (n = 28) were randomly assigned to one of four groups: 1) sham (operated but uninjured) injected with vehicle; 2) brain-injured injected with vehicle; 3) brain-injured injected with undifferentiated NT2 neurons; 4) brain-injured injected with transduced NGF-NT2 neurons. Cognitive function (learning) was evaluated with the Morris Water Maze at 4 weeks post-injury/surgery. Sham injured mice performed better than the brain-injured mice (p < 0.01). Cognitive function of the brain-injured group engrafted with NGF-NT2 neurons was significantly better than the injured group receiving vehicle (p < 0.05) or undifferentiated NT2 transplants (p < 0.01). These data suggest that ex vivo gene therapy may attenuate cognitive dysfunction following traumatic brain injury. Supported by a Merit Review grant from the Veterans Administration, NIH P01-NS08030, NIH RO1-NS49978, NIH DK42707 and NS38690

P112.

NEURAL PROGENITOR CELL TRANSPLANTS SHOW LONG-TERM SURVIVAL AND ENHANCE BEHAVIORAL RECOVERY IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY
Deborah A. Shear*, Matthew C. Taise*, David R. Archer, Stuart W. Hoffman, Yvonne D. Hauck, Michelle C. LePlaca, and Donald G. Stein, (1Dept. of Psychology, 2Neurology, 3Emergency Medicine, 4Pediatrics, Emory University, 5Dept. of Biomedical Engineering, Georgia Tech/Emory, Atlanta, GA. 6Field Neurosciences Institute, Saginaw, MI).

The goal of this study was to assess whether neural progenitor cell (NPC) transplants could enhance recovery from behavioral deficits resulting from traumatic brain injury (TBI). NPCs were derived from mouse cerebrocortical tissue containing a transgene for an actin promoter tagged with green fluorescent protein (GFP) and cultured as neurospheres in FGF-containing medium. NPCs were injected into the ipsilateral striatum of adult C57BL/6 mice 1 wk following unilateral cortical impact injury. Motor and spatial learning abilities were assessed on a rotodisk task and in a Morris water maze (MWM) task over a 12-month period. Significant improvements in motor abilities were observed in NPC-treated mice as early as one week and were sustained out to 12 months post-transplant. In addition, NPC-transplanted mice showed significant improvement in spatial learning abilities at 3 months, whereas an intermediate treatment effect was detected at 1 and 12 months. Following behavioral testing, animals were perfused and NPC survival, migration, and differentiation were assessed. Initially, NPCs remained near the injection site and subsequently migrated into the penumbra surrounding the injured hippocampus where they were observed at 3 and 12 months post-transplant. Confocal microscopy revealed that transplanted GFP+ NPCs co-label for NG2 but not for neuronal, astrocytic, or microglial markers, suggesting that these cells are NG2+ oligodendrocyte progenitor cells. In conclusion, transplanted NPCs survive in the host brain up to 12 months, enhance motor and cognitive recovery, and may play a role in remyelination following TBI.

Acknowledgments: Support for this project was generously provided by an NSF Fellowship (to DAS) and a Field Neurosciences Institute Research Gift (to DGS).
P113. ACTIVATED EGF SIGNALING AND TRANSPLANTED NEURAL STEM CELL MITILITY

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The epidermal growth factor receptor (EGF) has been shown to regulate migration in stem cells, through direct and indirect mechanisms. To test the hypothesis that activated EGF signaling enhances transplanted stem cell motility, we transduced neural stem cells with the constitutively active EGF (EGF-RIII). The ligand independent, constitutively active EGF-RIII receptor (and empty vector controls) was stably transfected using the lipofectamine reagent into the C17.2 mouse neural stem cell line. The C17.2 EGF-RIII cells (15,000 cells/ml 2ml per injection; one injection site per animal) and empty vector controls were then transplanted into anesthetized (sodium pentobarbital 60mg/kg), immuno-suppressed (Cyclosporin A 10mg/kg Lp injection), un.injected (n = 12/ cell type) adult Syrian-Dawley male rats and into rats subjected to lateral liquid perfusion (FP) brain injury (n = 12/ cell type). All stereotactic injections were done into the corpus callosum (Bregma +1.1ML, 2.22DV, -4.5AP) contralateral to the site of injury.

At 2 weeks post transplant, all animals were sacrificed under anesthesia and assayed by X gal immunohistochemistry and by immunoreactivity to rabbit anti-EGF-RIII/EGF-RIII using a gift of Albert Woll, M.D., Philadelphia, PA. At 2 weeks post transplant, only C17.2 EGF-RIII-overexpressing cells were found to survive and migrate extensively in all transplanted animals. In the animals subjected to lateral FP injury, transplanted C17.2 EGF-RIII stem cells were seen crossing the corpus callosum (migrating as far as 3 mm from the transplant site and entering the injury cavity. Many cells were found within the injury cavity as well. In none of the C17.2 empty vector transplants were cells seen to cross the corpus callosum. In naive (uninjured) animals, the empty vector C17.2 cells were also non-migratory. Membrane EGF-R III expression was seen to remain robust in the transplants after 2 weeks in vivo.

These results suggest that activated EGF signaling may contribute to a migratory phenotype in neural progenitor cells in vivo. By enhancing EGF signaling on stem cells in vivo, these highly migratory stem cells may represent a better source of cells for neuro-transplantation following CNS injury.

P114. INHIBITION OF NOGO-A IMPROVES RECOVERY OF NEUROMOTOR AND COGNITIVE FUNCTION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

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Severe traumatic brain injury (TBI) leads to long-term neuromotor and cognitive deficits, and limited recovery occurs in human patients. The inability of the central nervous system (CNS) to regenerate appears to be due, in part, to inhibitory molecules associated with myelin. The role of one of these myelin-associated proteins, Nogo-A, has been extensively studied. Nogo-A inhibits neurite outgrowth in vitro, and blockade of this molecule, in vivo, leads to functional recovery as well as regeneration and plasticity of the injured CNS.

We subjected rats to a lateral fluid perfusion (FP) brain injury, a well characterized model of TBI, and administered a novel and purified monoclonal antibody against Nogo-A (mab 11C7C7, kindly provided by Novartis, Basel Switzerland) for two weeks intracerebroventricularly (ICV). Rats were assessed behaviorally using several neuromotor function tests up to 4 weeks post injury. Brain injured rats receiving mAb 11C7C7 recovered significantly better than controls receiving a control antibody. Interestingly, a test assessing predominantly somatosensory motor function (adhesive paper test) was not influenced by the type of antibody used. Furthermore, Nogo-A inhibition significantly improved performance of brain injured rats in a spatial learning task in the Morris water maze at 4 weeks post injury. However, using antegrade tract tracing methods with biotinylated dextran amine (BDA), we were unable to observe an improvement, due to the type of antibody used. Furthermore, Nogo-A inhibition significantly improved performance of brain injured rats in a spatial learning task in the Morris water maze at 4 weeks post injury. However, using antegrade tract tracing methods with biotinylated dextran amine (BDA), we were unable to observe an improvement, due to the type of antibody used. Furthermore, Nogo-A inhibition significantly improved performance of brain injured rats in a spatial learning task in the Morris water maze at 4 weeks post injury. However, using antegrade tract tracing methods with biotinylated dextran amine (BDA), we were unable to observe an improvement, due to the type of antibody used.

P115. VOLUNTARY EXERCISE THERAPY AFTER TBI: A CRITICAL WINDOW OF RETURN TO FUNCTION

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Voluntary exercise leads to an upregulation of brain derived neurotrophic factor (BDNF) in the rat. This activity-induced enhancement of neuroplasticity may be considered for the treatment of TBI. During the first postinjury week, the brain is undergoing dynamic restorative processes and metabolic changes that will strongly influence the outcome of exercise. Therefore, the postinjury timing of exercise is crucial. To address this, rats were subjected to either sham injury (FP) and were housed with or without access to a running wheel (RW), from postinjury day 0-7 (acute) or 7-14 (delayed). Rats were cognitively assessed in the Morris Water Maze after RW exposure. As reported previously non-injured animals benefited from RW exposure. RW exposure alone proved to be beneficial in the delayed-FPI-RW animals, as indicated by a 52% improvement in the MMW, compared to the FPI-sedentary rats. However, cognitive performance in the acute FPI-RW rats was impaired. Whereas the sham exercised animals showed an improvement, the acute FPI-RW rats were impaired compared to all the other groups (p < 0.05). No difference was observed between the FPI and sham-sedentary rats. Exercise was related to increased levels of hippocampal BDNF in the sham (p < 0.05) but not in the acute FPI-RW rats. Increased levels of hippocampal synapsin I (p < 0.001) and cyclic AMP element-binding protein (CREB) (p < 0.001) were present in the FPI-sedentary rats at postinjury day 7. In this group, regression analysis indicated a strong relationship between phosphorylated CREB and phosphorylated synapsin (R-squared 0.9; p < 0.001). In the acute-FPI rats a decrease in phosphorylated synapsin I (p < 0.05) and CREB (p < 0.05) was seen. These results are opposed to those in the RW-sham, in which exercise was associated to an increase of synapsin I and CREB. These results suggest that even voluntary exercise can be deleterious when administered to soon after injury.

P116. UP-REGULATION OF THE CELL CYCLE/ INHIBITOR OF APOPTOSIS PROTEIN SURVIVIN IN ASTROCYTES AND NEURONS AFTER TBI IN RATS

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This investigation examined mRNA and protein up-regulation of the cell cycle/ inhibitor of apoptosis protein survivin after traumatic brain injury (TBI) in rats. Additionally, survivin cell type expression was determined. Levels of survivin mRNA and protein were significantly elevated in the ipsilateral cortex and hippocampus after injury as compared to sham injured control rats. These levels increased at 1 day, peaked at 5 days and returned to baseline by 14 days post injury in both regions. To determine if survivin up-regulation is correlated with the up-regulation of other cell cycle proteins, western blots of the cell cycle associated proteins survivin and p21 were compared. PCNA showed significantly elevated protein levels with a similar expression pattern to survivin. With immunohistochemistry (IHC), both neurons and astrocytes showed survivin immunoreactivity in the ipsilateral cortex. Additionally, survivin localized to neurons in the contralateral hippocampus and astrocytes in the ipsilateral hippocampus. Interestingly, although the majority of survivin positive cells were astrocytes that are known to proliferate after injury, a small population of survivin-positive neurons was identified. Future studies will investigate whether these neurons express other cell cycle proteins in an attempt to initiate a cell cycle related program in response to TBI. (Supported by DAMID-91-1965, DAMID-01-1-0765, NIH R01 NS39091 and NIH R01 NS40182).

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Neuronal precursors in the adult rodent anterior subventricular zone (SVZ) proliferate, migrate to the olfactory bulb via a restricted pathway known as the rostral migratory stream (RMS), and differentiate into neurons. A thorough understanding of these processes is necessary to utilize the SVZ as a source of neuronal and glial precursors for genetic manipulation, transplantation, or brain self repair following CNS injury. In the current experiment we sought to determine whether the normal migratory pattern of SVZ-originating neural precursors was altered following experimental brain injury. Anesthetized mice (n = 50) were subjected to controlled cortical impact (CCI) brain injury (5 m/s, 0.5 mm), injected intraperitoneally (i.p.) with 50 mg/kg bromoacetamide (BrdU) three times over a six-hour period beginning seven days post-injury, and then sacrificed at 0, 3, 7, 14, or 21 d post BrdU injection. Control mice were anesthetized and surgically prepared and received the identical BrdU administration paradigm without brain injury. Immunohistochemical analyses were performed with antibodies directed against BrdU, nestin (a cytoskeletal protein associated with stem cells), doublecortin (Dcx; a microtubule-associated phosphoprotein expressed in migrating neuroblasts); gliarial filament acid protein (GFAP), and class III beta-tubulin (TuJ1; a tubulin isoform expressed by immature and mature postmitotic neurons). In addition to the RMS and olfactory bulb, brain-injured animals Dcx+, BrdU+ and Nestin+, BrdU+ cells were localized to the anterior periphery of the injury cavity, organized in a spinal-like formation originating at the same ventricular location as the RMS, and enumerated by astrocytes after 7 d post BrdU administration. These data suggest that normal caes are required for directed migration of SVZ-residing neural precursors may be disturbed following CNS injury.

Supported, in part, by NIH NS40978, NS38803, GM34690, MH17168, and a Veterans Administration Merit Review grant.

P118.
GENDER DIFFERENCES IN COGNITIVE RECOVERY AFTER INTERVENTION WITH ENVIRONMENTAL ENRICHMENT FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY. Sokolski J1, Kline AE1, Zaffoni RD1, Dixon CE2, Wagner AR1 1Dept. Physical Medicine and Rehabilitation, 2Dept. Neurological Surgery, University of Pittsburgh, Pittsburgh, PA (US).

Environmental enrichment clinically after traumatic brain injury (TBI), and enrichment of the housing environment has been shown to improve spatial memory after experimental TBI in male rat models, and therefore may have some parallel to receiving rehabilitation. However, the impact of gender on how environmental enrichment affects behavioral performance after experimental TBI has not been studied. Therefore, the purpose of this study was to examine the therapeutic effect of environmental enrichment on post TBI recovery in both male and female rats. Male (n = 32) and normally cycling female (n = 35) Sprague-Dawley rats underwent either controlled cortical impact (2.7 mm, 4.0 m/s) or sham injury and were housed in either standard or enriched environmental conditions, which consisted of novel and social living conditions as well as gustatory, olfactory, tactile and visual stimulation. There were no differences in peri-injury plasma estrogen (n = 15) and progesterone (n = 16) levels for injured females in each of the housing conditions. Motor function was assessed both pre-injury and for the first 5 days after injury. Spatial memory was assessed beginning 14 days after injury using the Morris Water Maze task. Repeated measures ANOVA post-hoc analysis showed that enriched injured males exhibited significantly shorter latencies to find the hidden platform than standard injured males (p = 0.0165). Surprisingly, enriched injured females performed worse than enriched injured males on this task (p = 0.0221). Enrichment did not improve cognitive recovery in injured females, as they performed no differently than other injured groups in the standard housing environment. Enrichment did not affect motor performance for either males or females. These results suggest that environmental enrichment after TBI beneficially affects cognitive recovery for male, but not female rats. More work is needed to determine the effects of sex hormones and enrichment environments on cortical plasticity and specific tissue markers of neurotransmission that influence spatial memory.

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P119.
EXTRACELLULAR SIGNAL-RELATED KINASE/MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IS CRITICAL FOR ASTROCYTE PROCESS EXTENSION AND MIGRATION IN THE SETTING OF BRAIN INJURY. W. Shawn Carbonell† and James W. Mandell. (University of Virginia, Charlottesville, VA (US)).

Extracellular signal-related kinase (ERK/MAPK) is a member of the MAP kinase family involved in diverse cellular functions including apoptosis, motility, and differentiation. We have previously shown that reactive astrocytes in a variety of human neuropathologies exhibit chronic activation of ERK/MAPK. We hypothesized that activation of ERK/MAPK is involved in the induction/maintenance of reactive phenotypes. Accordingly, we characterized activated ERK immunoreactivitiy (pERK-IR) from 1h-30d after a forebrain-stab lesion (FSL) in the adult C3H/HeJ mouse. Perilesional neurons demonstrated pERK-IR primarily at the 1h timepoint, whereas staining in astrocytes was found at all timepoints peaking between 3d-7d. pERK-IR in astrocyte processes could be appreciated from 1d-30d. We tested the functional relevance of pERK-IR in cell processes in vitro with the specific MAP kinase inhibitor MEK1/2 inhibitor U0126 (20mM). MEK1/2 blockade inhibited process extension after replating of both primary astrocytes and C6 glioma cells. U0126 also attenuated growth factor-stimulated migration in a scratch-wound model. To evaluate the role of ERK/MAPK in astrocyte process extension and migration in vivo we administered a blood-brain-barrier permeant analogue of U0126, SL327 (100mg/kg IP; 2-3h half-life), to mice twice daily for 6d beginning 1d after FSL. Mice were sacrificed at 7d, 12h after the final dose. In vehicle-treated animals, glia arising from the medial wall of the ipsilateral lateral ventricle demonstrated polarized morphology suggestive of migration, pERK-IR in nuclei and leading cell processes, and were found throughout the septal parenchyma. These cells were also immunoreactive for phosphorylated ERK-receptor and ezrin, known mediators of cell motility. Corresponding glia from SL327-treated animals demonstrated nuclear pERK-IR, but were negative for ezrin or phosphorylated ERK-receptor. Further, they exhibited non-polar morphologies, lacked cell processes, and were clustered subependymally. Taken together, we demonstrate a critical role for the ERK/MAPK cascade in process extension and migration of astrocytes after brain injury.

P120.
CO-ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE, AND PRESENLIN-1 IN CULTURED AXONS FOLLOWING STRETCH INJURY. A. Iwami†, X.H. Chen, B.J. Pfister, D.F. Menney†, and D.H. Smith, (Depts. of Neurosurgery and Bioengineering, Univ. of Pennsylvania, Philadelphia, PA (US)).

We have previously found that amyloid-beta (A-beta) accumulates in damaged axons following brain trauma in humans and animal models. In Alzheimer’s disease (AD), A-beta is thought to be primarily produced via transmembrane cleavage of amyloid precursor protein (APP) by beta-secretase (BACE) and presenilin-1 (PS-1). However, the source intraxonal A-beta following trauma is unknown. Using a cultured axonal injury (CAI) system, we examined potential co-accumulation of A-beta with BACE and PS-1 in damaged axons. CAI was induced by rapid selective stretch of axons bridging a 2 mm gap between two populations of human neurons (N-terz-N). For each stretch, the rise time 20ms, duration <50ms, and axial strain of 75%. The cultures were then either fixed with 4% paraformaldehyde or frozen with selective protein extraction of the axons in the gap and in the culture media at 0, 6, and 24 h following injury. Double immunohistochemistry using highly specific antibodies was performed to detect co-localization of APP, A-beta, BACE, and PS-1. ELISAs were performed to detect an increase of A-beta(1-40) and A-beta(1-42) in extracts from the media and axons. We found that CAI induced accumulation of A-beta in axonal swellings remarkably similar in appearance to that observed in humans and animals following brain trauma. In addition, we observed that this A-beta co-localized with APP, PS-1, and BACE in axonal swellings. Moreover, we detected an increase of both A-beta(1-40) and A-beta(1-42) in the media and in homogenates of axons for at least 24h following injury. These results demonstrate that axonal trauma in vitro can induce the production of A-beta. Like AD, this process may depend on BACE and PS-1 cleavage of APP. Surprisingly, however, this activity appears to occur within the axonal membrane compartment. These data may have important implications for the link between brain trauma and AD. Supported by NIH grants AG21527 and NS38104.
P121.
IDENTIFICATION OF MULTIPLE DISTINCT PATHOLOGIC NUERONAL PHENOTYPES WITHIN DIFFUSELY INJURED BRAIN
Richard H. Singleton and John T. Pavlicek (Medical College of Virginia/VCU, Richmond, VA, US).

Although traumatic brain injury (TBI) is known to evoke axonal injury (TAI), resulting in delayed axotomy, little is understood regarding the neuronal somatic response to either the diffuse or the traumatic mechanical forces of injury or the retrograde sequelae of TAI. We have recently demonstrated that neuronal somata axotomized by TAI do not progress to cell death within 7 days postinjury, suggesting the potential for recovery. However, in these foci, a distinct population of neurons that did not sustain TAI revealed rapid degenerative changes suggestive of cell death. To better understand these differing primary somatic and delayed-secondary retrograde neuronal responses, we subjected rats to moderate central fluid percussion TBI followed by perfusion fixation at varying times over 30 days. Antibodies to amyloid precursor protein (APP) and the 70kD heat shock protein (HSP-70) and phosphorylated eukaryotic translation initiation factor 2 alpha (eIF2aP) were used to detect potential neuronal perturbation and recovery, and TUNEL as well as antibodies to single stranded DNA were used to detect apoptotic cell death. Fluoro-Jade (FJ) was used as a more generalized marker of cell death. Single and double labeling immunocytochemical strategies revealed TAI-linked somata that colocalized with eIF2aP and, infrequently, with HSP-70 in the early postinjury period. After 72h, the increased expression of both HSP-70 and eIF2aP reached to shams levels, suggesting recovery. Apoptotic labeling was rare, with no apoptotic cells correlating with either axotomized neurons or with HSP-70 or eIF2aP labeled somata. Some adjacent somata, however, stained positively with FJ within 7d postinjury, suggesting the presence of necrotic cell death. Collectively, these findings emphasize the occurrence of diffuse somatic injury following TBI that involves a spectrum of pathological change, ranging from cell perturbation with the potential for recovery to overt cell death.

P122.
RELATIONSHIP OF 40kD, 10kD, AND 3kD FLUORESCENT INDICATORS OF ALTERED AXOLEMMAL PERMEABILITY TO IMPAIRED AXOPLASMIC TRANSPORT IN TRAUMATIC AXONAL INJURY

Traumatic axonal injury (TAI) evolves within minutes to hours following traumatic brain injury (TBI). Previous studies have identified axolemmal disruption and impaired axoplasmic transport (AXT) as a key mechanism in the evolution of TAI. While initially hypothesized that axolemmal disruption led to impaired AXT, recent studies employing antibodies to amyloid precursor protein (APP) to identify impaired AXT and 40kD fluorescently-tagged dextran to identify axolemmal disruption suggest these processes occur within distinct populations of TAI.

Building on these studies, the current investigation employs smaller molecular weight (MW) dextrans to determine whether more subtle alterations of the axolemma may co-localize with impaired AXT. Specifically, rats were administered an intrathecal mixture of either 40kD+10kD or 40kD+3kD fluorescently-tagged dextrans, with brains subsequently prepared for APP immunofluorescence. APP and all MW dextrans consistently localized to two distinct classes of TAI. The first class demonstrated influx of all MW dextrans across a damaged axolemma, was thin and elongate, sometimes vacuolated, and revealed little progressive change over time. The second class was distinguished by the presence of APP alone within swollen axons at early time-points and APP + all MW species of dextran within disconnected axonal bulbs at later time-points. Interestingly, there was no co-localization of smaller MW dextrans with APP prior to disruption.

These studies confirm axolemmal disruption and impaired AXT are distinct events early in TAI. Further, these studies provide evidence that the process of impaired axoplasmic transport and subsequent axon degeneration leads to delayed axolemmal instability, rather than being a consequence of initial axolemmal failure. This finding underscores the need of multiple approaches to fully assess the axonal response to TBI supported by the Commonwealth Neurotrauma Initiative.

P123.
QUANTITATIVE DIFFUSION WEIGHTED IMAGING ANALYSIS OF CELL-PERMANT CALCIUM BUFFER INDUCED NEUROTROPHIC PROTECTION AFTER CORTICAL DESEVASCULARIZATION IN RATS
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An excitotoxic cascade resulting in a significant intracellular calcium load is thought to be the primary mechanism leading to neuronal death. One way to protect neurons from injury is through the use of cell-permanent calcium buffers (CPCBs). These molecules have been reported to be neuroprotective via their ability to increase the cell’s overall Ca2+ buffering load as well as by attenuating neurotransmitter release. We used the CPCB 2-amino-2-methyl-1-(3-nitrophenyl)-1-propanol (N,N,2-ti triethylammonium nitrate) (APTRA-AM), to determine its effectiveness in providing neuroprotection after a cortical devascularization injury. Injured animals were given two intravenous injections of saline, DMSO, or APTRA-AM at 1 and 12 hours after injury. Animals were imaged on a Bruker 4.7 T MRI using a diffusion-weighted imaging sequence prior to injury and at 12, 24, 48 hours, and 3 days after injury. Correlative histological and immunocytochemical studies were performed on euthanized rats 7 days after injury. In saline treated rats, a decrease in the apparent diffusion coefficients (ADC) over the injured area was observed by 12 hours after injury, and remained below pre-injury values throughout the next 7 days. DMSO treated rats also exhibited a decreased ADC within the injured area. In contrast, animals injected with APTRA-AM showed almost no change in the ADC of the injured area. APTRA-AM also significantly reduced infarct volume and inflammatory cell infiltration. The results presented here clearly demonstrate the effectiveness of APTRA-AM in preventing neuronal cell death and the accompanying inflammatory response which further contributes to our understanding of the mechanisms associated with post injury inflammation and infarct development in brain injuries.

P124.
SPINAL CORD OLIGODENDROGLOBLIA EXPRESS ACTIVATED CASPASE-3 FOLLOWING K+ INDOUCED DEPOLARIZATION AND NMMA EXPOSURE
S.A. Nottingham* and J.E. Springer, (Anatomy and Neurobiology, Spinal Cord and Brain Injury Research Center, University of Kentucky Medical Center, Lexington, KY, USA).

Despite evidence of widespread apoptosis of neurons and glial cells following spinal cord injury (SCI), the extra- and intracellular molecular signals that activate the apoptotic pathway remain poorly understood. Since after SCI, the spinal cord is exposed to numerous secondary insults, including elevated levels of glutamate that contribute to cell dysfunction and death. While glutamate mediated excitotoxicity is typically associated with necrosis, recent studies suggest a role for glutamate in apoptosis. In this present study, we examined the actions of glutamate by performing intrathecal injections of the selective glutamate receptor agonist, N-methyl-D-aspartate (NMMA), into uninjured rat spinal cord. Even though oligodendroglia are believed to contain only AMPA/ kainate receptors, immunohistochemical colocalization studies demonstrated a significant increase in the percentage of oligodendroglia exhibiting activated caspase-3 in comparison to c cSF control data at 4 hours following NMMA injection (p < 0.05, n = 6). At the later time points examined (24 and 96 hours following injection), there was no evidence of caspase-3 activation in this cell type. However, significant oligodendroglial loss was present at 96 hours following NMMA exposure in comparison to c cSF control data (p < 0.003, n = 6). In an attempt to better define the mechanism for the action of NMMA in oligodendroglia, we examined the effect of spinal cord depolarization induced by intrathecal injections of high K+ c cSF (50 mM KCl) on caspase-3 activation. Infusions of high K+ c cSF also resulted in caspase-3 activation in oligodendroglia, suggesting one putative and indirect mechanism by which NMMA may lead to caspase-3 activation in oligodendroglia. Supported by: PHS Grant NS40015 and KSCHIRT.
P125. DIFFERENTIAL GENE EXPRESSION PROFILING IN THE EMBRYONIC AND ADULT-INJURED SPINAL CORDS


The failure of descending pathways to regenerate after spinal cord injury (SCI) may be due to the failure of the injured spinal cord to express genes that promoted descending axonal growth and targeting during development and/or the expression of genes in the injured spinal cord that create an environment hostile to regeneration. An indication of the differing abilities of the embryonic and adult-injured spinal cords to support regeneration is offered by studies showing that while the adult spinal cord is inhospitable to the regeneration of brain-spinal cord connections, an embryonic spinal cord grafted into the site of injury promotes this regrowth and regeneration. We hypothesize that these differing regenerative abilities of the adult-injured spinal cord and the embryonic spinal cord will be reflected by differences in their gene expression. The purpose of this study was to identify these three classes of genes: 1. Genes expressed only in the embryonic spinal cord (may have an ameliorating effect on SCI recovery), 2. genes expressed solely in the injured cord (may have a maladaptive role in SCI) and 3. genes common to embryonic and injured but not to a normal tissue (indicative of the reaction to the presence of embryonic gene products). Three subtracted cDNA libraries were created to isolate these gene populations. A novel three-way approach to subtractive hybridization was used. To identify genes in each library, the subtracted cDNA populations were fluorescently labelled and used as microarray probes. Differentially expressed genes, as detected by clustering analysis of subtracted cDNA populations, include entire classes of genes (pro necrotic genes were found only in injured tissue) and different genes within a single class (different cell adhesion molecules were found in all three cDNA populations). Putative roles of differentially expressed genes in SCI is also discussed.

P126. RAPID FUNCTIONAL RECOVERY AFTER THORACIC SPINAL CORD INJURY IN YOUNG RATS

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Glutamate excitotoxicity contributes to secondary injury after trauma in the adult CNS. Glutamate receptor subunit protein expression is elevated during the first 2–3 weeks after birth as compared to that in adults. This relative "overexpression" of glutamate receptors could contribute to differences in both secondary injury processes and plasticity related to functional recovery in young animals. To investigate this hypothesis, we modified an adult model of spinal cord injury (SCI) for use in Sprague-Dawley rats at postnatal day 14–15. A laminectomy was performed at the T8–T9 vertebral level, a 1.5 mm diameter impounder was lowered onto the dura, and a contusion was produced with a 10 g weight dropped from heights of 2.5 or 5 cm. Hind limb function was evaluated 24 hours later and at 1, 2, 3 and 4 weeks with the Combined Behavioral Score to estimate overall hind limb sensory-motor function and the BBB rating scale for open field locomotion. Results showed that rats injured at P14–15 with a 5 cm weight drop (n = 10) exhibited significantly less recovery of function at 4 weeks than those rats injured with a 2.5 cm weight drop (n = 27). The degree of hind limb deficit at 4 weeks was similar to that previously described in adults with comparable SCI. However, animals injured at P14–15 exhibited a significantly faster rate of recovery than adults. Recovery in the young rats was maximal by 1–2 weeks as compared to 3–4 weeks in adults subjected to similar SCI. The rapid recovery in young rats suggests that this model may be useful to study potential mechanisms of recovery after incomplete contusion injury in the spinal cord. (NIH R01 NS 37733)

P127. BENEFICIAL EFFECT OF AN EARLY ANTI-INFLAMMATORY STRATEGY AFTER ACUTE SPINAL CORD INJURY: COMPARISON TO THE EFFICACY OF METHYLprednisolone


We propose that targeted inhibition of early inflammation due to hematogenous infiltration remains an important first step in neuroprotection after spinal cord injury (SCI). In this study we hypothesized that suppression of leukocyte extravasation early after SCI would lead to improved neurological outcomes. In accordance with our goal of reducing the early inflammatory response, we used a paradigm of treatment [saline or monoclonal antibody (mAb) to the αd subunit of B2 integrin or methylprednisolone (MP) or combined mAb/MP] administration in the first three days after clip-compression SCI at the 4th thoracic segment in rats. The outcome of treatment with this selective mAb was compared with that after treatment with the pleiotropic MP. We assessed neurological outcomes using BBB open field locomotor scores and evaluation of autonomic dysreflexia. We used histological methods to assess the amount of spared tissue and the pathological changes below the injury site. Mean arterial pressure (MAP) induced by colon distension was used to assess autonomic dysreflexia. Baseline MAP was similar in all groups (~110 mmHg). MAP increased by 37 ± 3 mmHg in untreated rats (n = 14), by only 27 ± 3 mmHg after mAb treatment (n = 11), by 26 ± 2 mmHg after MP (n = 7) and by 28 ± 2 mmHg after mAb/MP treatment (n = 7). mAb treatment improved BBB locomotor scores from 3 ± 1 to 6.7 ± 1 whereas MP or the mAb/MP treatment did not improve them. The amount of white matter was greater in mAb-treated rats throughout the lesion site than in untreated rats. MP alone had little impact on the lesion size. Addition of MP to the mAb treatment paradigm diminished the effect of the mAb on lesion size. In conclusion, the outcome after mAb treatment was clearly superior to that after corticosteroid therapy and demonstrates that early selective intervention in the inflammatory process after SCI can be highly beneficial. Support: Ontario Neurotrauma Foundation and ICOS.

P128. Olfactory Ensheathing Cells Promote Robust Axon Growth Following Compressive Spinal Cord Injury


Strategies to promote axonal regeneration and functional recovery after spinal cord injury (SCI) have included application of exogenous neurotrophic factors, neutralizing the inhibitory environment of the CNS, and implantation of various cell types at the site of SCI, such as olfactory ensheathing cells (OECs). We have utilized a clinically relevant model of compressive spinal cord injury in order to characterize the interaction between fetal rat OECs and injured spinal cord tissue. One week following injury using modified aneurysm clips, OECs were implanted into the cystic cavity, which had formed at the site of injury. Three weeks after injection, OECs occupied most of the cystic cavity, and were observed intermingling with reactive astrocytes. Double immunofluorescence for GAP-43 and neurofilament demonstrated numerous axons that had invaded the intraspinal grafts of OECs, and extended from the rostral and caudal portions of the cystic cavity. Ultrastructural examination of the cystic cavity demonstrated that implanted OECs were associated with both myelinated and unmyelinated axons. These observations provide the first direct evidence that a purified population of fetal rat OECs facilitate axon growth in a clinically relevant model of SCI. We hope that data from this investigation will provide novel insight into this type of cell therapy, as a clinically applicable technique that could be used to reduce functional deficits in humans with SCI.

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P129.
CYCLIC AMP INDUCES FUNCTIONAL REGENERATION-ASSOCIATED GENES AND REPRESSES ASSOCIATED GENES.
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Increasing cyclic AMP (cAMP) allows neurons to grow on myelin, which normally inhibits growth. While dorsal root ganglion (DRG) neurons extend short neurites on myelin, neurons growing in the presence of cAMP extend longer processes. cAMP levels are elevated in DRG neurons after peripheral lesion and during development, times when DRG neurons extend neurites on myelin. Protein kinase A inhibitors block the ability of cAMP to overcome inhibition. By comparing gene expression of neurons grown in the presence or absence of cAMP, we identified several genes expressed when neurites switch from an inhibited state to a growth state. We dissociated P5 rat DRGs and plated the cells on myelin overnight. At 1 hour to 18 hours after addition of dbcAMP or medium alone, the cells were harvested and their RNA was assayed on microarrays containing 5,000 spots of oligonucleotide probes. cAMP markedly induced expression of the pro-inflammatory cytokine IL-6. Real-time PCR showed 15-fold induction of IL-6 mRNA over 18 hours of cAMP treatment. IL-6 protein applied directly to DRG cells growing on myelin showed equivalent growth-promoting effects as cAMP. Although cAMP treatment repressed GAP-43 expression, GAP-43 is expressed at high levels in DRGs after peripheral nerve lesion and during development. Thus, cAMP induces regeneration-associated genes and allows growth on myelin while repressing GAP-43.

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P130.
NOVEL SYNTHETIC GRAFTS THAT ARE BIOCOMPATIBLE AND PROMOTE AXONAL REGENERATION AFTER SPINAL CORD INJURY.
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While synthetic grafts to promote peripheral nerve axonal regeneration have been widely investigated, their use in promoting spinal cord axonal regeneration after injury has been limited. We examined the biocompatibility of a novel synthetic hydrogel tubular device, composed of a rigid or malleable formulation of poly (2-hydroxyethyl metacrylate) (PHEMA), that can be biodegraded to provide improved hapticotic and chemotoxotactic cues for regeneration. Adult Sprague Dawley rats underwent complete spinal cord transection at T8 and repair with PHEMA tubes of two different elastic moduli: rigid (260 kPa; n = 8) or a malleable (178 kPa; n = 8). The cord stumps were inserted into the tube, fibrin glue was applied to the cord-tube interface, and a Preclude® membrane used for duraplasty. Controls (n = 4) underwent cord transection alone. Half the animals underwent axonal tracing with anterograde DiI and retrograde Fluoro-Gold. Survival times were 2, 4, or 8 weeks. Gross and histological examination of the spinal cords showed continuity of the tube and the cord stumps as early as 2 weeks. Continuity of neural tissue was more consistently seen with the rigid tube, and neurtomulli stained axons were visualized within the continuous neural tissue. Supraspinal serotonin axons were found growing into the graft and were found to enter the caudal spinal cord. Calcium deposits occurred more frequently on the external surface of the rigid tubules and, with both tube types there was minimal scarring at the tube-cord interface, and significantly less scarring at the tube-dura interface compared to the Gorex. For the first time, we have evidence of axonal regeneration in a rat after complete spinal cord transection using synthetic hydrogel tubes without a contained matrix. Present work is examining the effects of contained matrices on axonal regeneration and functional recovery.

P131.
NOGO-66 RECEPTOR ANTAGONIST PEPTIDE PROMOTES AXONAL REGENERATION AND FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY.
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After trauma to the adult mammalian CNS, axonal regeneration is minimal. Myelin-derived axon outgrowth inhibitors such as Nogo-A may account for this lack of CNS repair. The IN-1 antibody recognizes Nogo-A and promotes corticospinal tract (CST) regeneration and locomotor recovery. However, the limited specificity of IN-1 for Nogo and the non-specific antagonist myelin digestion has prevented a firm conclusion about the role of Nogo-66 or its receptor (Ngr). Here, we identify a peptide antagonist (Nogo Extracellular Peptide residues 1-40, NEP1-40) of the Nogo-66 Receptor derived from amino terminal fragments of the Nogo-66 domain. This antagonist binds to the Nogo-66 Receptor competitively with nanomolar potency but does not stimulate axonal growth cone collapse in the cultured dorsal ganglion cells. We delivered this peptide or vehicle intrathecally to adult rats at the site of a mid-thoracic dorsal hemisection injury via an osmotic minipump. The integrity of the descending CST was traced by biotin-dextran-amine injection into the motor cortex. The integrity of axotomeric raphespinal tracts was evaluated with immunostaining for 5-HT fibers. The administration of this NgR antagonist to spinal cord injury rats results in a significant regeneration of both CST and raphespinal axons, and remarkably improves locomotor functional recovery assessed with a standardized BBB score. The egressing from severed CST tracts following peptide treatment extends at least 1.5 cm caudal to the lesion. These findings reveal the central role of the Nogo-66 Receptor in limiting axonal regeneration after adult mammalian CNS injury, and NEP1-40 provides a potential therapeutic approach to treating traumatic CNS axonal injury.

P132.
TRANSPANTATION OF RODENT SKIN-DERIVED PRECURSOR CELLS ONTO RAT HIPPOCAMPAL SLICE CULTURE.

One approach to repair damaged central nervous system (CNS) is to transplant new neural cells to restore functional circuitry. As accessible candidate cells for transplantation, we have recently isolated multipotential precursor cells from dermis of the skin. These skin-derived precursor cells (SKPs) form spheres in the presence of adhesion and permissive culture conditions (with EGF and FGF) that are immediately dissociated, prelabeled with a fluorescent dye, and grafted onto the hippocampal slice. Alternatively, 5-7 days prior to transplantation, spheres were plated down in medium lacking growth factors, but supplemented with FBS. This condition significantly increased the number of neural cells in vitro. These cells were then, labeled, and grafted onto the hippocampal slice culture. 7-14 days after transplantation, SKPs prepared from skin containing medium proliferated more vigorously than those treated with FBS. Immuno staining revealed a robust growth of nestin-positive fibers that appeared to originate from FBS-treated SKPs, whereas little nestin immunoreactivity was seen in the untreated SKP graft. Furthermore, FBS-treated SKP grafts were immunoreactive for early neuronal markers like b III tubulin and HuC/D, and in some cases, for mature neuronal markers like MAP-2 and phosphorylated neurofilaments. Host hippocampal slices showed no adverse reaction to SKP grafts derived from either culture condition. These observations suggest that SKPs survive transplantation procedures and are capable of acquiring a neuronal phenotype in the grafted host environment. N.R.K. is supported by Christopher Reeve Paralysis Foundation.
P133.

PROGNOSTIC VALUE OF SPECT IN PATIENTS WITH POST-TRAUMATIC TRAUMENTORIAL HERNIATION

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Objectives: Cerebral perfusion disturbances are common in patients after the severe head injury, particularly in patients with transtentorial herniation and may influence outcome.

Material and methods: We present a group of 36 head injured patients admitted to neurosurgery with the syndrome of transtentorial herniation (GCS 3-5, homolateral dilated pupil and disturbed vital functions). There were 15 epidural, 19 subdural and two intracerebral hematomas. Mean age 40.8 years. All patients had urgent surgery and then continuous monitoring of ICP, CPP, blood pressure and jugular bulb oxygenation was instituted. Two postoperative CT and SPECT examinations were performed in each patient.

Results: 10 patients had visible ischemia on the first postoperative CT scan, 7 of them died. All patients except 3 had ischemia on SPECT (92%). Ischemia improved on the 2nd SPECT in 17 patients and 16 of them (94%) had a favourable outcome. On the other hand there were 16 patients with no change or even worse ischemia on 2nd SPECT and only 3 (19%) of them had a favourable outcome (p < 0.05). 12 out of 17 patients (70%) with improvement of perfusion on 2nd SPECT had a normal CPP during the whole posttraumatic course. On the other hand only 5 out of 16 patients (31%) with no improvement on SPECT had a normal CPP all the time.

GOS (mean follow up 12 month): 19 patients good, 3 moderately disabled, 1 severely disabled, 2 vegetative, 11 died.

Conclusions: SPECT is very sensitive to impair cerebral perfusion and may be helpful as a serial study. This might be beneficial in patients with reversible ischemia and all the effort has to be made to keep CPP on normal levels. Improvement of perfusion detected by SPECT is related with a good outcome. Moreover SPECT shows the real areas at risk of ischemia which might be chosen for brain oxygenity monitoring.

P134.

LEFT-RIGHT ASYMMETRY OF THE ESTIMATION OF CEREBRAL PERFUSION PRESSURE USING TRANSCRANIAL DOPPLER ULTRASOUND: A PPRELIMINARY REPORT

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Background: The estimation of cerebral perfusion pressure (cCPP) using transcranial Doppler (TCD) has recently been evaluated [1]. We investigated time trends in left-right asymmetry of cCPP and its correlation to CT scan patterns after traumatic brain injury.

Methods: In 25 sedated and paralysed head injured patients, arterial blood pressure (ABP) and intracranial pressure (ICP) were monitored continuously. Left and right middle cerebral arteries were sonicated (106 measurements) using a purpose built transcranial Doppler monitor (NeuroQ Ltd, Chestnut UK). NeuroQ's software is capable of on-line bilateral measurement of mean and diastolic flow velocities (FVM, FVD), and calculation of left-right cCPP following: cCPP = (ABPM + FVM / FVM + ICP) + 14 [2].

Results: The mean absolute value of left-right difference in cCPP (ΔcCPP) was 6 ± 5.8 mmHg. Daily observations showed that ΔcCPP progressively increased with time after head injury (ANOVA; p < 0.012).

Similarly, the absolute value of the difference between left and right FVM increased with time (ANOVA; p < 0.006). The ΔcCPP was significantly correlated with left-right asymmetry in FVM (r² = 0.61, p < 0.0001), and ICP (r² = 0.18, p < 0.04). Evidence of global brain swelling on CT, was significantly associated with an increase in absolute value of ΔcCPP (p < 0.05).

Comparing patients with and without midline displacement on CT (n = 4 and n = 21 respectively), showed that ΔcCPP was significantly greater (p < 0.04) for patients with midline shift, cCPP being higher on the side of the expanding brain.

Conclusion:
1. TCD is not only able to measure left and right flow velocities, but also to identify interspheric gradients of cerebral haemodynamics.
2. After head injury, left-right differences in estimation of CPP seems to follow a specific pattern.
3. Assessment of left-right asymmetry of cerebral haemodynamics should be of clinical significance.

References:

P135.

PERFUSION WEIGHTED MAGNETIC RESONANCE IMAGING (MRI) IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY.

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Data from a pilot study to apply Arterial Spin Labelled (ASL) Perfusion MRI to a well-characterized Controlled Cerebral Impact (CCI) mouse model of TBI is presented. Our aims were to detect relative changes in Cerebral Blood Flow (CBF) in the injured hemisphere (compared to the contra-lateral hemisphere) and to compare changes in perfusion brought about by three differing injury levels (mild, moderate, and severe).

Animals were anesthetized with Isoflurane (4% knock down, maintained at 1.5% in air/nitrogen mixture) during CCI and MRI imaging. Traditional MRI techniques and arterial spin labeled perfusion MRI were used to detect T2, T1 and cerebral blood flow changes in the initial two hours after differing levels of injury. Our data show that CBF at the site of injury is reduced after TBI in this model and that CBF changes scaled with the level of injury. Severe CCI produced the greatest change, leading to a decrease in CBF of 53% in the contusion and 18% in subcortical tissue in the injured hemisphere (compared to contra-lateral tissue). Moderate CCI caused a smaller decrease in CBF, 29% in the contusion, 7.3% in subcortical tissue, while mild CCI led to an even smaller change, 8.4% in the contusion and 4.9% in the sub-cortical tissue. This study demonstrates that MRI has utility in characterizing blood flow after injury, that CBF is reduced after injury and that the level of this perfusion deficit is directly related to the level of injury.

P136.

EFFECTS OF EARLY AND LATE INFUSION OF NOREPIEPHINEPHRINE ON CEREBRAL BLOOD FLOW, BRAIN TISSUE OXYGENATION, AND BRAIN EDEMA FORMATION IN BRAIN-INJURED RATS


Reduction of CBF during the early phase after traumatic brain injury (TBI) is followed by a later phase of normal to increased cerebral perfusion. Thus, a pharmacological elevation of mean arterial blood pressure (MABP) with the aim of improving posttraumatic cerebral blood flow (CBF) may exert different time-dependent effects on CBF, tissue oxygenation (pO2) and brain edema formation after TBI.

Thirty-seven male Sprague-Dawley rats were subjected to a focal controlled cortical impact injury (CCI). At 4 or 24 hours after CCI, MABP was increased to 120 mmHg for 90 minutes by infusing norepinephrine. In rats receiving physiological saline MABP remained unchanged. In the first series, pericontusional CBF was measured using a laser Doppler flowmetry scanning technique before CCI, before, during, and after the infusion period. In a second series, intracranial (ICP) and cerebral perfusion pressure (CPP) as well as intraparenchymal CBF and pO2 measured within the pericontusional cortex were recorded continuously before, during, and after nor-epinephrine infusion. At the end of each experiment the brain was removed to determine hemispheric swelling and water content.

At 4 and 24 hours after CCI intravenous norepinephrine significantly increased, in parallel to the CCF increase, pericontusional cortical perfusion and pO2, whereas pericontusional parenchymal CBF was only significantly increased at 4 hours after trauma. Hemispheric swelling and water content did not significantly differ between the norepinephrine and control animals either with early or late infusion.

Following CCI early and late norepinephrine induced elevation of MABP significantly increased CBF and tissue oxygenation without aggravating or reducing brain edema formation. There was no evidence for a norepinephrine-induced reduction in perfusion due to vasoconstriction in the cortical or subcortical brain tissue at the early or late timepoint after trauma.

Due to its hyperosmolar and hyperoncotic properties HyperheateTM might influence posttraumatic impaired cerebral perfusion and secondary brain damage. These possible effects following controlled cortical impact (CCI) injury in rats were investigated.

In 19 Sprague Dawley rats a moderate left focal cortical contusion was induced using the CCI. HyperheateTM (4ml/kg bw) was intravenously administered within 2 min following trauma in 8 animals. In control animals [n=8] physiological saline was administered. In all animals blood gases were drawn before and following infusion. Temperature and mean arterial blood pressure (MAP) were monitored continuously. 24 hours following trauma brains were removed and posttraumatic edema was quantified gravimetrically. In three additional animals Laser Doppler flowmetry was used to assess pericontusional cortical perfusion before and after trauma, after drug application as well as 4 and 24 hours after trauma.

Temperature and arterial blood gases were determined within physiological limits during the entire study. Following administration of HyperheateTM no significant changes in MAP could be observed. In HyperheateTM treated animals posttraumatic swelling (8.2±0.3%) was moderately decreased compared to the placebo (8.5±0.9%), a significant decrease which was significant increased in both groups in traumatized versus non traumatized hemispheres. There were no differences in water content in non traumatized hemispheres. In HyperheateTM treated animals water content in traumatized hemispheres was (79.9±0.05%) no significant changes versus placebo (80.1±0.08%) were observed. Pericontusional perfusion was only moderately reduced at 4 hours following trauma in HyperheateTM treated animals in comparison to significant hypoperfusion in placebo group.

HyperheateTM increases posttraumatic pericontusional perfusion while no significant effect on posttraumatic edema formation was shown. The effect on neuronal damage still needs to be clarified.


Blood flow-metabolism uncoupling is a well-documented phenomenon after traumatic brain injury but little is known about the direct consequences for the underlying tissue. The aim of this study was to quantitatively assess the topographic inter-relationship between local cerebral blood flow (LCBF) and metabolic rate of glucose uptake (LCMRglu) in the same rat after concussion injury and to determine the degree of correspondence with the evolving axonal injury.

Isoflurane-anesthetized rats were injured by controlled cortical impact over the left parietal cortex (4m/s velocity, 2mm deformation). Quantitative measurements of regional LCMRglu and LCBF were obtained at 3hr in the same rat from 18F-fluorodeoxyglucose (FDG) and I4C-iododeoxyuridine (14CguC) co-registered autoradiographic images, and compared to the density of damaged axonal profiles in adjacent sections using beta-amyloid precursor protein (BAPP) immunohistochemistry. Sham-injured rats were used for comparison of all data together with an additional 24hr injury group that was assessed for B-APP alone (all groups n = 6).

LCBF was significantly reduced over the ipsilateral hemisphere versus sham-controls, for example in the contusion core it was 26 ± 2.6 versus 86 ± 8.2 ml/100g·min-1·1, respectively and 29 ± 3.2 versus 67 ± 14.4, ml/100g·min-1·1 in the cingulum (P < 0.01). By contrast, LCMRglu was unaffected, apart from foci of elevated LCMRglu in the contusion margin versus sham-controls (86 ± 2.5 versus 65 ± 6.5 μmol/100g·min-1·1, respectively, P < 0.05). Flow-metabolism was uncoupled indicated by a significant 2-fold elevation in the metabolism/blood flow ratio within most of the structures analyzed in the ipsilateral hemisphere (P < 0.05). B-APP staining was evident even by 3hr, with significant increases in injured axon density by 24hr (P < 0.01). No staining was present in sham-injured rats. The increase in B-APP axon density was negatively correlated with LCMRglu and positively correlated to the metabolite/glucose ratio (r = 0.62 and 0.77, respectively, P < 0.001) indicating the critical dependence of axonal outcome on flow-metabolism in the acute injury stage.

P140. AMYLOID BETA 1-42 AND TAU IN CEREBROSPINAL FLUID AFTER SEVERE HUMAN TRAUMATIC BRAIN INJURY. G. Prant*, R. Beer, A. Kümpf, K. Engelhardt, E. Schmutzhard, H. Ulmer, and P. Deitrichammer. (Departments of Neurology and Biostatistics, University Hospital Innsbruck, Austria).

Background: Traumatic brain injury (TBI) is a recognized risk factor for Alzheimer's disease (AD). Related to histopathological changes in AD, amyloid beta 1-42 (Aβ42) levels are decreased and tau levels increased in cerebrospinal fluid (CSF).

Methods: CSF samples were collected from 29 patients with severe head trauma between 1 and 284 days post trauma. Aβ42 and tau levels were measured using sandwich ELISA techniques and compared with CSF levels in patients with cognitive disorders and headache.

Results: At all time points, concentrations of Aβ42 were significantly lower in TBI patients than in control groups. A statistically significant correlation existed for Aβ42 levels and outcome of patients. Both the cutoff of 230 pg/mL, the sensitivity of Aβ42 to discriminate between good outcome (GOS 4 and 5) and worse outcome (GOS 1 to 3) was 100% at a specificity of 82%. CSF tau levels were significantly higher in TBI patients compared with control groups. In patients with multiple CSF samples collected at various time points between 1 and 32 days after trauma, tau levels increased early after TBI, peaked 11 days post trauma and slowly decreased thereafter. Independent of outcome, all patients had normal tau levels when CSF was collected more than 43 days post trauma.

Conclusions: Aβ42 and tau may play a potential role in the pathophysiology of TBI. Furthermore, the results of our study suggest that Aβ42 might be a supportive early predictor for recovery after severe head injury.
P141.
TEMPORAL AND SPATIAL PROFILE OF BID CLEAVAGE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
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This study examined the temporal profile and cell subtype distribution of the proapoptotic protein BID from 6 hours to 7 days following cortical impact injury in the rat. Increased protein levels of the truncated active form of BID (tBID) were seen in the cortex ipsilateral to the injury site from 6 hours to 3 days after trauma. Immunohistochemical examinations revealed expression of tBID in neurons, astrocytes and oligodendrocytes from 6 hours to 3 days after TBI and concurrent assessment of DNA damage using TUNEL identified tBID immunopositive cells with apoptotic-like morphology in the traumatized cortex. Moreover, BID cleavage and activation of caspase-8 and caspase-9 occurred simultaneously in hippocampus, contralateral cortex and hippocampus up to 7 days after the injury.

Our results provide evidence of BID cleavage in the traumatized cortex after experimental TBI in vivo and demonstrate that tBID is expressed in neurons and glia. Further, our findings indicate that cleavage of BID may be associated with the activation of the initiator caspase-8 and caspase-9. Lastly, our data support the hypothesis that cleavage of BID contributes to the apoptotic degeneration of different CNS cells in the injured cortex.

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P142.
TEMPORAL AND SPATIAL PROFILE OF CASPASE-6 EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
R. Beer2, G. Franc1, K. Boguthard2, S. Krajewski3, J.C. Reed1, A. Bak1, T. Doeat4, N. Levental1, E. Schumichard1, and A. Kemppi1. (Department of Neurology, University Hospital Innnsbruck, Austria;2 The Burnham Institute, La Jolla, California, U.S.A.;3 Department of Neurosurgery, UCS University, Hungary.)

This study investigated the temporal expression and cell subtype distribution of the executioner caspase-6 and the cell death receptor p75NTR from 6 hours to 14 days following cortical impact-induced traumatic brain injury in adult rat. Western blotting analysis revealed increased immunoreactivity of caspase-6 and p75NTR in the cortex ipsilateral to the injury site from 6 hours to 3 days after trauma. Immunohistochemical examinations revealed expression of caspase-6 in neurons and glial cells from 6 hours to 3 days after TBI and concurrent assessment of DNA damage using TUNEL identified caspase-6 immunopositive cells with apoptotic-like morphology in the traumatized cortex. Moreover, double labeling experiments demonstrated expression of both, caspase-6 and the cell death receptor p75NTR in individual CNS cells after injury. In contrast, there was no evidence of caspase-6 expression and upregulation of p75NTR in the ipsilateral hippocampus, contralateral cortex and hippocampus at all time points investigated.

Our results provide evidence of caspase-6 expression and upregulation of the cell death receptor p75NTR in the traumatized cortex after experimental TBI in vivo and demonstrate that caspase-6 is expressed in neurons and glia. Further, our data indicate that expression of caspase-6 contributes to the apoptotic degeneration of different CNS cells in the injured cortex. Last, our findings indicate that upregulation of the cell death receptor p75NTR may be associated with the activation of the executioner caspase-6.

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P143.
MULTIDIMENSIONAL IMPAIRMENTS OF ATTENTION FOLLOWING PEDIATRIC TRAUMATIC BRAIN INJURY
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Pediatric traumatic brain injury (TBI) is a serious public health issue in the U.S., affecting approximately 200,000 children each year. Of the children who survive a TBI, up to 80% may experience persistent impairments in physical, behavioral, cognitive, and self-care functioning. Despite the high incidence of childhood TBI, the nature of associated cognitive impairments has been relatively unstudied. Neuropsychology strives to examine brain-behavior relationships by characterizing the cognitive impairments associated with damage to various brain regions. Frontal and temporal brain regions essential for attentional processing are particularly vulnerable to damage following TBI. Few studies have examined the multidimensional characteristics of attention within a pediatric TBI sample of varying severity levels. The current study examines possible attentional impairments in children/adolescents in the months following head injury (HI). Pilot data are presented for children/adolescents ages 6-16 classified with "mild" or "moderate-severe" HI, with groups comparable on age and gender. Participants were assessed once medically stable. IQ tests and the Test of Everyday Attention for Children (TEA-Ch), a standardized measure of three dimensions of attention (selective, sustained, and attentional control/switching) were generally administered within six weeks of HI. The moderate-severe group obtained a significantly lower Full Scale IQ compared to the mild group. Across the attention measures, the mild group consistently performed better than moderate group. Further, the moderate-severe group performed statistically worse on tasks of selective and sustained attention. No significant differences were noted on tasks measuring attentional control/switching. Results suggest that moderate-severe acute TBI is associated with specific types of attentional impairments that may have specific effects on the course of cognitive recovery. This study is unique in that it evaluates multiple dimensions of attention within the same test measure in acute HI. Future studies will examine the relationship between attentional impairments and other cognitive abilities within a longitudinal design.

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P144.
TEMPORAL PROFILE OF ALFA-1-PECTRIN BREAKDOWN PRODUCTS AFTER TRAUMATIC BRAIN INJURY IN IMMATURE RATS
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Previous studies have shown that calpains and caspase-3 like proteases play a key role in cellular dysfunction and/or cell death after brain injury. Current work in our laboratory has demonstrated the role of caspase-3 and calpain in protein breakdown with the formation of distinct neuronal breakdown products of alfa-II-pectrin (SBDFP) after TBI in the mature brain. Age dependent increase in caspase-3 activity suggests a relatively greater role of programmed cell death in the pathophysiology of traumatic and ischemic brain injury in the developing brain. In the present study, protein analysis was used to immunoblot specific proteolytic fragments of alfa-II-pectrin produced by calpains (145 kDa) and caspase-3 (120 kDa) proteases in the immature rat. Our experiments provide evidence for a role for both calpain and caspase-3 in the production of signature proteolytic fragments after TBI in immature rats. Increases in both the 120 kDa and the 145 kDa fragments of alfa-II-pectrin were detected as early as 15 minutes after injury and persisted for as long as 7 days. These results lay the ground to additional experiments aimed at further defining the relative contribution of each calpain and caspase-3 to the pathophysiology of TBI in the immature brain. (Supported by DAMID-01-1-9565, DAMID-O1-1-0765, NIH R01 NS39091 and the Howard Hughes Medical Institute Biomedical Research Support Program)
P145. ZINC CHELATION ALTERS THE MOLECULAR PROFILE OF STRESS SIGNALING PATHWAYS IN TRAUMATIC BRAIN INJURY
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Considerable evidence implicates zinc neurotoxicity in the pathogenesis of brain injury and neurodegenerative diseases such as Alzheimer’s disease (AD). In a transgenic mouse model of AD, zinc chelation significantly reduced brain b-amyloid deposition. This evidence, together with the known risk of traumatic brain injury (TBI) for development of AD, suggests that dysregulation of zinc homeostasis could contribute to the pathology of both diseases. We hypothesized that chelating zinc using calcium EDTA would alter the expression of stress response genes following fluid percussion TBI in rats. Zinc chelation had a salutary effect on temporal patterns of gene expression. Most notably, the chelation treatment enhanced the upregulation of neuroprotective genes after TBI. These included the heat-shock proteins HSP70 and HSP27, antioxidant genes such as cellular glutathione peroxidase-1, and heme oxygenase-1, which is activated by inflammatory and oxidant stress and has been shown to have a protective role in vascular wound repair. The expression of genes, such as the intermediate filament protein vimentin, that are involved in regenerative processes was also further upregulated by zinc chelation. After TBI alone, multiple members of the mitogen-activated protein kinase (MAPK) family that regulate downstream cell signaling pathways, including those involved in the pathophysiology of neurodegenerative diseases, are activated. Treatment with the zinc chelator had either no significant effect on these pathways or increased the expression of some of the MAPK genes, which have a positive role in cell survival. Finally, there was a salutary effect of zinc chelation on the expression of various cell cycle regulatory genes that are dysregulated following TBI.

P146. EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL mRNA EXPRESSION LEVELS OF CALPAINS AND CASPASES AFTER TRAUMATIC BRAIN INJURY IN RATS

Despite a preponderance of studies demonstrating gene expression and/or enzymatic activation of calpain and caspase proteases after traumatic brain injury (TBI), no studies have examined the effects of injury severity on these important families of cell death effectors after TBI. In addition, relative expression levels of these various gene products for a given injury is unknown. Thus, determination of the effects of injury severity on specific expression profiles will be critical to understanding the various pathways to parenchymal pathology. This investigation tested the hypothesis that different injury magnitudes cause different regional and temporal patterns of mRNA expression of mu- and m-calpain, calpastatin, caspases 3-, 8-, 9-, and bid after 1.0, 1.2, and 1.6 mm lateral cortical impact TBI in rats. Methods: Quantitative RT-PCR was used to compare effects of injury on mRNA levels in ipsilateral (injured) cortex and hippocampus from 6 h to 5 days post-injury compared to sham-injured controls. Results: TBI resulted in significant increases in all genes examined with highest expression in the cortex. Generally, higher injury magnitude caused higher gene expression. The most robustly expressed genes were bid, caspase-3, and -8. Caspase-9 was the lowest expressed gene and levels were undetectable in hippocampus. Interestingly, alpha calpain is known to be activated within minutes after TBI, mRNA expression of calpains was highest 72 h to 5 days post-TBI. Discussion: This study provides a detailed analysis of regional and temporal expression of calpains and caspases after TBI. Studies provide critical insight into distinct profiles of individual patterns of gene transcription during the evolution of the sub-acute response to TBI between various injury magnitudes. Studies comparing mRNA expression vs. enzymatic activity will be critical for developing gene and protein based therapies for treatment of TBI. (Supported by DAMD17-99-1-9655, DAMD17-01-1-0765, NIH R01 NS39091, NIH R01 NS40182, and USAMRMC)

P147. THE PREDICTIVE VALUE OF PROCALCITONIN AND S 100 B IN TRAUMATIC BRAIN INJURY
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Background: Procalcitonin (PCT) is a marker of posttraumatic inflammation but has recently also been associated with local hypoperfusion and secondary brain damage after traumatic brain injury. S 100 B is a well-known marker of traumatic brain injury. The object of this study was to compare the predictive value of plasma PCT and S 100 B in patients with traumatic brain injury for the development of septic inflammatory response syndrome (SIRS) and outcome.

Patients and Methods: 102 consecutive patients with traumatic brain injury (ISS > 23, GCS < 8) admitted to either of the two participating level II trauma centers were included in this prospective study. Plasma PCT and S 100 B were measured at admission and daily thereafter for a maximum of 21 days using commercially available immunoluminometric assays (Lumitest PCT, B.R.A.H.M.S.-Diagnostika GmbH, Henningsdorf, Germany and LIAMat Sangtec 100, Byk-Sangtec Diagnostika, Bromma, Sweden.). The courses of PCT and S 100 B were evaluated with regard to SIRS (patients with and without SIRS according to the Bone criteria) and outcome (survivors versus non-survivors).

Results: A total of 68 survivors (8 with SIRS, 60 without SIRS) and 34 non-survivors (9 with SIRS, 35 without SIRS) were evaluated. Though elevated PCT levels later than 24 hours after trauma were always associated with SIRS, we found no relationship between elevated PCT and outcome. In contrast, the course of S 100 B later than 24 hours after trauma was always associated with outcome. In all non-survivors, regardless of whether they had SIRS or not, S 100 B was either continuously elevated or increased at least 48 hours before death.

Conclusion: PCT may be a sensitive predictive indicator of SIRS but is not associated with outcome following traumatic brain injury. S 100 B, however, appears to be a sensitive predictive indicator of outcome.

P148. CONTEXTUAL FEAR CONDITIONING TO ASSESS COGNITIVE DYSFUNCTION IN BRAIN INJURED MICE

The Morris Water Maze (MWM) is commonly used to assess the cognitive effects of fluid percussion injury (FPI) in rodents. Special obstacles unique to mouse species that may complicate the interpretation of MWM experiments have been identified (Carbonell, 1998); for example a significant percentage of individuals may be poor swimmers or non-learners. The present study examined the potential of contextual fear conditioning (CFC) as a technique to circumvent these issues and assess hippocampus-dependent cognitive deficits in FPI-injured mice. Mice were trained in CFC, injured and then assessed for cognitive function 6-8 days post-injury. Injured mice displayed significant deficits with respect to sham-operated control mice in freezing behavior when tested in the training context. Injured mice demonstrated a 38% decrease in freezing compared to sham-operated controls over the 5 min retrieval test (15.2% ± 1% compared to 24.4% ± 2%, p < .05, n = 17, 18 for FPI and SHAM respectively). In previous experiments, injured mice tested in the spatial version of the MWM displayed significant increases in both latency and search error, a measure of deviation from the optimal swimming path to the goal platform (Carbonell, 1998), with respect to sham-operated controls. Mice in both behavioral assays displayed similar and significant deficits in hippocampus-dependent tasks. As CFC avoids some of the inherent difficulties of the MWM when applied to mice, we propose that CFC is an appropriate and accurate technique to assess hippocampus-dependent cognitive deficits in FPI mice.
P149.
A 4-AXES MODEL OF THE STRESS RESPONSE
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This paper proposes an expanded 4-axes model of the physiological stress axis to help identify cross-cultural risk factors for traumatic brain injury (TBI), and its sequelae. The 4-axes stress response model includes the interrelated fast-acting hypothalamic-pituitary-adrenal (HPA) axis, the immediately fast-acting pituitary-atherosclerotic (PO) axis, the slow-acting glucose-glutamine-GABA (GGG) axis, and the hippocampal-glutamatergic (HC) learning/memory axis. An example of the interrelatedness is the high density of cortisol receptors and the co-localization of glutamate and opioid peptides within the hippocampus. Also, plasma glucose levels have been identified as an early marker to predict survival after TBI, since the brain is continually permissive to glucose.

Two relevant studies address the cognitive impairments seen even in mild TBI: damage to the HC axis. In the first study, tetra game experts, tetrax games, showed similar memory consolidation after awakening from slow wave sleep. Since the amnesiac group had no recent recall, the results established a dual system for memory and learning, whose path, from hippocampus to prefrontal cortex, is unidirectional. In the second study, it was found that a minimum of 6 hours sleep, preferably 8, was needed for memory consolidation, and that an "all-nighter" severely blighted memory consolidation even post 3rd day.

An indirect value of the expanded 4-axes stress response model is that it is easier to identify multiple points at which risk factors for TBI act, for example, alcohol intoxication. Alcohol regulates the 4-axes stress response at two points: by potentiating the glutamate receptor and by inhibiting the GABA receptor.

TBI is a towering, unresolved, worldwide public health concern. In an expanded stress response model, ongoing surveillance for the underdiagnosed TBI, for improvement of the consequences of TBI, and for understanding risks for prevention can augment our evolving efforts.

P150.
INDUCTION OF HIGH PURITY OLIGODENDROCYTE CULTURES FROM HUMAN EMBRYONIC STEM CELLS
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The broad developmental potential and replicative capacity of embryonic stem cells promise a virtually unlimited supply of specific cell types for transplantation therapies. One of the current challenges in the field is the development of methods to selectively differentiate multipotential human embryonic stem (huES) cells to cell-type specific precursors. Here we describe the induction of high purity oligodendrocyte cultures from huES cells. huES cell lines H1 and H7 were provided by Geron Corporation and expanded in culture conditions that did not contain mouse fibroblasts. Neural differentiation was initiated in embryo bodies using retinoic acid and basic fibroblast growth factor (bFGF). Glat-restricted precursor differentiation was initiated by gradually transitioning the cells from huES expansion media to a glial-restriction media containing mitogens. Clusters were enriched by an overnight incubation on adherent substrate, dissociated, then plated a week later on adherent imaging chambers at low density for cell counting and immunostaining. These studies demonstrated greater than 95% pure populations of oligodendrocytes. Thus, huES cells can serve as a plentiful source of oligodendrocyte-lineage restricted cells, which may have use in transplantation regimes aimed at treating demyelinated states of the central nervous system. This study was supported by Geron Corporation and BioSTAR.

P151.
QUANTIFICATION OF DIFFUSION TENSOR IMAGING PREDICTS AXIAL AXONAL INJURY FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

Diffuse axonal injury (DAI), commonly observed following human traumatic brain injury (TBI), is well characterized in animal TBI models. Diffusion tensor imaging (DTI) characterizes molecular displacement in highly oriented tissues and allows calculation of displacement pathways (white matter). Case studies suggest DTI may be altered in injury or disease states. This is the first study to quantitatively compare DTI with a pathological outcome related to TBI—DAI.

Moderate-severe TBI in adult male rats was induced via controlled cortical impact. Fixed brains were excited and DTI of 1H in water measured at 17.6 Telsa using a spin-echo sequence with diffusion gradients applied in 7 directions using 3 weightings. The diffusion tensor of water was calculated and fiber tracts derived from the principal diffusion direction. Fractional anisotropy (FA) and directional coherence (DC) of specific white matter tracts were calculated. Number of injured axons, retraction ball immune-positive for NFP68, within specific tracts were counted and compared with DTI related parameters.

Derived fiber tracts were consistent with corresponding sections in a rat brain atlas. FA and DC values were inversely related to number of injured axons. These results indicate that DTI is a useful tool in assessing diffuse white-matter tract damage. Additionally, they suggest DTI quantification techniques may allow clinicians to better assess damage following TBI. We intend to extend this technology to in vivo. BSCIRT 2001, NIH P01 NS53702, P41 RR16105, NIH R01 NS50901, R01 NS40182, US Army DAMD17-99-1-5655.

P152.
RESPONSE OF NEURONS CULTURED IN TWO- AND THREE-DIMENSIONS TO DYNAMIC SHEAR DEFORMATION
D. Kacy Cullen and Michelle C. LaPlace. (Department of Biomedical Engineering, Georgia Tech. Atlanta, GA, USA).

In vitro models of traumatic brain injury (TBI) have evaluated post-injury alterations in cell biochemistry and viability by utilizing 2-D cell cultures, a contrast from the 3-D architecture of native brain that may lead to a deviation in the injury response. Fundamental differences exist between cells cultured in 2-D and 3-D in terms of cell morphology, cell-cell/cell-matrix interactions, response to biochemical stimuli, and gene expression (1). However, it is unknown whether a differential response to high rate shear deformation—the most prevalent type of deformation in TBI (2)—will exist between these configurations. Primary rat cortical neurons (E17) were plated in 2-D (2.5 × 10^5 cells/cm²), below an acellular 3-D extracellular matrix (ECM) gel), or 3-D (6.0 × 10^5 cells/cm³), distributed throughout an ECM gel) configurations. Cultures in these two configurations were characterized based on viability, neuronal purity, and neurite extension, and injured via high strain rate simple shear deformation (20s-1 or 30s-1, 0.50 strain).

Prior to injury, both 2-D and 3-D cultures formed mature neuronal networks consisting of ~97% neurons with no significant differences in cell viability at one-, two-, or three-weeks post-plating. However, neurons plated in 3-D (n = 3) had a 1.9-fold increase in the expression of Tau, a cytoskeletal protein associated with neurite outgrowth, versus 2-D cultures (n = 3; p < 0.05). After high rate shear deformation, cells cultured in a 3-D orientation (n = 17 at 20s-1, n = 14 at 30s-1) experienced a significant decrease in cell viability compared to cells in a 2-D orientation (n = 19 at 20s-1, n = 14 at 30s-1; p < 0.05). These results begin to establish fundamental characteristics of primary cortical neurons in 2-D and 3-D configurations and, furthermore, show differences in the response of these cell cultures to high rate shear deformation, suggesting a possible role for cell orientation and cytoskeletal protein expression in the response to a mechanical insult. Accurate cellular models of TBI are important to develop mechanically-driven intervention strategies and can therefore serve as valid pre-animal and pre-clinical test beds.

P153. ADAPTATION OF SENSORIMOTOR AND COGNITIVE TASKS FOR USE WITH MICE: EFFECTS OF CONTROLLED CORTICAL IMPACT INJURY AT VARIED INSULT LOCATIONS

Yelena K. Baskin, Annamarei J. Bronwell, W. Dalton Dietrich and Edward J. Green. (Departments of Psychology and Neurological Surgery, University of Miami, Miami, FL USA).

In an effort to characterize the behavioral deficits seen after traumatic brain injury (TBI), specific sensorimotor tasks, the gridwalk task and spontaneous forelimb use task (SFL), along with the Barnes circular maze spatial task, were adapted for use with the mouse. The SFL and gridwalk tests have not been used extensively in mice, particularly with forebrain insults. Male C57BL/6 mice were anesthetized and given parasagittal controlled cortical impact (CCI) injury or sham procedures, targeting right anterior, middle, or posterior locations relative to bregma (n = 9–10 per group). All injuries were performed using a 3mm impact tip at a velocity of 6.89m/sec, to a depth of 1mm. Animals were tested pre-operatively on the sensorimotor tasks, and post-operatively once per week for four consecutive weeks, and again at five months. There was no overall post-operative forelimb use asymmetry on the SFL task. However, significant contralateral forelimb deficits were observed in each task for at least one month post injury, depending upon insult location. Barnes maze testing during month two revealed significant TBI-related and insult location dependent deficits in spatial acquisition and on several probe trial measures, relative to shams. Histopathological analysis indicated that all TBI animals displayed cortical and striatal damage, with anterior TBI mice presenting with the most extensive striatal lesions and posterior TBI animals having hippocampal pathology. The present results demonstrate the effectiveness of these tests for use in evaluating behavioral deficits following TBI in the mouse. Supported by NS30291.

P154. PROGESTERONE IMPROVES BEHAVIORAL AND MORPHOLOGIC OUTCOMES AFTER TRAUMATIC BRAIN INJURY IN MICE

Douglas W. Lowery, Joshua E. Logan, Deborah A. Shear, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).

The beneficial effects of progesterone in rat models of traumatic brain injury have been well documented. With the development of progesterone receptor knockout mouse strains, there is increased interest in exploring the effects of progesterone on traumatic brain injury in a mouse model. This study aims to characterize the behavioral and morphologic effects of exogenous progesterone after traumatic brain injury in mice.

Male C57BL/6 mice were subjected to sham surgery or lateral impact cortical contusion. One hour after surgery, animals were injected intraperitoneally with vehicle (cyclolexdrin) or progesterone (8mg/kg, 16mg/kg, or 32mg/kg). Subsequent injections were given subcutaneously at 6 hours post-injury, and daily for 5 days. Behavioral outcomes were assessed using the rotord the Morris Water Maze (MWM). Rotord performance was measured one day before surgery, and at 7 and 14 days post-injury. MWM was assessed daily on days 15–28 post-injury.

No significant injury-induced deficits were observed on the rotord. MWM testing showed that the 8mg/kg and 16mg/kg doses of progesterone attenuated injury-induced cognitive impairment, whereas the 32mg/kg dose had no beneficial effect.

Collectively, these results suggest that the behavioral benefits of progesterone are conserved in this mouse model of TBI and that 16mg/kg/day of progesterone for 5 days is the optimal dose for facilitating recovery of function. Supported by NIH grants R01NS38864, R01NS40825, & R05HD040235.

P155. THE NEUROPROTECTIVE EFFECTS OF PROGESTERONE ARE ASSOCIATED WITH MODIFIED GENE EXPRESSION IN RAT CORTICAL IMPACT MODEL

Edward H. Pettus, David W. Wright, Stuart W. Hoffman, Donald G. Stein. (Emergency Medicine, Emory University, Atlanta, GA US).

Although progesterone has been shown to be therapeutic in animal models of traumatic brain injury, the cellular mechanisms behind this therapeutic neurosteroid remain unknown. Goal: characterize and quantify the influence of progesterone on post-injury gene expression related to cellular pathology and/or protection.

Adult male rats received either bilateral prefrontal cortical contusion or sham surgery. Post-injury and sham rats were given 25% Cyclolexdrin (vehicle), or 16mg/kg progesterone in vehicle. Treatments were given 1hr, 6hr, and 24hr post-injury. At 48hrs post-injury, the rats were killed, and brains extracted. Both frontal lobes were dissected out; right for edema measures and left for RNA isolation. The isolated RNA served as a template for cDNA, which was then used as a template for the labeled cRNA that was hybridized to the Affymetrix™ U34A (8800 genes) chip. Chips were scanned and analyzed in Emory’s core facility.

Results indicated that animals given vehicle had higher water content than either the sham-operated or post-injury rats given 16mg/kg of progesterone (p < 0.05) (previously presented). Rats given progesterone post-injury had modified expression of 768 genes with 96 genes increased, and 405 decreased versus those given vehicle post-injury. Genes were divided into groups related to: oxidative stress, inflammation, growth factors, neural and metabolic activity, cell death, cytoskeleton, and myelination. Progesterone decreased expression of pro-inflammatory cytokines, markers of metabolic activity, indicators of injury, and genes promoting apoptotic and necrotic cell death. Pregestosterone increased the expression of cytoskeletal and myelin proteins. The data shows complex gene modulation by progesterone that will be further explored with descriptive and quantitative studies of related protein distribution. Support: NIH/NINDS R01NS38864 and General-CologneRe.

P156. ANESTHESIA AFFECTS GENDER-RELATED FUNCTIONAL OUTCOME FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY IN RATS

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A number of studies have demonstrated that outcome in males and females are different following traumatic brain injury. Some report increased mortality and morbidity in males following trauma while others report that males do significantly better than females under certain conditions (1). These conditions involve type of anesthesia and outcome measure. In the present study we have used three different anesthetic protocols and four different outcome measures to determine how these parameters affect functional outcome following traumatic brain injury in male and female rats. Diffuse traumatic brain injury was induced in adult male and female animals using the impact-acceleration brain injury model (2). Mortality in female animals was no different than males when using halothane anesthesia, slightly less than males when using isoflurane anesthesia, but significantly worse than males under pentobarbital anesthesia. Female animals always performed better than males on rotord tests of motor outcome, with this effect being unrelated to anesthetic effects. Conversely, in cognitive tests using the Barnes Maze, only isoflurane-anesthetized females performed better than their male counterparts. Similarly, in an open field activity task, females always performed better than males after trauma, with isoflurane-anesthetized females doing significantly better than the halothane-anesthetized female group after injury. Our results suggest that female animals do better than males after diffuse traumatic brain injury, although this observation is dependent upon the type of anesthesia and the functional task employed. Isoflurane is particularly protective in females, pentobarbital is deleterious to female outcome, while halothane anesthesia has the least influence on gender-related outcome.

P157. A PARALLEL RANDOMIZED DOUBLE-BLIND MULTICENTRE CLINICAL TRIAL FOR THE EFFICACY AND SAFETY OF NALOXONE IN ACUTE TRAUMATIC BRAIN INJURY
Yuanli Zhao MD,1 Jiyaof Jiang MD,2 Li Li MD,1 et al. on behalf of the National Naloxone Study Group. (1Beijing Neurosurgical Institute. 2Shanghai Neurosurgical Institute).

This clinical trial was designed to test the efficacy and safety of naloxone in treatment of traumatic brain injury. It was organised by Chinese neurological society and Chinese Journal of Neurosurgery, which was carried out in 18 major neurological centres in China.

Methods: From 1999 to 2001, a randomised double blind prospective multicentre clinical trial was implemented to compare the difference of naloxone and saline in moderate or severe traumatic brain injured patients. Naloxone or saline placebo was intravenously given for 10 days and follow-up for 3 months. The dosage of Naloxone is 0.3mg/day/kg. Glasgow Coma Scale, Glasgow outcome scale, Karnofsky performance scale, motor function and verbal function were the index of assessment for patients' prognosis.

Results: A total of 330 cases were enrolled in our clinical trial and 511 cases met the need of statistics. The mortality of naloxone group and saline placebo group was 12.5% (22/175) and 17.5% (44/255) respectively (P < 0.05). Glasgow coma scale in naloxone group was significantly better than that in saline placebo group starting at 5 days after treatment (P < 0.05). Glasgow Outcome Scale, KPS, verbal and motor functions in naloxone group were also statistically better than those in saline placebo group with a mean follow-up period of 3 months (P < 0.05). In addition, naloxone did not show any side effects.

Conclusions: The data of our trial has confirmed that early application of naloxone for acute brain injured patients could significantly reduce the mortality and morbidity with side effects.

P158. DOWNREGULATION OF AMYLOID PRECURSOR PROTEIN (APP) mRNA EXPRESSION FOLLOWING POST-TRAUMATIC CYCLOSPORIN-A ADMINISTRATION
*James J. Donkin*, Corinna Van Den Heuvel1, John W. Finnie, Peter C. Blumberg2, Tim Kuchel3, Barbara Koszyca1, Jim Manavitz4, Nigel R. Jones1, Peter L. Reilly2 and Robert Vaid3 (Departments of Pathology and Neurosurgery, University of Adelaide, and Department of Neuropathology and the Veterinary Division, Institute of Medical and Veterinary Science, Adelaide, Australia).

Amyloid precursor protein (APP) mRNA and antigen are rapidly increased following traumatic brain injury (TBI) and the expression is further increased following administration of the neuroprotectant magnesium sulphate (MgSO4)[1]. The aim of these studies was to assess and quantitate the effects of another neuroprotectant, Cyclosporin-A (CyA), on APP mRNA expression in sheep brains after a controlled focal head impact. Two-year-old merino ewes were injured under isoflurane anaesthesia using the humane stereotaxic method as previously described in detail elsewhere [1]. Animals were then killed at 2 h or 6 h after injury, and their brains removed, sectioned and snap frozen in liquid nitrogen for PCR analysis. In contrast to the upregulation of APP previously observed with MgSO4, post-traumatic administration of CyA resulted in a rapid decrease in APP mRNA expression. CyA treatment caused a statistically significant 1.3 ± 0.1 fold decrease in APP mRNA in the central grey matter of CyA treated impacted sheep compared to untreated impacted sheep 2 hours post-injury (p < 0.05). A more profound reduction in APP mRNA synthesis (1.6 ± 0.2 fold) was evident at 6 hours (p < 0.001). These results show that CyA has a downregulatory effect on the increased APP expression caused by TBI. This has potential implications as a means of preventing APP overexpression and possibly the pathological processes underlying AD.


P159. THE NEUROPROTECTIVE EFFECTS OF PROGESTERONE AND ALLOPROGESTERONE AFTER CONTROLLED CORTICAL INJURY IN RATS
Myrtam J. Djeballi*, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).

We have previously shown that systemic injections of progesterone (16mg/kg) are neuroprotective, leading to improve behavioral outcomes following impact injury (CCI) to the frontal cortex. In order to determine if the neuroprotective effect is specific to this neurosteroid, we compared the effect of progesterone (16mg/kg) injections to different doses (0, 4, 8, and 16mg/kg) of progesterone metabolites, allopregnanolone. The vehicle for these substances was cycloexetrin.

Our results show that 24h after CCI, progesterone and allopregnanolone (4mg/kg) decreased both body weight and cortical neuronal loss compared with injured animals injected only with vehicle. The higher doses of allopregnanolone did not affect body weight loss, but surprisingly the number of spared cortical neurons was reduced as compared to those in injured-vehicle animals. This demonstrates that allopregnanolone at doses between 1-16mg/kg are toxic in our injury model. Under light microscopy we found that the number of apoptotic neurons was greatly reduced in rats given progesterone, while number of apoptotic neurons was only slightly reduced in the allopregnanolone-treated rats. Currently, histological assays using Akt and caspase-3 immunocytochemistry are in progress to determine the molecular anti-apoptotic mechanisms being affected by these neurosteroids. Additional behavioral assays will determine if there is a relationship between type of treatment, apoptosis, and behavioral outcome.

This study indicates that progesterone and allopregnanolone both inhibit apoptosis after injury, but progesterone seems to be more efficient in this effect. Supported by NIH grants 1R01NS40825, 1R01NS38664, & S03HD040295.

P160. PREGNENOLONE FACILITATES RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY
Melissa A. Arellano*, Robert M. Simkins, IV, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).

Past studies have shown that both progesterone and its metabolite, allopregnanolone, have been effective in promoting functional recovery following traumatic brain injury (TBI). Pregnenolone, a progesterone precursor, has been associated with neuronal microtubule formation, implicating its role in neural plasticity. In keeping with this we decided to examine the role of chronic administration of pregnenolone in promoting recovery of function following traumatic brain injury.

Male SD rats received either sham surgery or bilateral cortical impact injury to the medial frontal cortex. On surgery day, animals were injected intraperitoneally 1 hour after injury and subcutaneously (SC) 6 hours after with either pregnenolone (6.33 mg/kg) or vehicle (sesame oil). Injections were continued twice a day for 3 weeks post-injury. A baseline measure of bilateral sensory neglect was established one day prior to surgery and then evaluated post-operatively on days 6 and 20. Spontaneous motor activity was also assessed on post-injury days 2, 5, and 10 after injury. Spatial learning was measured using the Morris water maze (MWM) task for 2 blocks of five days, starting 36 hours after the end of the 3-week injection period. Animals were then perfused and their brains paraffin embedded for histological analyses.

Results of behavioral testing show that pregnenolone facilitates improved sensory performance in animals with bilateral cortical contusions. Although initially, pregnenolone-treated animals showed a deficit in MWM, by day 6 of testing, they showed enhanced performance compared to vehicle treated animals. Supported by NIH grants 1R01NS36804, R01NS40825, & S03HD040295 and a gift from GeneralColognere.
P161.

STERIODS IN SEVERE TBI: A META-ANALYSIS
Anne-Marie Guerguerian, Alexander Agthe, Sean Berenthal, Elizabeth Bradley, Christopher Consent and Susan Gerhardt. (Johns Hopkins Medical Institutions, Baltimore, MD, US)

Objectives: We systematically reviewed and quantitatively evaluated the literature to assess the efficacy of steroids in severe TBI to improve survival and neurologic outcome, in order to determine whether this therapeutic option was discarded prematurely.

Methods: PubMed, Embase, and the Cochrane Controlled Trials Registry databases were searched from 1964 to 2002. Randomized controlled trials (RCT) in English or German were extracted, involving adults and/or children with severe closed TBI, receiving either corticosteroids or placebo. Risk ratios (RR) of events were used to determine pooled estimates of risk using a random effects model.

Results: We retrieved 749 articles. Eleven articles remained for analyses involving 2611 subjects. Dexamethasone was used in seven studies, methylprednisolone in two, triamcinolone acetonide in one, and triliskalizol in one. The doses ranged from 17 mg to 2300 mg of dexamethasone equivalent. The pooled estimate RR of death in the steroid group compared to the placebo group was 0.93 (95% CI: 0.78–1.10, p-value: 0.41, homogeneity statistic 15.24 df: 9, p-value: 0.084) and RR of death and disability was 1.09 (95% CI: 0.87–1.35, p-value: 0.45, homogeneity statistic 37.86, df: 10, p-value: 0.000). A regression of the RR of death with the dexamethasone dose showed an increase of RR by 1.1% for every increase in 100 mg of dexamethasone (0.011, 95% CI: -0.02-0.04, p-value: 0.442) and the RR of death in the steroid group was 19% higher for patients receiving a dose above 500 mg compared to a dose below this value (0.19, 95% CI: -0.17-0.55, p-value: 0.311).

Discussion: Our analyses of the evidence show that steroids neither present a beneficial or detrimental effect on the risk of death of patients with severe TBI. We suggest that the pre-clinical experimental evidence has not been translated into a beneficial clinical endpoint because questions such as determining the efficacy of the dose and type of steroid remain unanswered. The information reported did not allow us to evaluate the effect of age, gender, and management strategies as confounders or effect modifiers on the outcome. To quantify their impact, reporting of adherence to therapeutic management strategies and documentation of clinical confounders during acute and rehabilitation phases are essential for ongoing trials.

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P162.

DELIBERATE MILD HYPOTHERMIA FOR TREATMENT OF SEVERE BRAIN INJURY
Roman Gollst1, Ivan Cundy1, Martin Smrcka2 (1Department of Anaesthesi- and Intensive Care, 2Department of Neurological Surgery, University Hospital Brno, Brno, Czech Republic).

Thirty patients with severe head injuries with Glasgow Coma Scale (GCS) score of 3-8 were enrolled into the study. The subjects were divided into two groups. The average age in the hypothermic group of 15 patients (10 subdural and 3 epidural hematomas, 2 brain contusions) was 35 years. The average GCS was 4.5 at the site of accident. The average age of the 15 patients (7 subdural and 7 epidural hematomas, 1 brain contusion) in the normothermic control group was 39 years with an average GCS of 4.5. All the patients in the hypothermic group and 11 patients in the hypothermic group underwent neurosurgery. The standard treatment was guided according to the European Brain Injury Consortium protocol. Cooling to a core temperature of 34°C in the hypothermic group was achieved by forced air cooling in combination with circulating-water mattress cooling (Blanketrol II, Climatherm Sub-Zo). The difference in the Glasgow Outcome Scale (GOS) between the hypothermic and normothermic groups of patients after six months was not statistically significant (p value 0.0643). In the hypothermic group, however, good neurological outcome (GOS 4 and 5) was reached in 13 patients (87%), which represents a 40% increase compared with the normothermic control group in which good neurological outcome was reached in 7 patients (47%). Mean normothermia ICP value of 18 ± 2 mmHg was significantly (p value 0.0007) reduced during mild hypothermia therapy to 12 ± 2 mmHg. Mean normothermia CPP value of 72 ± 3 mmHg significantly increased (p value 0.0007) during this time to 90 ± 4 mmHg with unchanged systolic arterial pressure (p value 0.0013).

Our results showed that mild hypothermia could be useful in improving the outcome and neurological recovery, especially in brain injured patients with surgical lesion on admission.

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P163.

REGULATION OF HYDROGEN PEROXIDE PRODUCTION BY BRAIN MITOCHONDRIA BY CALCIUM AND A BHF DEATH DOMAIN PEPTIDE
Gary Fiskum and Anatoly Starkov. (University of Maryland School of Medicine, Baltimore, MD, US).

Background: Abnormal accumulation of Ca2+ and exposure to pro-apoptotic proteins, e.g., Bax, is believed to stimulate mitochondrial generation of reactive oxygen species (ROS) and contribute to neuronal cell death during acute ischemic and traumatic brain injury. However, the mechanism by which Ca2+ or apoptotic proteins stimulate mitochondrial ROS production is unclear. This study tested the hypothesis that Ca2+ can either stimulate or inhibit mitochondrial ROS generation, depending on the source of electrons donated to the respiratory chain and the effects of Ca2+ on the retention of mitochondrial cytochrome c. We also tested the hypothesis that mitochondrial ROS production is stimulated by cytochrome c release elicited by exposure to Bax and a peptide containing a BH3 cell death domain.

Methods: H2O2 production by isolated rat forebrain mitochondria was monitored fluorometrically using Amplex Red in the presence of ATP and Mg2+ and different respiratory substrates. Mitochondrial membrane potential was monitored with the fluorescent dye Safranine O. Ca2+ transport was measured by monitoring the medium [Ca2+] using the fluorescent dye Calcium Green. Cytochrome c was quantified using ELISA.

Results: Ca2+ uptake suppressed H2O2 generation and reduced the membrane potential of mitochondria oxidizing succinate or glutamate plus malate. In the presence of the respiratory chain inhibitor rotenone, Ca2+ stimulated H2O2 production by mitochondria oxidizing ascorbate and induced the release of cytochrome c. In the absence of Ca2+, release of cytochrome c induced by BAX protein plus a BH3 cell death domain peptide also stimulated H2O2 production.

Conclusion: In the presence of physiological concentrations of ATP and Mg2+, Ca2+ accumulation suppresses mitochondrial ROS production by decreasing mitochondrial membrane potential. However, Ca2+ as well as Bax plus a BH3 domain peptide stimulate ROS production when conditions favor mitochondrial release of cytochrome c.

Support: NS43152 and ES11838.

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P164.

EFFECT OF DEXTROMETHORPHAN—A NON-COMPETITIVE NMDA ANTAGONIST—ON THE SECONDARY GROWTH OF A CORtical NECROSIS FROM FOCAL COLD INJURY

Objective: A cortical lesion induced by cold injury leads to an immediate burst of excitatory amino acids into the traumatic penumbra. Aim of the study was to investigate, whether the secondary growth of a cortical necrosis can be attenuated by the non-competitive NMDA antagonist dextromethorphan.

Material & Methods: Male SD-rats (n = 17) were anesthetized (N2O/air/oxygen) and ventilated. The tail artery/vein were cannulated for measurement of mean arterial blood pressure/blood gases and drug administration. Brain temperature was kept constant (37.0°C) by an electrode in the temporal muscle and feedback-controlled heating. Dextromethorphan (solved in NaCl) was given i.v. 20 min before trauma (20 mg/kg) followed by 10 mg/kg/h until sacrifice. The sham group received NaCl (4 ml/kg) followed by 2 ml/kg/h. After right parietal trephination, a standardized freezing lesion was induced onto the brain cortex. 24 h later, the animals were sacrificed and brains were removed for histology.

Results: At 24 h after focal cold injury (i.e. maximal necrosis spread in rats) sham treated animals had a cortical lesion with a necrosis volume of 3.82 ± 0.45 mm3. After treatment with dextromethorphan, the animals developed a necrosis of the cortex with a volume of 3.87 ± 0.93 mm3 (n.s.). In relation to the volume of the ipsilateral hemisphere, the cortical necrosis expanded to 5.6 ± 0.1 % in sham treated animals and to 5.9 ± 0.2 % in the dextromethorphan group. Throughout the observation period, mean arterial blood pressure remained constant (iamb: 82 ± 4 mmHg, dextro: 81 ± 3 mmHg) without statistically significant differences between the groups.

Conclusion: Administration of dextromethorphan prior to a focal cold injury to the brain does not attenuate the amount of tissue damage 24 h after trauma. Accordingly, it must be surmised that glutamate does not mediate secondary necrosis growth of a traumatic cortical lesion via the NMDA receptor.

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P165. NEURAL STEM CELL TRANSPLANTS FOLLOWING BRAIN INJURY IN RATS: CELLULAR SURVIVAL AND BEHAVIORAL CONSEQUENCES
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Certain members of the newly discovered family of intrinsic inhibitors of apoptosis (IAP) proteins can directly bind and inhibit caspases. However, IAPs have been shown to undergo cleavage by caspases in response to inducers of apoptosis, but the significance of IAP cleavage has not been established. One IAP family member that is of particular interest in gender studies is the X-linked inhibitor of apoptosis (XIAP) that undergoes cleavage following traumatic brain injury. Since estrogen has been shown to have anti-apoptotic properties, this study examined gender differences and the influence of estrogen on XIAP processing during apoptosis after TBI. Male (TBI-M, n = 6), female (TBI-F, n = 3), ovariecotoimized female (TBI-OVX, n = 5) and ovariectomized females supplemented with estrogen (TBI-OVX + E2, n = 7) Sprague-Dawley rats were intubated, anesthetized (70%N2O, 0.5% halothane, 30%O2) and subjected to a moderate (1.7-2.2 atm) fluid percussion injury (FPI). Animals were sacrificed 24 hours after FPI; cortical tissue (ipsilateral and contralateral) was dissected and analyzed for XIAP processing by immunoblot analysis and quantitative densitometry. Significant differences in XIAP cleavage in the ipsilateral cortex were found between groups (p < 0.05). Post-hoc analysis showed an increase in XIAP processing in both TBI-F and TBI-OVX + E2 compared to TBI-M and TBI-OVX (p < 0.05), indicating that more XIAP is cleaved following injury in intact females and estrogen supplemented ovariectomized animals than in TBI-M and TBI-OVX groups. Based on these data, we propose that estrogen may provide neuroprotection by regulating XIAP cleavage after injury. This regulation may be influenced by exogenous estrogen treatment. (NS 30291 & Eli Lilly and Co.)

P166. COMPARISON OF TWO PROTOCOLS TO DIFFERENTIATE BONE MARROW STROMAL CELLS INTO NEURONS OR GLIA
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Recent reports suggest that adult-derived bone marrow stromal cells (BMCs) become neuron-like after differentiation, implying that BMCs could be used for repair after CNS injury. However, in vitro protocols for neuronal differentiation of BMCs vary substantially and have not been systematically compared. This study replicated and compared the Woodbury et al., 2001 and the Deng et al., 2001 protocols for BMCs differentiation in sister cell preparations. BMCs were harvested from adult rat femurs/tibias, passaged at least 6 times, differentiated and processed for immunocytochemistry for NeuN and GFAP at various time-points. Immunoreactivity was analyzed hourly during the Woodbury differentiation and daily using the Deng method. To determine longevity of differentiated state, cells were then placed in their respective maintenance media or stem cell feed and evaluated by light microscopy for morphology as well as processed for immunoreactivity at 12, 24, and 72 hrs. An adaptation of unbiased stereological technique was used for cell counting. Although highly variable, results show a higher percentage of NeuN and GFAP positive cells in the Woodbury vs. Deng protocol as differentiation time increased. Additionally, cells removed from the maintenance media in both conditions had a significant decrease in expression for either NeuN or GFAP. In conclusion, although results show higher NeuN positivity using the Woodbury protocol, the percentage of differentiated cells is highly variable within each condition in both protocols. This suggests that BMCs have a large heterogeneity of potential and that maintaining a differentiated state is dependent on specific environmental conditions which may question the benefit of BMCs in replacement therapy. Supported by UC President’s Undergraduate Fellowship and UC Neurotrauma Research Initiative.

P167. THE EFFECT OF RUTHENIUM RED, AIDA AND MK-801 ON MITOCHONDRIAL MEMBRANE POTENTIAL (MMP) IN STRAIN-INJURED ASTROCYTES
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The purpose of these studies was to determine if astrocytes in neuronal plus glial cultures show a reduction in mitochondrial membrane potential (MMP) in response to strain (stretch) induced injury, as previously reported in pure astrocyte cultures, and to probe potential mechanisms which reduce MMP in astrocytes of mixed cultures. Rhodamine 123 fluorescence was used as an indicator of MMP. We found that astrocytes in injured mixed cultures displayed an identical drop in MMP to that in pure astrocyte cultures. Previous studies have shown that there is post-traumatic activation of group 1 metabotropic receptors (mGlur1) in astrocytes. However we found no effect of the mGlur1 antagonist AIDA on injured astrocyte MMP.

We have also previously shown that pretreatment with NMDA antagonists partially reduces the loss of MMP seen in neurons of strain-injured neuronal plus glial cultures (J. Neurotrauma 17: 957, 2000). Because neurons and glia signal to each other and NMDA receptors have recently been reported on astrocytes, we examined the effect of MK-801 pretreatment on astrocyte MMP in neuronal plus glial cultures. We found that MK-801 significantly increased uninjured astrocyte MMP by 20%, but did not effect the reduction in MMP found in injured astrocytes.

Following injury, intracellular Ca2+ is thought to be in part buffered by mitochondrial uptake. We used the mitochondrial Ca2+ - unporter inhibitor Ruthenium Red (RR) to determine if blockade of mitochondrial Ca2+ uptake might affect astrocyte MMP. RR pretreatment had no effect on uninjured astrocyte MMP, but reduced the post-traumatic decrease in astrocyte MMP by one-third. These studies suggest that post-traumatic mitochondrial Ca2+ influx may play a role in the reduction of astrocyte MMP, but do not support a role for mGlur1 or NMDA receptors in loss of astrocyte MMP in strain-injured neuronal plus glial cultures. Supported by NS-27214.

P168. GLIAL NEURONAL SIGNALING IN NEUROTRAUMA, STUDIED IN PRIMARY CULTURES

After a brain damage, nucleotides, nucleosides and glutamate (Glu) are released from the damaged region, and pH drops. These substances and altered conditions induce microglial proliferation, and activation of both microglia and astroglia with a resultant production of an array of molecules including cytokines. We report on astroglial-microglial interactions in a model system of primary cultures and co-cultures when Glu level is increased and pH decreased. We report on early microglial activation with the formation of vacuoles followed by astroglial and microglial interactions with cytokine production, and we also report on microglial process and network formations. The substances produced are highly neuroactive with effects on other cell types in the nervous system. Interleukin-1 beta (IL-1 beta) decreases connexin 43 expression, and thereby astroglial gap junction coupling. Tumor necrosis factor alpha (TNF-alpha) reduces astroglial glutamate uptake capacity. Potassium, released from microglial and astroglial cells further impairs the astroglial glutamate uptake capacity. Microglia can also produce and release toxic substances such as free radicals, superoxide radicals, and nitric oxide (NO) to participate in cytotoxic reactions. Transforming growth factor-beta (TGF-beta) inhibits the release of TNF-alpha, and vasoactive intestinal peptide (VIP), released from depolarized neurons, and adenosine, released from astrocytes participate in protective mechanisms. VIP induces the synthesis of astroglial derived neurotrophic factor (ADNP), which in turn induces the production of neurotrophin-3 (NT-3). Adenosine increases astroglial glutamate uptake capacity and stimulates the production of trophic factors of importance in the rebuilding of the nervous system, such as basic fibroblast growth factor (bFGF), nerve growth factor (NGF) and interleukin-1 (IL-1). In conclusion, close interactions between astroglia and microglia could be important during early phases of a brain injury and during neuroprotection.
P169. SIGNALING FROM ATP RECEPTORS TO ERK IN AN IN VITRO MODEL OF TRAUMATIC BRAIN INJURY
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Previously we reported that rapid, reversible stretch-induced injury of astrocytes cultured on deformable elastic membranes resulted in activation of extracellular signal regulated protein kinase (ERK), a key regulator of cellular proliferation and differentiation. When ATP released by injury was hydrolyzed by ectonucleotidase, ERK activation was significantly reduced, suggesting the involvement of ATP/PP2 receptors. To test this hypothesis, we studied the effect of P2 receptor antagonists on injury-induced ERK activation in primary cultures of rat cortical astrocytes. Treatment of astrocytes with uramin, a broad-spectrum P2 receptor antagonist, inhibited injury-induced ERK activation by 65%. Reactive blue 2, an antagonist of P2X2 receptors which are expressed on astrocytes and P2Y6 and P2Y12 which are not, was not effective as uramin. P2A3D, an antagonist of several P2X and P2Y receptors subtypes expressed on astrocytes, was partially effective. Brilliant blue G, an antagonist of P2X7 receptors, did not inhibit injury-induced ERK activation. In addition, pre-injury treatment with EOTA almost completely blocked activation of ERK, indicating that an influx of calcium is required for ERK activation. These results, as well as P2X2 agonist studies of ERK activation in uninjured astrocytes, suggest that ionotropic P2X receptor, perhaps P2X2 receptor, are selectively activated after injury. However, further studies are needed to identify the P2X subtype(s) involved and to evaluate the possible role of P2Y receptors. We conclude that activation of ERK by traumatic injury is mediated in part by ATP/PP2 receptors and suggest that this signaling pathway contributes to the development of gliosis after brain trauma. (Supported by the Department of Veterans Affairs.)

P170. DELAYED TREATMENT WITH DEHYDROEPIANDROSTERONE SULFATE ENHANCES GENE EXPRESSION RELATED TO NEUROPLASTICITY
Stuart W. Hoffman*, Edward H. Pattus, Robert M. Simkins, IV, Donald G. Stein. (Emory University, Atlanta, Georgia US).

Dehydroyepiandrosterone sulfate (DHEAS) has been previously shown to be a neurotropic/neuroprotective neurosteroid. Our recent results indicate that DHEAS can facilitate recovery of function after a bilateral control cortical impact (CCI) to prefrontal cortex when given after a 7-day delay and then daily prior to behavioral testing. To determine the effects of DHEAS administration on neuroplasticity during this period of recovery, we employed the use of DNA microarray technology. Starting 7-days after CCI, rats were injected with 10mg/kg of DHEAS (n = 3) or vehicle (n = 3) 1hr before testing on a water maze task. The injections and testing continued for the next 4-days for a total of 5 injections and 5-days of testing. Approximately 2h after the final trial of testing, the rats were killed and the frontal cortices that contained the injury were processed for RNA isolation. The isolated RNA served as a template for cDNA, which was then used as a template for the labeled RNA that was hybridized to the Affymetrix™ U34A (8800-genes) chip. Chips were scanned and analyzed in Emory's core facility.

The latencies to the platform over 3-days of testing was vehicle 66 ± 14 vs. DHEAS 44 ± 5. The results of the DNA microarray indicated that DHEAS treatments in these animals produced consistent increases in the expression of genes that are linked to neuroplasticity, such as MAP2, GAP43, and CaMKIIα when compared to injured-controls. In addition, the expression of genes that counter neuroplasticity (GAP43 and S100) were reduced. PCR and Western blot analyses are in progress. These results indicate that DHEAS promotes behavioral recovery by enhancing mechanisms related to neuroplasticity. Supported by NIH grants 5R03HD040295, 1R01NS40825, & 1R01NS38564.

P171. TIMING OF PHYSICAL EXERCISE FOLLOWING MILD TRAUMATIC BRAIN INJURY: IMPACT ON STROOP TASK PERFORMANCE

Functional recovery following traumatic brain injury (TBI) is variable and appears to be dependent on post-injury "critic periods" or windows of opportunity during which the recovery process is exquisitely vulnerable to intervention. While there has been much study of these issues using animal models, less is known about critical periods following human TBI. For example, the role of physical exercise after brain insult is believed to be important, but what remains is to identify the specific period of time, i.e., when exercise is introduced. Thus, the present, retrospective study was designed to delineate the window of opportunity for physical exercise following mild TBI. Cognitive functions (e.g. memory, attention, concentration, selective attention) were assessed using a neuropsychological battery comparing current functional level with initial post-injury performance on the same measures. Independent variables of interest were the timing, type and frequency of post-injury exercise. Results indicate that selective attention measures, such as the Stroop task, are impacted by post-injury exercise. Specifically, the earlier the participant initiated exercise within the first 12 months following TBI, the lower the Stroop interference score. These findings could not be accounted for by time since injury, age at time of injury or the use of certain other rehabilitation interventions. However, exercise type (aerobic vs. resistance training) and frequency appear to be important. Thus, the initiation of exercise within a critical time period after mild TBI can be correlated with improvements in certain aspects of cognitive function.

P172. THE DELAYED ADMINISTRATION OF DEHYDROEPIANDROSTERONE SULFATE PROMOTES RECOVERY OF FUNCTION AFTER CORTICAL IMPACT INJURY
Shardul Virmani*, Robert M. Simkins, IV, Donald G. Stein, Stuart W. Hoffman. (Emory University, Atlanta, Georgia US).

Traumatic brain injury (TBI) initiates destructive sequences of secondary events within the brain that lead to permanent cognitive and sensorimotor deficits. Previous research has shown that GABAergic neurotransmitters are neuroprotective. The goal of the current study was to show that dehydroyepiandrosterone-sulfate (DHEAS), a stimulatory neurosteroid, could facilitate recovery of function in male rats after delayed treatment following TBI. DHEAS has been found to play a major role in brain development by influencing the migration of neurons, arborization of dendrites, and formation of new synapses.

In our study, behavioral assays were conducted concurrently with DHEAS administration (0, 5, 10, or 20 mg/kg in 2-hydroxypropyl-β-cyclodextrin) starting seven days post-injury (PI). Behavioral assays included 10-day's of Morris Water Maze testing (MWM; 7d PI), 10-days of Greek-Cross (GC; 21d PI), Tactile Adhesive Removal (TAR; PI days: 6,13,20,27,34), and spontaneous motor behavior (SMB; PI days: 2,4,6,12,19,26,33).

Results showed an improvement in performance in all tasks among injured rats that received 5, 10, or 20 mg/kg DHEAS, yet, the most effective dose was 5 days post-injury. The most effective dose of DHEAs in the MWM was 10mg/kg, in the GC it was 20mg/kg, in the TAR was 5mg/kg, and all doses, except for vehicle, were effective at reducing injury-induced SMB. In no task did DHEAS treated animals perform worse than the injured-controls. In addition, DHEAS had no significant effects on behavioral performance in the sham-operates.

These results demonstrate that after a 7-day delay, the chronic administration of DHEAS to injured rats significantly improves behavioral recovery on both sensorimotor and cognitive tasks. Supported by NIH grants 5R03HD040295, 1R01NS40825, & 1R01NS38564.
P173.
TRAUMATIC PERIMESENCEPHALIC SUBARACHNOID HEMORRHAGE: A SIGN OF BRAINSTEM INJURY
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Objective: To evaluate the frequency, distribution, appearance, and clinical outcome of brainstem injury, as seen on MR, in a prospective study of patients with traumatic perimesencephalic subarachnoid hemorrhage (pSAH) seen on initial CT scan.

Methods: MR images were prospectively obtained in 38 patients with head injury who on initial CT scans showed pSAH. To identify the amount and location of pSAH, the CT scans of all patients were evaluated, and MRI findings were evaluated according to the presence, location and signal intensity of brainstem injury, and other combined intracranial injuries. Initial Glasgow coma scale (GCS) and Glasgow outcome scale (GOS), as noted on clinical records, were reviewed.

Results: Brainstem injury was demonstrated on MR imaging in 30 patients (79%). The majority of these lesions (76.7%) were located in the dorsolateral portion, and nonhemorrhagic lesions were more frequent (70%) than hemorrhagic.

In patients with brainstem injury, as seen on MR imaging, the GOS score was worse, especially in those with combined diffuse axonal injury in the corpus callosum and cerebral white matter.

The location and amount of pSAH seen on CT was not related with brainstem injury or clinical outcome.

Conclusion: The presence of pSAH in patients with acute head trauma, as seen on CT was thought to be an indicator of brainstem injury, and MR imaging was necessary. If such injury was identified on MRI, this was predictive of a worse clinical outcome.

P174.
LOSS IN CORRELATION BETWEEN ADMISSION GCS AND OUTCOME IN PATIENTS WITH MULTIMODAL BEDSIDE MONITORING
Balestrieri M, Steiner LA, Chatfield DA, Schmidt EA, Czonyka M, Pickard JD. (*Academic Neurosurgery and **University Department of Anaesthesia, Addenbrooke's Hospital, Cambridge, UK).

Background: Age and Glasgow Coma Scale (GCS) score on admission are considered important predictors of outcome after traumatic brain injury [1]. More recently data from computerized neuromonitoring systems have been shown to add relevant information for building prognostic models following head injury [2]. We investigated the predictive value of GCS and age in a large group of patients in whom multimodal bedside monitoring data were processed over the last ten years.

Methods: Data from 358 head injured patients collected between 1992 and 2001 were analysed retrospectively. Patients were grouped according to year of admission. GCS and Glasgow Outcome Scores (GOS) at six months were determined. Spearman's correlation coefficients between GCS and GOS were calculated for each year.

Results: On average 34 (±7 SD) patients were monitored every year. We found a significant correlation between GCS and GOS for the first 5 years (overall 1992–1996 r = 0.41; p < 0.00001; n = 183) and consistent lack of correlation starting from 1997 (overall 1997–2001 r = 0.09; p = 0.236, n = 175). In contrast correlations between age and GOS were in both time periods significant and similar (Age: 1992–1996 vs. 1997–2001: r = -0.24 vs. r = -0.24; p < 0.002).

Conclusions: Admission GCS has lost its predictive value for outcome in this group of patients from 1997 onwards. We can only speculate on which elements of our management have caused this, an inconsistency in obtaining GCS perhaps influenced by more aggressive pre-hospital treatment, as well as progress in clinical management may have influenced the relevance of GCS for outcome. The predictive value of GCS should be carefully reconsidered when building prognostic models incorporating multimodality monitoring after head injury.


P175.
DIFFUSION WEIGHTED MAGNETIC RESONANCE IMAGING OF EDEMA FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

Traumatic brain injury (TBI) is often associated with edema that can be examined using diffusion weighted imaging. We examined the development and extent of edema following liquid flux perfusion TBI in rats using diffusion weighted imaging (DWI). Baseline imaging was carried out in unjured adult, male Sprague Dawley rats on a Bruker 1T magnet with Paravision 3.0 software. Animals were anesthetized and mechanically ventilated, with image acquisition gated to respiration cycle. After TBI, DWI was carried out at 1, 2, 24 hr, 1–5, 9 and 10 weeks to examine the development of edema. Apparent diffusion coefficient (ADC) values were calculated for specific regions of interest, including hippocampus, parieto-occipital cortex, temporal cortex, retrosplenial cortex and thalamus, and compared with histopathological findings. ADC values in the hemisphere contralateral to TBI remained unchanged over 10 weeks of imaging. In contrast, a small, localized area of hypotension (i.e., decreased ADC) in the ipsilateral hippocampus developed 1–2 hr after TBI. With later imaging a large area of hypointensity (i.e., increased ADC) in the hippocampus, parieto-occipital cortex and temporal cortex began 3–4 weeks after TBI. Histopathological evaluations indicated that the late appearing hypointensity in the ADC map was due to fluid accumulation within enlarged ventricles and a region of cavitation at injury site. The small changes in the DWI and ADC maps immediately after TBI suggest that vasogenic edema is not a major consequence of LFP injury in rodent brain, and that accumulation of extracellular fluid associated with TBI is readily quantified by DWI. (Supported by NIH NS 39509, a UCD NMR Award and the UC Neurotrauma Research Initiative).

P176.
VASCULAR TUNNEL CREATION AND FURTHER SPACE WINNING METHODS IN THE TREATMENT OF AGGRESSIVE BRAIN SWELLING.
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Introduction: Decompressive craniectomy with durotomy, is a last resort therapy. Although the method successfully diminishes the ICP, partial or total vascular insufficiency occurs in the herniated part of the brain. The cause of the insufficiency is most likely due to the compression of the cortical veins and arteries, caused by shearing and pressure forces between the dural edge and brain tissue. Furthermore venous congestion may induce edema in the protruding parts of the brain.

Methods: The new surgical technique consists of a stellate type durotomy and the creation of a vascular tunnel, by supporting pillars, on both sides of the main cortical veins and arteries, between the dural edge and brain surface, with the aim that the vessels do not become compressed by the sharp dural or bone edge. The effect of the novel vascular tunnel technique was proven by measuring the blood flow of the protected and non protected veins with doppler UH, intraoperatorally. Further space winning surgical methods was applied by eccentricity and stretching the skin or let it open.

Results: Last two years 33 patients were operated with this method. All were in severe GCS 3 or GCS 4 status, with more than 30 mmHg ICP. In comparison with the traditional treatment, the mortality rate was reduced from 80% to 40% and, recovery (GOS4,5) rate increased significantly in these severe cases.

Conclusions: With this technique the ICP was significantly reduced and further edema and vascular insufficiency was prevented.

P177.

CONCEPT OF 'TRUE ICP' IN MONITORING AND PROGNOSTICATION IN HEAD TRAUMA

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Objective: Mean ICP is an important prognostic variable in severe head injury. However, absolute ICP would more predictive if we could take into account where on the Pressure-Volume Curve the 'working' pressure point is positioned. ICP above 30 mmHg may not be the danger when compensatory reserve is sufficient, while 20 mmHg, may be life-threatening when compensatory reserve is close to exhaustion. The RAP coefficient helps to monitor this reserve continuously. We propose a new coefficient, which contains information on both the absolute ICP and the pressure-volume compensatory reserve.

Method: ICP was monitored daily in 176 sedated and ventilated patients. The RAP coefficient was calculated as the running (6 minutes) correlation coefficient between slow changes in pulse amplitude and mean ICP. RAP has been demonstrated to have value 0 on the flat part of the Pressure-Volume Curve and +1 on ascending exponential part. Then RAP decreases to zero and then becomes negative when ICP is so high that it affects cerebrovascular pressure-reactivity. Coefficient tICP = ICP(1-rap) has been called 'true ICP'. It magnifies the critical values of ICP when cerebrovascular pressure-reactivity is exhausted and dampsens those states where absolute ICP is moderately elevated but vascular reactivity reserve is not affected.

Results: Both Mean ICP and RAP are independently correlated with outcome (ANOVA: ICP-GOS: F = 5.9; p < 0.007, RAP-GOS: F = 5.8p < 0.05). True ICP has a much stronger association with outcome: F = 8.8; p < 0.0001. The association between GCS and outcome was weaker: F = 4.8p < 0.04 and the association between outcome and CPP was not significant as a majority of patients were managed using CPP-oriented protocol.

Conclusion: The proposed variable is a more powerful predictor of the outcome following head injury than ICP or GCS. It is sensitive to both the rising absolute ICP and the critical change of the pressure-volume compensation.

P178.

ACCUMULATION OF CALPAIN AND CASPASE-3 CLEAVED AIF-SPECTRUM BREAKDOWN PRODUCTS IN CSF OF PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY


Several lines of evidence in animal models suggest that calpain may play a major role in traumatic brain injury. Moreover the role of caspase-3-protein as key executioner in mammalian apoptosis is well established. Nonerythroid (alpha II-spectrin (alpha-fodrin) is a cytoskeletal protein that is a substrate of both calpain and caspase-3 cysteine proteases. Cleavage of (alpha II-spectrin by calpain and caspase-3 results in accumulation of protease-specific (alpha II-spectrin breakdown products (SBDPs)) that can be used to monitor the magnitude and temporal duration of protease activation. We performed a longitudinal western blotting analysis from 3 TBI patients using monoclonal antibodies against (alpha II-spectrin (280 KD), calpain specific (145 KD) and caspase-3 specific (120 KD) SBDPs. Calpain SBDPs were present immediately after injury in all patients and gradually decreased but were still apparent up to 6 days after injury. Caspase-3 SBDPs were also evident immediately after injury in all patients but were not as pronounced as the calpain SBDPs. Caspase-3 SBDPs decreased in intensity and disappeared by the fifth day. Importantly, in one patient ICP increased at 18 h and 3 days and these ICP spikes were associated with concomitant increases in SBDPs (particularly calpain). Thus, (alpha II-spectrin and its calpain and caspase-3 specific SBDPs appears to be sensitive to secondary cerebral injuries. These preliminary data suggest that alpha II-spectrin-SBDPs may be useful biochemical markers of human brain injury, allowing for monitoring of specific proteolytic cascades known to play an important role in TBI and provide potentially sensitive markers for determining severity of brain injury and effects of therapy. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091, NIH R01 and NS40182).

P179.

MODELLING INTRACRANIAL PRESSURE INSULTS IN HEAD-INJURED PATIENTS USING ARTIFICIAL NEURAL NETWORKS

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The objective of this investigation was to model the incidence of intracranial pressure (ICP) insults in head-injured patients with the use of artificial neural networks (ANNs).

The BrainIT Multicentre project supplied data from 23 patients admitted to three neurosurgery units between 12/2001 and 4/2002. For each patient mean ICP and compliance were recorded every minute. All patients were aged over 16 and were excluded if they had undergone craniotomy or were dead on admission.

ICP insults were defined where within any 5 minute sliding window at least 3 of the measurements were greater then a threshold value (20 and 25 mmHg). Continuous, sub-threshold, ten minute sequences were marked, some of which were "positive sequences" followed by an insult after 5 minutes, and the remainder which were not ("negative sequences"). For each sequence the following were extracted: rates of change in ICP and compliance, standard deviations, maximum ICP, % time ICP > predefined sub-threshold value, minimum compliance, % time compliance < 0.8 ml/min/mHg, a flag indicating the sequence class. A dataset balanced between the two classes was created and divided into two parts, 70% for training an ANN, and 30% for testing performance. The process was repeated 20 times for each threshold value.

Where the thresholds were 20 and 25 mmHg the number of positive/negative sequences identified were 426/804 and 278/1092 respectively. The accuracies of the ANNs in classifying sequences from the test set were 71.6% (C95: 70.4-72.8) and 68.8% (C95: 65.6-71.1). The most important predictors were maximum mean ICP, and rates of change in ICP at later time points.

ANNs are a promising methodology for predicting ICP insults and could form the basis of an intensive care early warning system. Improved performance may be achieved by pre-processing the ICP signal, including other physiological indices in the model, and by detailed annotation of clinical interventions.

P180.

THE EFFECT OF MICROGLIA ABLATION FOLLOWING TRAUMATIC BRAIN INJURY IN MICE

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As a whole, whether activated microglia are acting as protectors or attackers to the neurons in response to the certain pathological condition such as trauma? In this study we used microglia-specific immunotoxin, Mac-1-SAPORN, at the time of injury to temporarily eliminate microglia to see the effects following penetrating traumatic brain injury in mice.

At 24 hours after injury, very few arborized microglia were observed around the injury site in microglia ablation group, while arborized microglia were widely distributed around the injury site or throughout the ipsilateral hippocampus and round-shaped microglia/macrophage were sparsely distributed along the needle track in microglia non-ablation group. At 7 days after injury, many arborized microglia were observed around the injury site or throughout the ipsilateral hippocampus even in immunotoxin treated (microglia ablation) group. Gliosis around the injury site was more evident in microglia non-ablation group at 72 hours and 7 days following injury. At 72 hours after injury, the neuronal cell loss became evident in the non-ablation group compared to that in ablation group. However, at 7 days after injury, no statistically significant difference was observed.

The microglia ablation with immunotoxin alleviated the neuronal cell loss in the dentate gyrus following penetrating traumatic brain injury in the mouse hippocampus. The microglia ablation also inhibited the hypertrophic change and proliferation of astrocytes following traumatic brain injury. We concluded that activated microglia in the acute stage of penetrating brain injury are considered to mainly act on the neurons suffering from injury as attackers through their phagocytic function and/or modification of astrocyte-neuron interaction.
P181.
OVEREXPRESSION OF X-LINKED INHIBITOR OF APOPTOSIS PROTEIN IMPROVES FUNCTIONAL RECOVERY AFTER CONTROLLED CORTICAL IMPACT INJURY IN THE MOUSE.
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Traumatic brain injury (TBI) initiates secondary injury responses that result in apoptotic cell death and neurological dysfunction. Caspases have been implicated in this process. X-linked inhibitor of apoptosis protein (XIAP) is an endogenous inhibitor of intrinsic caspase activation pathways, through its interactions with caspases 3 and 9. Recently, we evaluated whether delivery of XIAP by a non-replicating type 5 adenoviral construct would improve recovery after controlled-cortical injury (CCI) in the C57BL mouse. We injected the left hippocampus and overlying cortex of mice with either Adeno-LacZ, Adeno-XIAP or an equal volume of saline. Seven days later, animals were subjected to moderately-severe, left, lateral CCI. The number of foot-faults on a balance beam test of motor function was significantly reduced at 7, 14, 21 and 28 days after injury in Adeno-XIAP mice, compared to Adeno-LacZ or saline controls. Adeno-XIAP injected mice showed trends toward improved performance compared to controls in a Morris water maze test of cognition administered on days 14-18 after injury. These data indicate that XIAP significantly improves motor outcome after TBI, and lend additional support to the hypothesis that intrinsic apoptotic cascades may play an important role in cell death and neurological impairment after TBI.

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P182.
INFORMATION PROCESSING DEFICITS AND THEIR RELATIONSHIP TO NEUROIMAGING FOLLOWING MODERATE AND SEVERE TRAUMATIC BRAIN INJURY

Neuroendocrinological and neuropathological studies indicate that diffuse axonal injury (DAI) is a common consequence of traumatic brain injury (TBI) and that the amount of damage increases with injury severity. DAI particularly affects the frontal and temporal lobes, and the corpus callosum (CC). However, the amount of DAI required to cause detectable neuropsychological deficits in TBI patients is not yet known. Moreover, the information processing deficits that are likely to be caused by diffuse damage (measured by reaction time tasks; RT), and the functional integrity of the CC, are not routinely assessed in clinical settings. This study compared the performance of a group of 25 moderate to severe TBI patients with that of 25 matched controls on standard neuropsychological tests as well as visual and tactile KT tasks that required both compatible (intra-hemispheric processing) and incompatible (inter-hemispheric processing) responses. The latter tasks were designed to target the effects of DAI, including DAI to the CC. The neuropsychological data were also analysed in relation to morphometric analysis of the corpus callosum and the results discussed in terms of loss of interhemispheric connectivity.

P183.
“KEY HOLE” APPROACH FOR THE MANAGEMENT OF NEUROTRAUMA
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Minimally invasive surgery is being utilized recently for non-traumatic brain lesions, such as: removal of tumors, clipping of cerebral aneurysms and others.

Recently we enlarged the indication for the utilization of this procedure and we use it also in some trauma cases. Among other trauma cases we used the approach for draining acute epidural hematoma, repair of the anterior skull base and dural tear in cases of acute traumatic CSF leaks and removal of foreign body in penetrating brain injury.

We are reporting here with 5 such cases with excellent results. A few representative patients will be presented in detail.

P184.
DETERRIMENTAL ROLE OF BRADYKININ B2 RECEPTORS FOLLOWING CLOSED HEAD INJURY IN MICE
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The selective non peptide B2 receptor antagonist, LF 16-0687 Ms , and B2 receptor knock-out mice (B2R−/−) were used to investigate the role of bradykinin B2 receptors in traumatic brain injury (TBI).

TBI was produced using a weight-drop device. Neurological deficit (grip test) and brain water content (BWC) were evaluated 4 h post-trauma. Myeloperoxidase (MPO) activity and B1R, B2R, COX-1, COX-2 and iNOS mRNA levels (RT-PCR) were determined 24 h after TBI.

Intact mice had a grip test score of 28.9 ± 0.8 s. It was 12.3 ± 2.9 s (p < 0.001), 17.2 ± 3.0 s (NS), 21.1 ± 2.9 s (p < 0.05) and 17.4 ± 3.3 (NS) in traumatized mice treated s.c. with vehicle, 1, 3 and 10 mg/kg LF 30 min after TBI, respectively (n = 14-15). Neuroprotection was still observed when LF-treatment was delayed for up to 2 h after TBI. BWC was 79.8 ± 1% and 82.5 ± 0.2% (p < 0.001) in intact and injured mice, respectively. LF (3 mg/kg) reduced by 26% TBI-induced increase of BWC (p < 0.05). MPO activity increased from 0.017 ± 0.01 to 0.25 ± 0.05 U/g after TBI (p < 0.01). LF (3 mg/kg) reduced TBI-induced MPO activity by 50% (p < 0.01).

iNOS mRNA which was absent in intact mice was markedly induced after TBI and this was reduced by LF.

The grip test score was 30 s both in intact B2R+/− and B2R−/− mice (n = 8-10). It was 10.5 ± 0.5 s and 22.6 ± 3 s in injured B2R+/− and B2R−/− mice (p < 0.01), respectively (n = 7-12). However, BWC was unaltered and MPO activity was non significantly reduced by 26 % in injured B2R−/− mice. Blockade or deletion of B2 receptors produces a neuroprotection associated with a reduction of the acute inflammatory response. Therefore, blockade of B2 receptors might represent a promising pharmacological treatment of severe TBI.
P185.
SECONDARY GROWTH OF A CORtical NECROSIS FROM COLD INJURY IN WILD-TYPE AND INOS-DEFICIENT MICE
Michael Stoffel, Gerard Ran, Patrick Schäfer, Johannes Schramm. (Dept. Neurosurgery, Bonn, Bonn, DE)

Objective: (1) To evaluate the underlying mechanisms of secondary growth of a cortical necrosis from cold injury in animals with selective deletions of specific genes, this model was adapted for mice. (2) To test the hypothesis that the inducible NO-synthase is a mediator of this phenomenon of secondary brain damage.

Material & Methods: (1) CS7Bl/6x129 mice (n = 40) were subjected to a right parietal trephination. A lesion was induced to the cortex by focal freezing (−68°C, 15 min). 10 min, 24 h, 72 h, and 3 weeks thereafter, respectively, the lesion was measured histomorphometrically. (2) At the time of maximal lesion spread (111 days), the volume of the cortical necrosis was evaluated in iNOS−/− mice and their wild-type littermates.

Results: (1) Focal freezing produced a cortical lesion with a volume of 0.045 ± 0.014 mm3 10 min after trauma. This lesion was expanding to 0.342 ± 0.026 mm3 at 24 h and to 0.522 ± 0.09 mm3 at 72 h (p < 0.01 vs. 10 min). 3 weeks after trauma a glial scar was seen in the area subjected to cold injury. (2) Within 72 h after trauma, the cortical necrosis developed to a volume of 0.33 ± 0.04 mm3 in iNOS−/− mice and to 0.48 ± 0.04 mm3 in their wild-type littermates.

Conclusion: (1) The current cold injury model is highly suitable to investigate the pathophysiology of secondary necrosis growth from trauma in mice, since it produces a sharply demarcated lesion that expands massively after trauma and is limited to the brain cortex. (2) At the time point of maximal lesion spread, the volume of the cortical necrosis in iNOS−/− mice is only 68% of the necrosis volume in wild-type littermates. This supports the hypothesis that the iNOS-product acts as a mediator of secondary necrosis growth after trauma.

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P186.
RECOVERY MECHANISM OF TRAUMATIC CEREBRAL HEMIPLEGIA
Phillip Shun Wu,Chao Ying Wu. (Yu Huaing Ding Hospital, Yantai, Shangdong CN).

A cerebral hemiplegia comes from the cerebral ischemia of the ascending frontoparietal artery, which has 2 types: the tree-branch type (57.5%) and the candlestick type (42.5%). In the treatment of hemiplegias, the most useful arteries for a single anastomosis are:

* F-A arteries, frontal branch of the superficial temporal artery-posterior branch of the candlestick artery.

* P-P arteries, parietal branch of the superficial temporal artery-posterior branch of the candlestick artery.

The single anastomosis except for a slow recovery is the same to the double anastomosis. The pressure-flow in the superficial temporal artery was 600 mm H2O and 20 cc Per minute; in the ascending frontoparietal artery, 100 mm H2O and 10 cc Per minute. The high pressure-flow, the amount of which following anastomosis enters the ascending frontoparietal artery for a single anastomosis, was 10 cc Per minute, 600 cc Per hour, 14,400 cc Per day; for a double anastomosis, 28,800 cc Per day. The high pressure-flow, in the ascending frontoparietal artery, could dilate the closed cortical arteries and subcortical pressure splinters of distributing arteries to improve the microcirculation. This is the recovery mechanism. The cerebral hemiplegia is an neuronal pseudo-death as a facial nerve paralysis, and is a neuronal true-death as an infantile paralysis.

Now the 5 hemiplegic patients have completely recovered. They may raise their paralysed hands and lift their paralysed feet to walk towards man's world.

P187.
POSTTRAUMATIC LOCAL INFLAMMATORY CELLULAR INTERPLAY DETERMINES NEURONAL FATE
Itach Shaked. (Weizmann, Rehovot, Israel IL).

Inflammation following central nervous system (CNS) injury is still a subject of controversy, regarded by many as a detrimental process. Our group, however, have provided substantial evidence for immune involvement in neuronal protection. In this study we found that, the ability to resist the consequences of CNS axonal injury was characterized by the early onset of site-specific phagocytic activity and MHC class II expression. Our data suggest that post-traumatic CNS inflammation comprise a highly complex cascade of events, in which only a suitably timed and properly balanced innate immune dialog will lead to neuronal survival. Such a dialog was demonstrated in vitro, using rat primary cultures of microglia and astrocytes. In this experiment, both types of cells showed a specific ability to take up 14C-glutamate in a sodium-dependent manner, and their glutamate-scavenging abilities were dramatically enhanced after their pre-embolization with activated autoreactive T cells. These results shed some light on the role of inflammation after CNS injury, and compel us to modify our therapeutic strategy in favor of regulating the inflammatory reaction rather than suppressing it.

P188.
EXPRESSION OF EPHA7 SUGGEST ROLES IN SPINAL CORD INJURY PATHOPHYSIOLOGY
J.D. Figueroa1*, C. Wilson1, H. Gaskins2, S.R. Whittemore3 and J. Miranda1 (1Department of Pharmacology, University of Puerto Rico School of Medicine, San Juan, PR; 2Kentucky Spinal Cord Injury Research Center, University of Louisville School of Medicine, Louisville, KY USA).

The molecular mechanisms underlying processes involved in the growth cone response to spinal cord injury (SCI) are incompletely understood. In several SCI animal models, injured neurons have shown the intrinsic capacity to regenerate, but the microenvironment surrounding the lesion site have shown inhibitory and prevents axonal growth. During the past decade, the Eph receptor protein tyrosine kinase family and its cognate ligands, the ephrins, have emerged as key regulators of cell signaling processes including cell fate, axon guidance, synapse formation, and axonal pathfinding. More recently, it has become clear that in certain situations they can mediate different effects on cells, including adhesion. Developmental analysis of EphA7 regulation nurture the idea that the truncated receptors, which lack the kinase activity, act in endogenous, dominant-negative suppressors of the full-length EphA7 signaling. Therefore, in the absence of signaling these receptors could promote cellular adhesion. We hypothesize that alternative usage of different splice forms of Eph A7 tyrosine kinase receptor can mediate cellular adhesion or repulsion after neural trauma. We have examined the relative expression of EphA7 full-length and truncated isoforms in adult rats after injury using the NYU compression model and semi-quantitative real time PCR analysis. Standardized real-time RT-PCR analysis show increases in the full-length EphA7 expression 7, 14, and 28 days post-injury and these results were corroborated by immunohistochemistry. Immunoreactivity was observed in GFAP positive cells located in the ventral region of the white matter. Owing to the limited regenerative capabilities of the central nervous system, these results suggested that EphA7 full length might be involved in the establishment of the restrictive environment for axonal regeneration after SCI. This study is supported by NIH/NINDS (NS 39405), NSF-ESCOR (EPS-9874782), KSCHIRT (8-29), Norton Health Care, MBRS SCORE (2 SO6 6M8224).
P189.
TRANSPORT OF POLIOVIRUS RNA FROM THE PERIPHERY VIA SCIATIC NERVE AXONS RESULTS IN GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM.
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Poliovirus replicons are a unique RNA based gene vector capable of spatially and temporally localized effects on the injured spinal cord. Poliovirus replicons spatially localize to spinal cord motorneurons. Replicons induce a transient burst of foreign gene expression, initiating at 6 hours and peaking at 72 hrs after injection. In mice transgenic for the poliovirus receptor (prv mice), intramuscular injection of poliovirus replicons encoding gfp reveals expression in the ventral horn motorneurons. Expression is also found within the injected muscle, sciatic nerve, dorsal root ganglia (drg) and within fibers of the dorsal horn. No inflammation in the muscle or spinal cord and functional changes were noted.

Injection of naked (unencapsidated) poliovirus replicon RNA encoding gfp into the sciatic nerve of mice or rats also result in gfp expression within the spinal cord. The pattern of gfp expression within the muscle, sciatic nerve, drg and spinal cord was similar to that seen after intramuscular injection of encapsidated replicons. Transfection of the sciatic nerve eliminated gfp expression in the sciatic nerve and spinal cord. Thus, replicon RNA is transported via sciatic nerve axons back to the spinal cord, where RNA translation occurs primarily within the motorneuron cytoplasm. The ability of replicons to be transported from the periphery to the central nervous system, coupled with their unique gene expression profile, further enhances the potential uses of replicons for gene delivery of therapeutic proteins to the central nervous system. Supported by AI 25005 to CDM.

P190.
INFLAMMATORY CELLULAR RESPONSE AND CYTOKINES IL-1BETA, IL-6 AND TNF-ALPHA
Liquan Yang, * Corinna Van Den Heuvel, Peter Blumberg, Nigel Jones, Jim Manovis. (University of Adelaide, Adelaide, Australia).

The inflammatory response following spinal cord trauma plays an important role in the secondary spinal cord injury. We hypothesized that the pro-inflammatory cytokines IL-1beta, IL-6 and TNF-alpha produced by intrinsic cells in the spinal cord may act as messengers to coordinate the inflammatory cascade and the influx of neutrophils and monocytes to the site of damage and that the cytokine response should be greater in severe than in mild injury.

Neutrophils were not detected at 1 and 3 hrs after spinal cord injury, dramatically increased at 6 hrs postinjury primarily around blood vessels in the central gray matter and peaked at 1 day.

Macrophages were noted at 6 hrs and then progressively increased for the first 3 days postinjury. Activated microglia were found as early as 1 hr after contusion, increased dramatically at 1 day postinjury and frequently around axonal swellings and healthy neurons. RT-PCR showed an early and robust up-regulation of IL-1beta, IL-6, TNF-alpha mRNAs in spinal cord after severe contusion injury, maximal at 6 hrs postinjury with return to control levels by 24 hrs postinjury, the changes being quantitatively less in mild injury.

RT-PCR analyses together with histological observations suggest that intrinsic CNS cells, not peripheral inflammatory cells, are the main source for cytokine mRNAs because the peripheral inflammatory cells do not invade the injured spinal cord until 6 hrs postinjury, a time when cytokine mRNA levels have peaked and started to decline. Furthermore, our comparative RT-PCR analyses, showing significantly increased expression of pro-inflammatory cytokine mRNAs in severe injury in contrast to mild injury, support the hypothesis that cytokine up-regulation is an important factor in the generation of the severity of the inflammatory response and thus a suitable target for pharmacological intervention to attenuate this response.

P191.
HEMATOGENOUS MACROPHAGES EXPRESS CD8 AND DISTRIBUTED TO REGIONS OF LESION CAVITATION AFTER SPINAL CORD INJURY.

Historically, CD4 and CD8 antigens have been used to designate functionally distinct T-lymphocyte subsets. However, these antigens also have been described on macrophages in the normal and pathologic CNS. Signaling through CD4 or CD8 may impart unique functional attributes to macrophage subsets expressing these antigens. In the current study, the distribution of CD4 or CD8+ cells was evaluated within rat spinal cord following contusion injury. Survival intervals ranged from 6 hours to 6 weeks post-injury (n = 4-6/group). The data reveal divergent patterns of CD4 and CD8 expression on unique macrophage populations. Specifically, in addition to infiltrating lymphocytes, we observed sustained elevations of CD4 expression on microglia and macrophages throughout the lesion site and spared white matter up to 6 weeks post-injury. In contrast, CD8 was predominantly associated with hematogenous macrophages that are recruited from the blood during the first week post-injury. These cells were restricted to zones of necrosis and lesion cavitation. The hematogenous nature of the CD8+ macrophage was confirmed by immunohistochemical analysis in radiation bone-marrow chimeric rats and after intravenous injection of liposome encapsulated clodronate (a method for selectively depleting blood monocytes and hematogenous macrophages). Indeed, macrophage depletion caused a 20-40 fold reduction in CD8+ macrophage infiltration at the injury site. The restricted expression of CD8 on blood-derived macrophages and the limited temporal appearance of this molecule after SCI suggest that CD8 is actively regulated and could play a role in triggering the acute neurotoxic properties of recruited macrophages after SCI. This work supported by NS37846 (FGP).

P192.
HP184 INCREASES CONDUCTION VELOCITY IN THE DYSMYELINATED CNS OF THE LONG EVANS SHAKER (LES) RAT.

Demyelination of injured but surviving axons following trauma results in axonal conduction deficits and altered ion channel distribution. Conduction block in demyelinated fibers is believed to be at least partly due to the appearance of aminopyridine-sensitive potassium channels in areas of myelin loss. Potassium channel blockers, such as 4-AP, increase action potential duration and amplitude in demyelinated fibers and improve conduction of action potentials. In this study, HP184 was administered orally to adult mutant dysmyelinated Long Evans Shaker (LES) and normal Long Evans rats. Conduction velocity (CV) of evoked compound action potentials (CAP) was measured in vivo 2 hours after ingestion. While CV in the untreated LES rats (24.75 m/s) was 3.27 times slower than CV in normal myelinated untreated controls (80.88 m/s) HP184 had a positive effect on CV in treated LES rats. HP184 caused a 56.1% (38.63 m/s) increase in CV at 3 mg/kg body weight and 168.6% (66.48 m/s) increase at 10 mg/kg. HP184 improves conduction velocity of unmyelinated central axons and is therefore proposed as a treatment for symptoms of demyelination resulting from spinal cord injury. The new in vivo model of measurement of CV of evoked CAP developed for this study proved reliable and can be used in chronic studies requiring multiple electrophysiological measurements performed in the same animal.

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P193. BEHAVIORAL OUTCOME FOLLOWING GRADED UNILATERAL CERVICAL SPINAL CORD CONTUSSION IN RATS
John C. Gensel,* C. Amy Tovar, Frank P.T. Hamers, Michael S. Beattie, Jacqueline C. Brennahan. (Department of Neuroscience, The Ohio State University, Columbus Ohio USA)

The majority of human spinal cord injuries occur in the cervical cord, however, few animal models have examined the effects of contusion at this level. This study explores behavioral and histological outcomes of unilateral contusions at C5 using the MASCIS device. One advantage of this model is the ability to assess functional effects of gray and white matter injury. Unilateral injury also provides a within subject control, and sparing of bladder and respiratory function. Several well-characterized tests for forelimb and hindlimb function can be applied, including paw preference test (Liu et al., 1999), grooming test (Bertelli and Mira, 1993), Catwalk quantitative gait analysis system (Hamers et al., 2001), horizontal ladder test (Metz and Whishaw, 2002), and open field locomotor test (Basso et al., 1995).

Experiment 1: 6.25mm (n = 5) and 12.5mm (n = 5) unilateral contusion injuries were made at C5-6. Mild and moderate functional deficits were observed. In the open field, 6.25mm subjects recovered some ipsilateral forelimb plantar stepping over 6 weeks whereas 12.5mm subjects did not. For the cylinder test, 12.5mm subjects rarely used their ipsilateral limb while the 6.25mm subjects used their ipsilateral limb less than normal but more than the 12.5mm subjects. The grooming test revealed serious impairment after 12.5mm injuries that partially recovered over 6 weeks. Minimal impairment was observed in 6.25mm subjects, with complete recovery observed by 3 weeks. Histological analysis showed ipsilateral sparing of 70% and 43.7% of the cord area after 6.25mm and 12.5mm injuries respectively. Strong correlations were observed between spared tissue and contralateral paw preference (r² = 0.92) and grooming performance (r² = .74) were observed.

Experiment 2 is underway to evaluate the model for consistency of histological and behavioral outcomes (n = 10 per group); to test sensitivity to potentially neuroprotective agents, methylprednisolone and minocycline.

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P194. HEAT SHOCK REDUCES THE DEGREE OF LIPID PEROXIDATION FOLLOWING ACUTE SPINAL CORD INJURY IN RODENTS S.D. Christie, B. Cameau, R.W. Currie and I. Mendez. (Dalhousie University, Halifax, Nova Scotia CA)

Following acute spinal cord injury, a period of secondary metabolic injury ensues. One of the hallmarks of these changes is the ongoing oxidative stress that leads to lipid peroxidation. Reduction of lipid peroxidation is felt to preserve the remaining spinal cord ultrastructure and minimize the effects of the secondary injury. Heat shock has been shown in various models of injury to minimize the effects of oxidative stress. The objective of this project was to assess the effect of heat shock on lipid peroxidation in a rat spinal cord injury model.

Fifty female Wistar rats were used in this study. Twenty-five rats were heat shocked, temperature 40°C for 15 minutes, prior to lesion. Five animals from each heat shocked and normal animals were sacrificed prior to lesion. The remaining animals received a mid-thoracic complete spinal cord injury via clip compression. The animals were randomly assigned to a sacrifice time, either 4, 6, 12, or 24 hours post lesion. Spinal cord tissue was either processed immunohistochemically for hsp27 or assayed colorimetrically for malondialdehyde (Oxford Biomedical Research; Oxford, MI) as a marker for lipid peroxidation.

Immunohistochemical staining revealed increased expression of hsp27 in the neurons of both the heat-shock control and lesion-only animals at all time points. However, the animals that received a lesion after heat-shock displayed increased expression in both neurons and glia. Malondialdehyde levels were reduced by 35 and 70% in heat-shocked animals at 4 and 6 hours, respectively, when compared to lesion-only animals at the same time points.

In summary, we have shown that heat-shock prior to spinal cord injury induces hsp27 expression in both neurons and glia and leads to a reduction in the degree of secondary injury as measured by lipid peroxidation.

P195. THE EFFECT OF FK506 ON NITRIC OXIDE SYNTHASE ACTIVITY IN THE LACERATION MODEL OF SPINAL CORD INJURY
*Stephen P. MacNally, Peter J. Hamlyn, Patrick N. Anderson. (Selim Cellek, University College London, Dublin, IE)

The aim of this study was to investigate the alterations in spinal cord nitric oxide synthase (NOS) activity following an experimental spinal cord injury and to assess the effects of FK506 on this NOS activity. A blind study involving seven animal groups was initially performed (Set 1). Four groups underwent a T9 laminectomy and laceration spinal cord injury, with 2 groups receiving subcutaneous (s.c.) vehicle and the other 2 groups receiving s.c. FK506 (2.0mg/kg). Two uninjured groups received s.c. FK506. The 2 vehicle groups differed in survival time (6 and 16 hours post injection) as did the injured and uninjured FK506 groups. A control group consisted of uninjured and untreated animals. A citrulline assay was used to assess the cNOS and iNOS activities in each animal. The results did not reveal any significant changes in iNOS activity in any of the groups. The cNOS in all injured animals was increased at 6 hours and remained similarly elevated at 16 hours, but there was no significant difference between the vehicle and FK506 treated groups. The cNOS activity in the uninjured groups that received FK506 was unaltered at 6 and 16 hours compared to the controls. A further blind citrulline assay study (Set 2) was performed in vitro to observe the effect of three different forms of FK506 (base, oral and intravenous) at different doses (10µM, 100µM, 11µM and 10µM) on cNOS activity in cerebellar and spinal cord tissue. A negative control group (untreated) and positive control group (treated with the NOS inhibitor L-NAME) were utilized. The results did not reveal any significant differences between the FK506 groups and negative controls. Both Sets 1 and 2 results indicate that the neuroprotective mechanism of action of FK506 is probably not via an alteration in NOS activity.

P196. INCREASING DOSAGES OF FIBROBLAST GROWTH FACTOR-2 (FGF-2) DELIVERED NEAR THE SITE OF SPINAL CORD INJURY IMPAIRS FUNCTIONAL RECOVERY AND TISSUE SPARING IN RATS
A.G. Rabcevich, I. Fugac, University of Kentucky, Department of Physiology & Sanders-Brown Center on Aging, Lexington, KY USA)

We conducted a dose-response study of FGF-2 to characterize its efficacy for functional recovery and tissue sparing following contusion spinal cord injury using the Infinite Horizon Impactor. Immediately after T10 injury a catheter connected to an osmotic pump was inserted intrathecally at T13/L1 and advanced to T11 for continuous delivery of FGF-2 at 3µg, 15µg or 30µg per day versus control vehicle for 1 week (n = 6/group). Additionally, to test the in vivo influence of heparan sulfate (HS) on FGF-2 activity, others received the same dosages plus 10µg/ml HS (n = 6/group) versus HS alone (n = 12). Animals were tested for 8 weeks using the BBB locomotor rating scale and histologically assessed for volumetric tissue sparing of gray and white matter. All injured rats demonstrated an acute loss of hindlimb function followed by a recovery phase that peaked by 2-3 weeks. HS alone did not significantly affect recovery or tissue sparing, but animals that received 10µg FGF-2, with or without HS, demonstrated significant impairment in both acute and long-term hindlimb locomotor function compared to vehicle or HS. The remaining FGF-2 dosages also rendered somewhat lower BBB scores versus controls, except for a marginal improvement with 3µg FGF-2+HS. Accordingly, this group had the greatest amount of spared gray and white matter while the 30µg FGF-2 groups had the least. Since there were no significant differences among all groups in percent tissue sparing at the lesion epicenters, ongoing stereological studies are examining putative cellular alterations that may account for these observations.

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P197.
DEVELOPING NOVEL CALPAIN INHIBITORS: TAT-CALPA-STATIN
Tomoko Sengoku, Shin-Wei Shiu, Zineta Bondada. (James W. Geddes Spinal Cord and Brain Injury Research Center, Sanders Brown Center on Aging, and Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY US).

Following CNS injury, including spinal cord injury and ischemia, excessive calcium influx has been implicated in the ensuing secondary neuronal death. Calpains are calcium activated neutral proteases whose substrates include a wide variety of signaling, cytoskeletal, and life/death proteins. Following spinal cord injury, maximal calpain activation occurs within 1-2h and remains elevated for at least 24h, inhibiting calpain activity is a rational therapeutic target. However, synthetic calpain inhibitors are problematic due to limited cell permeability, short half lives, and inhibition of other proteases. Calpastatin, an endogenous calpain inhibitor is very potent and specific but does not cross cell membranes. By linking the 11 amino acid protein transduction domain of the Hiv Tat protein to green fluorescent protein (GFP) we developed a fusion protein that could be easily visualized in order to determine transduction capabilities. At low concentrations Tat-GFP did transduce primary rat hippocampal neurons but appeared to be localized within endosomes. By linking Tat and calpastatin we developed a novel calpain inhibitor that was functionally active and able to transduce neurons of primary cultures at low concentrations. At higher concentrations the Tat-calpastatin appeared to aggregate on the external surface of the cell surface. Our work with Tat-GFP and Tat-calpastatin reveals both the possible problems of fusion proteins as well as the potential of this novel calpain inhibitor.

P198.
POST-INJURY TREATMENT WITH Mn (III) TETRAKIS (4-BENZOIC ACID) PORPHYRIN IMPROVES FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY
V. Lokhande*, F. Bao† and D. Liu†. (Departments of Neurology and Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX US).

Manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBP) is a cell permeable superoxide dismutase mimetic and a broad spectrum scavenger of reactive species. The present study compares the effect of MnTBP and methylprednisolone—the only drug approved for clinic treatment of spinal cord injury (SCI) on neurological recovery after SCI. The rat spinal cord was injured at T10 vertebrae by dropping 10 and 1.25 cm down to the cord with a NYU device. The force of injury was digitally recorded on a PC equipped with data acquisition board. At 4 and 6 post-SCI, rats were treated with MnTBP (10 and 5 mg/kg, i.p., respectively), methylprednisolone sodium succinate (MPSS, 30 and 15 mg/kg, i.p., respectively), or saline as control. Functional recovery was evaluated by the standard Basso, Beattie and Bresnahan (BBB) test and inclined plane test on day 1 and every week until 9 week following SCI. The pre-trained rats were initially placed in open field and recorded BBB score based on hind limb movement, support, fore- and hind-limb co-ordination, paw and tail position. The inclined plane test is to test the rat's ability to maintain itself for 5 seconds on the maximum angle of a plane. MnTBP treatment significantly increased the scores of BBB test (p < 0.001) and inclined plane test (p = 0.01 - 0.007) compared to saline treatment. MPSS treatment did not significantly improve the scores by both tests, although the scores were better compared to controls. Therefore, MnTBP is superior to MPSS in enhancing neurological recovery following SCI, indicating that MnTBP is a potential therapeutic agent for reducing secondary SCI. Supported by NIH grants to D Liu (NS 34048 and NS 35119).

P199.
CHARACTERIZATION OF INTRASPINAL BONE MARROW STROMAL CELL TRANSPLANTS IN THE RAT SPINAL CORD INJURY MODEL
Daniel P. Arkeny* Dana M. McTigue, Lyn E. Jakeman and Bradford T. Stokes. (Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH USA).

Previously, we reported that transplanted marrow stromal cells support lesion site axonal regrowth and stimulate hindlimb astepping—a locomotor-like behavior in the injured rat spinal cord. Separately, we demonstrated that brain derived neurotrophic factor (BDNF) also provokes astepping following spinal contusion or transaction injuries, suggesting that BDNF activates the locomotor central pattern generators to induce the behavior. Therefore, we performed in vitro ELISA studies to explore the possibility that MSCs induce astepping by effecting the release of BDNF either directly or from local neurons. Specifically, we tested the supernatants from MSCs that were cultured alone and on a carpet of mouse spinal cord neurons. Because MSC transplants provoke astepping by 4 days after transplantation, we predicted that the cells produce BDNF prior to transplantation. However, we did not detect BDNF production by either MSCs alone or MSC/SC co-cultures, suggesting either that MSCs are not stimulated to produce BDNF release in artificial culture settings, or that the cells provoke astepping by another mechanism. Additionally, we transferred supernatants from cultured MSCs to trkB-expressing PC12 cells (trkB/PC12). Although neurite outgrowth was not as robust as when BDNF standard was applied, MSC supernatant treated trkB/PC12 cells displayed short processes, while those treated with conditioned media displayed none. These results raise the possibility that MSCs produce NT-4/5 or another factor, rather than BDNF. Additionally, we further examined MSC grafts in vivo, with the aim of better characterizing the morphology and composition of the grafts. We observed that grafts contained both lamina and fibroblast and appeared to direct regenerating axons to extend in the rostral-to-caudal orientation, rather than transversely across the cord. The grafts also contained significant amounts of PO-labeled Schwann cell myelin, but were largely devoid of MBP-positive central myelin. Collectively, these results support further examination of MSC transplants for SCI. Supported by NS 37321.

P200.
INFLUENCES OF ACUTELY TRANSPLANTED GLIAL RESTRICTED PRECURSOR CELLS ON THE CHRONIC LesION ENVIRONMENT FOLLOWING CONTUSIVE SPINAL CORD INJURY C.E. Hill††. C. Praschak†, M. Mayer-Praschak‡, M.D. Noble‡, M.S. Beattie*, and J.C. Bresnahan†. (Dept. of Neuroscience, OSU, Columbus, OH; Center for Cancer Biology, U. Rochester, Rochester, NY US).

Glial restricted precursor (GRP) cells are multipotent cells that can differentiate along oligodendrocyte and astrocyte lineages both in vitro and in vivo. We previously reported that GRP cells transplanted acutely into spinal cord contusion injuries and assessed after 8 days, were able to survive and migrate (based on nuclear labeling with Hoechst) and that GRP cells appeared to reduce neurologic recovery scoring and the expression of inhibitory chondroitin sulphate proteoglycans (CSPGs). Here we examine whether these alterations persist chronically and whether transplantation of GRP cells alters axonal responses. Cells isolated from transgenic rats that ubiquitously express the human placental alkaline phosphatase (PLAP) gene were isolated and transplanted into the contusion site immediately following injury. Similar to our previous results, 6 weeks after injury GRP cells are able to survive and migrate when transplanted immediately after injury. PLAP expressing GRP cells were observed within the lesion site. The majority of cells were confined to the lesion area, which was partially filled with cells, and some cells were observed in the white matter up to 5 mm rostral or caudal to the lesion center. We also examined the glial and molecular scar and the extent of axonal growth from CST and SHT fibers 6 weeks after injury and transplantation to determine if the alterations in scoring persisted in the longer term and whether these changes towards a more permissive environment and the presence of an immature astrocyte subtype could result in increased sprouting of descending aconas 6 weeks after injury. (Support: NS 38079).
P201.
ADENOVIRAL VECTOR-MEDIATED GENE TRANSFER OF BRAIN DERIVED NEUROTRPHIC FACTOR PROMOTES FUNCTIONAL RECOVERY AND AXONAL REGENERATION AFTER COMPLETE TRANSECTION OF ADULT RAT SPINAL CORD
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MATERIALS AND METHODS: We prepared adenoviral vectors encoding either beta-galactosidase (AxCALaZ) or brain-derived neurotrophic factor (AxCABDNF). The titers of the vectors were adjusted to 5x10^10 plaque forming units. COS cells were infected with the vectors and western blotting was performed to detect BDNF. Tissue samples were obtained from 8-week-old male Wistar rats. The spinal cord was completely transected at the T8 level. Immediately after the transection, 5ml of the vectors was injected into both stumps. AxCALaZ-treated rats were perfused transcardially with 0.1% glutaraldehyde and cryosections of the brain and spinal cord were made. X-gal histochemistry and immunohistochemistry were performed to evaluate transgene expression. Locomotor activity was evaluated using the BBB locomotor score. In AxCABDNF group, retranssection of spinal cord was performed 6 weeks after injury. For retrograde tracing, Fluorogold (FG) was injected into the lumbar enlargement 6 weeks after the transaction, and FG-labeled neurons in the brainstem nuclei were evaluated.

RESULTS AND DISCUSSION: Western blot analysis revealed that the conditioned medium from AxCABDNF infected COS cells contains BDNF. X-gal histochemistry revealed transgenic expression in the injected site and in the brainstem nuclei. Immunohistochemistry revealed transgenic expression in neurons and glial cells near the injected site, and in neurons of the brainstem nuclei. BBB locomotor score of AxCABDNF group showed significant recovery and the average score 6 weeks after injury was 6.0, although no recovery was observed in the AxCALaZ group. The average score 6 weeks after injury in AxCALaZ group was 0.4. Retranssection caused complete loss of the recovered hindlimb function.

Retrograde tracing revealed FG-labeled neurons in the red nucleus of AxCABDNF group although no FG-labeled cells were found in AxCALaZ group. Our results showed that adenoviral vector-mediated gene transfer of BDNF promotes axonal regeneration, resulting in functional recovery after complete transection of the spinal cord in adult rats.

P202.
TRANSPLANTED HEMATOPOIETIC STEM CELLS@FROM BONE MARROW DIFFERENTIATE INTO NEURAL LINEAGE CELLS AND PROMOTE FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY IN MICE
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Introduction: Recent evidence demonstrates that hematopoietic stem cell (HSC) fraction of bone marrow can differentiate into cells bearing neural markers in brain. In the present study, we employed a mouse model of spinal cord injury and transplanted HSCs from bone marrow into the injured spinal cords. Based on the results, we discuss a possible role of the transplanted HSCs on their functional recovery.

Materials and Methods: To purify HSCs, we collected total bone marrow cells from femurs of male Rosa26 mice. The mice were pretreated and exposed to beta-galactosidase ubiquitously. The cells were analyzed by FACS Vantage (Becton Dickinson) and, E-hat+ Sca1- Line cells were sorted, yielding primitive HSCs. Female C57BL/6J mice were subjected to a contusion injury of spinal cord using Farouque's technique. HSCs in phosphate buffered saline or buffer alone (control) were injected into the spinal cord 1 week after injury. We evaluated their functional outcome using hindlimb motor function score (Farouque, 2000) hybridization for Y chromosome was performed to detect cells derived from HSCs. To visualize the cellular co-localization of beta-galactosidase and cell-type specific markers, we employed double immunofluorescent staining.

Results: Significant recovery of hindlimb motor function score was detected in mice transplanted with HSCs compared with control. Histological analysis showed that the transplanted cells survived and differentiated into cells expressing neural marker. Discussion: HSC fraction of bone marrow offers several advantages for the clinical use of cell transplantation. Clinical uses of fetal embryonic and neural stem cells are limited both from immunological and ethical standpoints. In contrast, transplantation of patient's own bone marrow cells could circumvent the ethical issues related to cell transplantation from donors to recipients. Significant benefits of HSC transplants in SCI and other neurological injuries were reported. Our results suggest that transplanted HSCs from bone marrow may represent an effective strategy for the treatment of spinal cord injury.

P203.
NG2, p75 AND NEUROFILAMENT EXPRESSION AFTER SPINAL CORD INJURY IN RATS: DISTRIBUTION, CO-LOCALIZATION AND QUANTIFICATION
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Oligodendrocyte progenitors, found throughout the adult brain and spinal cord, are thought to be the source of NG2 chondroitin sulfate in the nervous system. We previously determined that NG2+ cells display protracted proliferation after spinal cord injury (SCI). Since NG2 is considered a potent inhibitor of axon growth, this increased level of NG2 may have important implications for regeneration. Currently, we sought to further evaluate NG2 expression after SCI by identifying the cellular source and determining the relationship of its distribution to that of axons within the injury site. Surprisingly, a considerable number of NG2+ cells were observed. We quantified the number of NG2+ cells 3 days post-injury, which remained elevated for 4 months. While p75+ profiles may include oligodendrocytes or axons, the majority had a bipolar phenotype and displayed immunoreactivity for S100b, suggesting they are premyelinating Schwann cells. Acutely, NG2+ p75+ cells were distributed in the ventral funiculus, while chronically they were found throughout the tissue and often were seen lining cavities and forming bands along septae within the cavities. A minority of NG2+ cells displayed fibrinogen immunoreactivity, suggesting they were fibrin-deposited on the meninges or peripheral nerves. NG2+ also was detected on macrophages and on myelinating Schwann cells within the spared white matter. Thus, many cells in addition to oligodendrocyte progenitors express NG2 after SCI. An unexpected finding was considerable overlap in the distribution of NG2 and neurofilament immunoreactivity along the septae on which axons grow after injury. This suggests that NG2 is involved in axon growth after SCI acting either as either a permissive or regulatory substrate.

Furthermore, the p75+/NG2+ cells along the septae may play a role in this process. Supported by NS37321.

P204.
PRECLINICAL TRIAL OF INTRATHecal GABAamide IN THE TREATMENT OF SPASTICITY
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The most effective current clinical treatment for severe spasticity is baclofen delivered intrathecally. Tolerance development and various side-effects emphasize the need for the development of a new drug that lacks these problems. GABAamide is an active metabolite of Progabide that has been tested extensively in animal studies and in clinical trials and found to be more effective in treating spasticity than baclofen. Its superior effectiveness was probably due to its action at both the GABA-A and GABA-B receptors while baclofen only influences the GABA-A receptor. However, systemic delivery of Progabide resulted in liver dysfunction in some patients. GABAamide can be delivered intrathecally at much lower doses than required for systemic delivery, thus avoiding the problem of liver dysfunction. Rats with chronic spinal cord injury produced using a weight-drop device were evaluated for spasticity every other day using a new test developed by our lab that was based on the Ashworth scoring system. Atlet or ESOX pumps connected to 1 French catheters were used to deliver the drug or vehicle intrathecally. Our drug cross-over studies [GABAamide (5 mg/kg), baclofen (15 mg/kg), saline] in rats confirm that intrathecal GABAamide and baclofen significantly reduce spasticity. In spastic rats treated with GABAamide intrathecally for 1 month, there was no evidence of the development of tolerance. Intrathecal delivery of GABAamide in normal animals did not produce any changes in sensorimotor function based on BBB locomotor scores and CBS testing. Our studies support the conclusion that GABAamide is a better alternative to baclofen in the treatment of spasticity. Supported by STTR (NIH) to Neurorecovery Inc.
P205. CELLULAR REACTIONS REMOTE FROM THE LESION SITE AFTER HUMAN SPINAL CORD INJURY

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Little is known about the cellular responses in the human spinal cord after traumatic injury. The growth associated protein GAP-43 and the transcription factor c-jun are believed to play a major role in the process of regeneration and they have been reported to be induced in axotomized neurons in a number of experimental models. In this study, we investigated the possible up-regulation of GAP-43 and c-jun in neurons of Clarke's nucleus (CN) in human post mortem material of patients who died after severe spinal cord trauma. By non-radioactive in situ-hybridization, a strong induction of GAP-43 and c-jun mRNAs could be observed bilaterally within CN neurons below the lesion site after short, but not after long survival times. Immunohistochemistry demonstrated the enhanced expression of 200kDa neurofilament protein in CN neurons below the lesion. These results confirm experimental data that even non-regenerating CNS neurons can up-regulate regeneration-associated genes such as GAP-43 and c-jun, which might reflect a transient regenerative capacity.

Furthermore, we have investigated the dynamics of Wallerian degeneration within white matter tracts. Neurofilament staining demonstrated a specific spatio-temporal pattern of axonal loss within degenerating fibre tracts which could be detected as early as 12 days close to the lesion. After late survival times the affected tracts were almost devoid of any NF staining. Between 5 weeks to 4 months, activated microglia were abundant within the corticospinal tract. These activated microglia were of the MHC class II positive and co-localized with the macrophage marker CD68 reflecting the phagocytic role. GFAP-staining revealed a late astrogial reaction throughout the area of the degenerating tracts leading to a long term deposition of a dense astrocytic scar.

Such studies on post mortem tissues are required to obtain a better understanding of human pathology after SCI and will elucidate the clinical relevance of experimental data.

P206. EARLY DECOMPRESSION AND ITS RELATION TO THE PHARMACOLOGICAL TREATMENT OF ACUTE CERVICAL SPINAL CORD INJURIES

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PURPOSE: The aim of the research was to examine the impact of timing on the effectiveness of decompression in traumatic spinal cord injury, especially in interaction with pharmacological treatment.

MATERIALS AND METHOD: 216 cervical fracture patients admitted to our tertiary SCI unit were studied retrospectively: 118 treated with immediate decompression, 84 with delayed surgery, and 14 not operated due to their clinical condition. Data was also collected on the administration of methylprednisolone. The timing of surgery and implementation of pharmacological treatment were determined by the time required for the patient to arrive in our unit after injury. Neurological condition was measured using the ASIA scale.

RESULTS: Patients who underwent late surgery had more severe spinal cord injuries initially than those operated. However, no differences in neuropotential improvement at 1-year follow-up were found for those who underwent early and late surgery. No correlation was found between pharmacological and surgical treatment.

CONCLUSION: The direct clinical benefit of early decompression and stabilization to improve the physiological environment include decreased hospitalization and faster neurological improvement in cases of cervical spine trauma with proven compression of the spinal cord, as well as earlier rehabilitation and mobilization. Over the long term, however, the neurological outcome in late surgery is comparable. The reported positive effects of methylprednisolone treatment are not influenced by surgical outcome.

P207. CONTINUOUS SPINAL CORD INJURY (SCI) INDUCED CHANGES IN CATHEPSIN B GENE AND PROTEIN EXPRESSION

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An increase in protease activity is a hallmark event of the secondary injury cascade following contusion SCI. Elevated levels of protease activity result in the degradation of cytoskeletal and myelin proteins essential for cellular function and survival. We have provided the first data that at least one member (CB) of the cathepsin protease family is upregulated by SCI. The excessive release and activity of cathepsin B, a ubiquitous lysosomal cysteine protease, has been implicated in several pathologies including tumor metastasis, arthritis and Alzheimer's disease. Our goal was to characterize the SCI-induced changes in cathepsin B expression. Following a T12 laminectomy and a moderate contusion (NYU device), the gene and protein profiles of cathepsin B in rats were examined using real-time PCR and immunoblots, respectively. Both the contusion-injured and the matched sham-injured animals exhibited elevated proenzyme (37kDa) protein levels at the lesion (LX) site, with significant differences between the two groups which could be detected as early as 12 days post-SCI. Furthermore, there was an increase in the active species of the protein with significant differences at 72 and 168 hr. post-SCI. For the 30 kDa form and at 48 and 168 hr. for the 23 kDa form. Cathepsin B protein levels were also affected in areas rostral (RLX) and caudal (CLX) to the injury epicenter. These levels differed significantly between animals at various post-SCI time points (24 to 168 hr). Real-time PCR revealed increases in cathepsin B mRNA levels following contusion SCI as early as 6 hr. post-SCI. These data indicate that SCI causes an upregulation of cathepsin gene expression and protein levels, which provides the basis for future studies to determine if these proteases are involved in the secondary injury cascade.

P208. DESTRUCTIVE CNS AUTOIMMUNE REACTIONS TRIGGERED BY SPINAL CORD INJURY ARE ASSOCIATED WITH THE PRODUCTION OF INFLAMMATORY CYTOKINES AND ENHANCED RECRUITMENT OF CD4+ T-LYMPHOCYTES

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T-lymphocytes are one of several immune cells that infiltrate the injured spinal cord. Previously, we demonstrated the injurious potential of myelin-reactive lymphocytes isolated from rats following spinal cord injury (SCI). We later confirmed this potential by showing that axonal injury and demyelination are exacerbated and neurological function is impaired after SCI in transgenic (Tg) mice enriched in myelin-reactive T-cells. Together, these studies indicate endogenous T-cells are activated by SCI and contribute to secondary injury. Still, other studies suggest myelin-reactive T-cells are neuroprotective. To further understand the role of CNS-reactive T-lymphocytes after SCI, we completed a time course analysis of the molecular cues necessary for lymphocyte entry and activation within the CNS. Specifically, mRNA levels of co-stimulatory molecules (CD80, CD86) and chemokines (IP-10, RANTES, MCP-1 and MIP-1α) were compared between Tg and nTg mice using quantitative real-time PCR. At 7 and 21 days post-injury, mRNA for co-stimulatory molecules (CD80 and CD86) and inflammatory chemokines (IP-10, RANTES, MCP-1) was dramatically increased in Tg and non-Tg mice. However, expression was significantly higher in Tg mice. For example, relative to uninjured control mice, RANTES mRNA was elevated 180-fold in Tg mice compared to a 33-fold increase in nTg mice. Increased molecular signal for T-cell recruitment during the first 3 weeks post-injury was accompanied by robust intraspinal accumulation of CD4+ T-cells. In SCI nTg mice, T-cells were localized to the injury site with few cells present in the rostral/caudal spinal cord. However, in SCI Tg mice, significantly larger numbers of CD4+ T-cells were found throughout the rostro-caudal extent of the injury with large infiltrates localized to regions of axon loss and demyelination. These data suggest that if unregulated, the chemokine and co-stimulation profile induced by SCI can initiate feed-forward amplification capable of evoking chronic and destructive CNS inflammation. This work was supported by NS07845 (PBG).
P209. EFFECTS OF TARGET CONTROL INFUSION OF PROPOFOL ON BURST SUPPRESSION AND BISPECTRAL INDEX IN HEAD INJURED PATIENTS

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The Bispectral Index (BIS; Aspect Medical, Inc) monitor quantifies hypnotic in the form of a BIS score, and cerebral metabolism as a burst-suppression (BS) percentage. The device is widely used to monitor anaesthetic depth and sedation, and could be assessing sedation and metabolic suppression following head injury. However, acute brain injury has effects on BIS scores [1], and propriety methodology used in the device has not been evaluated against more conventional measures in this population. We have investigated the relationship between propofol concentration, BIS and BS figures derived from the BIS monitor and conventional electroencephalography (EEG).

Methods: We studied 3 severely head-injured patients, aged 36 years (range 20–53) who required intensive care. Plasma propofol levels were standardized using a Target Controlled Infusion (TCI), commenced at least four hours before the start of the study. Sixteen lead EEG and BIS-A2000 monitors were sited to monitor BIS levels, BIS burst suppression ratio (BISBSR) and EEG burst suppression ratio (EEGBSR) at two target propofol concentrations.

Results: A change in mean (SEM) estimated target concentrations of propofol from 2.3 ± 0.3 to 4.2 ± 0.2 mcg/ml resulted in BIS values of 45.5 ± 2.9 and 37.7 ± 10.2, BISBSR values of 2.5 ± 1.5 and 20.9 ± 8.2, and EEGBSR of 0.0 and 4.9 ± 0.2 respectively. Propofol levels correlated to BIS scores. There was a significant correlation between Propofol levels and BISBSR (r²: 0.615) and between Propofol level and EEGBSR (r²: 0.766). While BISBSR and EEGBSR were significantly related (r²: 0.418; p < 0.05), there was inter-individual variability in the level of BIS assessed by the two techniques.

Conclusions: Current implementations of the BIS score do not correlate with target sedative infusions. While the BIS monitor does provide a measure of cerebral metabolic suppression, further work is required to relate this to more conventional measures.


P210. PREVENTING FLOW-METABOLISM UNCOUPLING ACUTELY REDUCES EVOLVING AXONAL INJURY AFTER TRAUMATIC BRAIN INJURY


We have previously presented evidence that the development of secondary traumatic axonal injury is related to the degree of local cerebral blood flow (LCBF) and flow-metabolism uncoupling. We have tested the hypothesis that augmenting LCBF in the acute stages after brain injury prevents further axonal injury.

Isotopic-aanesthetised rats were injured by controlled cortical impact over the left parietal cortex (4mm/s velocity, 2mm deformation). Quantitative measurements of regional local cerebral metabolic rate of glucose (LCMRglu) and LCBF were obtained in the same rat from 18F-fluorodeoxyglucose (30MBq) and 14C-lactate/14N-pyruvate tracer autoradiographic images respectively, and the density of injured axons from adjacent beta-amyloid precursor protein (β-APP)-immunostained sections. Data were acquired at 3hr post-injury with and without acetazolamide-induced CBF augmentation immediately following injury (150mg/kg, i.p.). Axonal outcome was assessed at 24hr in two further groups with and without CBF augmentation immediately following injury. Sham-injured rats were used for comparison of all data (all groups n = 6).

In CBF-augmented-injured rats, LCBF was significantly elevated above untreated-injured rats at 3hr, by ~2-fold in ipsilateral and contralateral regions (P < 0.05). LCMRglu was globally unaffected by acetazolamide compared to untreated-injured rats although it was no longer significantly increased from sham-injury control in the ipsilateral hemisphere. Isplacial LCMRglu/LCBF ratios were normalised by CBF-augmentation compared to untreated-injured rats from values 2-fold greater than in sham-controls. This demonstrated that β-APP-stained axons at 24hr were significantly reduced by CBF augmentation in most regions compared to the untreated-injured group at 24hr (P < 0.01). Furthermore, there was generally no significant increase when compared to the 3hr untreated-injured group, indicating that further axonal injury was prevented.

These data suggest that increasing post-injury CBF prevents axonal injury by diminishing the pronounced metabolic > blood flow dissociation that occurs in the acute stage of injury. This underlines the importance of maintaining flow-metabolism coupling immediately after injury in order to prevent further axonal injury.

P211. PHYSIOLOGICAL HETEROGENEITY MASKS HYPERRVENTILATION-INDUCED REDUCTIONS IN CEREBRAL OXYGEN METABOLISM IN HEAD INJURY


We have previously used positron emission tomography (PET) in patients with head injury to show that hyperventilation-induced reductions in cerebral blood flow (CBF) are associated with increases in regional oxygen extraction fraction (OEF). However, conclusive evidence of critical ischaemia requires the demonstration of hyperventilation-induced reductions in regional cerebral oxygen metabolism (CMRO2). We have used 15O PET to assess this.

Methods: PET was undertaken in 18 head-injured subjects, 2–5 days post-injury, at baseline and following hyperventilation. Maps of CBF, CMRO2 and OEF were calculated, coregistered to X-ray CT, and normalised to Talairach space. 15 Regions of interest (ROIs) covering the whole brain were defined on these metabolic images. Baseline data were collected in two frames to quantify the reproducibility of the technique, and estimate 95% confidence intervals (CI) to test whether changes in CMRO2 (dCMRO2) were real, or were the consequence of intra-subject variability.

Results: Reduction of PaCO2 from 36 ± 0.7 to 29 ± 0.6 mmHg led to significant and consistent reduction in CBF and increase in OEF in all subjects (p < 0.001). Despite an overall increase in CMRO2 with hyperventilation (p < 0.001), individual responses were highly variable. CMRO2 measurement was highly reproducible, and test-retest analysis showed that a dCMRO2 > 2.4 micro-mol/100g/min could be defined as significant (>95% CI), 43% of ROIs showed significant increases in CMRO2, but 30% of ROIs showed significant reductions in CMRO2, with significant reductions in one or more ROIs in 10 of the 18 patients studied (56%).

Conclusions: CMRO2 increases with hyperventilation may arise from increased neuronal excitability [1], but these increases may be attenuated or reflected if CBF reductions are critical. Summary statistics may mask this heterogeneity and miss critical regional ischaemia leading to CMRO2 reductions in many patients.


P212. EFFECTS OF CEREBRAL PERFUSION PRESSURE AUGMENTATION ON CAPILLARY-TISSUE OXYGEN GRADIENTS AFTER ACUTE BRAIN INJURY


We have previously shown [1] that increased capillary to tissue oxygen diffusion gradients exist after traumatic brain injury (TBI). We have used positron emission tomography (PET) and invasive tissue oxygen monitoring to characterize these gradients and determine their response to cerebral perfusion pressure augmentation.

Methods: We have studied 5 patients, within 5 days of a severe closed head injury, using 15O-PET to image cerebral blood flow (CBF), cerebral oxygen metabolism (CMRO2) and oxygen extraction fraction (OEF). Cerebral tissue PO2 was measured using a multiparameter sensor (NeurotrendTM, Codman), and cerebral venous PO2 (PvO2) was calculated from the OEF in a region of interest around the sensor. Capillary-tissue oxygen gradients (PvO2-PtO2) were calculated for each patient at baseline cerebral perfusion pressure (CPP) and after increasing the CPP by at least 20% using a norepinephrine infusion.

Results: Both OEF and Pto2 varied between patients, but OEF did not predict Pto2, which was determined, in large part, by the gradient between end-capillary and tissue Pto2 levels. An increase in CPP from 67.4 ± 3.1 mmHg (mean ± SD) to 88.7 ± 2.7 mmHg (p < 0.001) resulted in an increase in Pto2 (19.8 ± 9.3 mmHg). Despite the significant change in OEF or Pto2, this resulted in a variable reduction in the Pto2-PvO2 gradient (12.2 ± 10.6 to 7.6 ± 6.2 mmHg; p = 0.10). The magnitude of change in the Pto2-PvO2 gradient varied inversely with the baseline Pto2 (2 ± 0.88; p < 0.03).

Conclusions: Significant Pto2 gradients exist between the vascular and tissue ECF compartments in head injury. Early results suggest that CPP augmentation may partially overcome these oxygen diffusion barriers, and that such improvements may be more prominent in tissue where the baseline Pto2 is low. Further work is needed to understand the incidence, mechanisms and therapy of these novel pathophysiological processes in head injury.

P213. EARLY BRAIN SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IN PATIENTS FOLLOWING CRANIO-CEREBRAL TRAUMA
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Central nervous system (CNS) injuries are among most frequent sequelae of trauma. Patients with CNS injuries often require at least hospital observation, due to the diversity of clinical symptoms and poor prognosis in case of delayed or missed diagnosis combined with shortcomings and deficiencies in management. The aim of the study was to evaluate regional cerebral blood flow (rCBF) after minor cranio-cerebral trauma (Glasgow Coma Scale 13-15 points) by means of single photon emission computed tomography (SPECT). Three types of rCBF changes have been found: local perfusion deficits at the site of trauma (below the wound or cranial fracture), multifocal perfusion deficits and diffuse perfusion deficits. Among diffuse perfusion deficits the most common was a bilateral hypoperfusion of frontal lobes. Most focal perfusion deficits were not linked to the site of trauma but 48.8% were localized at the site of trauma or contralaterally. The most frequent localization was found in frontal and temporal lobes. The correlations between the rCBF deficits and neurological symptoms have been found. Brain SPECT demonstrates posttraumatic brain edema earlier, with a greater sensitivity and defines more precisely their extent than by means of computed tomography. Brain SPECT may be helpful in distinguishing patients simulating head trauma symptoms in an attempt to avoid legal problems or my conscience.

P214. CEREBRAL BLOOD FLOW AND BLOOD VOLUME RESPONSES TO CARBON DIOXIDE AFTER HEAD INJURY

Hyperventilation is commonly used to produce reductions in intracranial pressure (ICP), which are thought to be due to reductions in cerebral blood volume (CBV). There is concern that associated changes in cerebral blood flow (CBF) may result in cerebral ischaemia. The relative CBF and CBV responses to PaCO2 reductions have been poorly studied in patients with head injury. We have used O-15 PET to measure CBF, CBV and CO2 reactivity after head injury.

Methods: PET was undertaken in 18 head-injured subjects, 2-5 days post injury, at baseline and following hyperventilation. Maps of CBF and CBV were calculated, coregistered to X-ray CT, and normalized to Talairach space. Fifteen regions of interest (ROIs) covering the whole brain were defined on these maps. Regional CBF and CBV reactivity to changes in PaCO2 were calculated. Data are expressed as median (interquartile range).

Results: Regional CBF and CBV were variable and showed a positive correlation (r=0.24; p<0.001). CBF reactivity to CO2 in patients (2.8 (1.6 to 3.9) %/mmHg) was similar to published normal values, but CBV reactivity (0.1 (0.1 to -1.1) %/mmHg) was lower than published normal values (0.1 to -1.1). ICP reductions with hyperventilation (4.5 (2.6-5.2) mmHg) were unrelated to CBF or CBV changes, but were proportional to baseline ICP (r=0.49; p<0.0001). Regardless of baseline ICP (range: 5-30 mmHg) none of the regions showed CBF increases with hyperventilation.

Conclusions: The positive correlation between CBF and CBV supports a microcirculatory cause for ischaemia in head injury. CBF and CBV reactivity to PaCO2 in this population. The effect of CBV reductions on ICP depends on intracranial compliance, but regional CBF reductions may be the dominant effect of hyperventilation at this interval post-injury, even in patients with relatively high baseline ICP values. Reference: [1] J Trauma 1995; 39: 465.

P215. TRANS SODIUM CROCETINATE INCREASES OXYGEN DELIVERY TO MARGIN PARENCHYMA IN RATS ON OXYGEN SUPPLEMENTATION

In spinal cord injury (SCI) and traumatic brain injury (TBI), the primary injury largely determines a patient's neurological grade upon admission and thereby is the strongest prognostic indicator. However, secondary mechanisms of injury can exacerbate damage and limit restorative processes, and hence, contribute to overall morbidity and mortality. Hypoxia and hypotension are major causes of ischemia in both SCI and TBI. We investigated the effect of TSC on regional oxygen delivery to ischemic heart and liver tissue and in lower-flow states such as shock, but has never been studied in brain or neurologic disorders. We investigated the effect of TSC on brain oxygen delivery in order to determine indication for study in TBI and SCI with secondary hypoxic insult.

To this end, male rats were ventilated with either 21% or 100% FiO2. Femoral artery and vein cannulation was achieved and baseline arterial blood gas measures taken. Next, a small burr hole was drilled into the right parietal bone. A calibrated Li-Cor rat brain PO2 probe was inserted into the cortical parenchyma and PO2 in brain tissue (PtO2) plugged into the brain tissue oxygenation monitoring system. PtO2 was recorded over the course of an intravenous infusion of TSC or saline. Serial ADGs were also monitored.

TSC significantly increases PtO2 to brain in rats ventilated with 100% FiO2. A similar effect on oxygen delivery is not achieved in unventilated rats ventilated with 21%FiO2. O2 supplementation is the standard of care for hypoxia after neurologic insult. Because TSC increases oxygen delivery to brain in the presence of O2 supplementation, an investigation of the impact of TSC on oxygen delivery in TBI and SCI with secondary hypoxic insult is warranted. Supported by AO North America.

P216. REGIONAL EFFECTS OF CEREBRAL PERFUSION PRESSURE AUGMENTATION IN HEAD INJURED PATIENTS

Cerebrovascular autoregulation is often impaired after head injury. However, there is substantial pathophysiological heterogeneity in the injured brain, and global measurements may not predict the response of regional cerebral blood flow (rCBF) to augment cerebrovascular perfusion pressure (CPP). We have used positron emission tomography (PET) to investigate rCBF responses to a change in CPP.

Methods: Fourteen sedated and ventilated head injured patients were studied within 4 days of injury. CBF and cerebral blood volume (CBV) were measured using PET at a CPP of ~70 and ~90 mmHg. CPP was controlled using norepinephrine. Changes in CBF and CBV and the static rate of autoregulation (SRO) were calculated globally (gSRO) and regionally (rSRO) for 15 normalized regions of interest (ROI). ROIs were classified as lesioned or non-lesioned based on CT images.

Results: gSRO ranged from 37–101%; rSRO in 210 ROIs from 14 patients ranged from 20–109%. There was substantial spatial variation in autoregulation within patients, and rSRO was < 50% in 15% of non-lesioned ROIs. Regional dysautoregulation was commoner when global autoregulation was impaired: no patient with gSRO > 85% showed regions with rSRO < 85%, and rSRO < 50% was only found in patients with gSRO < 50%. Assessment of CBV responses to hypertension allowed definition of a subset of ROIs (23% of ROis with rSRO > 85%) that showed an increase in CBV > 10%, suggesting false autoregulation, possibly due to distal vascular compression leading to an increase in CBV but not CBF.

Conclusions: CPP augmentation leads to unpredictable changes in CBF due to heterogeneity of vascular reactivity, but global autoregulation is a useful indicator of regional autoregulation. CT scans underestimate the extent of cerebrovascular dysfunction after head injury. Assessment of autoregulation by CBF responses only may be confounded by false autoregulation.
P217.

INHIBITION OF NA+/CA++ EXCHANGE WITH KB-R7943 ATTENUATES EARLY ASTROCYTE LOSS IN HIPPOCAMPUS FOLLOWING FLUID PERCUTION BRAIN INJURY.


Astrocytes play a significant role in normal brain function including active neuronal-glia signaling and maintenance of homeostasis in the extracellular microenvironment. Early impairment of astrocyte function after traumatic brain injury (TBI) may compromise critical neuronal-glia interactions and thus may play a significant role in outcome after injury. Recent studies in ischemia (Mol Brain Res 68:29-41, 1999) as well as in TBI (J Neurotrauma 18:1165, 2001) indicate astrocyte loss in selectively vulnerable brain regions. Overload of intracellular calcium is thought to be a major cause of cellular damage following TBI. We examined the effects of KB-R7943, a novel inhibitor of the reversed Na+/Ca++ exchanger, on early astrocyte loss in hippocampus.

KB-R7943 (0, 0.2, 2, 15, or 40 nmol) was infused into the lateral ventricle of male Sprague-Dawley rats for 1 hr prior to lateral fluid percussion TBI. At 4 hr after TBI, rats were euthanized and glial fibrillary acidic protein (GFAP) immunohistochemistry was performed. Counts of astrocytes were made in the dorsal hippocampus CA2-3 sectors from coronal sections between Bregma -2.12 through -4.80 mm using stereological procedures. Astrocyte counts in the contralateral hemisphere were not significantly different between groups (65,135 ± 2103). Ipsilateral astrocyte counts were significantly higher in the 15nmol (58,442 ± 372) and 40nmol (53,684 ± 1254) KB-R7943 groups compared to the vehicle-treated group (34,170 ± 3732).

Inhibition of the Na+/Ca++ exchanger reduced astrocyte loss in the hippocampus after TBI. These data suggest that TBI may cause reversal of the Na+/Ca++ exchanger, which could be detrimental to astrocyte survival in selectively vulnerable brain regions after TBI. Supported by NIH NS29955 & UC Neurotrauma Research Initiative.

P218.

CSF ACCUMULATION OF CALPAIN-SPECIFIC aLI-SPECTRIN BREAKDOWN PRODUCTS ARE ASSOCIATED WITH INJURY MAGNITUDE AND LESION VOLUME AFTER TRAUMATIC BRAIN INJURY IN RATS.


There currently exists no definitive diagnostic tests of traumatic brain injury (TBI) to help physicians determine the seriousness of injury, the extent of cellular pathology, or to guide appropriate therapeutic administration. Although we recently reported that calpain-specific aLI-spectrin breakdown products (SBDPs) accumulate in CSF after TBI (Pike et al., 2003, J. Neurochem. 78:1297-1307), correlation of SBDP levels with injury magnitude and outcome is not known. The purpose of this study was to examine SBDP accumulation in brain and CSF at two levels of lateral controlled cortical impact TBI (1.0 mm and 1.6 mm) in rats at 2, 6, and 24 hours after injury. In addition, SBDP levels at each injury magnitude were correlated with rotarod performance on days 1-5 post-TBI, and with lesion volume at 28 days post-TBI (by T2-weighted MRI). Results: Accumulation of SBDPs in brain and CSF was highest after 1.6 mm injury at all time points. Both 1.0 mm and 1.6 mm groups had significantly greater CSF levels of SBDPs than the control group (p < 0.05, p < 0.01 respectively). In addition, SBDP levels were associated with both rotarod performance and with lesion volume where each was greater after 1.6 mm injury than 1.0 mm injury. Conclusions: This study indicates that CSF levels of calpain-specific SBDPs are sensitive to injury severity and that acute levels of SBDPs in CSF (hours) are associated with delayed measures of behavioral outcome (days 1-5) and lesion volume (28 days). These findings support the use of SBDPs as biochemical markers of cellular pathology, injury severity, and outcome after TBI (Supported by DAMD17-99-1-565, DAMD17-01-1-0765, and NIH R01 NS39091, and NIH R01 40182).

P219.

TISSUE-TYPE TRANSGlutaminase DISTRIBUTION AND EXPRESSION AFTER TRAUMATIC BRAIN INJURY.


Tissue-type transglutaminase (TG) has been implicated in various diseases including neurodegenerative disease. Tissue transglutaminase (TG) is a calcium-regulated enzyme and intracellular Ca++ overload is triggered following traumatic brain injury (TBI). Therefore, we analyzed the expression of TG after TBI in a rat cortical impact model. Western blot analysis has demonstrated an increase in TG protein expression after TBI. In ipsilateral cortex, peak induction of TG protein (561% ± 16%) was observed five days after injury, with expression remaining elevated after two weeks. Lesser TG protein induction was observed in hippocampus (194 ± 9%) five days after injury. The TG protein induction was supported by northern blot and semi-quantitative PCR transcript analysis that demonstrated peak induction three days after injury in ipsilateral cortex with a small induction in hippocampus. Semi-quantitative PCR analysis of TG mRNA demonstrated a peak induction three days after injury in ipsilateral cortex (414% ± 21% of control, n = 3), while in hippocampus, maximal induction was observed one day after injury, 196% ± 14% of control. Further, to elucidate the cell subtype distribution of TG immunofluorescence was performed. Studies have demonstrated increased expression of TG in both neuron and astrocyte cell population after injury, however, the expression was stronger in astrocytes than in neuronal cells. Future studies are in progress to determine (the role of TG in injured cells) whether TG positive cells are involved in apoptosis or proliferation. These findings will lay the groundwork for elucidating the functional role of TG in TBI or any other CNS related injury and may result in the development of effective treatment strategies. (Supported by DAMD 17-99-1-9565 and NIH RO1 NS 39091)

P220.

RECOVERY OF SPEECH-SOUND PRODUCTION SKILLS IN VERY YOUNG CHILDREN AFTER SEVERE TRAUMATIC BRAIN INJURY.

Thomas P. Campbell, P. David Adelson, Christine A. Doolinghan, Jane Janosky. (University of Pittsburgh and Children's Hospital of Pittsburgh, Glenshaw, PA US).

The current literature provides little information on the recovery of speech skills in children following severe traumatic brain injury. No data exist on the relationship between age at time of injury and speech recovery in these children.

In this investigation, we examined the rate and level of consonant mastery in 30 children who sustained severe traumatic brain injury (TBI) between 15 months and 10 years of age. Percentage of Consonants Correct was calculated and plotted over twelve monthly speech samples beginning when the child produced at least 10 intelligible words, and compared to a normal Percentage of Consonants Correct growth curve.

Results showed that children who were relatively older (i.e., >49 months) at the time of injury tended to display Percentage of Consonants Correct values that approached the normal performance curve in a shorter period of time than the children injured at younger ages (<49 months). In addition, older children were generally less variable across the 12 sampling sessions than younger children. Despite improvements in consonant production for the majority of these subjects over the 12 testing sessions, prosodic and voice aspects of speech production remained compromised in 83% of the subjects at the final session, suggesting underlying motor defects. These findings do not support the traditional view that earlier onset of neurological injury results in greater recovery. The need for predictive models of speech outcomes that include age at injury, extent of neurological injury, and severity of oral-motor dysfunction will be discussed. This research was funded by a grant from the National Institute on Deafness and Other Communication Disorders awarded to the first author (RO1-DC0 3608).

1302
P221.
EXPERIENCE-DEPENDENT LOSS OF PLASTICITY IS RESTORED AFTER DELAYED EXPOSURE TO AN ENRICHED ENVIRONMENT.

It has previously been shown that developing rats exposed to an enriched environment (EE) immediately following brain injury fail to show experience-dependent plasticity (Finerman et al., 2000). In this study, entry into an EE was delayed to 14 days after lateral fluid-percussion injury (FPI) to determine the time window during which developmental plasticity is lost. Rats were reared in EE for 30 days. After EE exposure rats were trained in the Morris Water Maze task (MWM) for five days. Both the sham-enriched rats and the FPI-enriched rats showed a significant improvement in the rate of learning compared to the standard-housed rats (p < 0.05). A probe test performed one week after MWM training showed that both the FPI-enriched and FPI-standard-housed rats spent significantly less time swimming in the target area compared to the sham animals (p < 0.01). In addition, the FPI-enriched rats took significantly more time to initially reach the target area compared to all the other groups (p < 0.05). Exposure to EE did not affect any influence on delayed probe performance in the sham animals. These behavioral results indicate that plasticity is restored by PND 14, as indicated by the rate of learning. However, injury-induced memory impairments persist after exposure to EE. Supported by: University of CA BIRC, NS30308, NS27544, NS38978.

P223.
DIFFERENCES IN APOE GENOTYPE ON SECONDARY INSULTS AFTER TRAUMATIC BRAIN INJURY.
Imran Libaqui, Lawrence T. Dunn, Ian R. Piper, Graham M. Teasdale* & James A.R. Nicoll (Department of Neurosurgery, University of Glasgow, Scotland, UK; Division of Clinical Neurosciences, University of Southampton, UK).

Objectives: The aim of the study was to determine the influence of APOE genotype on secondary insults after Traumatic Brain Injury (TBI).

Methods: Sixty-two of 262 genotyped patients admitted to a neurointen
care unit with TBI over a 4 year period had monitoring data on intracranial pressure (ICP), blood pressure (BP), oxygen saturation and cerebral perfusion pressure (CPP). Demographic, management and outcome data including admission Glasgow Coma Scale (GCS) and 6 month Glasgow Outcome Score (GOS) were recorded using a standardised proforma for each patient. APOE genotype was determined using polymerase chain reaction. Monitored data was validated manually. Insults were identified using established definitions and total insult duration as a fraction of valid monitoring time and frequency of insults were recorded.

Results: Eighteen patients had one or more APOE ε4 alleles. Hypotension and CPP insults were more frequent in the APOE ε4 group (c2 = 6.236, df:2, p = 0.04 and c2 = 48.9, df:2, p = 0.00 respectively). The duration of hypotensive and CPP insults was also longer in the APOE ε4 group although this failed to reach statistical significance. There were no significant differences between the groups for insults related to ICP or hypoxia.

Conclusions: Hypotensive and CPP insults were more frequent amongst patients with an APOE ε4 allele. Differences in the acute response to TBI may be at least partially responsible for the worse outcome that had been observed in individuals with an APOE ε4 allele.

P224.
HIGH-DENSITY HIGH-THROUGHPUT TISSUE MICROARRAY PROFILING OF NEURODEGENERATIVE ALTERATIONS IN EXPERIMENTAL TRAUMATIC BRAIN INJURY.
Goodman JC, O’conner C, Magdon SL, Robertson CS, and Fuller GH (Baylor College of Medicine and M.D. Anderson Cancer Center, Neurapraxia and Neurotrauma Programs, Houston, TX).

Head injury sets into motion a complex neurochemical cascade that may worsen the initial in
jury and render the damaged tissue more vulnerable to secondary damage from ischemia and hypoxia. These neurochemical alterations may be subject to pharmacological intervention. Studies of these processes have been hampered by the lack of high-throughput techniques that al
low rapid analysis of large numbers of tissue samples from experimentally produced brain in
jury. Very recent advances in high-density, high-throughput molecular profiling technologies de
veloped for tumor analysis may be directly transferrable to the study of non-neoplastic dis
cases such as head injury. One of the most promising of these techniques is tissue microarray (TMA), in which small tissue cores from hundreds of individual tissue samples are composited into a single parasitic block from which microtome sections can be cut and subjected to a wide range of analytical procedures, including routine immunostaining, immunohistochemistry, and a wide range of immunoperoxidase and fluorescent in-situ hybridization and PCR techniques. Ex
isting technology permits the manual or automated compositing of individual tissue cores ranging from 0.6-2.0 mm diameter with 0.1 mm spacing between array elements. TMAs permit the simultaneous staining and analysis of up to 1000 different tissue samples on a single 45 x 20 millimeter glass slide.

We constructed a TMA of cerebral cortex cores taken (pallidum and caudate) in a con
trolled cortical impact site from rats 80 days after an controlled cortical impact site from rats 80 days after an controlled cortical impact site from rats 80 days after an controlled cortical impact site from rats 80 days after an controlled cortical impact site from rats. The donor block comprised archival material from previous experiments in which the animals were sacrificed 14 days after injury. Duplicate punch sam
ples were obtained from each site to yield a total of 300 tissue cores arrayed in a single rep
lication (TMA) block. From this TMA block, 40 unstained sections were cut. The first and last sections were stained with hematoxylin & eosin and the TMA block was refrigerated for future use. Using two of the unstained sections, we performed glial fibrillary acidic protein (GFAP) and NADPH-diaphorase (a marker for macrophage activation) immunohistochemistry. Strong GFAP immunore
activity was seen in approximately 50% of the cores, and in 50% of these GFAP-positive cores the immunoreactivity localized to the sides of the cortical impact. No significant NADPH im
munoreactivity was present in any core. The remaining unstained slides and the TMA block are now archived for additional studies. In addition, the STA-27 punch order program has also been saved, permitting easy automated construction of additional TMAs from the archived donor block study set as needed.

This proof-of-principle study demonstrates the technical feasibility of using tissue microwave
ning for proteomic expression profiling in traumatic brain injury. Tissue microarrays facil
itate the preservation of valuable tissue resources by virtue of their "tissue expansion" property and permit an increase in the number of experiments that can be performed on limited and sometimes improvable tissue samples by at least two orders of magnitude. High-density high
throughput TMAs provide a powerful new tool for the dissection of molecular and cellular events occurring in traumatic brain injury.

(The support of NIH ROI-NS36806 is gratefully acknowledged.)
P225.
GENE EXPRESSION FOLLOWING HUMAN TRAUMATIC BRAIN INJURY BY MICROARRAY ASSAY

Human data accumulated over the past decade has validated animal studies showing gene expression after traumatic brain injury (TBI), including up-regulation of c-fos, Jun B and HSP 70. We tested the hypothesis that mRNA expression following human TBI characterized by microarray assay (MA) would be consistent with previous work and uncover novel gene expression. Methods: This study was conducted with IRB approval. Percutaneous tissue from TBI patients was analyzed against normal tissue removed during surgery for non-traumatic indications using MA. Experimental and control cDNA probes were hybridized against >5000 gene segments on GSP2000 human GeneFilter® and quantified with Pathways 4D® software.

Results: To date we have analyzed samples from five TBI patients against control samples. We identified upregulation of c-fos (4 of 5), Jun B (4 of 5) and HSP 70 (3 of 5) achieving statistical significance in several cases. In other comparisons glyceroldehyde-3-P, dynacin, 4, B crystallin, and myelin basic protein were up-regulated, while GFAP was down-regulated, in TBI compared to control patients.

Discussion: These results further address the validity of current animal TBI models by using MA to demonstrate specific, differential gene expression in human TBI. Further study will provide new insight into the pathophysiology of TBI. Support by the L.M. Thomas, M.D. Fund, to D.B.M., by NIH R15-D05179 to L.N.I., and an NIH-NCCRR (RCMI) grant to the Univ. of Texas at El Paso.

P226.
THE EXPERIMENTAL STUDY ON EXPRESSION AND ACTIVATION OF CAPSASE 3 AFTER ACUTE BRAIN TRAUMA
Shuyan Yang, Xinyu Yang, Jianning Zhang. (Department of Neurosurgery, General Hospital of Tianjin Medical University, Hejing, Tianjin CN).

To analyze the role of Caspase 3 on delayed neuronal death. Experiments were based on rat diffuse brain injury model. The neuronal DNA injury in cortex and hippocampal was observed by TUNEL stain. The mRNA and protein expression and enzyme activation of Caspase 3 were observed by northern blot, in situ hybridization, immunohistochemistry stain and western blot. Special Caspase 3 enzyme inhibitor were given to observe the therapeutic effect.

After impact, neurons with positive TUNEL stain appeared 2 hours after severe injury, most peaked at 24 hours, last till 7 days. Northern blot shows that the Caspase 3 mRNA expression increased and peaked on 24 hours, 3 times higher than the controls. In the area of cortex and hippocampal, positive mRNA stain neurons appeared most distinct on 24 hour. With the antibody for Caspase 3 P20 subunit, the active Caspase 3 expression peaked on 1–3 days. The electrophoresis band of PARP degradation would be seen by western blot. Caspase 3 enzyme inhibitor decreased apoptotic neuronal death, but had no effect on Caspase 3 P20 subunit expression.

After brain trauma, there were increases on Caspase 3 mRNA and protein expression and enzyme activation, accounting for neuronal DNA injury or apoptosis. Using special Caspase 3 enzyme inhibitor can apparently decrease the delayed neuronal death.

P227.
CXCR CHEMOKINES MAY CONTRIBUTE TO INFLAMMATION IN SUBARACHNOID HEMORRHAGE AND ITS CONSEQUENCES
Norihito Shirakawa*, Takeharu Tani. (National Zentsuji Hospital, Zentsuji, Kagawa JP).

Activated neutrophils are thought to be involved in neuronal injury following subarachnoid hemorrhage (SAH). CXCR chemokines, interleukin (IL)-8 and epithelial neutrophil activating peptide (ENA)-78, are responsible for activation of neutrophils and for neutrophil chemotaxis to site of injury. To understand the importance of these chemokines in the pathogenesis after SAH, we have examined the production of these chemokines in cerebrospinal fluid (CSF) from 19 patients with SAH and in 70 control patients without central nervous system lesions. We also investigated the expression of mRNAs of IL-8, CXCR1 and CXCR2 by RT-PCR and immunocytochemically the expression of IL-8 protein in CSF leukocytes from patients with SAH.

Significantly increased levels of IL-8 (p < 0.001) and ENA-78 (p < 0.001) were detected in the CSF of patients after SAH compared with levels in the CSF of control patients, and the levels of both chemokines correlated with neurologic severity at admission assessed by Hunt & Kornik grading and neurologic outcome by Glasgow outcome scale. The elevated leukocyte count in CSF collected by ventricular drainage correlated with the levels of IL-8 (p < 0.01) and ENA-78 (p < 0.05). We followed time-related changes in concentrations of the CXCR chemokines in 7 patients with SAH for up to 30 days, and found peak concentrations of them within 10 days after the onset of injury. Furthermore we detected mRNAs of IL-8 and CXCR1 and CXCR2 in CSF leukocytes and IL-8 protein in macrophages and in polymorphonuclear leukocytes in CSF.

These findings suggest that IL-8 and ENA-78 may contribute to inflammation in SAH and its consequences through leukocyte activation and chemotaxis.

P228.
SYSTEMIC ANTI-INFLAMMATORY REACTION AFTER BRAIN INJURY
Ch. Wociachowski, N. Daberko, S. Rupprecht, H.D. Volk. (Department of Neurosurgery, Berlin, Germany).

Local release of pro-inflammatory cytokines as well as an increased ICP after brain injury may activate the hypothalamic-pituitary-adrenal (HPA)-axis and the sympathetic nervous system (SNS) and induces a systemic anti-inflammatory response syndrome. In order to analyze the mechanisms of a systemic immunodepression resulting from cerebral inflammation and an increased ICP we established different animal models using intra-cerebroventricular (icv) or intra-hypothalamic (ih) infusion of the pro-inflammatory cytokines TNF-α and IL-1β and increasing continuously the ICP using an subarachnoid placed catheter. Interestingly, icv and ih infusion of IL-1β but not TNF-α produced distinct signs of central nervous system (CNS) inflammation. Correspondingly, icv and ih infusion of IL-1β generated an increase of neutrophils and a decrease of lymphocytes in blood. This could be reduced by hypophysectomy (HPX) and completely blocked by administration of the β2-adrenoceptor antagonist propranolol. Furthermore, icv and ih infusion of IL-1β produced a diminished TNF-α secretion capacity. This could be reversed by HPX. Finally, icv infusion of IL-1β caused a temporal elevation of the endotoxin-induced IL-10 secretion. This effect was antagonised by propranolol suggesting an involvement of the SNS. Moreover, increased ICP and bolus IL-1β into the brain are able to induce systemic IL-10 release. This effects could be likewise blocked by application of the β2-receptor-antagonist propranolol suggesting that sympathetic activation modulated also the systemic IL-10 release. Interestingly, brain injured patients showed also high IL-10 concentration in plasma and the IL-10 levels were associated with the severity of the injury. Finally high IL-10 levels correlated with systemic immuno-depression and increased risk of infectious complications. So we conclude, that increased ICP and cytokines in the brain can produce vegetative disturbances with sympathetic activation. Catecholamines are able to induce an IL-10 release from white blood cells. This may lead to systemic immuno-depression and infectious complications in brain-injured patients.
P229.  
TRAUMATIC BRAIN INJURY (TBI)-INDUCED SPASTICITY: MONOAMINE CHANGES AND POSSIBLE MECHANISMS.  
Prody Bote*, Ronald Parmer, Justin Parker, Ronald L. Hayes, and Floyd J. Thompson, (Dept. of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL).  

Little is known regarding the fundamental neurobiology of TBI-induced spasticity that could guide the development of successful treatment strategies. These studies were conducted in Sprague Dawley rats with moderate controlled contusion cortical injuries (CCI), that had just completed three-months of spasticity and behavioral studies (see poster: Thompson et al. 2002). The purpose of the present studies was to investigate two monoamine neural substrate systems known to influence spinal reflex excitability. Fluorescent immunocytochemistry (ICC) was applied to pons-medullary and lumbar spinal cord tissue to investigate TBI-related changes in the expression of immunoreactivity (IR-) in noradrenergic (NE) cell clusters in the locus ceruleus (LC) and fibers in L4-5 spinal segments, as well as in the serotonergic (5-HT) cell clusters in the raphe and fibers in the spinal L4-5 segments. A significant reduction in LC NE cell number was detected in the TBI specimens in conjunction with significantly decreased number of IR-NE positive fibers in the ventral horns compared to that of control specimens. At the same time, a robust hyperinnervation in the dorsal raphe serotonergic positive cells was detected in the TBI specimens, compared with time-matched sham control specimens. Similarly, hyperinnervated IR-5HT positive fibers were also detected in the dorsal, ventral, and intermediolateral column of L4-5 spinal cord of TBI specimens. We hypothesize that this marked asymmetry between noradrenergic and serotonergic expression both in the brain and spinal cord following TBI could substantially contribute to the development of the robust tonic spasticity observed in these animals (see companion poster). Supported by the Brain and Spinal Cord Injury Rehabilitation Trust Fund.

P230.  
SIMPLE MORPHOMETRY OF AXONAL SWELLINGS CANNOT BE USED IN ISOLATION FOR DATING LESIONS AFTER TRAUMATIC AXONAL INJURY.  
Gentlemen SM1, Leclercq PD2, Stephens SM3, Murray LS, McIntosh TK1, Graham DP1, (1Division of Neuroscience, Faculty of Medicine, Imperial College, London; 2University of Glasgow, Glasgow, UK; and 3Neurosurgery, University of Pennsylvania, Philadelphia, PA USA).  

Objectives: Disruption of fast axonal transport due to traumatic brain injury results in the accumulation of b-amyloid precursor protein (APP) in axonal swellings. Using image analysis we have tested the hypothesis that the size of axonal swellings correlates with survival time after injury.  

Materials and methods: Paraffin sections of the corpus callosum from 63 cases of fatal head injury were stained for APP and counterstained with haematoxylin. Three different measurements were made of the APP-immunoreactive axonal swellings: i) minimum and ii) maximum Feret diameters, or iii) area.  

Results: Linear regression revealed a significant correlation between survival time and the minimum Feret diameter (p < 0.0001) and the area (p < 0.001) of axonal swellings.  

Conclusions: The findings are in agreement with a previous study showing a significant correlation between axonal swelling size and survival time. However, it is suggested that the large variability in swelling size within individual cases and the heterogeneity of the original trauma seriously compromises the utility of such information in the timing of lesions.

P231.  
COGNITIVE IMPAIRMENT WITH MENTAL FATIGUE DURING RECOVERY FROM NEUROTRAUMA. CELLULAR MECHANISMS FOCUISING ON ASTROGLIAL DYSFUNCTION IN GLUTAMETERGIC NEUROTTRANSMISSION.  
Lars Rönnbäck and Elisabeth Hansson, (Institute of Clinical Neuroscience, Göteborg, SE).  

During rehabilitation after brain injury, patients often suffer from mental fatigue, specifically having difficulty with attention, concentration, and learning. Glutamate, the most extensively studied excitatory neurotransmitter in the nervous system, is indispensable for information intake and processing within the brain. After glutamate has elicited its effects on the postsynaptic and adjacent glial membrane receptors, the astroglial cells, one of the supporting cells in the brain, remove excess glutamate from the extracellular space. The extracellular concentration of glutamate must be low in order for glutamatergic neurotransmission to be effective. According to our hypothesis, one underlying mechanism at the cellular level for this mental fatigue could be a reduced capacity of the astrocytes to clear the extracellular space of glutamate. We have developed cell culture conditions that make us able to study in primary culture, and co-cultivation, of different cell types, effects of slightly increased glutamate levels when astroglial glutamate uptake is impaired. We present results on a slight microglial activation and production of substances, tumor necrosis factor alpha (TNF-a) and interleukin-1b (II-1b) or altered conditions (slight acidification) after glutamate levels in culture medium has been increased over time.

P232.  
DIFFERENTIAL PEPTIDE DISPLAY AND ANALYSIS IN CSF AND PLASMA FOLLOWING TRAUMATIC BRAIN INJURY.  
M.U. Schlüter1, M. Heine2, M. Sbardello1, H. Tümen1, A. Appel2, T. Brinker1, (Department of Neurosurgery, 1Hanover Medical School and 2Nordstadt Hospital; 3BioVisioN AG, Hannover, Germany).  

CSF peptides reflect cerebral protein metabolism, blood-brain- and blood-CSF-barrier function, and have regulatory functions. The applied Pep tidometerTM technique involves a) high performance liquid chromatography to separate samples in 96 fractions, b) MALDI-TOF mass spectrometry for peptide detection and c) unique software solutions for image generation and statistics. Quantifiable peptide maps displaying the full range of peptide structures were created.  

We studied the peptide pattern of rat CSF and plasma in general and the changes of peptide composition following controlled cortical impact injury (CCI). 24 CSF and plasma samples, respectively, were analyzed from normal animals, from sham operated and trauma rats at 1h, 4h, 24h, and 7d after CCI. Maps displayed ~3600 and ~5000 peptide signals in CSF and plasma, respectively. A large increase in selected CSF peptide signals was seen at 1h and 4h reflecting initial disturbance of barrier function. Statistical time course analysis furthermore revealed 9 peptides in CSF and plasma each, which were significantly changed over the whole 7d and each showed a different pattern of intensity changes over time.  

This indicates in CSF, besides a remarkable overall stability of the CNS milieu after trauma, barrier-related as well as and metabolic changes, and in plasma, a systemic response to isolated brain injury. Sequence analysis for identification of these isolated peptides is currently underway. This novel technique opens a new window for insights in posttraumatic peptide metabolism and function of CNS barriers.
P233. TRAUMATIC BRAIN INJURY INDUCED SPASTICITY: NATURE, MAGNITUDE, AND TIME-COURSE  
Floyd J. Thompson*, Prodip Bose, Ronald Parmer, Justin Parker, Ronald L. Hayes. (Dept. of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL).

Traumatic brain injury (TBI) produces major health problems impacting the lives of 1.5 to 2 million people in the United States each year. Spasticity is one of the most significant challenges associated with rehabilitation following moderate to severe TBI. These problems are further exacerbated by the lack of understanding of many essential aspects of this condition. As a first step, our studies have developed a model that incorporates controlled contusion brain injuries (CCI) in adult Sprague-Dawley rats followed by unequivocal measures that quantify cognitive, spasticity, and vestibulolator function. Cognitive deficits in these animals were consistent with those previously reported for moderate CCI. Velocity dependent ankle torques and triceps surae EMGs were measured in awake animals over a broad range of rotation velocities (49–612°/sec) before and at weekly intervals following injury. A significant increase in velocity dependent ankle torque and associated EMGs were observed at all the rotation velocities from week 1 through 10 weeks following injury (repeated measures ANOVA). Mean increases of 108% in the peak torque and 177% in peak EMG magnitude at 612°/sec were observed at week-10 postinjury. Spasticity magnitude was only 50% predictive of the magnitude of anterograde memory deficit. The TBI-induced spastic animals also showed significant vestibulolator deficit tested on rotarod. These TBI-spasticity patterns, including changes in ankle extensor electrophysiology, differed from those observed in previous studies of spasticity following experimental thoracic spinal cord compression. These studies represent the first quantitative investigation of the nature, magnitude, and time course of the development of spastic hypertonia following TBI in an animal model. Supported by the Brain and Spinal Cord Injury Rehabilitation Trust Fund.

P234. ESTROGEN REGULATION OF XIAP PROCESSING FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT  
HM Brantley*, GP Alomar, G Latocha, WD Dietrich, RW Keanen. (Neurotrauma Research Center, The Miami Project to Cure Paralysis, Deps. of Neurosurgery and Physiology and Biophysics, University of Miami School of Medicine, Miami, FL USA).

Certain members of the newly discovered family of intrinsic inhibitors of apoptosis (IAP) proteins can directly bind and inhibit caspases. However, IAPs have been shown to undergo cleavage by caspases in response to inducers of apoptosis, but the significance of IAP cleavage has not been established. One IAP family member that is of particular interest in regard to the X-linked inhibitor of apoptosis (XIAP) that undergoes cleavage following traumatic brain injury. Since estrogen has been shown to have anti-apoptotic properties, this study examined gender differences and the influence of estrogen on XIAP processing during apoptosis after TBI. Male (TBI-M, n = 6), female (TBI-F, n = 3), ovariectomized female (TBI-OVX, DY-3) and ovariectomized females supplemented with estrogen (TBI-OVX + EST, n = 7) were subdivided into three groups. At 3 days post injury, animals were randomized to receive either vehicle treatment or estrogen (1000 mg/kg, s.c.). At 24 hours after injury, animals were sacrificed and tissue samples were collected. Brain tissue samples were then analyzed for XIAP expression using immunoblot analysis and quantiative densitometry. Significant differences in XIAP cleavage in the ipsilateral cortex were found between groups (p < 0.03). Post-hoc analysis showed an increase in XIAP processing in both TBI-F and TBI-OVX + EST compared to TBI-M and TBI-OVX (p < 0.05), indicating that more XIAP is cleaved following injury in intact females and estrogen supplemented ovariectomized animals than in TBI-M and TBI-OVX groups. Based on these data, we propose that estrogen may provide neuroprotection by regulating XIAP cleavage after injury. This regulation may be influenced by exogenous estrogen treatment. (NS 30291 & Eti Lilly and Co.)

P235. NEURON-GLIA COMMUNICATION: METALLOTHIONEIN EXPRESSION IS RAPIDLY INCREASED BY ASTROCYTES IN RESPONSE TO NEURONAL INJURY  
RS Chung*, J Dittman, PA Adlard, IC Vickers, MI Chudi and AK West. (NeuroRepair Group, University of Tasmania, Hobart, Tasmania AU).

Metallothioneins (MTs) are stress-related proteins, which respond to numerous stimuli including cytokines, metals and various chemical agents. We examined MT-I and MT-II expression following scratch wound injury in primary rat embryonic neuron/astrocyte co-cultures and pure astrocyte cultures. Following injury in mature neuron/astrocyte co-cultures (21 days in vitro), MT-I and II were rapidly induced in astrocytes aligned along the injury site and staining was observed in both cell bodies and processes. At later time points, almost all astrocytes were MT-I and II immunoreactive, suggesting that a chemical or physical signal spreads from the injury site. In uninjured controls, MT-I and II staining was either absent or localised solely to the nucleus of a small number of astrocytes. MT was not found in neurons at any time point investigated. Intriguingly, an equivalent scratch wound injury in pure astrocyte cultures resulted in no change in MT-I and II expression, indicating that the upregulation of MT is specifically induced by neuronal injury and subsequent neuron-astrocyte communication. Further, focal cortical brain injury in anaesthetised adult rats also resulted in a similar pattern of MT-I and II induction, suggesting that our co-culture model will be valuable in confirming the mechanism by which astrocyte gene expression is modulated by neuronal injury. We are currently investigating the involvement of several neuron-derived factors in this response.

P236. THE EFFECT OF CYCLOSPORIN A UPON MITOCHONDRIAL FUNCTION AND ENERGETIC METABOLISM FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY  
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Introduction: Pre- and post-injury Cyclosporin A (CsA) administration has shown neuroprotective properties by ameliorating mitochondrial damage associated with nerve axotomy in rats (NAA) reduction and ATP loss, two sensitive markers of mitochondrial dysfunction and bioenergetic impairment.

Methods: Adult male Sprague-Dawley rats were exposed to Impact Acceleration TBI (2m/550g) and randomized into the following two experimental groups: Intrathecal (I.T.) CsA/Vehicle treated (n = 12), Intravenous (I.V.) CsA/vehicle treated (n = 24) and Sham (n = 8). I.T. treatment consisted of post-injury (30 min) intrathecal bolus of CsA or Vehicle (0.15 ml, 10 mg/kg), I.V. treatment consisted of post-injury infusion of 20 and 35 mg/kg CsA or Vehicle. HPLC analysis of whole brain samples sampled 6 hours post-injury for levels of NAA and ATP.

Results: I.T. CsA delivery demonstrated significant neuroprotection bunting a 32% NAA reduction (p < 0.001) and restoring 30% of ATP loss (p < 0.005). The 20 mg/kg I.V. dose failed to ameliorate the biochemical damages. The 35mg/kg I.V. infusion showed 36% NAA recovery and 40% ATP restoration (p < 0.001).

Conclusion: CsA is capable of blunting NAA reduction and restoring ATP. Intravenous infusion of 35 mg/kg appears to be the most effective therapeutic strategy. These findings contribute to the notion that CsA achieves neuroprotection preserving mitochondrial integrity and provide a rationale for the assessment of CsA in the clinical setting where MR Spectroscopy can monitor NAA and ATP in brain injured patients.

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P237. GENE DELIVERY OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) PRIOR TO TRAUMATIC BRAIN INJURY: DIFFERENTIAL EFFECTS ON ANATOMY AND BEHAVIOR
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Traumatic brain injury (TBI) results in significant long-term disabilities in millions of patients, yet no treatment exists. This study is the first to test neurotrophic factor gene delivery to the cortex in an animal model of TBI— the controlled cortical impact (CCI). GDNF protein is neuroprotective in experimental models of stroke, Parkinson’s disease and in hippocampal cells following CCI. Here, we investigate whether administering a GDNF gene via an adenovirus (AdGDNF) to the penumbra of the CCI one-week prior to the injury can be neuroprotective and ameliorate behavioral deficits. Adult male rats received two injections of an adenoviral vector harboring GDNF (5 x 10^8 particles in 4 μl total) into the cortex medial and lateral to the site of injury. One week later, a CCI was administered over the forelimb sensorimotor cortex. Controls received CCI only or a control vector and CCI. Behavioral testing (foot fault and limb-use) was performed on days 0, 2, 4, 7, 10 and 13 post-injury. Rats were sacrificed on day 14. Serial sections through the contusion area were analyzed with NIH image to quantify contusion volume. AdGDNF treatment resulted in significantly smaller contusions (p < 0.05) suggesting that AdGDNF is neuroprotective. However, this neuroprotection did not result in a significant decrease in behavioral deficits. AdGDNF slightly decreased deficits on the foot fault at all time points, and on limb use on day 2. However, there were no significant decreases in limb use at all other time points. Although AdGDNF significantly decreased the size of the contusion it did not significantly decrease behavioral deficits suggesting that although the neuronal perikarya in the contusion survived these were not fully functional. Future work will focus on elucidating these differential effects. Supported by NIH-NI0157 (M.B.), Shaw Fund (M.B.), Carver Fdn (Univ. Iowa Vector Core), NIH-NS4258301 (D.K.) DePaul University Research Council and College of Liberal Arts & Sciences (D.K.)

P238. EXAMINATION OF THE ROLE OF N- AND PQ-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL BLOCKERS IN TRAUMATIC BRAIN INJURY PRODUCED BY LATERAL FLUID PERCUSSION
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Voltage sensitive calcium channels (VSCCs) are major sources of cellular calcium (Ca^{2+}) entry. Ca^{2+} regulates several processes critical for normal cell function such as cellular excitability, neurotransmitter release, and gene expression. Intracellular Ca^{2+} overload has also been implicated in the pathogenesis of neuronal loss after traumatic brain injury (TBI). Past studies have shown neuroprotection by both N- and PQ-type VSCC blockers in rodent models of ischemia. Few, if any, studies have been carried out using TBI models. We examined the role of N- and PQ-type VSCCs in the pathophysiology of TBI using the lateral fluid percussion (LFP) injury model in rats. Immediately after injury; twenty microliters containing 50, 100, or 200 nmol of SNX-185, a N-type VSCC blocker, 10, 25, 50 or 100 nmol of AgaIVA, a PQ-type VSCC blocker, or ACSF-vehicle was injected into the CA3 subregion of the hippocampus. Histological assessment of neuronal degeneration was visualized in brain sections using cresyl violet and Fluoro-Jade staining. Behavioral assessments were carried out using beam walk, inclined plane, radial arm maze, and Morris Water Maze. Compared to control, rats treated with 100 pmol of SNX-185 or 10 pmol of AgaIVA showed both a significant decrease in neuronal degeneration and improved behavioral outcome. Doses above 50 pmol AgaIVA showed toxicity. Our data indicate that both SNX-185 and AgaIVA may be neuroprotective, implicating both N- and PQ-type VSCCs in the pathophysiology of TBI. Blockage of VSCCs after TBI may have important therapeutic potential. (Supported by NIH NS39090 and the UC Neurotrauma Research Initiative)

P239. ASSESSING THE GLOBAL BURDEN OF PATHOLOGY IN HEAD INJURY USING 2D PQ HISTOGRAMS AND DIFFUSION TENSOR IMAGING
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Diffuse axonal injury (DAI) results in changes in the mean apparent diffusivity and diffusion anisotropy of tissue water as seen on magnetic resonance imaging (MR) diffusion tensor imaging (DTI). DAI can be widespread and subtle, extending beyond image abnormalities seen on conventional CT or MR. We describe novel graphical tools for visualizing and quantifying the topological abnormalities across the entire brain, making no assumptions regarding their location. Methods: Acute DAI was undertaken in five volunteers and five patients with acute head injury on a 3 Tesla MR system, using a pulsed gradient spin echo, echo planar imaging sequence. The diffusion tensor was computed on a voxel-by-voxel basis, and deconvolved to its isotropic (p) and anisotropic (q) components. These variables were used to provide a 2-D graphical representation of DTI abnormalities across the brain. Summary statistics for p and q included the peak (mode) location, mean values (p*,q*), and the full width half maximum ranges of distribution (dp,dq). Results: Volumes showed highly consistent pq plots, with coefficients of variation for the above parameters ranging from 1.4 to 12.9%. Acute head injury resulted in marked inter-individual variations in these parameters, with increases in isotropic diffusion (probably representing ischemic vasogenic edema), and/or a significant reduction in the number of voxels with high q values, suggesting loss of white matter anisotropy due to DAI. Follow up imaging showed partial resolution of these abnormalities in some patients. Conclusions: PQ histograms provide a easily comprehensible graphical depiction of the global burden of ischemia and axonal injury, and can be used to obtain parametric measures of these pathologies. Further work is required to clearly define the pathological correlates of these imaging abnormalities, and select individual parameters that best quantify the variable pathology in this patient population.

P240. REDUCTION IN THE FORMATION OF CEREBRAL EDEMA FOLLOWING FLUID PERCUSSION INJURY FROM FREE RADICAL SCAVENGER AND NSAID COMBINATION THERAPY

Combination therapy consisting of the free radical scavengers vitamins C and E and the non-specific cyclooxygenase (COX) inhibitor ibuprofen administered 30 minutes post fluid percussion injury (FPI) have been shown to reduce neurological and motor deficits following experimental brain injury. This treatment is hypothesized to reduce the availability of arachidonic acid for conversion to vasotoxic prostaglandins by COX via the protective action of the free radical scavengers vitamins C and E. Vitamin C in addition to acting as a general free radical scavenger also converts the radical form of vitamin E back to an active radical scavenger. Further benefit may be derived from protection of cellular structures by the scavengers. Ibuprofen reduces the conversion of arachidonic acid by the inhibition of COX resulting in less vasotoxic prostaglandins, and a reduced inflammatory response.

The aim of this study was to determine the effect of this treatment on the formation of cerebral edema 24 hours post injury. Long Evans rats were subjected to severe FPI (3.1 atm. mean duration of unconsciousness 175 sec) centered over the left hemisphere midway between bregma and lambda. Thirty minutes post FPI rats received either 10mg/kg vitamin C, 45mg/kg vitamin E, and 10mg kg ibuprofen or vehicle treatment. Twenty four hours post-FPI the rats were rated on forelimb flexion. Treated subjects demonstrated significantly less deficits compared to the vehicle treated group (<0.05). Following neurological assessment, the subjects were sacrificed and brains removed and dissected into defined regions. The sections were freeze dried for 72 hours and edema levels determined using the wet/dry method. The brains of the untreated subjects had significant levels of edema in the ipsilateral cortex, hippocampus, and thalamus. Rats receiving the combination treatment displayed significantly less edema in the ipsilateral cortex (<0.01) and ipsilateral hippocampus (<0.05). No effect was observed in the thalamus.
**P241. SODIUM AND CALCIUM EXCHANGE FOLLOWING IN VITRO MECHANICAL AND/OR ISCHEMIC INJURY IN ASTROCYTES**


Mechanical brain injury is clinically often followed by ischemia but the mechanisms of each insult are difficult to separate. The effect of mechanical injury alone or followed by ischemia on astrocyte intracellular Na⁺ and Ca²⁺ concentrations ([Na⁺][Ca²⁺]) was examined using fluorescent imaging of Fura-2-AM or SBFI-AM in cultured astrocytes. Following loading, astrocytes were perfused with standard solution (NORM), stretch-injured, and imaged for 30 min. If applicable, cells were then exposed to hyperoxic, acidic, ion-shifted Ringer's (HAIR, Bondarenko and Chesler, 2001) for 5 min., and reperfused with NORM buffer for 30 min. while imaged.

Cell viability was evaluated by propidium iodide uptake (PI). Mild, moderate and severe stretch injury increased [Na⁺][Ca²⁺] by 2, 3 and 5-fold, respectively. When mild injury was followed by mild HAIR, [Na⁺][Ca²⁺] increased by nearly 5-fold, [Ca²⁺] increased by 4-fold, and PI uptake increased 5-fold suggesting that the combination injury is more damaging than either insult alone. Application of KB-R7945, an inhibitor of reversed Na⁺/Ca²⁺ exchange, significantly reduced injury-induced increase in [Ca²⁺] and PI uptake in the moderate stretch injury, but not in mild or severe stretch, or combined mild stretch+HAIR. This suggests that the Na⁺ load following mild stretch injury may not be sufficient to reverse the Na⁺/Ca²⁺ exchange, but that Na⁺ load following moderate stretch injury may drive the exchanger in the reverse direction. Additionally, we hypothesize that the severe stretch injury and the combination of mild stretch+HAIR may involve additional mechanisms of tonic imbalance beyond reversal of Na⁺/Ca²⁺ exchange. Supported by UC Neurotrauma Research Initiative, NIH NS 29995.

**P242. DELAYED TREATMENT OF HEMOGLOBIN NEUROTOXICITY**

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Hemoglobin is an oxidative neurotoxin that may contribute to cell injury after CNS hemorrhage. Prior studies have demonstrated that coontaminant treatment with iron-chelating antioxidants prevents its neurotoxicity. However, the efficacy of these agents when applied hours after hemoglobin has not been determined, and is the subject of the present investigation. Consistent with prior observations, an increase in reactive oxygen species generation, as detected by 2’,7’-dichlorofluorescin oxidation, was observed in cultures exposed to hemoglobin alone. However, this oxidative stress developed slowly. A significant increase in the dichlorofluorescin signal compared to control, untreated cultures was not observed until four hours after addition of hemoglobin, and was followed by loss of mitochondrial integrity and propidium iodide staining. Treating cultures with the 2-aminomethoxy U74500A or the ferric iron chelator deferoxamine four hours after initiating 1h treatment markedly attenuated reactive oxygen species production within 2 hours. Continuous exposure to 5 μM hemoglobin for 24 hours resulted in death of about three-quarters of neurons, without injuring astrocytes. Most neuronal loss was prevented by cocontaminant treatment with U74500A; its effect was not significantly attenuated if treatment was delayed for 2–4 hours, and it still prevented over half of neuronal death if left delayed for 4 hours. Similar neuroprotection was produced by delayed treatment with deferoxamine or the lipid-soluble iron chelator phenanthroline. None of these agents had any effect on neuronal death when added to cultures 12 hours after hemoglobin. In contrast, delaying treatment of glutamate-exposed neuronal injury for two or more hours resulted in complete loss of the protective effects of U74500A and MK-801. These results suggest that hemoglobin is a potent but slowly-acting neurotoxin. The delayed onset of hemoglobin neurotoxicity may make it an attractive target for therapeutic intervention.

**P243. ENHANCED NEURONAL DIFFERENTIATION OF TRANSPLANTED NEURAL STEM CELLS INDUCED BY IN SITU ADMINISTRATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR RESTORES NEUROMOTOR FUNCTION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS**

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We have demonstrated that neural stem cell (NSC) transplantation together with in situ administration of brain derived neurotrophic factor (BDNF) could enhance differentiation to neuronal phenotype in rat cortical ablation model. The present study was undertaken to examine whether similar neuronal differentiation of transplanted NSCs was induced by BDNF administration following traumatic brain injury (TBI) and restored neuromotor functions. Adult male wistar rats were deeply anesthetized and cortical contusion was induced in the unilateral sensorimotor cortex by controlled cortical impact device. Seven days following injury, BrdU-labeled human fetal nucleus-derived NSCs (approximately 10 × 10⁴ cells/animals) were stereotactically transplanted into pericontusional areas together with injection of BDNF-soaked gel foams into the contusion cavities. Controls animals were given saline-soaked gel foam with NSCs transplantation. Two weeks after the transplantation, neurormotor function was evaluated by rotaed test and sacrificed. The survival and differentiation of transplanted cells were examined immunohistochemically by NeuN, MAP2, GFAP, vimentin, BrdU. The BrdU-positive surviving cells could be detected in the pericontusional areas in both BDNF-treated rats and controls. In controls, the majority of the transplanted cells expressed GFAP or vimentin immunoreactivities, indicating differentiation to glial lineage. In contrast, the cells expressed the neuronal marker, NeuN increased significantly in the pericontusional areas in the BDNF-treated rats. In addition, the rotaed test demonstrated that attenuation of motor deficits was observed in BDNF-treated rats compared to controls. These results indicated that in situ administration of BDNF enhanced neuronal differentiation of transplanted NSCs, which may lead to the functional recovery.

**P244. BRAIN-DERIVED NEUROTROPHIC FACTOR ADMINISTERED IN GEL FOAM ENHANCES THE NEURONAL DIFFERENTIATION OF TRANSPLANTED NEURAL STEM CELLS IN RAT ABLATION MODELS**

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It has been demonstrated that the neural stem cells (NSCs), when transplanted into damaged neural tissues, the majority of the cells differentiated to into glial phenotype, but not neurons. In order to reconstruct the damaged neural circuits by NSCs transplantation effectively, promotion of neuronal differentiation is crucial. The present study was undertaken to examine whether brain-derived neurotrophic factor (BDNF)-soaked gel foam applied into ablation cavities could enhance the neuronal differentiation of transplanted NSCs. Adult male wistar rats were deeply anesthetized and the unilateral sensorimotor cortex was ablated by sucking force. Immediately after injury, BrdU-labeled rat fetus-derived NSCs (approximately 10x10⁴ cells/animals) were stereotactically transplanted in the area adjacent to the cavities with insertion of BDNF-soaked gel foam within the ablation cavities. Control animals were performed NSCs transplantation with saline-soaked gel foam. Two weeks after injury, survival and differentiation of transplanted cells were examined by NeuN, MAP2, GFAP, vimentin, BrdU immunohistochemistry. The BrdU-positive surviving cells could be detected in the regions in both BDNF and non-BDNF treated rats. Saline-treated rats, most of the transplanted cells expressed glial markers, little number of the cells showed neuronal marker NeuN. In contrast, in BDNF-treated rats, a large number of NeuN-positive cells existed in the area adjacent to the cavities, indicating the upregulation in neuronal differentiation of the transplanted NSCs. These findings indicates that BDNF could enhance the neuronal differentiation of NSCs, even administrated exogenously.
P245.
SPATIAL AND TIME DEPENDENT DIFFERENTIATION OF NEURAL STEM CELLS TRANSPLANTED IN THE LESION INDUCED BY TRAUMATIC BRAIN INJURY
Moro Nobuhito, Fukushima Matsumichi, Sama Takeshi, Koshinaga Morimichi and Katayama Yokoichi (Department of Neurosurgical Surgery, Nihon University School of Medicine, Tokyo, Japan).
In contrast to in vitro studies, it has been demonstrated that transplanted neural stem cells (NSCs) showed significant glial differentiation and the neuronal differentiation was restricted, suggesting that the temporal and spatial alteration of microenvironments in vivo may strongly influence their differentiation. In the present study, we examined the temporal and spatial pattern of NSCs, transplanted into damaged brain induced by traumatic brain injury (TBI) and determined the optimal timing and sites of transplantation, which showed pronounced neuronal differentiation.

Male wistar rats were deeply anesthetized and cortical contusion was induced in the unilateral sensorimotor cortex by controlled cortical impact device. BrdU-labeled human fetus-derived NSCs (approximately 10 × 10^4 cells/animals) were stereotactically transplanted into three individual sites (contusion core, pericontusional area, contralateral side of cerebral cortex) at 0, 3, 7, 14 days following injury. Transplanted cells survived in all animals. The marked migratory response could be observed in the animals which received NSCs transplantation at 0 and 3 days after injury, however, the majority of the cells showed glial marker, such as vimentin or GFAP. In the animals received NSCs transplantation at 7 days after injury, accumulation of the transplanted cells into pericontusional areas and a large number of cells expressed neuronal marker immunoreactivity, such as NeuN. The most prominent cell accumulation and neuronal differentiation could be observed at which the NSCs were transplanted into pericontusional areas. The present study indicates that differentiation of transplanted NSCs depends on the in vivo microenvironment and prominent neuronal differentiation achieved at subacute phase following injury.

P246.
GABA-A RECEPTOR SUBUNIT ALTERATIONS FOLLOWING TRAUMATIC BRAIN INJURY ARE NORMALIZED BY AN NMDA ANTAGONIST
Traumatic brain injury (TBI) produces an acute phase of neuronal excitation followed by a chronic phase of degenerated neuronal function. TBI-induced elevations in intracellular calcium concentrations ([Ca^2+]) may trigger mechanisms which drive changes in GABA-A receptor protein synthesis and expression. In study 1, Western blot analysis revealed no injury-induced alterations in protein expression for the GABA-A receptor β3 subunit at 3h, 24h, or 7 days post-injury. A significant increase in α1 protein was found 24-hours following injury and persisted for at least 7 days. Since the α1 subunit is primarily located on interneurons, this may imply a dysfunctional increase in interneuronal inhibitory tone during the chronic phase of injury. In study 2, pre-injury injections of MK-801 were used to block calcium influx through the NMDA receptor. This treatment normalized α1 protein expression 24h following injury. NMDA-mediated calcium influx may, therefore, be responsible for triggering the cascade that results in increased GABA-A receptor α1 protein expression. These studies indicate that specific subunits of the GABA-A receptor are altered by TBI, these alterations are likely driven by excessive [Ca^2+]i, and these changes may ultimately contribute to receptor dysfunction during the chronic phase of injury.

P247.
CHANGES OF BENZODIAZEPINE RECEPTORS IN PATIENTS WITH NEUROPSYCHOLOGICAL DEFICITS IN THE CHRONIC STATE AFTER TRAUMATIC DIFFUSE BRAIN INJURY / PET STUDY
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<Background and Purpose> Traumatic diffuse brain injury, including diffuse axonal injury, can cause neurochemical deficits such as problems with attention, memory, and information processing. Cortical localization of these higher brain functions has been controversial. Conventional neuroimaging techniques such as MRI (magnetic resonance imaging) and CT (computed tomography) can not show lesions causing these deficits. We have already reported that decrease of cerebral metabolic rate of oxygen (CMRO2) was seen in frontal lobe of these patients. The aim of this study was to clarify the relationship between distribution of benzodiazepine receptors of brain (BZR) and neurochemical impairment in the chronic state of traumatic brain injury.

<Materials and Methods> Six right-handed patients (18–63; mean 32.5 y.o.) with neuropsychological impairments and without aphasia, agnosia or motor weakness of extremities in the chronic stage (27.6 months) after traumatic brain injury, who had no abnormal lesion on MRI, were included in this study. PET scans were obtained using 11C-flumazenil to evaluate distribution of BZR of brain. The distribution pattern was compared to healthy volunteers (n = 2, 27.5 y.o.). Neuropsychological tests and 150-gain PET study were also evaluated.

<Results> Two patients with mild dysfunction of memory and attention had no abnormal changes of BZR distribution compared to normal control. On the contrary, BZR distribution in the frontal and parietal lobe relatively decreased in patient with moderate disturbance of memory and attention (n = 2). The other two patients had severe neuropsychological deficits and also had diffuse decrease of BZR in brain. There was a tendency that areas with decreased BZR activity were corresponded to areas with decrease of CMRO2.

<Conclusion> Cerebral BZR distribution in patients with moderate or severe disturbance of neuropsychological function decreased relatively in the chronic stage. This could be a good index of higher brain dysfunction as same as decrease of CMRO2.

P248.
REGIONAL PHYSIOLOGICAL ALTERATIONS IN INHIBITORY SYNAPTIC TRANSMISSION IN FLUID-PERCUSED MOUSE HIPPOCAMPUS
Traumatic brain injury (TBI) patients suffer cognitive deficits including impaired learning and memory. We have used the fluid percussion injury (FPI) model of TBI to study the putative mechanism(s) underlying these impairments in mice. Previous studies have revealed regional alterations in hippocampal excitability, i.e., dentate gyrus (DG) hyperexcitability and CA1 hypoexcitability. The present study was undertaken to examine whether changes in tonic inhibitory tone may precipitate alterations in the delicate balance between excitatory and inhibitory neurotransmission that is crucial for normal hippocampal function. Using visualized slice patch clamp techniques to test the above hypothesis, we recorded miniature inhibitory post-synaptic currents (mIPSCs) in the DG and area CA1 regions of the mouse hippocampus one week post-FPI and compared this activity to that recorded in sham and naive animals. The median mIPSC amplitude was significantly smaller in DG neurons from FPI mice than those recorded in sham and naive animals (~26.81 ± 2.7 pA; ~39.04 ± 7.1 pA, FPI and controls, respectively, p < 0.05, unpaired t-test). The 50% decay time (τ50) of mIPSCs in DG neurons from FPI animals was not significantly different from those from control animals (7.4 ± 1.5; 9.9 ± 2.0 ms, for FPI and control DG neurons, respectively). Conversely, the median mIPSC amplitude in CA1 neurons from FPI mice was significantly greater than that from sham and naive mice (~35.57 ± 0.9 pA; ~28.86 ± 2.0 pA, for FPI and controls, respectively, p < 0.05). In similar fashion to the DG, mIPSC τ50s were not significantly different in CA1 neurons from FPI animals compared to controls (7.75 ± 2.0 and 6.83 ± 1.3 ms for FPI and control CA1 neurons, respectively). These data support our hypothesis that changes in tonic inhibition in FPI mouse hippocampus may account for altered regional excitability.
P249.
PERIPHERAL NERVE TRANSPLANTATION IN THE ADULT CENTRAL NERVOUS SYSTEM: RECONSTITUTED NERVES, ALLOGRAFTS AND THE EFFECTS OF IMMUNOSUPPRESSION
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Peripheral nerve (PN) graft studies in the CNS of injured adult animals usually involve the use of autologous tissue. Clinically however, such an approach may not be optimal due to additional functional deficits that result from harvesting host PN material. We have tested new approaches to CNS repair using PN bridges, including the use of (i) freeze-thawed PN reconstituted with cultured Schwann cells (SCs), (ii) PN allografts and (iii) immunosuppression.
PN was grafted onto the cut left optic nerve (ON) of anesthetized (halothane) young adult rats. In the reconstituted nerve study, PN was rendered acellular by freeze-thawing and was repopulated ex vivo with neonatal SCs, adult SCs, or adult olfactory ensheathing glia (OEG). These studies were performed in Fischer rats. Regeneration of axons from injured retinal ganglion cells (RGCs) was assessed with retrograde tracing methods three weeks after transplantation. No regrowth was seen in control cell-free nerves or PN reconstituted with neonatal SCs or adult OEGs. In contrast, PN seeded with cultured adult SCs supported RGC axon regrowth: These adult SCs could be either SCs of donor- or host-derived.
In allograft PN studies, nerve was taken from Dark-Agouti (RT1a) rats and grafted onto transected ON of Lewis (RTII) rats. Without immunosuppression there was no RGC axon regeneration into allografts. However in the presence of cyclosporin-A or FK506 (given daily) axon regrowth was seen.
We were surprised to find, in control autograft studies in Lewis rats, that immunosuppression decreased the amount of regeneration into autologous PN grafts. This was a strain-specific effect; daily intraperitoneal injections of cyclosporin-A or FK506 in Fischer rats with PN-ON autografts resulted in significantly increased regrowth. These data show that there are potential alternatives to using autologous PN tissue and highlight the importance of knowing the immune status of an animal (or patient) when attempting to repair CNS injuries.

P250.
IMPROVED NEURAL REMODELING AND ENHANCED NEURAL STEM CELL PERSISTENCE AMIDST CASPASE-3 INDEPENDENT APOPTOSIS IN ADULT BAX DEFICIENT MICE FOLLOWING CONTROLLLED CORTICAL INJURY
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Numerous studies have implicated apoptosis as one mechanism for cell death seen following traumatic brain injury (TBI). More recently, it has been shown that neural stem cells are involved in cortical and hippocampal remodelling induced by TBI. To examine the role of apoptosis in this process we have analyzed TBI in mice lacking the proapoptotic gene, Bax. We find that adult Bax knockout mice have similar numbers of apoptotic cells within the hippocampus immediately following controlled cortical injury when compared to wildtype littermates. However, whereas apoptosis in wildtype animals occurs via a caspase-3 mediated process, in Bax knockout mice caspase-3 activation is totally absent. We also observe significant tissue preservation in Bax deficient animals three weeks following injury. Our data demonstrate that the apparent improvement in neural remodelling seen by attenuating Bax signalling is likely due to the consequence of increased numbers of neural stem cells in the adult and not due to inhibition of apoptosis.
We further provide support for the emerging notion that apoptosis following injury can occur via parallel pathways that are caspase independent.

P251.
RESPONSE OF THE SUBVENTRICULAR ZONE TO TRAUMATIC BRAIN INJURY
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The brain's ability to produce neurons throughout the lifetime of an individual (neurogenesis) is an important phenomenon to pursue in the treatment of brain injury. Previous studies in rat demonstrated that the number of cells within the subventricular zone (SVZ) of the adult brain increased as a result of degeneration in the brain. This study aims to examine this phenomenon following a controlled cortical impact (CCI) or adult mice. Adult male mice were subjected to a CCI over the frontal region of the sensorimotor cortex. Mice were perfused 3 days post-injury, and coronal sections (30 μm) of the brain were then cut and stained using cresyl violet. Digital pictures of the SVZ were obtained at 40X sections containing a cortical injury or from corresponding sections in control mice. Using NIH Image the thickness of the SVZ was measured at different levels in both hemispheres. Results demonstrate a significant difference in the overall thickness of the SVZ in injured mice, when compared to non-injured mice (CCI = 22 ± 1mm; control = 15 ± 1 mm; p < 0.05). This increase was significant in the dorsal SVZ, but not in the ventral-striatal SVZ, and was limited to the SVZ ipsilateral to the injury. There was no significant increase in the thickness of either the dorsal or the ventral SVZ on the side contralateral to the injury in injured mice. These results indicate that the SVZ responds to a CCI and that this response is limited to the dorsal SVZ ipsilateral to the injury. Future studies will examine the nature of this increase by examining cell number and type as well as the migratory ability and functional roles of these cells.

P252.
DECOMPRESSIVE SURGERY FOR SEVERE TRAUMATIC BRAIN INJURY, EXPERIENCE IN HAMAD MEDICAL CORPORATION, DOHA- QATAR
Dr El Fazah Bashiri1, Dr A. Hamid Mohamad, Dr Ali Raza. (Neurosurgery Unit, Hamad Medical Corporation Doha, Qatar).

Objective: This study examines the role of non-traditional decompressive surgical procedures in treating severe Traumatic Brain Injury (TBI) with associated life-threatening intracranial hypertension. In addressing the control of the latter, the procedures, comprise temporary removal of the craniotomy bone flap with or without frontal or temporal lobectomy. Existing acute epidural, subdural or intracranial haematoma was removed at the same time.

Methods & Results: We retrospectively analysed the data of 529 cases of severe TBI treated in Hamad Medical Corporation in Doha-Qatar, during the period between January 1997–December 2001. The AANS Management Guidelines were followed in treating the patients who were all evacuated to the hospital where the Neurosurgical team was involved in their management from the time of arrival to the Accident & Emergency Department. Out of the 82 patients who had surgical treatment (15.5% of the total), 48 underwent a decompressive surgical procedure; 27 cases had removal of the craniotomy bone flap, 10 cases had frontal or temporal lobectomy and 11 cases had both procedures. They were all males with an age range of 5 years—59 years (average; 27.1). The majority (30/48 = 62.5%) scored below 9 on Glasgow Coma Scale with 42 cases (87.5%) falling within Newcastle Outcome Prediction Groups 5 and 6 (poor groups). On follow up assessment using the Glasgow Outcome Scale, 64% of the patients were found to have had a favourable outcome (Good Recovery or Mild Disability) at 3 months to one year post injury with a 29% Mortality. This compared favourably to the predicted outcome.

Conclusion: Decompressive surgery, in the way performed in our unit, was found useful in treating life-threatening post traumatic intracranial Hypertension as it significantly improved outcome in those selected patients whose conditions were found to be refractory to standard therapeutic modalities.
P253.
GENDER IN RELATION TO OUTCOME OF MILD-TO-MODERATE TRAUMATIC BRAIN INJURY
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Males sustain traumatic brain injury (TBI) nearly twice as frequently as women, although women are reportedly at greater risk for developing psychiatric disturbances after TBI, which may complicate recovery and outcome. Gender differences for psychiatric disorders and outcome after mild-to-moderate TBI (MTBI) were assessed 6-months postinjury, using the Structured Clinical Interview for DSM-IV, Glasgow Outcome Scale-Extended (GOS-E), Short Form-36 (SF-36), and Community Integration Questionnaire (CIQ) in 55 female and 111 male patients. The groups were comparable for Glasgow Coma Scale score, age, education, and injury severity. Rates of posttraumatic stress disorder (PTSD), depression, and postconcussional disorder were at 33%, 8%, and 9% in males and 18%, 20%, and 16% in females, respectively. Females met DSM-IV-criteria for PTSD (p = 0.0001) nearly six times as often as males, and DSM-IV-criteria for depression (p = 0.040) twice as often. Within GOS-E outcome categories, a greater proportion of females (13/19, 68%) than males (11/31, 35%) functioned at the lowest level in the moderate disability (p = 0.04) category; no differences were noted within the good recovery category. Assessment of resumption of previous role activities (CIQ) showed that females functioned at lower levels than males for Productivity (p = 0.013) and Social functioning (p = 0.037), but had better resumption for Home functioning (p < 0.0001). On the SF-36, females reported their mental health functioning as worse (p = 0.035) than males. In summary, neurological damage associated with MTBI may compromise the patient = capacity for effective stress management, which may be manifested more in females and contribute to their development of psychiatric disorders and worse functional outcome.

P254.
IN VIVO AND IN VITRO EVIDENCE OF CYTOSKELETAL AND SYNAPTIC PROTEIN ALTERATIONS FOLLOWING REPEATED MILD TBI
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Neuronal cytoskeletal alterations in the cortex and brain stem following single mild TBI (sMTBI) and repeated mild TBI (rMTBI) have been well-documented. In contrast to sMTBI, rMTBI-related cognitive deficits have been shown to be more closely associated with altered hippocampal function. Changes in cytoskeletal structure are often associated with synaptic alterations (e.g., learning). However, alterations in synaptic proteins have not been investigated following rMTBI. We used in vivo and in vitro models of rMTBI to characterize cytoskeletal and synaptic protein alterations in the hippocampus following rMTBI.

Immunohistochemistry (IHC) was used to assess protein distribution, while immunoblot (IB) was used to assess protein levels in both models. IHC results showed decreased distribution of MAP2, particularly within the CA2 region. In contrast, IB expression of denatured MAP2 and NF200 showed increased levels of these proteins. IHC distribution of two synaptic proteins, synaptophylin and synaptophysin, showed overall decreased staining, but increased staining in neurons with decreased somatic distribution of MAP2. These results suggest a compensatory response of cytoskeletal and post-synaptic proteins to stabilize soma-dendritic architecture that may contribute to rMTBI-related learning and memory deficits through decreased plasticity. Supported by: BSCRTP 2001, NWO-MW, NWO-PIONIER, NIH ROI NS09091, ROI NS40182, US Army DAMD17-99-1-9555.

P255.
CEREBRAL BLOOD FLOW AND BRAIN NITRIC OXIDE LEVELS AFTER TRAUMATIC BRAIN INJURY, HEMORRHAGE AND HYPERTERTONIC ARGinine RESUSCITATION
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Traumatic brain injury (TBI) reduces cerebral blood flow (CBF) (1,2) and hypotension after TBI increases mortality (3). The nitric oxide (NO) synthase-thromboxane L-arginine or hypertonic resuscitation improves CBF after TBI and hypotension (3,4) but the effects of resuscitation with hypertonic L-arginine have not been studied.

Rats were anesthetized, intubated and ventilated with isoflurane in O2-air. Rats were prepared for TBI (2), laser Doppler flowmetry (2) and measurement of brain tissue NO levels using an ECO-NO electrode system (5). Rats (n = 6 per group) were randomly assigned to receive sham, moderate (2.0 atm) or severe (3.0 atm) TBI and hemorrhoage to mean arterial blood pressures of 60 mmHg for 45 minutes and then resusciation with 0.9% NaCl or hypertonic L-arginine (100 or 300 mg/kg L-arginine in 1800 mL hypertonic saline). CBF and brain tissue NO levels were measured for 4 hrs after resuscitation.

CBF remained constant after sham-injury but decreased and remained below baseline after saline treatment. In the hypertonic arginine-treated rats after either moderate or severe TBI, CBF returned nearly to baseline during hypertonic arginine infusion and remained higher than CBF in the saline treated rats. Brain NO levels remained constant in the sham-injured rats but decreased after TBI and hemorrhagic hypotension. Hypertonic arginine increased NO levels in the severe TBI and hemorrhage group. Neither saline nor hypertonic arginine improved NO levels after moderate TBI and hemorrhage.

These results suggest that hypertonic arginine resuscitation improves CBF through mechanisms that may be unrelated to brain tissue NO levels, especially after moderate brain trauma.


P256.
CYTOSKELETAL PROTEIN DEGRADATION AND NEURODEGENERATION EVOLVES DIFFERENTLY IN MALES AND FEMALES FOLLOWING EXPERIMENTAL HEAD INJURY
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The resulting neuropathological degeneration that occurs following a traumatic brain injury (TBI) is a consequence of both immediate and secondary neurochemical sequelae. Protosynthesis of cytoskeletal proteins, triggered by calcium-mediated events, is believed to be a particularly significant contributor to TBI-induced neuronal death. The objectives of this study were to 1) quantitatively describe, over a post-traumatic time course, the relationship and mechanisms of cytoskeletal degradation (Western blot) and neurodegeneration (silver staining) in male and female mice following a moderately severe weight-drop head injury; 2) to evaluate gender differences in the response to TBI and, 3) to examine the potential therapeutic window for future pharmacological treatment strategies. In male and female mice, we report a close correlation in the time courses of neurofilament M (NFM) protein degradation and e-spectrin breakdown products (SBDP 150 and 145) with the peak magnitude of neurodegeneration, as quantified by silver staining. Evidence from the increased patterns of SBDPs suggests that both calpain and caspase-3 are involved. In general, males incurred peak protein degradation and neurodegeneration within 3 days after injury, while in females, this did not occur until as late as 14 days. The neuroprotective effects of estrogen are believed to be key factors in the superior outcome of female vs. male mice following TBI. In mice, the therapeutic window of opportunity for pharmacological intervention aimed at limiting cytoskeletal degredation might be as much as 24 h following injury. Evidence of a pre-treated time course of cytoskeletal degradation, especially in females, suggests a potential for an extended treatment-duration following TBI.
P257.
TIME COURSE OF BRAIN TISSUE HYPOXIA IN THE PENUMBRA REGION AFTER TRAUMA.
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Brain tissue oxygen tension (PbO2) probes measure oxygen in only a small volume of tissue. Debate continues about whether such monitors should be inserted after brain injury in "normal" regions or in tissue that is "at risk," e.g., adjacent to traumatic lesions. This study investigated PbO2 measurements in the penumbra of traumatic intracranial lesions (TICLs).

Data from 156 patients were analyzed for transient decreases in PbO2 below 10 mm Hg without an identifiable cause, i.e., CPP < 60 mm Hg, SaO2 < 95%, or end-tidal CO2 < 25 mm Hg.

In 120 patients (77%), PbO2 probes (Licox, Integra NeuroSciences) were inserted in the penumbra of lesions: near contusions in 58, in brain underlying evacuated subdural hematomas (SDHs) in 47, or in brain underlying evacuated epidural hematomas (EDHs) in 15. In the remaining 36 patients, probes were inserted in grossly normal tissue. Twenty-five patients exhibited transient ischemic episodes beginning 25 ± 15 hours after injury (range 5-61 hours) and lasting 19 ± 19 hours (range 4-88 hours). During these episodes, PbO2 decreased from 27 ± 8 mm Hg to 6 ± 3 mm Hg and subsequently returned to baseline. Ischemic episodes occurred only in penumbra (12 near contusions and 13 under evacuated SDHs), MAP, ICP, ECo2, SpO2, and SyO2 did not change during the episodes. In all 25 patients, CT scans revealed hypodensities around the PbO2 probes despite initially normal densities. Six months postinjury, 73% of patients with transient PbO2 decreases had poor outcomes on a dichotomized Glasgow Outcome Scale. The mortality rate was 36%.

The occurrence of regional ischemia in the penumbra of TICL later after injury is a relatively common phenomenon and may contribute to poor outcome. Placing PbO2 probes near contusions or in brain underlying evacuated SDHs may improve detection of transient regional ischemia.

P258.
THE INFLUENCE OF SOCIAL AND CULTURAL FACTORS ON THE INCIDENCE AND SEVERITY OF TRAUMATIC BRAIN INJURY - A COMPARATIVE STUDY OF TRAUMATIC BRAIN INJURY IN DOHA, QATAR AND NEWCASTLE UPON TYNE, UK.
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Objectives: This study evaluates the geo-social factors that contribute to the incidence and outcome of the severely head injured patient by the first direct comparison of head injury outcome between a western centre and that of a developing country.

Method: A retrospective analysis of head injuries admitted to two centres (Doha and Newcastle) over a five year period (January 1997–December 2001) was carried out comparing demographic details, etiology, severity of injury and management outcome. The mode of participation of victims was identified and the geo-social factors that contributed to the etiology and severity of head injury were evaluated in detail. Data analysis was with Spss version 11.

Results: There was a significant male preponderance in Doha (12m:1f) compared with Newcastle (2.7m:1f). The peak age group in Newcastle for all head injuries was 0–10yrs, whereas in Doha the peak age was 20–30yrs. Road traffic accident accounted for nearly 70% of all head injuries in Doha whereas falls accounted for nearly 50% of all head injuries in Newcastle. Overall the good outcome was 74% in Doha and 81% in Newcastle (NS). The proportion of patients presenting in coma and mortality (26%) in Doha was more than twice that in Newcastle. A recent change in the law in Qatar allowing female drivers has not significantly increased the incidence of head injury.

Conclusion: The combination of powerful cars and good roads with a young population has resulted in an excessive mortality in Doha compared with Newcastle. Recent changes in the law in Qatar have not significantly altered the incidence of head injury related to road traffic accidents. The high male preponderance in Qatar reflects the relatively protected role women enjoy in this society.

P259.
HEALTH-RELATED QUALITY OF LIFE AND POSTCONCUSSIONAL DISORDER SIX MONTHS FOLLOWING MILD TO MODERATE TRAUMATIC BRAIN INJURY.
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Few studies have been conducted investigating Postconcussional Disorder (PPCD) in mild to moderate traumatic brain injury (TBI) six months post-injury. HRQOL was measured using the SF-36 and its subscales. Out of 166 patients, 19 met DSM-IV criteria for PPCD. The PCD and no-PCD groups did not differ in any demographic variable. Those in the PCD group were more often involved in motor vehicle accidents (MVA) than those in the no-PCD group (p < 0.04). The Injury Severity Score (ISS) was significantly lower (p < 0.03) in the PCD group vs. the no-PCD group. The patients with PPCD reported significantly poorer HRQOL on the Mental and Physical composite scales (p < 0.001) of the SF-36. PCD patients also reported significantly poorer HRQOL on all physical and mental subscale scores (p < 0.001). Patients with PCD had poorer global outcome as measured by the Extended Glasgow Outcome Scale (GOS-E; p < 0.0001). These results suggest that PPCD symptoms are reported at 6 months post-injury and represent a source of significant impairment both from the patient’s perception of their HRQOL and a more objective measure of global outcome. Early interventions to reduce the severity of PPCD symptoms may improve both HRQOL and global outcome following mild to moderate TBI.

P260.
SCULL BASE MISSILE INJURIES.
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Missiles used in war conflicts cause extensive destructions of the skull and brain due to their higher kinetic energy. Not even directly but kinetic energy transfer trough the skull can cause "discontinuous" fractures at the distance from entry wound and not in continuity with the fracture of the vault. These wounds that traverse parasinal sinuses and destroy skull base are likely to be contaminated. The rates of the CNS infection and CSF fistulas would be expected to be higher than in other penetrating cranioencephalic missile injuries. During treatment of such wounds we stress necessity of early diagnosis of CSF fistulas, and early operation with watertight dural closure.

The record of 312 casualties with missile injuries of the brain have been analyzed in the period of six years, with attention to the skull base fractures and the complications as CSF fistulas and infections. 45 of them developed CSF fistula, 15 (33%) on the wound side, 23 (51%) presented as rhinoliquorhea and 7(15%) as otoliquorhea. 6 patients (13%) developed infectious complications. 15 developed facio-orbito-cranial injuries with skull base fractures. 6 of them (40%) died, 3 (20%)developed CSF fistula and 2 (13%) meningitis.

The rate of infection did not exceed the rate associated with other cranioencephalic missile injuries, but the rate of CSF fistulas was twice as high. Skull base missile injuries are specific neurosurgical entity because of the high rate of CSF fistulas. They require emergency operation attempting the dura reconstruction of the skull base. With presented strategy and operative approach, the incidence of the infectious complications in skull base missile injuries remains low.
P261. HYPERBARIC OXYGEN THERAPY IN HEAD INJURY: AN ANIMAL MODEL OF BRAIN CONTUSION
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Background and Purpose: Cerebral contusions (CC) represent the most frequent traumatic lesions and the most common indication for secondary surgical decompression. Peri-infarct ischemia has been commonly proposed as one of the responsible mechanisms of deterioration. The purpose of this study was to design a reproducible and reliable model of cerebral contusion in rats, to investigate the physiology of peri-infarct secondary brain damage and evaluate the value of hyperbaric oxygen therapy (HBO) in the treatment of these lesions.

Methods: Four groups of 5 Sprague-Dawley rats each were included in this study. All animals were prepared and operated upon under general anesthesia induced by barbiturates. A 2 mm burr hole was drilled in the parietal region and a hollow connector with a vacuum apparatus was attached. A negative pressure of 0.47 ATA was applied to the unexposed cortex for 10 seconds. Animals were sacrificed after 4 days. Histological sections showed localized gross tissue loss in the cortex at the injury site, along with hemorrhages. In all cases, the severity of secondary brain damage was assessed in successive peri-infarct layers by numbering cells stained by Toluidin and Caspase 3 preparations. The study protocol: group I—control, group 2—HBO initiated 2 hours after injury and thereafter twice a day for 4 days (2.8 ATA for 90 min); group 3—peroxisome proliferator (PDI) of 30% of the host organ; group 4—PDI and HBO.

Results: Vacuom injury produced hemorrhagic lesions very close to traumatic CC in clinical situation. The size and morphology of the lesion at the vacuum site proved to be reproducible. At group 1, the peri-infarct region was characterized by a large number of apoptosis cells enhanced by Toluidin and Caspase (12.24% of the cells in a distance of 0.5 mm from the necrotic area). In group 2, there were less apoptosis cells in the peri-infarct area (4.7%, p < 0.0001). Hypoxia showed to worsen the outcome (31.75%, p < 0.001). As in group 2, HBO therapy decreased the extent and severity of secondary brain damage (9%, p < 0.003).

Conclusions: Our study shows that the vacuum model of brain injury is a reproducible model of cerebral contusion and further helps understanding mechanism of secondary extension that may account for clinical deterioration in some patients. Our results suggest HBO may limit the extent of secondary brain damage in CC and suggest further experimental studies.

P262. PREDICTORS AND INCIDENCE OF POSTTRAUMATIC SEIZURES IN CHILDREN AFTER BRAIN INJURY
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In this study we evaluate the incidence of early and late seizures after head injury in children under 18 years admitted to our hospital during 1995 and 2001 (6 years). The purpose was to find out factors correlating with a high risk of developing posttraumatic seizures. In our study 10.9% of the children developed seizures whereas 42.3% had early (during the first week) and 57.6% late (after the first week) seizures. Factors that showed a significant higher incidence for the development of seizures and should alert the physician, were the severity of the head trauma and a GCS of 3-8. These factors correlates with those of other studies. In contrast to many studies we found out that the incidence of posttraumatic seizures was significant higher in patients older than 12 years (12-16 and 12-18). Most of the late seizures were nonconvulsive diagnosed on a snapshot-EEG during the follow-up examination of the patients. We suppose that the EEG-examination in head injured children is important to find out these patients with epileptic potentials without clinical symptoms like convulsions because the epileptic changes of the EEG could worsen the diagnosis and clinical outcome of the children in accordance to studies are evaluated. We do not recommend the administration of antiepileptic drugs after the occurrence of a first early or late convulsion because no one of our patients had a second one. The prophylactic use of anticonvulsants in children who show risk factors of developing seizures is recommended in other studies and was not subject of our study.

P263. TRAUMATIC BRAIN INJURY AND HEMORRHAGIC HYPTENSION INCREASE POST-SYNAPTIC ZN2+ ACCUMULATION IN RATS
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Accumulation of ionic zinc (Zn2+), which contributes to neuronal injury after experimental cerebral ischemia (1.2) and weight-drop TBI(3) can be demonstrated by staining with N-[6-methoxy-2-quinolyl]-para-toluenesulfonamide (TSQ), a fluorescent dye with a high specificity for Zn2+(1). We hypothesized that fluid percussion TBI would increase Zn2+ accumulation and that subsequent hemorrhagic hypotension would cause further accumulation.

Rats were anesthetized (1.5% isoflurane), prepared for fluid percussion TBI(4) and randomly assigned to 1 of 4 groups (n = 6/group): sham TBI, hemorrhage to a mean arterial pressure of 60 mmHg for 45 min followed by reinfusion of shed blood; moderate TBI (1.8 atm) or TBI plus hemorrhage and reinfusion. Six hours after TBI, rats were reanesthetized and decapitated. The brains were frozen and 20 um sections were stained with TSQ. TSQ-positive neurons were counted by a blinded observer.

Few TSQ-positive neurons were observed in sham-injured rats and rats subjected only to hemorrhage. TBI alone significantly increased TSQ-positive neurons in the cerebral cortex, CA3, hilus, and dentate gyrus. The combination of TBI plus hemorrhagic hypotension significantly increased TSQ-positive neurons in comparison to TBI alone in the cerebral cortex, CA3, hilus, and dentate gyrus.

These results suggest that neural injury after TBI is mediated in part by Zn2+ accumulation that is exacerbated by post-TBI hemorrhagic hypotension. Reducing Zn2+ accumulation may reduce neuronal injury after TBI.


P264. ANALYSIS OF GENE EXPRESSION FOLLOWING ACUTE SPINAL CORD INJURY

Gene expression patterns offer a definable criterion on which to base the functional relevance of specific proteins to disease pathology. Our overall hypothesis is that genes whose expression is significantly or reproducibly altered following spinal cord injury (SCI) represent important candidates for intervention strategies as well as in the elucidation of mechanisms critical to cell injury/death pathways associated with SCI and other CNS disease states. We have employed the fluorescence-based quantitative method for the real time detection of PCR amplification (real time PCR) to identify gene expression patterns affected by SCI. We have selected gene families that may have a relevant impact on SCI for analysis and have evaluated the expression of over 90 known genes at acute time points post SCI in an established rat spinal cord contusion model. These families/groups include the Egr family of tyrosine kinase receptors and ligands; insulin-like growth factors, receptors and binding proteins; oncogenes and tumor suppressors; cell cycle regulators; interleukins and interferons; DNA binding proteins, transcription factors and regulators and extracellular cell signaling and communication proteins (i.e. growth factors, cytokines, chemokines). The gene expression patterns were evaluated at 12 and 24 hr post injury compared to sham controls. 49 genes had a greater than 2 cycle number difference between at least one injury time point and control. 12 genes exhibiting the largest change with a 5 or greater cycle number difference compared to controls were also analyzed at 2 and 6 hr post injury. Of the families and groups presented in this study, the most dramatic gene expression changes are observed 12 hr post injury from chemokines such as the macrophage inflammatory proteins 1a and 1b. These gene expression data are evaluated within cellular pathways and gene families to determine whether gene expression patterns collectively are relevant to SCI and other disease states.
P265. EXPRESSION OF SEMAPORININA IN THE RAT SPINAL CORD TRAUMA COMPRESSION MODELS
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Numerous studies have provided evidences that axonal growth and guidance is regulated by attractive and repulsive molecules present in the extracellular microenvironment of the growth cone. SemaphorinINa (Sema3A) has been described to function as a chemorepulsive molecule in directing growing axons to their target. However, its role in the spinal cord injury has not been well characterized. We demonstrate here that Sema3A is acutely up-regulated after transection of the rat spinal cord.

Material and Methods: 8 weeks Wistar rats were subjected to spinal cord transection and they were sacrificed 6h, 12h, 24h, 3d, 1w, 2w, and 1m after injury. Digoxygenin labeled in situ hybridization (ISH) for Sema3A and immunohistochemistry for Neu-N were simultaneously done in each cyrosection. We also performed double immunofluorescence study for Sema3A and Neu-N.

Results: In ISH, positive signal for Sema3A was detected in the gray matter near the transected site 6h after injury and sustained in the same level 24h after injury. 3 days after injury, signal intensity gradually decreased and only a slight signal was detected 1m after injury. Sema3A mRNA and protein expressing cells coincided with neurons which were immunoreactive for Neu-N antibody.

Discussion: In the present study, Sema3A was acutely up-regulated in the neurons near the transected site. The time course and localization of Sema3A expression in spinal cord transection model is similar with that in middle cerebral artery occlusion model as previously reported.

P266. "DECOY" INTERVENTION IN NF-KAPPA B ACTIVATION AFTER SPINAL CORD CONTUSION INJURY

Spinal cord injury triggers an inflammatory response that may be responsible for the observed pathophysiology. An early lesion event is transient and robust increases in IL-1 beta levels, which contribute significantly to augmentation of cyclooxygenase 2 (COX-2) and the inductive form of nitric oxide synthase (iNOS). Both COX-2 and iNOS stimulate production of reactive oxygen species (O2-, OH-, and NO.). IL-1 beta stimulates NF-kappaB activation, the transcription factor known to up regulate COX-2 and iNOS translation. Using injections of "decoy" oligonucleotides at the site of contusion containing the consensus DNA sequence found in the COX-2 promoter region, we found prompt, dynamic, and transient uptake of labeled "decoys" into both the cytoplasm and nuclei of resident cells. Further, we showed a selective modulation of NF-kappaB protein binding, as seen by electrophoretic mobility shift assay, as well as selective effects on iNOS and COX-2 expression at the site of injury. These data are consistent with the hypothesis that NF-kappaB transcriptional regulation of these proteins represents elements in the pathophysiology and recovery of mammalian spinal cord after contusion injury. Supported by NINDS Grant NS-39161 and Shriners Hospital Grant 8710.

P267. EVALUATION OF A FORCEPS COMPRESSION MODEL OF SCI IN RATS.

A reliable, high throughput animal model of spinal cord injury (SCI) would greatly facilitate the assessment of the numerous compounds with potential for reducing secondary damage or enhancing recovery mechanisms. We have evaluated a forceps compression model of SCI in rats (based on Gruner et al., 1996) that produces a graded injury as assessed by functional and anatomical outcome measures. Following laminectomy of T9/T10 vertebrae, the spinal cord of adult rats, anesthetized with isoflurane, was injured by a brief compression to a space of 0.9, 1.3 or 1.7 mm using modified coverslip forceps. The whole operation required approximately 15 minutes from incision to closing. Control animals received a sham injury. Compression distance correlated with open field locomotor behavior, at 12 weeks post-SCI: the 0.9 mm compression produced a moderate, yet more severe injury than the 1.3 mm compression. The 1.7 mm compression resulted in a mild injury that recovered to approximately the behavioral score of sham injured animals, by 12 weeks post-injury. Histological analysis reveals that the compression injury produces tissue cavitation and regeneration characteristic of spinal cord contusion injury. In separate experiments, lesion volumes were determined at 24 hours post-SCI from spinal cord tissue sodium and potassium ion measurements using atomic absorption spectrophotometry (Young, 1992). Lesion volumes were found to correlate to injury severity in a graded manner. The forceps compression model appears to be a rapid and reliable method to induce graded spinal cord injuries in vivo that will enhance the capacity and ability to test, assess and identify agents with therapeutic benefits in SCI.

P268. EARLY ANTI-INFLAMMATORY TREATMENT ATTENUATES NEUROFILAMENT DEGRADATION AFTER SPINAL CORD INJURY.

The pathogenesis of spinal cord injury (SCI) involves a series of responses resulting in further destruction of nervous tissue after the primary injury. For example, lipid peroxidation and calpain proteolysis, are exacerbated by the immune response and cytokine-stimulated macrophages. Previously, we correlated improved autonomic and locomotor function with white matter sparing after an early anti-inflammatory treatment with an antibody to the aD subunit of b2 integrin. This attenuates leukocyte infiltration into the injured cord by blocking the interaction between vascular adhesion molecules and cell surface b2 integrins. We determined the effect of the aD-antibody treatment alone, or in combination with methylprednisolone (MP), on neurofilament degradation, gliosis and production of transforming growth factor (TGF)-b1. After SCI, TGF-b1 initially has pro-inflammatory actions, but by 14 days post-SCI its role becomes more neuroprotective, inhibiting proteases and cytokine/cytokine secretion from macrophages. Rats underwent severe clip-compression SCI at the 4th thoracic segment followed by saline, anti-aD, or anti-aD/MP treatments at 2, 24, and 48 hours. Western blot analysis of neurofilament (NF200) demonstrated a major loss of protein in the lesion at 7 and 14 days post-SCI compared to the same segment in uninjured cord. Anti-inflammatory treatment markedly reduced this loss, indicating decreased calpain-mediated degradation of NF200. Northern blot analysis of total RNA extracted from uninjured cord and an antibody 7 days post-SCI revealed a significant increase in TGF-b1 mRNA levels after SCI. TGF-b1 protein, quantified by ELISA, increased from 7.4 ± 3.4 pg/mg total protein in uninjured spinal cord to 42.8 ± 11 and 28.5 ± 6.0 in the lesion at 7 and 14 days post-SCI, respectively. TGF-b1 protein levels were not altered by the anti-inflammatory treatment. In summary, anti-inflammatory treatment suppresses degradation of neurofilament protein, indicating marked neuroprotection. This effect was not mediated by modulating the changes in intraspinal TGF-b1 protein levels after SCI. Support: ICOS Corporation and Ontario Neurotrauma Foundation.
P269.

EARLY SELECTIVE ANTI-INFLAMMATORY TREATMENT BLOCKS THE DEVELOPMENT OF CHRONIC PAIN AFTER SPINAL CORD INJURY

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Chronic tactile allodynia is a frequent painful complication of spinal cord injury (SCI) with poorly understood mechanisms, likely involving plastic changes within the injured cord and loss of descending inhibitory pathways. Secondary damage after SCI can impact on these changes by causing death of axons and second order neurons. We proposed that a selective anti-inflammatory treatment, delivered during the first 48 hours after SCI, would minimize the development of this chronic pain. A monoclonal antibody (mAb) to the 2D subunit of the B2 integrin of leukocytes was used to block early macrophage/neutrophil infiltration into the injured cord. A clinically-relevant moderate clip-compression SCI in rats was used to generate an incomplete thoracic (T12) lesion. In allodynia testing sessions, the dorso trunk or plantar hind paws were probed ten times using 5 or 10 g Semmes-Weinstein hairs. Rats were acclimated to a testing chamber and tested for allodynia prior to SCI; no behavioural responses suggesting pain were elicited. Rats were tested again at two, three and four weeks after SCI. At these three periods, stimulation of the trunk appeared noxious in untreated rats (n = 7) in response at 3 x 1, 5 x 1 and 10 x 1 of 10 stimuli, shown by flinching, escape and/or vocalization. The mAb treated rats (n = 8) responded only to 0.2 x 0.5, 5 x 1 and 1 x 1 of 10 stimuli. Painful responses to paw stimulation appeared as abrupt withdrawal, licking or shaking of the paw and vocalization. At the three testing periods, the untreated rats responded as if 1 x 0.4, 3 x 1 and 2 x 1 of 10 stimuli were noxious. The mAb-treated rats responded only to 1 x 0.1, 1 x 0.4 and 4 x 0.1 of 10 stimuli. The mAb treatment also improved locomotor BBB scores from 6 x 0.1 to 10 x 0.3 at four weeks after SCI. In conclusion, selective inhibition of the early inflammatory response can reduce disabling chronic pain after SCI. Support: Ontario Neurotrauma Foundation and ICOS Corporation.

P270.

EVALUATION OF CONDITIONS FOR CALPAIN INHIBITOR IN THE RAT SPINAL CORD: EFFECTIVE POST-INJURY INHIBITION REQUIRES INTRASPINAL MICROINJECTION

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Calpains (calcium-activated cysteine proteases) are strongly implicated in the secondary damage that follows contusion injury to the spinal cord. Calpains are activated within a few minutes following injury and their elevated activity persists for 24h, thereby providing a reasonable window of opportunity for post-injury inhibition. Previous studies demonstrated decreased axonal damage and neurofilament proteolysis with post-injury intravenous administration of relatively low concentrations of the calpain inhibitors leupeptin, E-64-D, and calpeptin. We sought to determine if conditions under which calpain inhibitors were administered in previous studies resulted in effective calpain inhibition, and to identify conditions that result in significant calpain inhibition following spinal cord injury. Conus intrinsic spinal cord injury was produced in female Long-Evans rats using the NYU impactor at the 12.5-25 mm height setting. The results demonstrate that intravenous administration of 1mg/kg E-64-D or 250 µg/kg calpeptin does not inhibit total calpain activity in the rat spinal cord, measured using a BODIPY-FL labeled casein assay. Intravenous 20 mg/kg MDL28170 resulted in mild but significant calpain inhibition and a modest decrease in the proteolysis of calpain substrates a-spectrin and MAP2. Intraspinal microinjection of 50 nmoles MDL28170, either 30 min prior to or 20 min following contusion injury, resulted in a more robust inhibition of total calpain activity and significant attenuation of a-spectrin breakdown and MAP2 proteolysis. The calpain inhibition was within 2h after drug administration, but was still evident at 48h. Together, the results demonstrate that, using currently available calpain inhibitors such as MDL28170, direct microinjection is necessary to achieve the drug concentration required for effective calpain inhibition and to decrease the injury-induced proteolysis of calpain substrates.

P271.

TRANSPLANTATION OF NEUROTROPHIN-EXpressING FLIBROBLASTS INTO CHRONIC CONTUSION CAVITIES

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Chronic contusion injuries are characterized by a cystic cavity surrounded by a spared rim of white matter. Damaged axons with retraction bulbs persist at the cavity edges with long regenerative processes occurring even 3-6 months after SCI (Hill et al., 2001). Although most treatments for SCI focus on the acute pathology, it is possible that fibers persisting chronically at the lesion edge could be a therapeutic target. Few studies have intervened chronically, but there is evidence that application of neurotrophins to chronic injuries promotes cell survival and regeneration of supraspinal axons (Grill et al., 1997; Ye & Houle 1997; Houle and Ye, 1999). In addition, transplanted genetically-modified fibroblasts have been shown to improve behavior and enhance fiber outgrowth when applied to acute lesions (Liu et al. 1999). Here we report the results of 2 studies in which fibroblasts were transplanted into long-term stable lesions (8-9 weeks post SCI) and behavioral recovery and axonal growth were examined for 10-14 weeks following transplantation. In an initial pilot study animals received transplants of control fibroblasts (n = 5) or fibroblasts that expressed BDNF and NT-3 (n = 6). Animals in the pilot study improved in open field locomotion (mean BBB score 11.2 pre-transplantation, 13.7 post transplantation) following transplantation of fibroblasts (with or without neurotrophin expression); this result was not replicated in a subsequent comprehensive double-blind study (n = 50). These studies show successful transplantation of cells into chronic contusion sites in the rat with long-term cell survival and fiber growth into the transplants, suggesting that transplantation may be a realistic option for the treatment of chronic spinal cord injuries. (Support: ISRT, NS 38079)

P272.

DELAYED GRAFTING OF FETAL SPINAL CORD TISSUE ENHANCES EARLY GRAFT SURVIVAL AND DEVELOPMENT IN THE INJURED ADULT RAT SPINAL CORD

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When placed into acute lesions, intraspinal grafts of fetal spinal cord (FSC) tissue exhibit a dramatic attrition of donor cells by 4d post-transplantation (PT). Surviving cells (i.e., presumptive stem cells and lineage-restricted neuronal and glial precursors) then rebound to give rise to lesion-filling tissue masses. This loss of tissue may limit the extent of early host-graft interactions and affect the development of functional neural phenotypes. Since it is known from other work that by introducing an interval between injury and grafting, more robust transplants can be obtained, the goal here was to determine whether a post-injury delay can significantly reduce the rate of initial donor tissue loss seen with intraspinal FSC grafts. Adult-S-D rats received E14 FSC grafts into homieal cavities made at spinal C4. The animals were then distributed between three transplant groups: acute 10d delay, and 30d delay graft recipients. At 4d PT, tissue specimens were prepared for light microscopy and morphometric analysis. In acute graft hosts, the transplants consisted of small islands of cells apposed to either neighboring gray matter, vascular profiles, or the pial surface. With a 10d delay, however, the grafts were significantly larger than controls. 30d delay grafts were significantly larger than both controls and 10d grafts. It thus seems that the delayed grafting paradigm enhances the initial survival or rebound rate of these grafts, particularly with a delay of 30d. This result may be a reflection of increased connectivity within the lesion site over time. By virtue of the stem/precursor cell nature of these fetal grafts, the present observations may prove relevant to other transplantation paradigms using other donor cell types. Studies are in progress to determine whether reduced donor tissue loss alters the developmental dynamics of these fetal transplants. (Supported by the Mark F. Overstreet Chair for Spinal Cord Regeneration Research)
P273.  
NMDA RECEPTOR ACTIVATION AS A BASIS FOR INCREASED VULNERABILITY: OLIGODENDROCYTES IN CONTUSED SPINAL CORD  
Excitatory amino acid receptors play an important role in normal neurotransmission as well as in pathological changes and neural cell death that occur after spinal cord injury (SCI). Evidence also shows that spinal glia are involved in creation and maintenance of pathological pain. The purpose of this study was to investigate activation of NMDA receptors using phospho-NMDA1 glutamate receptor subunit expression after long-term SCI recovery (one month). A contusive injury was produced at T8 using the NYU impactor (10 gm, 12.5 mm drop). Antibodies to phospho-NMDA1 glutamate receptor subunit (Upstate Biotechnology) were used to detect activated NMDA receptors in dual staining experiments with markers for neurons, oligodendrocytes or microglia identified cell types expressing NMDA receptors. Four weeks after SCI, more immunoreactivity for phospho-NR1 was observed in gray and white matter than in sham control animals. An increased number of oligodendrocytes was seen in both gray and white matter of the injured cord, while the number of neurons and microglia was decreased after injury. A large proportion of oligodendrocytes in the white matter were found expressing increased levels of activated NMDA receptors after injury. This observation suggests that upregulation of glutamate NMDA receptors in oligodendrocytes may play a role important in chronic pathological processes, including increased pain transmission, neural reorganization and plasticity following long-term spinal cord injury. These studies suggest a new target for pharmacological treatment of SCI and control of pain. (TIRR-Mission Connect, Spinal Cord Research Foundation, NS11235 and NS 30161) 

P274.  
INHERENT LOKOMOTOR DIFFERENCES IN MOUSE STRAINS IMPACT RECOVERY AFTER SPINAL CORD INJURY  
D.L. Hassenzahl*, L.C. Fisler, P.G. Popovich, and D.M. Bassar. (Ohio State University, Columbus, OH USA). 
Recently spinal cord injury (SCI) research has expanded to mouse models due to the availability of transgenic and knock-out animals. Between-strain comparisons of mice found significant differences in activity level, neuroanatomy, and physiological function (Wahlsten, 2001), suggesting that differences in motor function of strains may exist. Coordinated locomotion may be an important parameter to investigate in mice because it is a sensitive indicator of injury severity. The purpose of this experiment was to determine if mouse strains use different locomotor patterns normally and which strains may be more vulnerable to SCI-induced locomotor deficits. We examined 4 mouse strains and a hybrid cross commonly used in generating transgenic animals: Balbi/c (n = 14), C57BL/6 (n = 18), C57/BL10 (n = 6), B10.PL (n = 6) and an F1 (C57/BL6 female X 129S6/SvEv male cross, n = 14). Mice received a 0.5 mm contusion at T8 with the Electromagnetic Spinal Cord Injury Device. Locomotion was measured prepost with the BBB and a subset of mice (n = 3-6/gp) was tested on a walkway apparatus that automatically calculates gait parameters. Preoperatively, greater external rotation was noted in some strains during gross locomotion. Quantitative measures showed significant differences (p < 0.02) in diagonal forelimb-hindlimb (FL-HL) coordination between C57/BL6, Balbi/c and Fls even though they walked at the same velocity, (C57/BL6: HL precedes FL by 1.64 ± 2.15%, Balbi/c: HL trails FL by 4.70 ± 0.38%; Fls: HL and FL move almost simultaneously 1.67 ± 0.51%). Balbi/c and C57/BL6 also tended to have longer HL, swing longer than other strains (p = 0.05 and 0.09). After SCI, C57/BL10 and B10.PL showed a greater rate and extent of recovery on the BBB than other strains. Quantitatively, C57/BL10 had greater residual deficits in FL-HL coordination than B10.PL (p < 0.05) despite similar lesion severities. 
Strain differences in locomotion exist in normal and SCI mice. Attribution of behavioral differences to treatment or genetic manipulations in mice must be done with caution. NS7846 CRPS D1-0200-2. 

P275.  
FORMATION OF COLLAGENOUS SCAR IS A COMMON FEATURE FOLLOWING TRAUMATIC AND ISCHEMIC INJURIES TO THE CENTRAL NERVOUS SYSTEM  
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Formation of a collagenous wound healing scar resp. basement membrane (BM) has been shown to impede axonal regeneration after mechanical transection of brain and spinal cord fiber tracts in rat. Here we show by use of specific tissue processing protocols that such a collagenous BM forms not just after transection but also following contusion and compression injuries to the brain and spinal cord as well as after ischemic insults such as focal or global stroke. These data suggest that inhibition of axonal outgrowth may underlie the same principles after penetrating injuries as well as following blunt or ischemic lesions. Therefore, we consider the collagenous wound healing scar as an obstacle for compensatory functional plasticity in traumatic and ischemic brain lesions. Suppression of collageneous scarring may thus provide a strategy to promote axonal regeneration and/or plasticity in traumatic and ischemic CNS injuries. 

P276.  
EFFECTS OF SEROTONERGIC DEPLETION IN BALB/C MICE ON LOCOMOTOR AND PUDENDAL REFLEXES IN INTACT AND CHRONIC SPINAL CORD DENTICED RATS.  
Gregory M. Holmes, Jacqueline C. Bresnahan, Michael S. Beattie. (The Ohio State University, Columbus, OH US). 
In spinal cord injured (SCI) animals, depletion of the external anal sphincter (EAS) produces a profound increase in muscle EMG responses. Following contusion injury (25mm; NYU device), consistent and parallel recovery of locomotor and pudendal (rectetile and EAS) reflex function occurs in animals over 6 weeks post-SCI. We have reported that the reduction, and subsequent return, of fibers labeled for serotonin (5-HT) immunofluorescence mirrors the recovery of function in these animals. This study sought to determine if the observed sprouting of 5-HT fibers mediates the recovery of pudendal reflexes after contusion. To test the efficacy of eliminating descending serotonergic inputs, intact males were administered intracerebral (IC) injections of the neurotoxin 5,7 DHT. At three days post-IC injection, the latency to the first penile erection was significantly reduced in animals with a confirmed reduction in spinal immunofluorescence. However, the magnitude and duration of EAS responses to distension were not affected. Locomotor function, as measured by BBB and computerized walkway were likewise unaffected. Preliminary data on female rats with SCI (25 mm T10 injuries, tested at post-operative days 2, 7 and 21 for BBB locomotor function and EAS reflexes) revealed that SCI yielded a predicted disruption of locomotor function and hyperreflexia of the EAS after distension followed by recovery over time. Surprisingly, the 5-HT synthesis inhibitor p-CPA (200mg/kg IP) did not re-introduce EAS hyperreflexia but instead markedly diminished EAS responses. (Support: NIH, NS-31193). 

1316
BILATERAL HYPEREXCITABILITY OF LUMBAR DORSAL HORN NEURONS FOLLOWING UNILATERAL THORACIC HEMISECTION-BASED FOR "PHANTOM" NEUROPATHIC PAIN
Claude E. Hulsebosch+, B.C. Haines, W.D. Willis (University of Texas Medical Branch, Galveston, TX US)

Spinal cord injury (SCI) results in chronic central neuropathic pain (CNP) that persists in the majority of patients. One possible mechanism for the sustained hyperexcitability of dorsal horn neurons, termed central sensitization, is the maintenance of changes in excitatory amino acid and peptide elements. Another mechanism, denervation supersensitivity, produced by the interruption of tonic descending inhibition, may confer membrane voltage changes that increase the activation state of other tonic receptors. To test this, using adult male Sprague-Dawley rats (n = 8), we obtained extracellular single-unit recordings of multireceptive (MR) dorsal horn neurons from L3-L5 immediately before, and 45 minutes after (n = 10 neurons per side of the cord) a unilateral T13 hemisection. Background activity and evoked responses to innocuous and noxious cutaneous stimuli (brush, press, pinch, 3.84, 9.96, 204 mN) were recorded, and peripheral receptive fields were mapped. We report a statistically significant increase in all measured parameters on both ipsilateral and contralateral sides of the cord, causal to the level of lesion. Ispilaterally, the responsiveness of MR neurons was significantly increased compared to the contralateral side. We propose that one mechanism of below-level pain syndromes described by patients with SCI after SCI is release from tonic inhibition and subsequent development of hyperexcitability, as a result of receptor plasticity. (Supported by: Mission Connect of TIRR. NIH grants NS 11255 and NS 39161.)

VENTRAL Funiculus LESIONS OF THE SPINAL CORD PRODUCE LASTING SENSORY BUT NOT MOTOR DEFICITS
E.A. Lewis², L.C. Fisher, V.McGoughy, D.L. Hassenzahl, P.G. Pappovich, D.M. Basile. (Ohio State University, Columbus, OH US)

Optimization of locomotion after spinal cord injury (SCI) depends on understanding the function of spared motor and sensory systems. Traditionally, descending systems in the ventral funiculus (VF) were thought to control spinal cord locomotion but recent studies cast doubt on this role (Loy '02, Brustein '93). Making precise, permanent lesions of axons in the VF has been problematic and limits our understanding of these systems. A novel approach to VF lesions may be a zymosan-induced inflammatory response which creates discrete lesions in the CNS. The purpose of this study is to determine the cellular response, lesion development and behavioral consequences of VF zymosan injections.

Sprague-Dawley rats (n = 10) received zymosan injections (2-1.125) in the VF and survived 1, 3 or 6 weeks. Behavioral testing for locomotion, reflexes, and sensation was conducted preoperatively and weekly postoperatively (po). Lesioned tissue sections were stained for neurofilament, myelin, T lymphocytes and macrophages. Plastic sections (1um) were stained with toluidine blue. Macrophage and T-lymphocyte responses peaked at 3 weeks and remained above baseline levels at 6 weeks postop. Axonal loss occurred by 1 wk with lesion size peaking at 3 wks. A thin astroglial scar evident at 3 wks on plastic sections became thicker over time and contained a few remyelinating axons. Zymosan produced significant but transient declines in BBB scores 5-dpo (p < .05). Pronounced and lasting hyperexcitability developed after VF lesions using monofilaments (p < .001). During proprioceptive placing, knee flexion angular extension was greater at 1 wk po (p = .003), indicative of hypertetreflexia. Zymosan injections into the VF produced permanent, precise lesions without damage to other areas by initiating a localized inflammatory response. Given that few sensory axons were damaged by VF lesions, the pronounced and lasting sensory changes suggest that firing thresholds of lumbar interneurons which integrate sensory and motor input are reset to lower levels. NS37846, N553798.

TRANSIENT SUPPRESSION OF FIBROUS SCAR AFTER ACUTE SPINAL CORD INJURY IN RAT LEADS TO MASSIVE AXONAL REGENERATION
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Acute traumatic injury of the CNS results in formation of a basement membrane (BM) in the lesion core and a glial scar characterized by reactive astrocytes in the periphery of the lesion. BM can be visualized by immunohistochemical staining with anti-collagen IV and anti-laminin antibodies. Due to its characteristic meshwork structure it is named fibrous scar or cicatrix and can be clearly distinguished from the glial scar with respect to spatial distribution and molecular and cellular composition. There is much evidence that fibrous scar BM serves as a scaffold to bind putative growth inhibitory and repellent molecules besides acting as a permissive mechanical barrier for regrowing axons in the CNS.

We developed a strategy to suppress BM biosynthesis at the injury site in rat spinal cord. After either scrotal wire knife lesions at T8 or dorsal hemisections with microsissors, the "anti-scarring treatment" (AST) leads to a significant suppression of collagen IV deposition compared to untreated animals within the first 12 days after lesion. AST consists of multiple injections of an iron-chelator (decarboxyphosphatidylethanolamine derivative BPY-DCA) into the lesion site, together with application of solid 8-Bromo-cAMP and longer lasting slow release of BPY-DCA through a synthetic copolymer at the top of the lesion. The 12 days time window with fibrous scar reduction proved to be sufficient to allow cortico-spinal axons to grow through the lesion site extending into the caudal spine through white and gray matter areas.

Our results show that the formation of the basement membrane in the fibrous scar after acute spinal cord injury is a major impediment for CST fiber regeneration. Supported by DFG.

CELL PROLIFERATION AND SURVIVAL CHRONICALLY AFTER SPINAL CORD INJURY
Laila J. Zaat, Jean R. Wrathall. (Georgetown University, Great Falls, VA US).

Following spinal cord injury (SCI), about half of the oligodendrocytes and astrocytes in the residual white matter at the injury site are lost by 24 hours. However, chronically after injury, the density of these cells in this region is equal to controls. This suggests that glial cells in the spinal cord are replaced after SCI. We have previously demonstrated that by 3 days, cell proliferation is significantly increased in the grey and white matter of the injured spinal cord as compared to laminectomy controls. To study the fate of cells dividing in response to injury, we performed SCI on adult female rats at the T8 level using a standardized contusion model. Animals received two BrdU injections (8.7 mg/kg) each day, on days 2, 3, and 4, with 2 hours in between injections. At 6 weeks after injury, spinal cords were fixed by perfusion and the tissue was analyzed using immunocytochemical detection of BrdU. We found that by 6 weeks, some cells that had been labeled 2-4 days after SCI were still present in the residual white matter, as well as in the central lesioned area. Double immunocytochemistry showed that a number of the BrdU positive cells also expressed nestin, PDGF-Ra, or NG2, markers characterizing glial precursor cells. These results suggest that a number of cells that are stimulated to divide in the first week after SCI do not undergo many additional cell divisions, but survive and remain in an immature state for many weeks after injury. (Supported by NIH F31 NS43019-01 and ROI NS55647).
P281.
HYDROGEN PEROXIDE ELEVATED BY SPINAL CORD INJURY INDUCES AND A METALLOPORPHYRIN ATTENUATES OXIDATIVE DAMAGE
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We demonstrated previously that hydrogen peroxide (H2O2) concentrations increase significantly after spinal cord injury (SCI). In this study, H2O2 was administered into the rat spinal cord through a microcannula at the concentration and duration produced by SCI for 10 h. H2O2-induced oxidation of proteins and DNA was characterized by immunohistochemical staining with anti-2,4-dinitrophenyl (DNP) and anti-8-hydroxy-2-deoxyguanosine (8-OHdG) antibodies. H2O2-induced peroxidation of membrane phospholipids was determined by measuring malondialdehyde by microdialysis sampling and HPLC analysis. The numbers of DNP- or 8-OHdG-positive cells counted along the cannula track were both significantly higher in the H2O2-exposed group than in ACSF controls (p < 0.001 and < 0.001, respectively). H2O2 significantly increased malondialdehyde production (p = 0.03). Mn (III) tetraalkyl (benzoic acid) porphyrin (MnTETBP)—a cell-permeable superoxide dismutase mimetic and a broad spectrum reactive species scavenger—administered through a second cannula (2.5 mM in ACSF) significantly reduced H2O2-induced oxidation of protein, DNA and membrane lipids (p = 0.02, 0.03, and 0.02 respectively). This is direct in vivo evidence that SCI-produced levels of H2O2 cause oxidative damage to major cellular components and MnTETBP effectively reduces H2O2-induced oxidative damage. (Supported by NIH grants NS 34048 and NS 35119).

P282.
ASSESSMENT OF POSSIBLE STRAIN—DEPENDENT DIFFERENCES IN MICE FOLLOWING SPINAL CORD INJURY
Isabella Fogaccia, Lisa M. Benjamin, Stephen W. Scheff. (Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY USA).

Experimental spinal cord injury (SCI) results in a rapid and significant pathophysiology throughout a large rostral-caudal extent of the spinal cord. In order to probe the molecular mechanisms of SCI, research interests have been to employ genetically engineered mouse lines. These transgenic mice utilize a number of different background strains. The present study was undertaken to assess possible strain-related differences in response to SCI. Four different inbred strains (C57BL/6, C57Bl10, BALB/c, FVB/N) and one outbred strain (ICR) of mice. All animals were 7-8 wks of age when subjected to a moderate SCI (50 kinesia at T10-11 utilizing the Infinite Horizon Impactor®). Seven days following injury, spinal cords were assessed for changes in morphology. In every injured animal there was an obvious bilateral bruising of the spinal cord at the time of injury. Initial analyses demonstrated strain-dependent differences in the injury length and volumes of spared gray and white matter. Although the age of the mice was equivalent, there were some significant strain-dependent differences in the animal size and weight that might account for the observed differences. Accordingly, we assessed possible strain-related differences in the naive spinal cords and subsequently revealed injury-induced changes compared to each strain’s naive spinal cord values. This new morphologic analysis failed to reveal any strain-related differences following SCI. These results support the idea that the basic morphologic changes observed following SCI are common to the most widely used mouse strains and underscores the need to utilize appropriate controls when assessing the results. Supported by KCHRT #9-20 and SCRF 2085-02.

P283.
IS THERE AN ACQUIRED CHANNELOPATHY CONTRIBUTING TO AUTONOMIC CONDUCTION DEFICITS FOLLOWING SPINAL CORD INJURY?
K.C. Hayes* and M.G. Fehlings. (Lawson Health Research Institute, London, Ontario; Krembil Neuroscience Centre, The Toronto Western Hospital, Toronto, Ontario, CA).

Axonal ion channel dysfunction (channopathies), as a result of immunological factors, or alterations in the expression of genes encoding ion channel properties, has emerged as an important element of the pathophysiology of conduction deficits in a variety of neurological disorders. We hypothesize that an acquired (traumatic) channopathy, distinct from axonopathy or myelinopathy, contributes to central and peripheral conduction deficits following spinal cord injury (SCI). Evidence is drawn from immunologic studies of chronic SCI patients revealing elevated serum and/or cerebrospinal fluid titers of proinflammatory cytokines and/or autoantibodies known to alter potassium (K+) or sodium (Na+) channel conductances, as well as studies of trauma-induced modifications in the expression of genes encoding K+ and Na+ ion channel properties in animal models of SCI. The presence of axonal ion channel dysfunction would compound conduction deficits due to co-existent myelinopathy or axonopathy. If proven, the acquired channopathies may help explain various clinical phenomena such as functional deficits that exceed those expected from the degree of frank neurological injury, bladder dysfunction or sensory paresthesias. Acquired channopathies may be remediable by targeted immunomodulatory therapy, gene therapies that target ion channel proteins, or agents that modify ion channel conductance, eg. 4-aminopyridine.

P284.
WHIPLASH INJURY OF THE NECK: CLINICAL SYNDROME OR MALINGERING?
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Background: Minor spine injuries, vastly predominated by whiplash injuries of the neck, tend in later few years to occupy each year more time in the ambulatory practice of our neurosurgeons. Increasing number of these types of injury and more complicated diagnostic and therapeutic protocols represent a growing financial burden especially to the health organisations and the insurance companies protest an epidemic of financial compensation claims. The physician is so often forced into the disagreeable position to arbitrate between the compensation-seeking malingersers and patients. A correct decision is almost unreachable goal in a population of these patients with predominantly subjective leading symptoms and lack of clearly distinctive criteria so esteemed by surgeons.

Conclusion: We could not even dare trying presenting all the aspects of the problem, but compared some data dealing with the epidemiology, diagnostic and therapeutic procedures used in clinical practice with the problem of chronic residual symptoms in medicol-legal practice. Results: The data compared referred to years 1996 and 2000. We noticed the marked increase in number of the injured seeking the medical help in urgency, as well as in ambulatory controls (~50%). The same percentage of the litigation is registered (<20%). The data showing leading acute symptoms (pain, radicular impairment), diagnostic tools, therapy (immobilisation, non-steroid analgesics, physical treatment), work absence (~10 weeks), and residual complaints (discomfort, minor impairments of physical activities) remained similar.

Conclusion: Although aware of both sides of the problem, we kept the "physicians attitude" to the injured: belief that most of the patients do suffer the symptoms (although somewhat aggravating) and need diagnostic and therapeutic medical approach. but we urge for clear-cut criteria both in urgency (QTF protocol, cervical spine X-rays) and in follow-up (symptom quantification - neck muscles and mobility, radicular, cervico-cephalic, and other symptoms, and correct evaluation of instrumental findings).
P285. HETEROGENEITY OF REGIONAL CEREBRAL BLOOD FLOW FOLLOWING SEVERE HEAD INJURY
Maria C. Briones-Galang*, Roman Hlacky, Shasti S. Prahara, Yavuz S. Silay, Alex B. Valadka, Claudia S. Robertson. (Neurosurgical Intensive Care Unit of Ben Taub General Hospital, Houston, TX, USA).

Local probes, such as tissue pO2 and microdialysis, are being widely used to monitor patients with traumatic brain injury (TBI). Often the probe is placed in a relatively uninjured area of the brain, assuming that this will provide a measure of global CBF. The purpose of this study was to analyze the distribution of cerebral blood flow (CBF) after TBI to provide an understanding of what information could be expected from a local monitor.

CBF was measured using xenon-enhanced computed tomography (CT) in 76 patients within 12 hours after severe TBI. For each patient, CBF was calculated in 80 standard cortical regions of interest (ROIs). The distribution of CBF in these ROIs was analyzed.

The average CBF for all ROIs studied was 35 ml/100g/min, but the values ranged widely from 1 to 151 ml/100g/min. The type of injury was the most important factor in determining the distribution of CBF. With Diffuse Injury 1, CBF was normally distributed, and was between 30-60 ml/100g in 72% of ROIs. CBF was below 20 ml/100g/min in only 8% of ROIs. With Diffuse injury 2 and 3 and with mass lesions, the median CBF was lower and the range of CBF in the ROIs was wider. With Diffuse injury 4, CBF was uniformly decreased, with 90% of all ROIs having a CBF between 10 and 30 ml/100g/min. Other factors that determined the distribution of CBF were age and the level of ICP at the time of the CBF measurement.

CBF within the brain varies widely in most patients after TBI. Depending on the placement of a local probe within the brain, the values obtained may or may not reflect global CBF values.

P286. COMPARISON OF SINGLE VOXEL AND MULTIVOXEL MR SPECTROSCOPY IN PREDICTING 3 AND 6 MONTH NEUROLOGIC OUTCOME AFTER TRAUMATIC BRAIN INJURY
Barbara A. Holshouser, Karen A. Tong, Lori Shutter. (Loma Linda University Medical Center, Loma Linda, CA, US).

Methods: We reviewed 27 patients, aged 15 to 79 (mean 33 years), at an average of 7.2 days after sustaining severe TBI (initial Glasgow Coma Scale ≤ 8). Seven control subjects, aged 17 to 49 (mean 27 years) were also evaluated. With a 1.5T MR scanner, two SVS (8 cc) were acquired in normal appearing brain regions: mid-occipital gray matter (GM) and parieto-occipital white matter (WM), using a short echo time (TE = 20msec) stimulated echo acquisition mode (STEAM) sequence. SVS spectra were processed using LCMRv2 model software to obtain peak areas for N-acetylaspartate (NAA), creatine (Cre), choline (Cho) and metabolite ratios. MR spectra was acquired using a long echo time (TE = 144 msec) point resolved spectroscopy sequence (PRESS) in a 10 mm thick slice at the level of the corpus callosum. Spectra for each voxel were processed to obtain mean metabolite ratios, then averaged to obtain a pooled mean metabolite ratio (Total) for each patient and control. Three clinical outcome groups (good recovery, moderate disability and severe disability/vegetative state) based on the Glasgow Outcome Score (GOS) were assigned at 3 and 6 months following injury.

Results: For 3 and 6 month outcomes: SVS metabolite ratios (GM and WM NAA/Cho and Cho/Cr) showed significant differences between controls and the severe/V5 group only. (ANOVA: p = .01–.05). MR "Total" metabolite ratios (NAA/Cr, NAA/Cho, Cho/Cr) showed significant differences between controls and all three outcome groups (p = .001–.05). Additionally at 6 months, MRI could distinguish between disability groups.

Conclusion: The MRI pooled metabolite ratios were better able to distinguish outcome groups than SVS ratios, particularly at 6 month outcomes.

P287. SIGNIFICANCE OF A REDUCED CEREBRAL BLOOD FLOW WITHIN 12 HOURS AFTER SEVERE HEAD INJURY.
Claudia S. Robertson*, Roman Hlacky, Charles F. Contant, Alex B. Valadka (Baylor College of Medicine, Houston, TX, USA).

Posttraumatic hypoperfusion is a well-known feature of traumatic brain injury pathophysiology. "Ischemic" levels (<18 ml/100g/min) of cerebral blood flow (CBF) occur in 1/3 of patients within 6-12 hours after injury. However, the underlying cause of this hypoperfusion is not so clear. The purpose of this study was to examine the factors associated with an early reduction in global CBF.

77 patients with severe head injury who underwent measurement of CBF using xenon enhanced computed tomography (xCT) within 12 hours after injury were included in this study. Global CBF, physiological parameters at the time of CBF measurement, and outcome measures were analyzed.

Global CBF averaged 35.8 ± 16.4 ml/100g/min. Nine patients had an average global CBF < 18 ml/100g/min (11.8 ± 5.5 ml/100g/min); the remaining 67 patients had a global CBF of 39 ± 15 ml/100g/min. Initial ICP was greater than 20 mmHg in 90% of patients, and greater than 30 mmHg in 80% of patients in group with CBF < 18 ml/100g/min, compared to 33% and 16%, respectively, in the nonischemic patients. Mortality was 90% at the time of ICU discharge, and at 6 months post-injury in patients with CBF < 18. Mortality in the patients without global ischemia was 17.9% at discharge and 19.4% at 6 months after injury. In contrast, the major factor associated with a reduction in global CBF in the range of 18-40 ml/100g/min was the volume of tissue with rCBF < 18 ml/100g/min (i.e. the volume of regional ischemia).

In patients with CBF < 18 ml/100g/min, intracranial hypertension plays a major causative role in global ischemia. Treatment would most likely be directed at controlling ICP, but the early, severe intracranial hypertension probably indicates a very severe brain injury. For levels of CBF between 18 and 40 ml/100g/min, the presence of regional ischemia was a more important factor in reducing global CBF.

P288. SERIAL QUANTITATIVE PROTON SPECTROSCOPIC FINDINGS IN SEVERELY BRAIN INJURED PATIENTS CORRELATE WITH OUTCOMES
Lori Shutter*, Karen A. Tong, Austin Collahan, Barbara A. Holshouser. (Loma Linda University Medical Center, Loma Linda, CA, US).

Intro: Proton magnetic resonance spectroscopy (MRS) is a sensitive non-invasive technique used to measure changes in brain metabolites. The purpose of this study is to quantify serial metabolite changes following severe traumatic brain injury (TBI) to determine if MRS is useful to predict long-term outcome.

Methods: We prospectively studied 42 patients, aged 14 to 79 (mean 34 years), who sustained severe TBI (initial Glasgow Coma Score ≤ 8). Using 6-8 local MRS sites, MRS was obtained within 16 days after injury and repeated at 6 months (n = 32). Two single voxel spectra were acquired in normal appearing brain: one in occipital gray matter (GM) and a second in parieto-occipital white matter (WM). All spectra were processed using the LCMRv2 technique to quantify peak areas for N-acetylaspartate (NAA), creatine (Cr), choline (Cho), glutamate/glutamine (Glx) and myo-inositol (Irn). Peak area metabolite ratios were also calculated. MRS data were correlated with the Glasgow Outcomes Score (GOS) at 6 months following injury. Patient outcomes were divided into two groups: favorable outcome (good recovery and moderate disability) and poor outcome (severe disability, vegetative state and death).

Results: Results of logistic regression analysis on initial MRS data showed that elevated GM Cho (p < 0.05), WM Cho (p = 0.01) and GM Glx (p < 0.01) quantitative levels are significantly correlated with poor outcomes with odds ratios of 5.4, 2.2 and 5.8 respectively. Elevated GM and WM Cho/Cr, and decreased WM NAA/Cho also showed significant correlation with poor outcomes (p < 0.01). Follow-up MRS showed continued NAA decline and persistent Cho and Irn elevation in the poor outcome group. Linear discriminant analysis found that GM and WM NAA, Cre and Cho correctly predicted 86% of outcomes. Combining MRS data with clinical information raised the predictive ability to 93%.

Conclusion: MRS correlates with long-term outcome, and provides additional information for patient management.
P289. ASSESSMENT OF FLOW VOLUME IN THE INTERNAL CAROTID ARTERY. CORRELATION WITH 133XENON CEREBRAL BLOOD FLOW
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Objective: Monitoring of cerebral blood flow in acutely brain injured patients is an essential component of critical care monitoring. The purpose of this study was to evaluate a new device coupling angle independent dual beam flow and digital Doppler technology (FlowGuard) for the assessment of the blood flow volume (BFV) in the carotid artery (ICA) in normal volunteers and acutely brain injured patients.

Methods: ICA-BFV (ml/min) and diameter (mm) in 30 healthy volunteers were measured by means of FlowGuard and duplex ultrasound and compared. Then BFVs in the ICA assessed by FlowGuard were compared with measurements of the cerebral blood flow (CBF, ml/100g/min) obtained using the clearance of Xe133 and AVD2O measurements. Sixteen CBF studies performed in eight acutely brain injured patients were compared to BFV rates in the ICA.

Results: BFV was satisfactorily recorded in 28 normal subjects (66.6% failure). ICA mean BFV was 277 ± 25 ml/min (range: 239-338) with a mean diameter of 5 ± 0.5 mm (range: 4.1-6.1). ICA-BFV proved to be significantly higher (N = 17, 284 ± 21 ml/min) in subjects younger than 35 years than in subjects older than 35 years (N = 11, 267 ± 25 ml/min, p = 0.003). ICA diameter measured by the FlowGuard correlated with the results of the Duplex ultrasound (r = 0.94, p = 0.0001). In head injured patients, BFV showed a strong correlation with global CBF measurements (r = 0.91, p = 0.0001) as well as hemispheric CBF; right r = 0.89, p = 0.0001, left r = 0.92, p = 0.0001. The mean error in CBF estimation by means of BFV was 13.6 ± 8.5% (range 3-31%). The BFV also correlated with the AVD2O, r = −0.51, p = 0.08 while CBF r = −0.39, p = 0.22.

Conclusions: This study showed that BFV measurements using the FlowGuard can be easily performed and implemented in the critical care environment. This preliminary study suggests that CBF can be estimated at patient's bedside with a reasonable accuracy.

P290. IMPROVED MRI DETECTION OF HEMORRHAGIC SHEARING INJURIES IN ADULTS USING SUSCEPTIBILITY WEIGHTED IMAGING (SWI): CORRELATION WITH SEVERITY AND OUTCOME

Purpose: To compare a new high resolution susceptibility weighted imaging (SWI) technique to conventional gradient recalled echo (GRE) imaging in the ability to detect hemorrhage suggestive of diffuse axonal injury (DAI), to determine severity of injury and to predict outcome after traumatic brain injury (TBI).

Material & Methods: Eleven patients were imaged early after trauma. A standard MR protocol was performed, including conventional gradient echo (GRE) imaging. A high resolution SWI technique was also performed using a 3D GRE sequence that incorporates post-processing to enhance signal loss from hemorrhage. Number and volume of hemorrhagic lesions demonstrated by both methods were compared. Extent of hemorrhage was also compared to initial Glasgow Coma Score (GCS) as well as Glasgow Outcome Score (GOS) at 1, 3 and 6 months after trauma.

Results: Hemorrhagic lesions on SWI were more visible than on conventional GRE. SWI detected approximately 3.8 times more lesions and 2.5 times more lesion volume than GRE. Number of SWI lesions had an inverse relationship with initial GCS. In addition, number of SWI lesions were related to severity of clinical outcome at 1 month: patients in a vegetative state (n = 3), with severe disability (n = 5), and with moderate disability (n = 3) had a mean number of 157, 77, and 51 lesions respectively. When dichotomized into two outcome groups, the number of mean lesions on the initial MRI study were consistently higher in the poor outcome group compared to the good outcome group, when assessed clinically at 1, 3, and 6 months after injury.

Conclusion: The SWI technique significantly improves visibility of hemorrhagic injuries. Extent of hemorrhagic lesions were related to initial GCS as well as severity of subsequent outcome, suggesting that SWI can improve diagnosis of hemorrhagic brain injuries and potentially predict outcome after TBI.

P291. CEREBRAL HEMODYNAMICS AFTER CORTICAL IMPACT INJURY IN THE eNos KNOCKOUT MOUSE
Alex B. Valadares, Roman Hutsky, Leela Cherian, J. Clay Goodman, Claudia S. Robertson. (Boyle College of Medicine, Houston, Texas US).

Nitric oxide (NO) generated by endothelial nitric oxide synthase (eNOS) plays an important role in regulating basal cerebral blood flow (CBF). The purpose of this study was to investigate the role of eNOS in maintaining CBF after cortical impact injury (CCI).

Three groups of animals were studied: wild-type controls (C57BL6) (WT; n = 12), eNOS-deficient mice (eNOS/−; n = 12), and eNOS-deficient mice treated with L-arginine (eNOS/−Arg; n = 12). The mice were anesthetized with isoflurane, intubated, and mechanically ventilated prior to CCI (3 m/sec, 1.5 mm deformation). Five minutes after injury, saline was administered to the WT and eNOS/− groups; L-arginine (300 mg/kg) was administered to the eNOS/−Arg group. Arterial blood pressure (ABP), intracranial pressure (ICP), and laser Doppler-CBF (LD-CBF) at the impact site were monitored for two hours after injury.

Baseline ABP was significantly higher in eNOS/− animals than in WT animals (89 ± 10 mm Hg vs 69 ± 6 mm Hg, p < 0.001) and remained higher even after injury (p of group effect < 0.001, time effect < 0.001, group × time effect < 0.001; two-way repeated measures ANOVA), with no difference between eNOS/− and eNOS/−Arg groups. Although baseline ICP was the same in all groups, ICP after injury was significantly higher in the eNOS/− animals (group < 0.001, time < 0.001, group × time < 0.001). Immediately after CCI, LD-CBF decreased to 34 ± 13% of baseline in WT animals and 19 ± 11% of baseline in eNOS/− animals. LD-CBF was consistently lower in eNOS/− animals compared to WT animals (group < 0.001, time < 0.001, group × time < 0.001), with no effect of L-arginine administration.

NO generated by eNOS plays an important role in the maintenance of CBF following CCI. Absence of eNOS prevents L-arginine from effecting an improvement in CBF after CCI.

P292. BEHAVIORAL DEFICITS FOLLOWING LATERAL FLUID PERCUSSION INJURY IN THE RAT PUP DUE TO CELLULAR DYSFUNCTION AND NOT CELL DEATH
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Traumatic brain injury (TBI) is the number one cause of pediatric death and disability in the United States. Surprisingly, research into the mechanism of development post-TBI pathophysiology is lacking. Evidence in several adult models of TBI suggests that hippocampal cell death and behavioral dysfunction are correlated. Following a mild percussion injury (FPI), however, dysfunction can be detected without obvious neuronal death. Elevated glutamate is well characterized during the acute phase following FPI in developing rats. The CA3 region of the hippocampus, having the highest glutamate receptor density and an elevated resting membrane potential, is particularly susceptible to glutamate excitation and excitotoxic neuronal death.

To test the hypothesis that deficits following FPI in post-natal day 19 pups are not related to neuronal loss, stereological cell counts were performed in the CA3 region. Estimation of the total number of cells in CA3 of injured (n = 8) and control (n = 3) pups two weeks following injury revealed no difference between groups (260285 ± 62982 and 254229 ± 60684 respectively). Similarly, within individual animals there was no difference in neuronal number between the ipsilateral and contralateral CA3 (260285 ± 62982 and 245233 ± 48695 respectively). This data supports the hypothesis that FPI in the developing rat leads to cellular dysfunction and indicates that at least some of the deficits reported following TBI are not related to cell death.
P293.
CELLULAR LOCALIZATION AND ALTERATIONS OF INHIBITORS OF APOPTOSIS AFTER TRAUMATIC BRAIN INJURY.
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Regulation of cell type expression of inhibitors of apoptosis (IAP) was examined in the normal rat brain and in brains subjected to moderate traumatic brain injury (TBI) using paraxial fluid-percussion brain injury model (1.7-2.2 atm). Immunohistochemistry combined with confocal microscopy were used to determine distribution and cell type expression of XIAP and cIAP-1. Quantification of XIAP-positive cells in the hippocampus of normal and traumatized rats was performed by stereological techniques. XIAP was present exclusively in neurons and localized to the perinucleus region and cell soma. cIAP-1 was expressed in processes and in the cell soma of large neurons in cortical layer IV, whereas hippocampal and thalamic neurons demonstrated differential cellular expression. Some oligodendrocytes in the corpus callosum expressed cIAP-1, but this IAP was undetectable in other glial cells. Traumatized brains showed dramatic redistribution and alterations in cellular and regional expression of XIAP and cIAP-1. XIAP immunoreactivity decreased significantly (P < 0.001 vs sham) in both hemispheres early after injury. By 24 hours the levels of XIAP increased significantly (P < 0.001 vs 1 hr and 6 hr groups), but did not reach those of sham controls. In contrast, cIAP-1 expression increased immediately after injury in neurons located primarily around the lesion epicenter. Astrocytes in the cortex and hippocampus showed robust cIAP-1 immunostaining. Expression arrays (GEArray, Superarray, Bethesda, MD) of apoptotic genes demonstrated increased mRNA expression of cIAP-1, cIAP-2, tumor necrosis factor receptors-1 and -2, casper (FLICE-like inhibitory protein) at 24 hours after TBI. Our data demonstrate that in the normal adult rat brain XIAP and cIAP-1 are expressed in a lineage-specific manner and in different brain regions. TBI induces redistribution and alterations in levels of mRNA and proteins in the apoptotic and anti-apoptotic pathways that may contribute to the pathophysiology after injury. Supported by AHA Grant 0215133B to G.L., and by NS 20591-10 to W.D.D.

P294.
THE EXPERIMENTAL OBSERVATION ON DELEY NEURAL DEATH AFTER ACUTE BRAIN TRAUMA.
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OBJECTIVE: In order to observe the phenomenon of delayed neuronal death. METHOD: These experiments were mainly based on rat diffuse brain injury model. Cultured newborn rat hippocampal neurons were tested for delayed neuronal death in vitro. The conditions of tissue and cell injury were observed under the microscopy and electron microscopy. The neuronal DNA injury in cortex and hippocampus was observed by DNA-Ladder and TUNEL stain. RESULT: Histological examination showed that the neurons presented degenerative changes; electronmicroscopy examination showed that the neuronal apoptosis and necrosis would be seen in the cortex and hippocampal, most serious on 24 hours. DNA Ladder would be seen and most distinct on 24 hours after severe injury. Mostly neurons with positive TUNEL stain represent neuronal apoptotic change. In vitro, 24 hours after neuron thrust injury, the neuron died by necrosis; some neurons died by apoptosis when the normal neuron cultured by the culture liquid from the injury neuron. CONCLUSION: Delayed brain injury happened after acute brain injury due to lot of second injury factors, and mostly exhibited by necrosis and apoptosis, given priority to apoptosis.

P295.
AGE-DEPENDENT RESPONSE TO SCALED CORTICAL IMPACT IN THE PIGLET
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Introduction: To investigate whether maturational stage influences the brain's response to mechanical trauma, a scaled cortical contusion model was developed which delivers a rapid volume of indentation proportional to changes in brain mass and dimensions with growth. Piglets of three different ages at injury were studied using serial magnetic resonance imaging (MRI) scans, histology, and immunohistochemistry. Methods: Anesthetized piglets at 5 days (infant), one month (toddler), and four months of age (adolescent) underwent craniectomy, dural opening, and direct frontoparietal cortical impact with the scaled indentation device. Injury magnitude was chosen to cause a clinically asymptomatic but visible lesion. Serial MRI studies at 24 hours, 7 days, and one month post-injury were obtained. Histology and immunohistochemistry at 6 hours, 7 days, and one month post-injury were performed to investigate processes relevant to cell death, repair, and regeneration. Lesions were compared among ages by expressing size as a ratio between the lesion and the contralateral uninjured hemisphere. Results: Lesions involving the cortex, white matter, and periventricular region were seen on histology and MRI. Despite comparable injury inputs, at seven days post-injury, histologic lesions were smallest in the youngest subjects, intermediate in the "toddler" age group, and largest in the adolescents. Differences in the time course and magnitude of swelling were seen on MRI, with the youngest (infant) subjects having the earliest peak, the "toddler" group having the most marked swelling, and the adolescent age group having the latest peak. Age-dependent differences in repair and regeneration processes were also seen, including generation of new cells in the subventricular zone. Conclusion: The response of the gyrencephalic brain to focal mechanical trauma differs with age at injury. These differences have implications both to clinical care and to strategies for influencing cell death pathways and in promoting repair and regeneration after traumatic brain injury.

P296.
DIFFERENCES IN ICP-AND CARDIOVASCULAR RESPONSE IN DEVELOPING VERSUS ADULT RATS FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY.
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Objective: Diffuse brain swelling and malignant brain edema following traumatic brain injury is a unique feature in the pediatric age group. In the present study the influence of diffuse traumatic brain injury on intracranial pressure (ICP) changes and cardiovascular response is investigated in developing rats and compared to findings in adult rats. Methods: Diffuse brain injury was produced in intubated and ventilated 19-23 days old Sprague-Dawley rats (N = 8) using a modification of the Marmarou-model (1.5m/100g). Mean arterial blood pressure recordings and intracranial pressure recordings were performed osseously. The results were compared to readings in adult animals (N = 10) subjected to a 1.5 m/500 g injury. Results: In the developing rat MABP decreased from 77.1 ± 16.8 mmHg to 50.9 ± 28.5 mmHg immediately following injury. Within 3 min it started to recover without reaching base-line values within one hour (56.5 ± 17 mmHg). No significant ICP-increase was determined and mortality rate was 50% within one hour following injury. In the adult rat only a minor decrease of MABP was determined 3 min following injury (from 139.5 ± 9.3 mm Hg to 119.5 ± 11.5 mm Hg) reaching base line levels within 15 min. All adult animals recovered following trauma with no relevant ICP-increase within one hour post-trauma. Conclusions: The results of the present study indicate a pronounced affection of the brainstem in developing versus adult rats resulting in a higher mortality. These findings may contribute to the different response of the young brain to traumatic brain injury.
P297. MICROARRAY ANALYSIS OF CELL TRAFFICKING GENES AFTER TRAUMATIC BRAIN INJURY: THE EFFECTS OF HYPERthermia AND HYPERthermia
T Suzuki*, JS Truettner, OF Alonso, WD Dietrich. (Department of Neurological Surgery, Neurotrauma Research Center, The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL USA).

Several lines of evidence suggest a pathogenic role of inflammation in brain trauma. Recent data have reported the importance of temperature on the early accumulation of polymorphonuclear leukocytes (PMNL) after traumatic brain injury (TBI). Mechanisms underlying these temperature effects remain to be clarified. The purpose of this study was to utilize expression arrays (GEArray, Superarray, Bethesda, MD) of cell trafficking genes to screen for changes in expression of these genes after TBI. In addition, the effects of post-traumatic hypothermia (33°C) and hyperthermia (39.9°C) were investigated. Male Sprague-Dawley rats underwent moderate fluid percussion brain injury (1.8 to 2.2 atmospheres). Four hours after injury, the injured cortex was removed and total RNA extracted. 32P labeled cDNA probes were generated by reverse transcriptase and hybridized to the arrays. Following exposure, signals were analyzed by Phosphoimager. Expression of the genes on the array was then compared between the various experimental groups. At 3 hrs after TBI, a number of genes were shown to decrease compared to sham operated controls. For some genes, post-traumatic hypothermia appeared to alleviate the injury-induced decrease in gene expression. These include Ncam2, NCAM, Lamb1, integrin β, cathepsin B, collagen β2, caveolin, catenin β, and basigin. In contrast, post-traumatic hyperthermia appeared to increase gene expression of integrin β, cathepsin B and collagen β2 compared to normothermic levels. Finally, expression of some genes was increased by both hypothermia and hyperthermia compared to normothermic TBI including tenasin C, Timp1 and CD44. Taken together these results suggest that microarray analysis of gene expression may be useful in elucidating the molecular mediators of inflammation after TBI and clarify specific genes that are sensitive to post-traumatic temperature manipulations. NS42133 and NS30291.

P298. EXPRESSION OF P2 PURINERGIC RECEPTORS IN RAT CORTEX AFTER MODERATE TRAUMATIC BRAIN INJURY.

Extracellular levels of ATP are increased after brain trauma. This increase in ATP release is thought to be involved in the initiation of reactive gliosis via stimulation of ATP P2 purinergic receptors. However, little is known about the expression of these receptors after traumatic brain injury (TBI). In the present study, we have investigated the expression of metabotropic P2Y and ionotropic P2X receptor subtypes in rat cortical tissue 1 (n = 9), 3 (n = 8), and 7 (n = 9) days after TBI or sham (n = 8) procedures. Fluid percussion brain injury (1.8–2.1 atm) was produced in anesthetized Sprague Dawley rats. At various periods after TBI rats were killed and cortical samples for ipsilateral and contralateral hemisphere dissected and frozen for analysis. Messenger RNA (mRNA) levels of P2Y1, P2Y2, P2Y4, P2X1, P2X3 and P2X7 were measured either with ribonuclease protection assay (RPA) or relative quantitative reverse transcription-polymerase chain reaction (RT-PCR). P2 receptor expression in cortical tissue from the injured side was compared with the uninjured side. After moderate TBI, there were no statistically significant changes in the mRNA levels of the P2Y receptor subtypes studied (P2Y1, P2Y2, P2Y4), although an increase in P2Y1 and P2Y2 was observed at day 3. For the P2X subtypes studied, a significant increase in expression of P2X1 and P2X7, but not P2X3, receptors was observed at day 1. These results indicate that the expression of distinct P2X receptor subtypes is increased by moderate TBI and suggest that this response may be involved in reactive gliosis in vivo.

P299. THE INFLAMMATORY RESPONSE AFTER TRAUMATIC BRAIN INJURY IN MALE AND FEMALE RATS AS ASSESSED BY cDNA ARRAYS
JS Truettner*, OF Alonso, WD Dietrich, HM Bramlett (Department of Neurological Surgery, the Neurotrauma Research Center, and Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL USA).

Pro-inflammatory cytokines such as TNF-α, and IL-1β have been shown to increase rapidly after traumatic brain injury (TBI) and to be involved in mediating the inflammatory response. However, it is unknown whether there are gender differences in the inflammatory response after TBI. In this study, inflammatory response cytokine expression arrays (GEArray, Superarray, Bethesda, MD) were used to screen for changes in expression of 23 known genes in the inflammation pathway. The use of a gene array specifically targeting components of the inflammatory cascade provides a useful tool for determining future targets of this cascade for therapeutic intervention.

Male and female Sprague-Dawley rats underwent moderate fluid percussion brain injury (1.8–2.2 atm). Three hours later, the injured cortex was removed and total RNA extracted. 32P labeled cDNA probes were generated by reverse transcriptase and hybridized to the arrays. Following exposure, signals were analyzed by Phosphoimager. Expression of the genes on the array was compared to a sham operated male.

Members of the interleukin superfamily (including IL-1α, IL-1β, IL-2, IL-6, IL-10), transforming growth factor family members (TGF-α, TGF-β1, TGF-β2, TGF-β3), chemokine GRO-1, transcription factor MEF-1, and lymphotixin B (LT-β) were upregulated in the TBI animals as compared to sham controls at 3 hours after TBI. In reference to potential gender differences, TGF-b1 and MEF were higher and IL-2 and LT-β lower in traumatized females versus males. Traumatic brain injury initiates a complex inflammatory cascade that involves the increased expression of many different genes. Whether these responses act beneficially, detrimentally, or in combination remains to be elucidated. The time course of this response as well as the influence of gender on the expression of these and other genes is being studied. Supported by NIH grants NS42133, NS30291, and Eli Lilly & Co.

P300. INTERLEUKIN-16 RELEASE FROM CD8-POSITIVE T LYMPHOcyTES FOLLOWING TRAUMATIC BRAIN INJURY.
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Interleukin-16 (IL-16) is expressed in a number of pathological conditions, including autoimmune disease and infection with human immunodeficiency virus. IL-16 induces the chemoattract and regulates the activation of CD4 positive cells. Using flow cytometry to detect intracellular cytokine and enzyme-linked immunosorbent assay to quantitate cytokine in patient plasma, we report that IL-16 is released from pre-formed intracellular depots by peripheral blood CD8-positive T cells immediately after severe traumatic injury. Both the extent of T cell release of IL-16 and peripheral blood IL-16 levels were found to be greater in more severely-injured patients, particularly those suffering a traumatic brain injury. In addition, the kinetics of IL-16 release was found to coincide with a transient decrease in peripheral blood CD4/CD8 T cell ratio. Release of IL-16 from CD8-positive T lymphocytes in vitro could be demonstrated by culture of normal donor peripheral blood leukocytes with epinephrine. T lymphocyte function is known to be compromised after severe traumatic injury, presumably to limit the adaptive immune response to self tissues. Release of IL-16 by peripheral blood CD8-positive T cells may be a mechanism to regulate CD4 T lymphocyte functional activity in the post-traumatic peripheral circulation.
P301.
S100 BETA PROTEIN RESPONSE IN ASTROCYTES AFTER HUMAN BRAIN CONTUSION
Gilberto Oehman da Silva*, Gerson Chadi, Almir Ferreira Andrade and Raul Marino Jr. (Division of Neurosurgery, Hospital das Clínicas, University of São Paulo Medical School, São Paulo, São Paulo Brazil).

S100 beta protein, a calcium-binding protein, present mainly in astrocytes, that exerts paracrine trophic effects of several neuronal populations. After human brain injury serum concentration of S100 beta increases. However, there is not direct information about the S100 beta protein response in the brain after Central Nervous System injury. Compressed brain tissue were obtained from 18 consecutive patients undergoing surgery for trauma contusion 6 hours to 6 days after trauma. S100 beta protein were analysed by immunohistochemistry. Twelve hours after trauma S100 beta protein was first detected in astrocytes and then increased progressively until 6 days, both in temporal and frontal contusions. Therefore, astrocytes can increase S100 beta protein expression after human brain contusion according to the serum concentration increase. The knowledge of trophic response after brain trauma can be helpful in development of new therapeutic approaches that can modulate glial and neuronal responses. Acknowledgements: Laboratory of Neuruggeneration, Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo. São Paulo, Brazil.

P302.
HYPOTHERMIA REDUCES THE ACTIVITY OF NF-KB AFTER PARASAGITTAL FLUID-PERCUSSION BRAIN INJURY.
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Post-traumatic hypothermia has previously been reported to attenuate the early inflammatory response to brain trauma. We investigated whether hypothermia exerts its effect by inhibiting the activation of the nuclear factor kappa B (NF-kB), a transcriptional activator that is essential to the expression of genes that are involved in the development of inflammation in the brain. Moderate traumatic brain injury (TBI) (1.8-2.2 atm) was induced in rats by a fluid percussion (F-P) device. In the first phase: intracerebral expression of NF-kB was studied using the electrophoretic mobility assay at 3- and 7-days after injury. In the second phase: rats underwent moderate F-P brain injury followed immediately by 4 hr of post-traumatic normothermia (37°C) or hypothermia (33°C) and were then killed. Ipsilateral and contralateral cerebral cortical regions were then assayed for NF-kB activation. Results indicate that on post-trauma days 3 and 7, the activity of NF-kB was increased in the ipsilateral cortex at 3-days after TBI and at both ipsilateral and contralateral cortices at 7-days after TBI. Post-traumatic hypothermia reduced NF-kB activity at both time points in ipsilateral and contralateral sites tested (p < 0.05). Post-traumatic temperature is a critical factor for determining NF-kB binding activity after brain trauma. Impairment of stimuluss-induced transcription factor activity may contribute to the reduced inflammatory response and the neuroprotective effects of early post-traumatic hypothermia. (Supported by NINDS # 1P50NS30291).

P303.
TRAUMATIC FRONTAL LOBE INJURY IN RATS CHRONICALLY AFFECTS T-MAZE ALTERNATION: EFFECTS OF CYCLOSPORIN A.
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Experimental injury of frontal lobes using a controlled cortical impact (CCI) model results in necrotic cavitation in frontal and medial frontal cortex, sparing hippocampus. In an effort to establish a cognitive assay sensitive to cortical damage in the absence of hippocampal damage, we tested Long Evans hooded rats in a working memory paradigm following frontal cortex damage. A T-maze alternation task was selected for its sensitivity to medial frontal cortex injury. Rats were pre-trained to run the alleys, and beginning a week after bilateral frontal lobe injury they were trained to alternate alley choice for a food reward (10 trials daily for a maximum of 24 days). Rats were required to meet or surpass a criterion of 90% alternation on 2 consecutive training days. Total trials-to-criterion and total errors-to-criterion were measured. Performance of CCI rats were significantly impaired when compared to sham controls; committing more errors and requiring more trials to learn the task. Our laboratory has reported significant neuroprotection with post-injury administration of Cyclosporin A (CsA). To determine if CsA treatment may affect recovery on this cortically dependent cognitive task, CCI rats were separated into 2 groups: CCI alone, and CCI + CsA (at a dose regimen previously shown to be neuroprotective in the CCI model). CCI + CsA treated rats required no fewer trials and made no fewer errors to criterion than CCI alone rats. We conclude that this task is sensitive to enduring cortical damage in the absence of hippocampal damage and is therefore a good candidate for inclusion in a battery of tests of cortically dependent cognition in the rat. Supported by NIH NS39828 & KSCIRKT #9-20.

P304.
LONGITUDINAL ANALYSIS OF THE DICHTOMIZED GLASGOW OUTCOME SCALE SCORE
Charles F. Contant*, Delvido Long, Steve Plath, H. Julia Hannay, (Baylor College of Medicine, Houston, TX US).

The Glasgow Outcome Scale score (GOS) is well known. It is often used as the outcome measure for clinical studies, including clinical trials. The purpose of these analyses was to examine factors related to the GOS as measured at three time periods, instead of at a single time point. We have used a method that is well known in the statistical literature, but not often applied in neuropsycha. The GOS was assessed at one, three and six months after injury. The following admission data were evaluated: age, pupillary reactivity, gender, presence of a gunshot wound, Marshall classification of the ER CT scan, and the ER motor component of the GCS. The GOS was dichotomized into two groups: Poor (Dead, PVS and SD) and Good (MD and GR).

A multi-level model was fit to the data. The logit of Poor outcome used as the dependent variable, where Poor outcome was assumed to follow a binomial distribution, resulting in a logistic regression like model. The predictors were all included as “fixed” effects, but a random baseline probability of being in the Poor outcome group was given to each patient. Data from 114 patients treated at Ben Taub General Hospital were used. In the initial analysis, age, gender and gunshot wound were removed from the model. The remaining variables were then evaluated using a Markov Chain Monte Carlo (MCMC) method. This is a simulation method for evaluating complex models of longitudinally measured dichotomous variables. Following 300,000 simulations, stability in the estimated coefficients was obtained, and these were evaluated. Pupillary reactivity, ERCT and ER GCS were found to be significantly associated with Poor outcome. A significant time effect was found. The random baseline probability was also significant. While computationally intensive, the MCMC can provide valid estimates for a dichotomy evaluated over time.

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P305.

ASSESSMENT OF TRAUMATIC AXONAL INJURY IN THE CORPUS CALLOSUM: A COMPARISON OF THE CORONAL AND SAGITTAL PLANES.

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Objectives: In conventional coronal sections of corpus callosum the automated assessment of axonal bulb size is complicated by the presence of filled axons. We have investigated axonal pathology in the sagittal plane to determine if this reduces the confounding factors for image analysis.

Materials and methods: Paraffin sections were cut in the coronal and sagittal planes from the corpus callosum of 7 cases of closed head injury (survival times from 12 hrs to 5 mths). Sections were immunostained using an antibody raised against amyloid precursor protein (APP). Digital images were captured and the size of axonal swellings determined using a previously developed algorithm.

Results: Assessment of the immunostaining in the sagittal plane revealed that the majority of profiles were round or oval in shape, with few axonal "tails". However, a new confounding factor was the heterogeneity of the staining intensity within individual axons, which posed a different set of problems for image analysis. Furthermore, quantitative analysis revealed no change in axonal diameter with increasing survival time in this small sample.

Conclusions: The use of sagittal sections of the corpus callosum does not confer any advantage over coronal sections in the measurement of axonal swelling size.

P306.

LONGITUDINAL ANALYSIS OF THE DISABILITY RATING SCORE FOLLOWING TRAUMATIC BRAIN INJURY

H. Julia Hannay*, Steve Plath, Delvada Long, Charles F. Contant. (Baylor College of Medicine, Houston, TX US).

The Disability Rating Scale (DRS) described by Rappaport has been suggested as a possible outcome measure for clinical studies, including clinical trials. The DRS is a thirty-point scale with death at one end (0) and no disability at the other (0). The purpose of these analyses is to evaluate the relationship of the DRS to the generally used predictors of outcome that are derived from studies using the Glasgow Outcome Scale (GOS) as the outcome.

The DRS was measured at one, three and six months following injury in 144 patients from Ben Taub General Hospital. Extensive quality assurance of the DRS and GOS was performed. The DRS scores were used as the dependent variable in a multilevel longitudinal linear model. The independent variables were age, pupillary reactivity in the Emergency Center (EC), gender, presence of a gunshot wound (GSW), the Marshall classification of the EC CT scan and the motor component of the GCS measures in the EC (ECCSME). Random effects of time were included in the model so that each patient had their own "slope" over time.

The model containing all the independent variables was fit, and those variables which were not significant at the p < 0.10 were removed and the model refit. Gender (p = 0.59), GSW (p = 0.44) and age (p = 0.37) were removed. The final model was highly significant. The effects of pupillary reactivity (p < 0.001), EC CT (p < 0.01) and ECCSME (p < 0.06) were retained in the model. These relationships are consistent with those found in multivariable logistic regression models using the GOS, and indicate the DRS may have utility as an outcome measure. The use of the longitudinal modeling with random effects provides increased statistical power and further insight into the changes over time.

P307.

GENDER SPECIFIC ACTIVITY AND FOOT-FAULT PERFORMANCE ON THE GRID TASK AFTER CASTRATIONS AND OVARECTOMIES


During locomotion on the grid-foot fault task, we have female rats to be significantly more active than their male counterparts. This increase in activity is correlated with an increased number of foot-faults during the trial. Previous literature suggests that the difference in activity may be hormonally mediated. In order to test this hypothesis we took male and female Long-Evans rats, approximately 100 days of age and performed castration procedures on male rats and ovariectomies on female rats. Additional subjects were used as normal male and female controls. After a 30-day recovery period the subjects were tested on the grid-foot fault task along a regular two-month testing schedule. During the sixty-second videotaped trial the total activity, as measured by line crossings, and number of footfaults per limb were quantified. Results of line-crossing activity showed female rats, either normal (Mean of summed activity 46.8) or operated (Mean sum 46.8), to be more active than normal male (Mean sum 35.5) or operated male (Mean sum 42.6) counterparts. For left forelimb fault performance, normal females showed mean sum of 15.0, and normal males 8.5. Operated females presented a mean sum of 18.8 and operated males 18.0. Right forelimb fault performance was similar, with normal females presenting a mean sum of 14.4 and normal males 9.5. Operated females presented a mean sum of 18.8 and operated males 13.6. The data show a trend for operated males to show a slight increase in activity compared to normal males, and operated subjects, both male and female, to trend towards greater forelimb fault performance on the grid task. Supported by a research grant from the New York College of Osteopathic Medicine.

P308.

CEREBRAL AMYLOID ANGIOPATHY AND TRAUMATIC BRAIN INJURY: THE EFFECT OF APOE EPS GENOTYPE

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Objectives: Possession of APOE e4 is associated with poor outcome after traumatic brain injury (TBI). Cerebral amyloid angiopathy (CAA) is characterised by the accumulation of Aβ peptide in the walls of cerebral blood vessels. The major clinical manifestation of CAA is spontaneous intracerebral haemorrhage for which e4 is also a risk factor. We hypothesised that e4 carriers have worse outcome after TBI in part due to CAA related susceptibility to haemorrhage.

Materials and methods: We have determined the frequency of CAA and the extent of the haemorrhagic pathology in relation to APOE genotype in 88 autopsy cases of TBI. Results: CAA was present in 7 of 40 e4 carriers compared with 1 of 48 non-e4 carriers (p = 0.021) with 6 (4 with CAA) of the 40 e4 carriers being homozygotes. There was also a tendency for patients with CAA to have more severe contusion injury (median contusion index 19 versus 14.5, p = 0.23).

Conclusions: Among head-injured patients (i) CAA occurs predominantly in APOE e4 carriers (ii) patients with CAA tend to have more severe contusions and (iii) this association may explain in part why e4 is associated with poor outcome after head injury.

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P309.
TETRAHYDROBIOPTERIN AND L-ARGININE AFTER CORTICAL IMPACT INJURY IN RATS
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This study compared the effects of tetrahydrobipterin (BH4), an essential co-factor for nitric oxide synthase (NOS), and L-arginine on cerebral blood flow after severe controlled cortical impact injury (CCI) in rats.

Fasted Long Evans rats were anaesthetized with isoflurane and subjected to severe (5 mm/sec, 3 mm deformation) CCI II. Rats received L-arginine (300mg/kg), BH4 (10mg/kg), or saline 5 min after injury. Laser Doppler flow (LDF) and nitric oxide (NO) were measured at the impact site for 2hr after injury.

In saline-treated rats, LDF decreased to 28% of preinjury values and NO decreased by 20+3.8nM. L-arginine increased LDF to 65% of preinjury levels. BH4 augmented LDF to 75% of preinjury values. Both L-arginine and BH4 normalized tissue NO concentrations.

Since both L-arginine and BH4 restore tissue NO levels after CCI, this study suggests that uncoupling of NOS contributes to the low tissue levels of NO that occur in the contused tissue. An increase in CBF accompanies the restoration of normal NO levels suggesting that the low NO levels have a role in the secondary ischemia that occurs in contused tissue.

P310.
A META-ANALYSIS TO DETERMINE THE SIGNIFICANCE OF SKULL FRACTURE AS A RISK FACTOR FOR INTRACRANIAL PATHOLOGY IN THE PAEDIATRIC POPULATION
Joel Deason*, John Batchelor. (Manchester Royal Infirmary, Manchester, Manchester UK).

Objectives: Triage of minor head injuries in children in the UK relies heavily on the skull radiograph. This has been largely replaced by Computed Tomography in the USA. We sought to perform a meta-analysis of the paediatric literature to assess the significance of skull fracture and intracranial pathology (ICP).

Methods: The literature was searched using Medline, Embase and the Cochrane Database. Reference lists were cross checked. Once all papers had been searched a common odds ratio was determined.

Results: 12 papers (see poster for references) were identified as satisfying criteria for inclusion in the meta-analysis. Data was extracted from these papers relating to either positive CT scan or positive ICP and the presence of a skull fracture. An Odds Ratio was calculated for each paper and a common odds ratio was calculated using the Mantel-Haenszel test with a pooled estimate.

The Pooled results of the papers gave a total sample size of 12,684. The common odds ratio was 2.151 (1.85 to 2.48) therefore suggesting a positive correlation between skull fracture and intracranial pathology.

The pre-test probability of intracranial pathology was 7.2%. After a SXR the post-test probability of intracranial pathology if skull fracture was not found was 4% and if a skull fracture was found the post-test probability was 16%.

Conclusions: There is a significant correlation between skull fracture and intracranial pathology shown in the literature but due to the low incidence of intracranial pathology the finding of a fracture only increases the probability of ICP from 7.2% to 16%, which in our opinion is of little use in the triage of children with minor head injury.

We are currently completing a prospective study of 22,000 children with head injury in the UK to provide an alternative decision rule to replace the SXR based protocol.

P311.
EFFECTS OF DIETARY CREATINE ON NEUROCHEMICAL MAKERS OF SECONDARY INJURY FOLLOWING TRAUMATIC BRAIN INJURY
H.S. Dillon*, L.M. Benjamin, T.J. Carbery, S.W. Schoff. (Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY USA).

Biochemical alterations following traumatic brain injury (TBI) include lactate acidosis and phospholipid degeneration, leading to the generation of free fatty acids (FFA) and lactic acid, markers of cellular injury. We have previously shown that animals fed a creatine (Cr) enriched diet demonstrate enhanced neuroprotection following TBI. To further characterize the neuroprotective qualities of dietary creatine we studied neurochemical changes in cortex and hippocampus following a moderate injury. Adult rats were fed either a control or Cr-supplemented diet (0.5%, 1%) for 2 weeks prior to TBI. At 30 min or 6 hr after injury, animals were subject to in situ brain freezing and tissue processed for levels of lactate and FFA. At 30 min post TBI, lactate was significantly increased in all tissues ipsilateral to the injury. Animals fed Cr-diet had significantly lower levels although elevated compared to sham controls. Accumulation of FFA was also significantly diminished at 30 min post injury in Cr-diet animals and in many cases not significantly different from sham controls. At 6h post-injury, levels of lactic acid were significantly elevated following TBI. Creatine-fed animals showed less lactate than animals fed the control diet. Levels of FFA were significantly higher at 6h in the control diet animals with Cr-diet animals less than control diet but above sham levels. In most regions, animals fed a 1% Cr-diet demonstrated lower levels for both lactate and FFA than animals fed a 0.5% Cr-diet. These results support the idea that a Cr-enriched diet can provide substantial neuroprotection in part by suppressing the accumulation of lactic acid and FFA. The fact that a 1% Cr-diet was more protective and a 0.5% diet suggest a possible dose response intervention. Supported by NIH NS39828 and KSHIRT #0-20.

P312.
The Pitfall of Brain Hypothermia Management in Neuro-Trauma Patients
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Purpose: The effectiveness of hypothermia to the brain injury has been demonstrated in many experimental animal models. However, clinical trials of hypothermia for severe brain injury are still controversial. Why these different results occur? Without understanding of these mechanisms, the clinical hypothermia treatment will be fail. We have studied about pitfall on the brain hypothermia treatment in experience of 11 years, retrospectively.

Clinical studies: One hundred fifty cases of GCS < 6 neurotrauma patients were treated by brain hypothermia. All of these patients were cared with monitoring of brain tissue temperature, internal jugular venous temperature, core temperature, 5O2, cardiac output, oxygen delivery and extraction ratio, ICP, BBB dysfunction (CSF/serum albumin ratio < 0.01), and hemoglobin function. The brain hypothermia, in 20 cases, managed with monitoring of brain tissue glutamate, lactate, glucose, and glyceral by micro-dialysis technique and also hypothermia-pituitary adrenal (HPA)-axis neuro-hormones changes in CSF and blood were studied. The pitfall of brain hypothermia was focused all of brain hypothermia management.

Results: The brain injury mechanism is not similar with human to experimental animal models in severe brain injury. The 20-100 times severe catecholamine surge was recorded that was not observed anesthetized experimental animal models. This excess stress to HPA axis produced more than 250mg insulin resistance hyperglycemia, and no effect of oxygen inhalation by difficulty release of oxygen from binding hemoglobin with reduced hemoglobin DP0. Therefore, normal control of ICC, CPP, CBF and PaO2 is not enough management. The precise control of serum glucose between 30-140mg/dl prevents these pitfall. Especially, metabolic shift from glucose to lipid at lower than 34A of brain temperature, produces more easily increasing of serum glucose increasing and brain tissue lactate. Uncontrolled serum glucose make much worse at 32-31A than 34.4A of brain temperature. The complication of pneumonia under presence of severe BBB dysfunction (CSF/serum albumin ratio < 0.01) fails the hypothermia treatment. Proinflammatory cytokines easy go into the injured brain tissue and produces uncontrollable increasing of neuron toxic glutamate. The prevent of immune dysfunction by intraventricular control of brain temperature between 32.34A, replacement of pituitary hormones, and control of serum albumin < 3.5g/dl are important. The 2 days short duration of hypothermia make stop of progression of brain damage thus restoration. The re-progression of injury mechanism at norming make much worse by over lapping of rewarming stress in severe brain injury. Without evidence of recovery of brain damage, rewarming is not indicated.

Conclusion: The management of brain hypothermia with control of above pitfalls produces excellent clinical results.
BONE MARROW STROMAL CELL TRANSPLANTED TO TRAUMATICALLY INJURED RODENT BRAINS MAY AID IN THEIR RECOVERY THROUGH PRODUCTION OF NERVE GROWTH FACTOR

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Recent reports demonstrated that bone marrow transplantation in brain parenchyma improved neurological outcomes in a rodent model of traumatic brain injury (TBI) (Neuroreport 12:559-63, 2001). We found that intraventricular injection of bone marrow stromal cells (BMSCs) in TBI mice reduced the brain lesion. However, the underlying mechanisms for the beneficial effects of BMSC transplantation are not clear. In this study, 4 groups of 5 ICR mice were used: 1) TBI with BMSC injection; 2) TBI with PBS injection; 3) sham-injury with BMSC transplantation; 4) sham-injury with PBS injection. BMSCs were harvested and cultured from the green fluorescent protein (GFP) transgenic mice and were transplanted (2 x 10^5/10 ul PBS/mouse) into the ipsilateral ventricle at 5th post contusion impact injured mouse. At 13 and 45 days post-transplantation, mice were euthanized, CSF was collected and frozen brain sections were processed for data analyses. By 45d, the transplanted BMSCs migrated to the boundary of the injured area. Histological analyses showed that tissue lesion volume was significantly (P<0.05) reduced in group1 compared with group2. NGF ELISA was demonstrated significantly (P<0.01) higher NGF levels in CSF samples from group 1 and 3 at either time point compared with group 2 and 4, respectively. We conclude that NGF production may contribute to the protection effects of BMSC transplantation in TBI mouse brains. Characterization of NGF producing BMSC is currently in progress. (Supported by NIH grant ROI-NSSS55032-05 and ATP grant 004949-0074).

EFFECTS OF HYPERTHERMIA AND ALKALIZING AGENTS ON BRAIN INJURIES IN RATS WITH ACUTE SUBDURAL HEMATOMAS

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Brain ischemia is the leading pathophysiological mechanism in the development of secondary brain damage after acute subdural hematoma (SDH). Hypothermia has been employed as an effective cerebroprotective treatment on brain injuries, but the control of the general condition is very difficult under hypothermia, and various severe complications have been reported. Cerebral acidosis in the ischemic area is one of the important factors augmenting the brain edema formation. Tri-(hydroxymethyl)-aminomethane (THAM) has been used as an alkalinizing agent for acidosis on brain injury and is reported to be effective. In the present study, we used a rat acute SDH model to assess the effect of mild (35 centigrade) hypothermia and THAM combined treatment on brain water content, brain ischemia, and blood-brain barrier (BBB) permeability at 4 hours after hematomia induction. Mild hypothermia did not significantly reduce the brain water content beneath the hematoma (79.5%) compared with normothermia (80.2%), but mild hypothermia combined with THAM presented a significant reduction (78.78%; P<0.01). Combined with mild hypothermia and THAM treatments significantly reduced the brain edema (31.3%) compared with normothermia and also reduced the brain water content beneath the hematoma (79.5%) compared with normothermia (80.2%). Furthermore, volume of Infarction at 24 hours after the hematoma induction (54mm³: P<0.01) was significantly smaller by the combined treatment compared with normothermia (70 mm³). The present findings indicate that mild hypothermia of 35 centigrade combined with THAM presents a potent cerebroprotective strategy. The protection of the BBB is one of the possible cerebroprotective mechanisms in this rat acute SDH model.
P317.
DIETARY CREATINE SUPPLEMENTATION ENHANCES COGNITIVE RECOVERY FOLLOWING EXPERIMENTAL RAT BRAIN INJURY

The food supplement creatine is neuroprotective against several different brain insults including, ischemia, oxidative stress and traumatic brain injury. In rats, chronic administration of creatine significantly reduces the extent of brain damage caused by a cortical contusion injury (CCI), possibly through stabilization of mitochondrial integrity. This study tested whether the tissue sparing effect of creatine is associated with enhanced functional recovery following brain injury. Adult male rats received dietary supplementation with 1% creatine for 2 weeks prior to a 1.5mm CCI injury, 2 weeks following CCI or in both the pre-and post-surgical intervals. Starting on the 15th day following CCI, the animals were tested in the Morris Water maze (MWM) for 4 trials per day, on 5 consecutive days. Following the last trial on the fifth day, the platform was removed and animals were re-tested. CCI caused significant impairment in MWM performance, which was attenuated by creatine when administered in the combined pre-and post-surgical intervals. Creatine enhanced performance in both the acquisition and probe test phases of testing. Creatine supplementation did not enhance cognitive performance in sham-operated animals. Following behavioral testing, brains were prepared for quantitative analysis of alpha 7 nicotinic receptor expression. 1.5mm CCI caused a significant reduction in the density of hippocampal and cortical alpha 7 nicotinic receptor binding, which was reversed by chronic creatine administration. These results indicate that the neuroprotective effects of creatine are associated with enhanced cognitive recovery following CCI. Attenuation of CCI-induced neurochemical changes may contribute to creatine-induced functional recovery. Supported by the Kentucky Spinal Cord and Head Injury Research Trust and NIH (NS39828 to SWS and NS42196 to JRP).

P318.
AGE RELATED EFFECTS OF ACUTE NMDA BLOCKADE ON FUNCTIONAL OUTCOME AFTER CONTROLLED CORTICAL IMPACT IN IMMATURE RATS

Rationale: Anti-excitotoxic strategies have been shown to paradoxically exacerbate neuronal death after traumatic injury in the developing brain. We sought to investigate the age-related effects of N- methyl-D- aspartate (NMDA) blockade on functional outcome after experimental traumatic brain injury (TBI) in the developing rat.

Methods: Using our contemporary models of controlled cortical impact (CCI), postnatal day (PND) 7 and 17 Sprague Dawley rats were injured (left, frontoparietal, 4 m/sec, 1.75 or 2.0 mm deflection, and 3 or 6 mm tip respectively) and treated acutely with a single dose of MK-801, 30 min pre-CCI, i.p. (0.25, 0.5, or 1.0 mg/kg) vs vehicle. Morris water maze (MWM) performance was then evaluated on post-injury days (PND) 11-17.

Results: In PND 7 rats, while a single, pre-injury dose of 0.25 mg/kg neither worsened nor improved MWM performance after CCI, higher doses of 0.5 and 1.0 mg/kg significantly worsened MWM performance. In contrast, in PND 17 rats, dose escalation from 0.25 to 0.5 mg/kg significantly improved MWM function as compared to vehicle; though this beneficial effect was lost with further dose escalation to 1.0 mg/kg.

Conclusion: Acute treatment of PND 7 rats following CCI with an NMDA antagonist adversely impacted functional outcome while NMDA blockade with MK-801 in PND 17 rats improved MWM function. These findings further support our hypothesis that there are critical age-related injury responses to therapies. Potential therapeutic modalities for pediatric TBI must be evaluated experimentally across a broad range of developmental ages prior to clinical trials.

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P319.
ENDOTHELIN-1 CONTRIBUTES TO AGE DEPENDENT G PROTEIN IMPAIRMENT AFTER BRAIN INJURY

Previous studies have observed that endothelin-1 (ET-1) concentration is elevated in CSF and contributes to impaired cerebral hemodynamics following fluid percussion brain injury (FPI) in an age dependent manner. This study was designed to characterize the effects of FPI on the vascular activity of two activators of a pertussis toxin sensitive G protein, mastoparan and mastoparan-7, as a function of age and the role of ET-1 in such effects in newborn (1-5 days old) and juvenile (3-4 weeks old) pigs equipped with a closed cranial window. Mastoparan (10-8, 10-6M) elicited pial artery dilation that was blunted more by FPI in newborns vs juvenile pigs (9 ± 1 and 16 ± 1 vs 3 ± 1 and 5 ± 1%, newborn; 9 ± 1 and 15 ± 1 vs 6 ± 1 and 9 ± 1%, juvenile). Similar results were observed for mastoparan-7 but the inactive analogue mastoparan-17 had no effect on pial diameter. BQ123 (10-6M), an ET-1 antagonist, partially restored impaired mastoparan dilation after FPI in the newborn but not in the juvenile (3 ± 1 and 5 ± 1 vs 7 ± 1 and 11 ± 1%, newborn; 6 ± 1 and 9 ± 1 vs 6 ± 1 and 10 ± 1%, juvenile). These data show that G protein activation elicits cerebrovasodilation that is blunted following FPI in an age dependent manner. These data suggest that ET-1 contributes to G protein activation induced dilator impairment post insult in an age dependent manner.

P320.
INCIDENCE AND PROGRESSION OF INTERCELLULAR CA2+ WAVES IN ASTROCYTES SURROUNDING AREAS OF MECHANICAL INJURY
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One possible avenue of communication between astrocytes during mechanical injury is through calcium waves, which are propagating increases in cytosolic calcium concentrations in cells. In this study, we examine the calcium waves that occur in astrocytes populations adjacent to regions of astrocytes subject to mechanical stretch. Astrocytes were cultured on flexible membranes and loaded with the calcium fluorescent dye fura-2. Cells were stretched at one of three magnitudes-low (1-2%), moderate (3-4%), and high (12-17%). Cells stretched at the low magnitudes experienced no significant increase in cytosolic calcium levels (p > .144) but were able to induce calcium waves in cells adjacent to the injury area, leading to a 1.5-2 fold increase in fluorescent ratio in the unstretched cells (p < .001). Cells stretched at higher levels undergo an ATP-independent calcium rise and also initiate calcium waves in the adjacent region. Apynase, which hydrolyzes ATP, attenuated the calcium wave in the unstretched region but had no effect on calcium changes in the stretched cells. The dramatic increase in astrocytic [Ca2+] at very low levels suggests that astrocytes can coordinate a response even under the mildest forms of injury, and pose and additional factor that can affect neuronal signaling following mechanical injury. Funds were provided by NIH NS 35712 and HD 41699.
P321
THE EFFECTS OF VITAMIN B3 (NICOTINAMIDE) ON BEHAVIORAL OUTCOME FOLLOWING BILATERAL FRONTAL CORTEX CONTUSION INJURY IN THE RAT.
M.R. Hoane* and S.L. Akstulewicz. (Brain Injury Laboratory, Department of Psychology and Program in Neuroscience, East Carolina University, Greenville, NC USA).

Previous studies have shown that administration of vitamin B3 (B3) in stroke models significantly reduced the size of infarction and improved functional recovery. The present study evaluated the effect of administration of B3 on recovery of function following traumatic brain injury (TBI), incorporating the bilateral medial frontal cortex contusion injury model. Groups of rats were assigned to B3 (500 mg/kg) or saline (1.0 ml/kg) treatment conditions and received contusion injuries or sham procedures. Drug treatment was administered 15 min and 24 h following injury. Rats were examined on a variety of tests to measure sensorimotor performance (bilateral tactile removal), skilled forelimb use (staircase test), and cognitive ability (reference and working memory) in the Morris Water Maze. Preliminary results indicated that administration of B3 following injury significantly reduced the behavioral impairments observed on the bilateral tactile removal test, but not on skilled forelimb use. The acquisition of reference and working memory tests was also significantly improved compared to saline-treated rats. Examination of the brains revealed that administration of B3 significantly reduced the size of the lesion compared to treatment with saline. In addition, examination of glial fibrillary acidic protein (GFAP) expression around the lesion revealed that B3 significantly reduced the number of GFAP+ astrocytes. Our results indicate that B3 administration significantly improved behavioral outcomes following injury, reduced the size of the lesion, and reduced the expression of GFAP. These findings suggest that B3 may have therapeutic potential for the treatment of TBI. This research was supported by an ECU Faculty Senate Research/creative Activity Grant.

P322
THE EFFECT OF AGE ON SENSORIMOTOR AND COGNITIVE RECOVERY FOLLOWING BILATERAL FRONTAL CORTEX CONTUSION INJURY IN THE RAT
L.A. Lasley*, S.L. Akstulewicz, and M.R. Hoane. (Brain Injury Laboratory, Department of Psychology and Program in Neuroscience, East Carolina University, Greenville, NC USA).

The elderly are one of the most at risk populations for sustaining traumatic brain injuries (TBI). However, the effect of age is rarely studied in animal models of TBI. The present study evaluated the effect of age on recovery of function following bilateral medial frontal cortex injury. Groups of young (2.5 months old) and old (~14 months old) rats received either bilateral frontal cortex contusions or sham procedures. The rats were tested on a variety of tests to measure sensorimotor performance (bilateral adhesive tactile removal test), skilled forelimb use (staircase test), and the acquisition of a reference and working memory task in the Morris Water Maze (MWM). Preliminary results indicated that bilateral frontal cortex injury produced significant impairments on the bilateral adhesive tactile removal test, staircase test, and on the acquisition of a reference and working memory task compared to sham controls. Aged rats that received cortical contusions were significantly impaired on the bilateral adhesive tactile removal test, staircase test, and on the acquisition of a reference memory task compared to young rats. There was no effect of age on the acquisition of the working memory task; however, the aged rats had received extensive pre-operative spatial memory training 4-5 months prior to injury. This pre-operative training may have prevented the acquisition impairment in working memory in the MWM. Our results indicate that aged rats respond to brain injury differently than young rats. An ECU Faculty Senate Research/creative Activity Grant supported this research.

P323
SECONDARY CEREBRAL ISCHEMIA-INDUCED CA1 HIPPOCAMPAL CELL DEATH: LATERAL VS CENTRAL FLUID PERCUSION INJURY IN RAT.
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Secondary insults after fluid percussion injury are known to exacerbate cell damage particularly within the hippocampus. Secondary cerebral ischemia has been shown to enhance cell death following central fluid percussion injury (CFPI). However, markedly different results have been reported following lateral fluid percussion injury (LFPPI) (Otto, J Neurotrauma 18:1131, 2001). To compare these models, male Sprague-Dawley rats were subjected to moderate CFPI or LFPPI and 8 min forebrain ischemia was carried out at 1h following injury. The animals were divided into the following 4 groups: (1) LFPPI + ischemia, (2) CFPI + ischemia, (3) LFPPI alone and (4) CFPI alone. Mean arterial blood pressure and blood gases were monitored. To assess the neuronal cell damage in the CA1 region, animals were sacrificed at 7 days following injury and the surviving cells were counted stereologically in cresyl-violet-stained sections. In all injured animals, secondary ischemia resulted in additional cell loss within the CA1 region of the hippocampus only when PC02 levels during the secondary insult were below 30 mmHg. Within these animals, CFPI+ischemia resulted in more cell death within the hippocampus compared to LFPPI+ischemia (number of surviving cells; Left: 71 ± 19 vs 131 ± 48, Right: 88 ± 32 vs 279 ± 34). Not surprisingly, LFPPI+ischemia resulted in more cell death within the ipsilateral hippocampus compared to the injury alone (number of surviving cells; 121 ± 48 vs 247 ± 29, p < 0.05). Secondary cerebral ischemia never resulted in more surviving cells compared to injury alone. Preliminary work indicates that the CFPI results in low levels of ATP in the CA1 region up to 2h. Additional ATP measurement using bioluminescence will confirm if secondary ischemia creates an additional loss of energy, which may explain the injury-induces vulnerability to secondary ischemia. [Supported by NS27544, NS30308, NS37363]

P324
CEREBRAL SPINAL FLOW (CSF) DYNAMICS IN PATIENTS WITH POSTTRAUMATIC HYDROCEPHALUS: PHASE-CONTRAST MRI DATA.
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Objective: to demonstrate the efficiency of phase-contrast MRI with cardiovascular for controlling results of ventriculoperitoneal shunting (CSF dynamics evaluation) in patients with open posttraumatic hydrocephalus and in the nearest postoperative period.

Material and methods: All MR-examinations were performed using high-field MRI before and after surgery: MR study included: T1-W1, T2-W1, phase-contrast MRI in sagittal orientation and across aqueduct. The values of linear and volume velocities of CSF in 3-d ventriculostoma and cerebral aqueduct were evaluated. We examined 11 patients with posttraumatic open hydrocephalus. All examinations were performed before and immediately after shunting, and during following 2 weeks in every 3-4th day.

Discussion: Results of dynamic examination of the patient with posttraumatic hydrocephalus: mean linear velocity amplitude (LVA) was 22.1 cm/sec before and 12.3 cm/sec after shunting (5.6 ± 0.7 cm/sec in norm). The mean CSF amounts in cerebral aqueduct, moving per one cardiac-cycle, stroke volume (SV) were 560 ml before surgery, 170 ml after surgery (56 ± 25 ml in norm). Clinically positive reaction was marked. In our experience CSF pulsation considerably exceeded the normal value (P < 0.001), thus making it possible to prognosticate favourable outcomes after surgery.

Conclusion: Phase-contrast MRI at the level cerebral aqueduct showed the decreasing of CSF pulsation after shunting for open posttraumatic hydrocephalus. These alterations remained for 2 weeks after surgery in all patients.
P325.
DIFFUSE AXONAL INJURY IN INTENTIONAL INFANT INJURY SYNDROME VICTIMS IS ACCOMPANIED BY EVIDENCE OF EXTERNAL TRAUMA TO THE HEAD

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Although intentional brain injury in infants was initially postulated to be due to violent shaking of the head, the need for head impact has recently been argued. The phrase "shaken baby syndrome" has been used frequently while others have preferred "shaken impact syndrome." We prefer "intentional infant injury syndrome" (IIS) since it does not presuppose a mechanical mechanism. Pathological findings in non-survivors of IIS commonly include subdural (SDH) and subarachnoid hemorrhages (SAH), bilateral retinal hemorrhages (BHR), cerebral edema and diffuse axonal injury (DAI) with minimal signs of external trauma. Antibodies to beta-amyloid precursor protein (b-APP) are rarely detected in DAI in adults and we sought to determine whether b-APP immunostaining might detect DAI in suspected IIS. Hospital records and police reports were used to identify abuse cases. Autopsies on 10 children documented external and internal evidence of head injury. IIS was suspected in 9 cases. One death was due to motor vehicle crash (MVC) and one was due to sepsis. B-APP immunostaining of selected brain regions was performed without knowledge of whether injury was intentional or not. b-APP staining was not observed in the MVC victim or in one suspected IIS victim with skull fracture. Little b-APP staining was found in the sepsis victim. Of the remaining 7 cases, b-APP staining of dystrophic axons and retraction bulbs was observed in corpus callosum and thalamus. All 7 victims presented with head or facial contusions, but without skull fracture. SDH and/or SAH and brain edema were seen in all cases. Bilateral RH was noted in 6 of 7 cases.

Conclusion: b-APP immunostaining can identify DAI in suspected cases of IIS. While the post mortem hallmarks of IIS were found, they were invariably accompanied by external evidence of abuse. This preliminary study suggests a positive correlation of DAI with external impact to the head.

P326.
QUANTIFICATION OF SECONDARY BRAIN DAMAGE AFTER CONTROLL-D CORTICAL IMPACT IN MICE


Introduction: The most important constituents of secondary brain damage from traumatic brain injury (TBI) with contusions are brain edema formation and delayed contusion expansion. In order to quantify secondary brain damage after the controlled cortical impact (CCI) model we investigated contusion volume and brain edema formation during the first 24 h after trauma.

Materials & Methods: Male C57Bl/6 mice (n = 48) were craniotomized and subjected to CCI (8 m/s, 1 mm). The craniotomy was closed thereafter. Brain water content and contusion volume were assessed 6, 12, 24, and 48h and 15 min. 6, 12 and 24h after trauma, respectively.

Results: Brain water content increased continuously from 78.1 ± 0.4% in sham operated animals to a maximum of 81.1 ± 0.7% (p < 0.05) in the ipsilateral hemisphere 24 h after CCI. No significant increase was detected on the contralateral side. The contusion was clearly demarcated already 15 min after trauma (19.4 ± 4.0 mm3); 6 and 24 h later the contusion increased to 131% (25.4 ± 3.1 mm3); p < 0.05 vs. 15 min) and 171% (33.2 ± 5.0 mm3 6 h) of its initial volume 15 min after CCI (100%), respectively.

Conclusion: The size of a cortical contusion is expanding significantly after closed head CCI. This secondary contusion expansion is paralleled by brain edema formation. Our data demonstrate on a quantitative basis that parenchymal loss in the vicinity of a cortical contusion is an ongoing process and amenable to therapy due to its delayed character.

P327.
ROLE OF BRADYKININ B2 RECEPTORS FOR SECONDARY BRAIN DAMAGE AFTER TRAUMATIC BRAIN INJURY IN MICE


Introduction: Bradykinin B2 receptors may be involved in the pathophysiology of traumatic brain injury (TBI). However, direct evidence is missing yet. In the present study we investigated contusion volume. Brain edema and functional outcome of bradykinin B2 receptor knockout (B2 K0) and wild type (WT) mice after experimental TBI.

Mice: B2 K0 and WT mice (n = 7 each) were subjected to controlled cortical impact injury (CCI: 8 m/s, 1 mm indention). Brain water content and contusion volume were assessed after 24 h and after 7 days. Hind paw misplacements during beam walking were counted daily 4 days before and 7 days after CCI. WT mice had a contusion volume 10 days after CCI.

Results: Deletion of the B2 gene resulted in a reduction of contusion volume by 33% as compared to WT mice (9.1 ± 1.4 mm3 vs. 13.5 ± 4.5 mm3; p < 0.02). In B2 KO mice cerebral water content 24 h after trauma was reduced from 81.1 ± 0.7% in WT mice to 79.6 ± 0.4% (−51%; p < 0.05). Functional outcome was significantly better on day 5 post trauma in B2 KO mice as compared to WT mice (6.7 ± 3.2 and 12.2 ± 5.8 foot misplacements, respectively (p < 0.05).

Conclusion: Bradykinin B2 receptors mediate brain edema formation, loss of brain parenchyma, and loss of function after TBI. Therefore the bradykinin B2 receptor might represent a good target molecule for the development of drugs against secondary brain damage after TBI in man.

P328.
INCREASED HIPPOCAMPAL CA3 VULNERABILITY TO LOW LEVEL GLUTAMATE ANALOGUE, FOLLOWING LATERAL FLUID PERCUSION INJURY


It is still debated whether high extracellular levels of glutamate are a cause or a consequence of secondary neuronal damage. We used a subletal dose of the glutamate analogue kainic acid (KA) to determine whether a secondary acute increase in neuronal activity exacerbates anatomical damage in vulnerable hippocampal regions following a mild lateral fluid percussion (LFP) injury.

KA (9mg/kg) was injected intraperitoneally in sham (n = 7) and LFP (n = 16) injured rats 1 hour following injury. An equivalent volume of saline was injected in LFP injured (n = 5) rats. Histological damage (7 days) in the dorsal hippocampus (CA3, CA4, and hilar regions) was assessed (LFP+KA; n = 8, LFP+saline; n = 5, sham+KA; n = 5, and naive; n = 3) by two dimensional cell count. Seizures were rated by Racine classification in the same subgroup. Hippocampal activation 15 minutes following KA injection was assessed by glucose metabolic rates (CMRglc; umol/100g/min) using [18F]-fluorodeoxyglucose in LFP+KA (n = 4) and sham+KA (n = 2) rats. Following FPI+KA the ipsilateral side exhibited a 62.7, 75.7 and 52.1% decrease in CA3, CA4 and hilar neurons respectively compared to naive rats. These CA3 and CA4 neuronal counts were also significantly decreased compared to LFP+saline and sham+KA groups. The contralateral Racine score in LFP+KA and sham+KA groups was 4 and 2 respectively (p < 0.015). CMRglc in CA3 following LFP+KA was 121.8 ± 3.9 (mean ± 5D) ipsilaterally and 71.5 ± 10.8 contralaterally (p < 0.0012). No changes were found in the BBB permeability measured by 14C-aminoisobutyric acid (AIB) in CA3, CA4 and hilar regions.

We conclude that the low level presence of kainic acid acutely after LFP dramatically increases the extent of hippocampal activation and induces a striking loss of ipsilateral CA3 pyramidal neurons. (NINDS 30306; NS02089).
P329.  
INTRACELLULAR CALCIUM SIGNALING IS PERTURBED IN ASTROCYTES AND MICROGLIA ISOLATED FROM HYDROCEPHALIC RATS.  

Hydrocephalus is often a secondary pathology associated with traumatic brain injury (TBI). Despite the prevalence of hydrocephalus in many neurological conditions, we know very little about the biochemical alterations in brain cells that lead to the development of hydrocephalus. In these experiments, mixed organotypic cultures of brain cells were isolated from 1-2 day old rat pups from either spontaneously hydrocephalic H-Tx strain or control Sprague-Dawley rats. Organotypic cultures consisted of a mixture of all brain cells, including astrocytes, microglia, and neurons. Using Fura-2 microspectrophotometry and high speed digital imaging, calcium-mediated signal transduction pathways were examined in astrocytes and microglia from H-Tx and control rats. Astrocytes in cultures prepared from H-Tx rats exhibited a dramatically increased intracellular free calcium ([Ca2+]) elevation in response to glutamate, as compared to control astrocytes, suggesting that glutamate-mediated [Ca2+] signaling is enhanced in H-Tx rats. Similar results were observed in microglia from H-Tx rats, in which the [Ca2+] elevation elicited by glutamate was also increased as compared to controls. Intracellular calcium signaling was associated with thapsigargin, which elicits release of calcium from intracellular stores in the endoplasmic reticulum, followed by capacitative influx of extracellular calcium. Astrocytes and microglia cultured from H-Tx rats displayed an increased thapsigargin-stimulated [Ca2+] elevation, as compared to controls, suggesting that the capacitative calcium signaling pathway is enhanced in hydrocephalus. These results provide insights into the signal transduction mechanisms activated in hydrocephalus and suggest potential targets for intervention. Supported by NS49449 and Wade Center at HRI.

P331.  
PATHOGENESIS OF "BRAIN LOW T3 SYNDROME" IN PATIENTS WITH SEVERE BRAIN INJURIES  

OBJECTIVES: A conceptualization of the crucial role of thyroid hormones in adult brain based on the discovery of the thyrogenic system and strict brain thyroid homeostasis (BTH) regulation has involved in recent years. The goal of this study was to evaluate changes in BTH (based on TSH, T4, T3, free T4, free T3, TBO) and to correlate changes with biochemical markers of tissue injury (protein S100, neuron specific enolase) and the acute inflammatory response (TNF-α, IL-1β, IL-6).

METHODS: Serum of thyroid function, tissue damage and inflammation in serum and CSF were evaluated by radioimmunoassays in 128 patients with traumatic brain injury (GCS <8) and in 75 patients with aneurysmal subarachnoid hemorrage (48% Hunt-Hess score III-V). Patients were evaluated in both the acute and chronic phases of injury.

RESULTS: A significant decrease in T3 (p < 0.001) was the most consistent finding across all patients especially during the periods of hemotoma expansion, ischemia/hypoxia, vasospasm and brain edema, as documented by CT, MRI, TCD and oximetry (SjO2) data. There was a direct correlation between T3 level and the patients' clinical condition (GCS, speech and motor function, presence of meningeal signs (p < 0.05). Low T3 levels correlated with increasing plasma and CSF cytokine, S100 and NSE levels (p < 0.01). T3 level normalization was strongly correlated with a favorable clinical outcome, ranging by Glasgow Outcome Scale and by Burdenko Psychiatric Assessment Scale (p < 0.001).

CONCLUSION: The changes of T3 levels indicate significant disturbances in BTH following severe brain injury. Good outcomes correlate with restoration of BTH as indicated by a normalization of FT3 and T3 levels. On the basis of these findings we propose the idea of 'Brain Low T3 Syndrome', which is independent from whole organism stress response. Besides our results, optimal T3 monitoring and corresponding hormonal therapy may contribute neuroprotective effect in the management of seriously brain injured patients.

P330.  
A COX2 INHIBITOR ATTENUATES CASPASE-3 ACTIVATION AND COX2 EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT  
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Understanding the details of neuronal "death pathways" may lead to new treatment paradigms in diseases from Alzheimer’s and Parkinson’s to central vascular accidents and traumatic brain injury (TBI). Neural cells are isolated from the periphery by the blood brain barrier, use limited substrates for energy, ankyrins and electrophysiological traits. Consequently, many genes expressed in the central nervous system function differently than in the periphery. Cyclooxygenase-2 (COX2) is one such gene that has recently become the subject of intense investigation. However, the role of COX2 in neural development and neuropathology has yet to be determined. Increased COX2 expression has been observed with TBI, cerebral ischemia, seizures, as well as chronic neurodegenerative conditions. We have found that DPU, a COX2-specific inhibitor, improves functional recovery in a rat model of TBI. In addition, this inhibitor protects neurons from glutamate-mediated neurotoxicity in cerebellar granule cell cultures. Our molecular findings indicate that glutamate receptors mediate COX2 mRNA induction in these neurons. We hypothesize that COX2 contributes to excitotoxic cell death following brain injury. Thus, a COX2 inhibitor should reduce cell death in vivo. Using the model of lateral cortical impact TBI, we show that treatments with DPU that improve behavioral recovery are neuroprotective in vitro and show an attenuation of caspase-3 activation. In addition, both IHC and immunoblot results indicate an attenuation of COX2 gene expression in brain regions associated with functional deficits following traumatic brain injury. This combination of in vitro and in vivo preclinical studies suggest exciting potential for this agent in the pharmacological treatment of TBI, and support the consideration of a Phase I clinical trial.

P332.  
CEREBRAL OXYGENATION AND RESPONSE TO HYPOXIA IN ACUTE BRAIN DAMAGE  
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Introduction: Cerebral oxygen tension (Pito2) is measured to assess oxygen availability to the brain. Pito2 depends on various factors, as probe positioning and arterial oxygen tension.

Aim of the study is to assess Pito2 and Pito2 response to hypoxia in patients where Pito2 probe was placed close to focally damaged tissue at the CT scan (Focal), compared with patients in whom the probe was in normally appearing tissue (Non focal).

Materials and Methods: Twenty-seven patients (14 Females, 43 + 17 years old) suffering from TBI (12) or SAH with a median motorGCS of 5 were studied. Monitoring included ICP, MAP, SjO2 for AVD02 calculation and tissue oxygen tension catheter (Licox GMS, Germany and Neurorad, Codman UK). Pito2 probe position was assessed by CT scan. Sixty-three hyperoxia tests were performed by increasing inspired oxygen fraction to 100%. Pito2 has been indexed for PaO2 (Pito2/PaO2 index) and Pito2 response to hypoxia was calculated as (Pito2 plateau—Pito2 at baseline)/(PaO2 at plateau—Pito2 baseline).

Results: The Pito2/PaO2 index was 0.13 + 0.08 in the focal group (12 patients) compared to 0.24 + 0.16 in the non focal group (15 patients; p < 0.05). There was a relation between the magnitude in Pito2 response and baseline Pito2/PaO2 values (R2 = 0.335 P = 0.001 Slope 0.67). Pito2 response was 0.9 ± 0.06 in 10 focal and 0.25 ± 0.16 in 15 non focal patients monitored (p = 0.005).

Conclusions: Pito2 was lower and Pito2 hyperoxia response was weaker when Pito2 was measured at the margin of focally damaged tissue compared to CT normally appearing tissue. This difference may reflect a greater amount of vascularity and therefore an increased intercapillary distance, in perifocal tissue. Although Hyperoxia tests could help in better understanding cerebral regulation, this response can be largely predicted by baseline Pito2 values.
P333.
MILD FLUID PERCUSSION INJURY LOWERS THE THRESHOLD TO KAINIC ACID-INDUCED SEIZURES WHICH IN TURN ELICITS RECURRENT INCREASES IN GLUTAMATE AND ENERGY DEMAND IN VULNERABLE TISSUE
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It is hypothesized that early seizures may precipitate adverse events in the traumatically injured brain primarily due to an increase in energy demand. We used a low dose of kainic acid (KA) to address the role of neural activity with respect to metabolic changes following a mild lateral fluid percussion (LFP) injury. KA (9mg/kg) was injected intraperitoneally in sham (n = 5) and LFP (n = 6) injured rats 1 hour following injury. An equivalent volume of saline was injected in LFP injured (n = 6) rats. Two CMA/12 microdialysis probes were placed into the cortex and perfused with saline (2microl/min). Samples were collected at 10 minute intervals, 1 hour before and 4h after LFP. Electroencephalogram (EEG) was recorded simultaneously.

No EEG evidence of spontaneous seizures after LFP was detected. LFP resulted in a glucose dialysate decrease ranging from -13 and -33% (duration: 4h) and in lactate dialysate increase up to 137% (duration: 30 min), indicating an increase in glycolytic metabolism. A glutamate spike up to 44% was detected immediately following injury, KA-induced ictal activity occurred in 3/5 sham + KA animals but was not associated with significant changes in neurochemistry. However, EEG seizures were detected in all LFP + KA acid animals and were associated with multiple glutamate spikes up to 154% (duration: 30 min) and further lactate increase up to 109% (duration: 70 min). We conclude that LFP injury lowers the threshold to KA-induced seizures. In this phase, the EEG-seizures induce glutamate release and an additional demand for energy and may play a detrimental role on cells already in a state of metabolic derangement. (Support: NINDS 30306; NS02089).

P334.
ROLE OF DECOMPRESSION CRANIOTOMY FOR SECONDARY BRAIN DAMAGE AFTER TRAUMATIC BRAIN INJURY IN MICE
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Introduction: Decompression craniotomy is a well known clinical treatment option for increased intracranial pressure (ICP), however, its role for the prevention of secondary brain damage, e.g. delayed contusion expansion, is not known on a quantitative basis.

Materials & Methods: Male C57/B16 mice (BW 25-28g; n = 56) were subjected to controlled cortical impact injury (CCI, 8 m/s, 1 mm indentation). In half of the animals (n = 18) the craniotomy was left open, in the other half it was closed. Animals (n = 6 per group) were sacrificed 15 min, 24 h and 7 days after CCI for quantification of contusion volume. A functional test battery was performed daily.

Results: 15 min after CCI contusion volumes were not different in animals with open or closed craniotomy (22.1 ± 1.4 mm3 vs. 22.1 ± 4.4 mm3, respectively). 24 h after CCI the contusion volume of the mice with intact skull increased by 37% (p < 0.05), while the craniotomized animals did not have larger contusions (18.3 ± 5.3 mm3; n.s.) as compared to 15 min after CCI. After 7 days the mice had large cavities at the site of CCI making direct comparisons with the findings after 15 min and 24 h difficult. Function (locomotion, walking, nesting) was improved in craniotomized animals.

Conclusion: The volume of a cortical contusion expands during the first 24 h after CCI only in animals with closed skulls where ICP can develop. In craniotomized mice where an increase in ICP does not occur secondary contusion expansion is completely prevented. Early craniotomy may therefore be one of the most potent procedures for the prevention of secondary contusion expansion.

P335.
THE CNS MICROVASCULAR PERICYTE RESPONSE TO HYPOXIA
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In response to fluctuations in environmental oxygen the cells of the blood brain barrier (BBB) undergo a number of complex adaptive measures in order to maintain tissue homeostasis and hemostasis. These adaptive responses are particularly important when the balance of oxygen availability and utilization is altered as a result of the pathophysiology of CNS disease. At the microvascular level oxygen responsive signaling mechanisms involving both the endothelial cell (EC) and the pericyte (PC) regulate angiogenesis, vascular permeability and metabolism. We have investigated very early responses of the CNS PC following in vitro exposure to hypoxia. freshly isolated rat cerebral microvessels were either cultured or sub-cultured to produce primary PC and EC. Cells or microvascular fragments were exposed to low oxygen for various periods of time using the GasPak 100 hypoxia system (Becton Dickinson and Company Sparks, MD). Within 15 minutes of exposure to low oxygen (1%) PC synthesize and release the cyclopentenone prostaglandin PGD2. Increased PGD2 and the dehydration product D12PGJ2 were detected by HPLC and by immune techniques. PGD2 was not synthesized by EC. Using PCR technology we have discovered that PC use the hematopoietic form of PGD synthase (PGDase) rather than the lipooxigenase form. PC constitutively express three of the five alternate splice variants of vascular endothelial cell growth factor (VEGF) mRNA. Exposure to low oxygen did not significantly alter mRNA levels but did increase synthesis and release of VEGF protein. Addition of either D12 PGJ2 or 15-deoxy D12,14 PGJ2 to PC under normoxic conditions increased the synthesis and release of VEGF protein in a dose dependent manner. In conclusion, results suggest that PGD2 produced by the CNS PC is an early signaling molecule in regulation of the angiogenic response to hypoxia.

P336.
ACID-SENSING ION CHANNELS IN ACIDOSIS-INDUCED NEURONAL INJURY

Acidosis is a common feature of ischemia and traumatic brain injury. Our previous studies have shown that activation of acid-sensing ion channels (ASICs) likely contributes to acidosis-induced neuronal injury. Here we explored the possibility that ischemic treatment may in turn modulate the activity of ASICs. Cultured mouse cortical neurons were subjected to oxygen-glucose deprivation (OGD) in an anaerobic incubator and the currents through ASICs were recorded in both control and OGD-treated neurons. Following 1h OGD treatment, the amplitude of ASIC currents was markedly increased, while desensitization of the currents was significantly decreased. In addition, OGD treatment induced a leftward shift of pH dose-response curve. Similar potentiation of ASIC currents and the shift of pH dose-response curve were observed in the same neurons following simple glucose removal or addition of metabolic inhibition agents such as NaCN (0.1-3.0 mM), rotenone (10 µM) or oligomycin (2.5 µg/ml). Substituting glucose with 2-deoxyglucose, a non-hydrolysable analogue of glucose, mimics the enhancement by glucose removal. An increase in intracellular calcium is not required in the potentiation of ASIC currents as the inclusion of 10 mM BAPTA in the pipette solution did not eliminate the potentiation. The enhancement by the addition of NaCN or glucose removal was however diminished in outside-out patch configuration, indicating the involvement of second messenger in the modulation of ASICs. With functional homomeric ASICs expressed in cos-7 cells, removal of glucose or addition of NaCN only potentiated the currents mediated by homomeric ASIC1a, without affecting the currents mediated by ASIC1b, ASIC2a or ASIC3 subunits. LDH assay demonstrated that addition of NaCN (1 mM) substantially potentiated the neuronal injury induced by incubating neurons with pH6 solution. Enhancement of ASIC responses by metabolic inhibition suggests that activation of ASICs in ischemic conditions may cause more injury than in acidic condition alone.

1331
P337.

OVEREXPRESSION OF RAT HEAT SHOCK PROTEIN 70 REDUCES NEURONAL INJURY AFTER TRANSIENT FOCAL ISCHEMIA, TRANSIENT GLOBAL ISCHEMIA, AND KAINIC ACID-INDUCED SEIZURE

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Our lab has previously demonstrated that mice overexpressing rat heat shock protein 70 (Hsp 70 g mice) showed less infarction than wild type controls after permanent focal ischemia. The purpose of this study was to determine whether neuronal injury is reduced in HSP70 Tg mice after transient focal and global ischemia, and kainic acid (KA)-induced seizure.

Adult male mice (28-36 g) were used for this experiment. Transient focal ischemia was produced by middle cerebral artery occlusion (MCAO) using intraluminal suture cannulation (n = 18). Infarct volume was assessed 24 hours after 30 minutes MCAO. Transient global ischemia was produced by 25 minutes bilateral common carotid occlusion (BCCAO) (n = 16). KA (10mg/kg) was administered subcutaneously and seizure activity was monitored (n = 20). The number of ephaptic neurotransmitters was assessed in CA1 72 hours after BCCAO and in CA3 24 hours after KA administration.

Infarct volume after transient MCAO was significantly less in HSP70 Tg mice than in WT mice (9.1 ± 5.7 mm3 vs. 22 ± 16.8 mm3; P < 0.05). The number of ephaptic neurotransmitters in CA1 after BCCAO was significantly decreased in HSP70 Tg mice than in WT mice (949.1 ± 1095.5 vs. 2406.9 ± 1380.3 mm3; P < 0.05). The number of ephaptic neurotransmitters in CA3 after KA injection was significantly reduced in HSP70 Tg mice compared with WT mice (35.8 ± 45.3 vs. 119.4 ± 121.1; P < 0.05).

Results suggest that HSP70 is neuroprotective and reduces excitotoxic cell death after transient ischemia, and after KA-induced seizures. Induction of DNA laddering in HSP70 Tg mice indicated that HSP70 reduced apoptosis in these in vivo injury models.

P338.

HYPOXIA CHANGES AKT PHOSPHORYLATION IN SUPERFUSED RESPIRING NEONATAL RAT CEREBROCORTICAL SLICES

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The serine-threonine kinase Akt (protein kinase B), which can critically alter the balance between survival and apoptosis, is activated by phosphorylation. Phospho-Akt is also involved in the regulation of glucose metabolism. Western blot quantifications of phospho-Akt, which prevents apoptosis by inactivating caspases and other targets, were evaluated after hypoxia in superfused, respiring, neonatal rat cerebrocortical slices using a protocol approved by the UCSF Committee on Animal Research. 350 μm thick slices were acquired from P7 Sprague-Dawley rats and superfused with 37°C oxygenated artificial cerebrospinal fluid (ACSF). Thirty minutes of hypoxia were induced by stopping the ACSF flow. Recovery occurred during 4 h superfusion with oxygenated ACSF.

Western blot intensities of phospho-Akt were moderate before hypoxia (control), nearly undetectable at the end of hypoxia, clearly detectable after 0.5 h of recovery, and greater than control after 1.5 and 4 h of recovery. Total Akt (phosphorylated and unphosphorylated) showed no change during and after hypoxia. Parallel 31P NMR studies at 14.1 Tesla were done with respiring superfused slices to examine phosphocreatine (PCr) and ATP levels at times corresponding to Akt measurements. PCr and ATP, nearly undetectable at the end of hypoxia, recovered quickly but incompletely after hypoxia. Reductions in phospho-Akt during hypoxia were consistent with a general unavailability of high energy phosphates (unchanged PCr and ATP). Results suggest that the time course of high levels of phospho-Akt in the recovery period after hypoxia require further study. The following NIH support is gratefully acknowledged: R01 GM34767 (Litt), P50 NS14543 (Chan) and R01 NS25372 (Chan).

P339.

ACCUMULATION OF CALPAIN AND CASEPASE-3 CLEAVED aI-SPECTRIN BREAKDOWN PRODUCTS IN CSF AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS


Although numerous biochemical markers of brain injury are correlated with outcome, a major limitation of current biomarkers is an inability for identifying specific neuropathological cascades operative in the injured brain. Identification of biomarkers elevated in CSF in response to brain injury that offer insight into specific pathological neurochemical events will provide critical information for emergency triage and will guide administration of therapeutic compounds. Non-erythroid aI-spectrin is a cytoskeletal protein cleaved by calpain and caspase-3 proteases to signature spectrin breakdown products (SBPDs). Although calpain-specific SBPDs are detected in CSF after traumatic brain injury (TBI) (Pike et al., J. Neurochem., 2001, 78:1297-1306), CSF levels of SBPDs has never been examined after cerebro ischemia. Methods: Transient focal cerebral ischemia in rats was produced by middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion. Ipsilateral (injured) and contralateral (uninjured) cortex and CSF were collected at 24, 48, and 72 h post occlusion. Results: Following MCAO, native aI-spectrin protein was decreased in brain tissue and increased in CSF up from 24 h to 72 h after injury. Calpain- and caspase-3 specific SBPDs were increased in brain (ipsilateral side) and CSF after injury. Levels of calpain-specific SBPDs were greater at each post-injury time point than the caspase-3-specific SBPD. Levels of these proteins were undetectable in CSF of uninjured control rats. Conclusion: Transient focal MCAO injury results in increased brain and CSF levels of calpain- and caspase-3-specific SBPDs. Importantly, MCAO injury resulted in greater CSF levels of caspase-3 SBPD than was observed after TBI by our laboratory. Thus, use of specific SBPDs as surrogate biomarkers may provide a powerful tool for discriminating concussive vs. ischemic injury and provide critical insight into specific patterns of protease activation after CNS injury. (Supported by DAMD17-99-1-9695, DAMD1701-1-0765, NIH ROI NS39091, NIH ROI 40182 and USAMRCM)

P340.

TISSUE-TYPE TRANSGLUTAMINASE EXPRESSION FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION


Tissue-type transglutaminase (TGt) has been implicated in neurodegenerative diseases and in protein aggregation associated with neurodegenerative disease. In this study, we have demonstrated induction of tissue-type transglutaminase in response to ischemic injury achieved by transient occlusion of the right middle cerebral artery. The area of infarcted tissue was revealed by decrease in TTC staining. Maximum decrease in TTC staining was observed 3 days after injury with recovery of TTC staining after 3 days. This suggests that maximum infarction was 3 days after occlusion. Western blot analysis has demonstrated increased expression of TGt-L (70Kda) protein with no detectable expression of TGt-S (70Kda) after ischemia. In ipsilateral cortex, peak induction was observed 5 days after injury (525% ± 10% of control), while lesser (TGt-L protein induction was observed in hippocampus after five days (196% ± 8% of control). To measure the mRNA transcript levels of TGt-L and TGt-S in rat cortex and hippocampus after traumatic brain injury a semiquantitative PCR was used. Results show that (TGt-L and TGt-S mRNA transcripts are induced after injury. In ipsilateral cortex both forms of TGt peaked on day 5 after injury with TGt-L transcript level being higher (95%) than that of TGt-S transcript (60%) after 14.1 Tesla were done with respiring superfused slices to examine phosphocreatine (PCr) and ATP levels at times corresponding to Akt measurements. PCr and ATP, nearly undetectable at the end of hypoxia, recovered quickly but incompletely after hypoxia. Reductions in phospho-Akt during hypoxia were consistent with a general unavailability of high energy phosphates (unchanged PCr and ATP). Results suggest that the time course of high levels of phospho-Akt in the recovery period after hypoxia require further study. The following NIH support is gratefully acknowledged: R01 GM34767 (Litt), P50 NS14543 (Chan) and R01 NS25372 (Chan).
P341.

NUCLEAR FACTOR-KAPPA B DECOY OLIGODEOXYNUCLEOTIDES CAN REDUCE THE ISCHEMIC SPINAL CORD INJURY OF RAT
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Purpose: Recently, it is reported that the cia element decoy oligodeoxynucleotides (ODNs) against nuclear factor-kappa B (NF-kB) block the activation of genes, which mediate ischemic injury. To evaluate the effect of NF-kB decoy ODNs on the spinal cord ischemia, we studied with rat spinal cord ischemia / reperfusion model.

Methods: Female Wister rats were used (B.W. = 200 to 250 g, n = 30). The animals were anesthetized with intraperitoneal injection of pentobarbital (30mg/kg). Temporal spinal cord ischemia (SCI) model was produced with the method described by Kanellopoulos. A 2Fr Fogarty catheter was inserted into the descending aorta via left carotid artery then the balloon was inflated for 10 min. Hemaggulutinating virus of Japan -liposome complex with fluorescein isothiocyanate-labeled NF-kB decoy ODNs was injected through the femoral artery during ischemia of spinal cord. Three hours, 3 days, 7 days after SCI, the spinal cords were removed at the lumbar enlargement level. The mRNA levels of factors related with ischemic-reperfusion injury at three hours were estimated by a real-time polymerase chain reaction method. Immunohistochemical study was performed using anti MAP2 antibody and anti ED-1 antibody to evaluate the degree of the neuronal damage and the infiltration of macrophages.

Results: The strong signals of the fluorescein were recognized in the spinal cord. The mRNA levels of tumor necrosis factor-alpha, interleukin-1 beta, intracellular adhesion molecule 1 and cyclooxygenase-2 were significantly reduced by the NF-kB decoy. The administration of NF-kB decoy reduced the number of infiltrated macrophages about 44% on 3 days and the infarct area about 30% on 7 days after SCI.

Conclusions: The introduction of NF-kB decoy reduced the spinal cord damage after ischemic injury. This strategy with NF-kB decoy may provide a useful tool for ischemic injury to the central nervous system.

P342.

DIFFERENTIAL EFFECTS OF HYPERBARIC OXYGENATION ON TISSUE NECROSIS AND ATP CONTENT FOLLOWING FOCAL CEREBRAL ISCHEMIA
J. Woitzik*, T. Karatzis, A.M. Mautes, L. Schilling (Department of Neurosurgery, University Hospital Marzahn. University of Heidelberg; *Neurosurgical Research Laboratory, University of Hamburg/Saar, DE).

Objective: Failure of energy metabolism and ATP synthesis may play a pivotal role in tissue necrosis following cerebral ischemia. The aim of the present study was to determine the neuroprotective effect of normo- and hyperbaric oxygenation and its relationship to the ATP content of the tissue.

Methods: In male SD rats anesthetized with isoflurane a focal ischemia was produced by an 8 hour unilateral occlusion of the middle cerebral artery (MCAO) using an intraluminal thread. After MCAO animals were kept in normal air (control), 100% oxygen (normobaric oxygenation, NBO) or 100% oxygen including a 1 hour hyperbaric period (HBO). Brains were serially cut, developed for planimetric analysis of tissue necrosis and ATP content and subjected to volumetric analysis.

Results: Lesion volume was 303 ± 54 mm^3 (mean ± SD) in controls and 258 ± 56 mm^3 in NBO animals. Volume of ATP loss amounted to 72.9 ± 19.7% of necrotic tissue. In the HBO group tissue necrosis was significantly smaller than in controls (187 ± 67 mm^3, p < 0.05) while the volume of ATP loss was markedly increased to 208.6 ± 38.4% of necrosis (p < 0.01 vs. NBO).

Conclusion: Normobaric oxygenation during an 8 hour period of permanent MCAO in rats does not salvage tissue from necrotic death. In contrast, an intermittent period of HBO protects tissue from early ischemic death and simultaneously increases the tissue volume displaying low levels of ATP. The pathophysiological meaning of this dissociation may reflect increased consumption resulting in neuroprotection or delay of ischemic cell death with ATP loss heralding growth of ischemic damage.

P343.

SELECTIVE HIPPOCAMPAL CA1 NEURONAL ACIDOPHILIA (RBC, RBC) CHANGE IN CASES OF SUDDEN DEATH FROM TRAUMA
Barbara Kotzyna, *Peter C Blumbergs. (Department of Pathology, University of Adelaide and Neuropathology Laboratory, Institute of Medical and Veterinary Science, Adelaide, AU)

Neuronal red cell change, the hallmark of hypoxic-ischaemic injury, is considered to be only reliably identified in immersion formalin fixed brains after a period of 4-12 hours. The nuclear changes and cytoplasmic eosinophilic eosinophilia seen in red cell change mapped the result of excitotoxic calcium mediated activation of endonucleases and proteases. We reviewed the neuronal appearance in the CA1 region of the hippocampus in 74 patients who had died suddenly as a result of trauma, and compared them to a group of 77 patients who had died in hospital as a result of non-traumatic medical and surgical conditions. Two independent observers examined haematoxylin and eosin (H&E) stained paraffin sections of the CA1 region in the hippocampus in each case and determined whether the neurons appeared normal, showed typical red cell change or were abnormal but without the typical features of red cell change. Neuronal red cell change was present in 58.0% of cases of sudden death due to trauma, compared to 10.7% in control cases; in contrast the CA1 neurons appeared normal in 56.3% of control cases but only 14.5% of trauma cases. These findings suggest that neuronal red cell change may develop over a very short period of time in cases of sudden traumatic death and is probably related to acute global cerebral ischaemia as the change occurred as frequently in the infarct region as in the group who died of non-brain related injuries as in the group who died of traumatic brain injury (61% and 59% of cases respectively).

P344.

DNA MICORARRAY ANALYSES OF GENE EXPRESSION CHANGES UNDERLYING CHRONIC CENTRAL PAIN IN SPINAL CORD INJURY
Nesic, O.*; Xu, G-Y; Johnson, K.M; McConnell, R.I; McAdoo, D.J.; Hulsebosch, C.E. and Perez-Polo, R.J. (Department of HBC&G, UTMB, Galveston, TX USA).

The rodent model of chronic central pain (CPP) is assessed using somatosensory tests of paw withdrawal responses to mechanical punctate (von Frey hairs), and measurements of response threshold for mechanical stimuli. By using K-means clustering, we divided injured rats in two groups: only one showing statistically significant increases in mechanical alldynia ("pain group") 28 days after contusion spinal cord injury (SCI). Locomotor recovery, measured in open field tests (BBB scores) for both groups of rats were indistinguishable, suggesting that the injuries to SC were equivalent for all rats. To characterize the gene expression changes underlying the development of CPP in SCI, we used Affymetrix DNA microarrays to analyze injured spinal cords (above the site of injury, TS) and thalamus of rats (n = 4) in the "pain" group and compared them with expression profiles of injured spinal cords and thalamus of rats in the "non-pain" group. Transcriptional changes in SC and thalami of rats with CPP included upregulation of inflammatory molecules, downregulation of molecules with "analgesic" effects (IGFII, somatostatin, opioids) and changes typical for tissues exposed to severe oxidative stress. Especially complex was the alteration in ion channels/transmitter receptors composition in spinal cords of rats showing mechanical allodynia. Novel findings were that neuropetides involved in olfaction, hormone receptors and regulators of cell adhesion/neurite outgrowth may have a role in CPP development.

Supported by NINDS NS 39161.
P345.
DATA MINING IN SCIGENES, THE DATABASE OF SPINAL CORD INJURY-RELATED GENES

SCIGenes is a searchable database containing information about genes whose expression is affected by spinal cord injury or nerve injury. It combines information about sequences, injury type, and protein function. Each entry includes links to sequences, fields describing the type of injury, the cell types affected, the polarity of the change in gene expression, and the temporal aspects of this response. It also includes links to functional information about each gene product. It supports data mining operations, making it possible to look for patterns of gene expression changes across all entries. For example, a search strategy for genes whose expression increases within the first two days after peripheral nerve transaction returns a set of growth factors and their receptors, transcription factors, and neuronal plasticity genes. SCIGenes is updated continuously, and users are encouraged to submit their own data at http://scigenes.uky.edu. Supported by an award from the Kentucky Spinal Cord and Head Injury Research Trust.

P346.
REDUCING THE T LYMPHOCYTE RESPONSE TO SPINAL CORD INJURY DECREASES SECONDARY DEGENERATION AND FUNCTIONAL DEFICIT
Rafael Gonzalez, Janette Olazer*, Michael T. Liu, Thomas E. Lane and Hans S. Keirstead. (University of California, Irvine, Irvine, CA USA).

Injury to the central nervous system (CNS) is followed in all instances by secondary degeneration, which leads to progressive tissue loss and cystic cavitation. Cellular and humoral immune responses have been implicated as mediators of secondary degeneration, and the expression of specific leukocyte chemoattractants has been shown to precede immune cell influx into the injured CNS. However, regulation of the cascade of proinflammatory molecule expression and immune cell recruitment into the traumatized CNS is poorly understood. Here we show that the lymphocyte chemoattractant CXCL chemokine ligand (CXCL) 10 is upregulated following dorsal hemisection and crush injury to the adult mammalian spinal cord, and that antibody neutralization of CXCL10 in injured animals dramatically reduces the CD4+ T lymphocyte invasion that normally occurs after trauma. This treatment resulted in a near elimination of secondary degenerative tissue loss and significantly reduced locomotor deficits. We conclude that CXCL10 plays a critical role in the recruitment of CD4+ T lymphocytes to sites of spinal cord injury, and that a reduction of the robust CD4+ T lymphocyte response to CNS injury significantly benefits tissue preservation and functional outcome following spinal cord injury. This project was funded by the Reeve-Irvine Research Center and the Roman Reed Foundation.

P347.
CHARACTERIZATION OF A RAT CERVICAL CONTUSION MODEL
YS Cha*, DD Pearce2, AE Marcillo3, MB Bunge1, WD Dietrich2,3. (The Miami Project to Cure Paralysis, Degs. of Cell Biology and Anatomy2 and Neurological Surgery3, Univ. of Miami School of Medicine, Miami, FL USA; 2St Vincent’s Hospital, The Catholic Univ. of South Korea, Seoul).

Numerous animal injury models currently exist that attempt to reproduce the pathophysiology of human spinal cord injury (SCI). However, these various paradigms do not represent the most prevalent type of SCI, contusive trauma to the cervical spinal cord. Whereas several cervical contusion studies have been performed, the number is a small percentage of the research conducted using SCI models. Because both (1) the different ways of injuring the spinal cord (compression, contusion, transaction) induce different processes of tissue damage and (2) the architecture of the spinal cord is not uniform, there is a need to use a model that is more clinically applicable to human SCI. Therefore, in the beginning study we have characterized a rat model of contusive, cervical SCI using the Electromagnetic SCI Device (Ohio State University) to induce injury by spinal cord displacement. The moderate contusion injury was performed at cervical level C6, using the circular flap tip of the impactor (made of methylmethacrylate, 4mm diameter) to transduce a force of 3 kdyne (as indicated by the force transducer). This results in slight dimpling of the dura dorsally and provides a consistent starting point from which displacement was measured (0.80mm displacement injury with a single, brief displacement of <20 m/sec). Analysis of the histopathological and behavioral consequences of SCI was performed over a 9-week period. Traumatized animals developed severe forelimb and hindlimb paralysis. Quantitative assessment of motor performance included BBB evaluation, hanging, climbing, and gripping tests for upper body strength and inverted plane, gridwalk and footprint analysis. Over the study period some degree of improve in motor function was observed. Histological assessment demonstrated a reproducible pattern of gray and white matter necrosis in terms of lesion size and cellular composition. This model of cervical SCI should allow the testing of novel neuroprotective and reparative therapies. (Supported by NIH PO1 38665)

P348.
IMPLANTATION OF SKIN-ACTIVATED BLOOD-BORNE MONOCYTES TO SPINALLY CONTUSED RATS: RECOVERY OF MOTOR ACTIVITY AND REDUCED CYST FORMATION.

Severe spinal cord injury leads to irreversible sensory and motor deficits due to the primary insult and to secondary degeneration that it causes. It was shown that in rats with transected spinal cords local implantation of peripheral nerve-activated blood-borne monocytes can induce functional motor recovery. In the present study we locally implanted skin-activated blood-borne monocytes to contused spinal cord of adult rats. The activated blood-borne monocytes, when compared to non-activated monocytes showed increased production of cytokines and elevated expression of surface molecules characteristics of antigen presenting cells. The implantation of skin-activated monocytes resulted in an improved recovery of motor activity and in a reduction of cyst formation when compared to spinally contused non-treated rats. The effect was noticed when the activated monocytes were injected even two weeks after the contusion. The results of this work further support the contention regarding the role of the inflammatory response, if well controlled, in recovery from CNS insult.
P349.
THE SERUM AND CEREBROSPINAL FLUID ELASTASE ACTIVITY DURING TREATMENT OF SPINAL CORD INJURY BY PERFORATOR
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(Moscow Medical Academy, Moscow, RU).

The previous experimental researches show that the blood substitute with

gas transporting function, known as "Perforator" (PF) is capable of reducing
spinal cord (SC) damage and protecting medullar tissue against ischemia
and secondary metabolic degeneration in acute spinal cord injury (SCI). PF
is emulsion of perfomrelchyl cyclohexil-iypentin stabilized by Proxanol
268. It oxygen solubility is 6–7 vol. %. PF accelerate the process of O2 de-

delivery and CO2 elimination, increase cerebral blood flow. The clinical

studies showed that the immediate local SCI oxygenation and IV infusion by PF

in acute SCI after decompression surgery results recuperation of the leg mo-
tor function to 12 of 20 paraplegic patients in contrast of 5 of 18 same pa-
tients without PF treatment.

The aim of the present study was to clarify the relationship between such
positive results and the level of SCI patient's serum and cerebrospinal fluid
(CSF) elastase activity (EA). The EA was measured before and 3 weeks af-
after treatment by L. Vasser and E. Blout (1972) method. The SCI patients clas-

sified into 3 groups: macrodex solution subdural and IV infusion treatment of
acute SCI (1st group, 18 patients); oxygenated PF subdural and IV infu-
sion treatment of acute SCI (2nd group, 20 patients); 3–4 months post-in-
jury only IV PF treatment (3 group, 14 patients). The EA was detected at
150±10.3 nM/min/mI in normal serum and was detected in normal

CSF. The EA was detected as 315±56.1 in serum and 21.8±1.2 nM/min/mI in

CSF after SCI. The EA was of no change significantly in mostly patients of 1st group.
The serum EA was declined to 182.4±1.6

58.1 nM/min/mI and to 43.4±1.2 in CSF in cases of the successful PF treat-

ment in 2nd group. The return of walking ability was observed 2–4 weeks post-surgery in these cases. The level of liquid EA was no change in unsuc-

cessful cases. A decrease of serum EA till 213±38.1 nM/min/mI was ob-

served in 3 chronic conditions group without real clinical results. Conclu-

sion: The EA may be reduced by PF high absorb capacity. On the other side,
the early tissue cord oxygenation and recuperation of SC microcirculation by PF

therefore leads to the partial improvement of SCI patients.

P350.
DOES MILD INTRAOPERATIVE HYPOTHERMIA LEAD TO IN-
CREASED COMPLICATIONS IN EPILEPTIC SPINAL SURGERY?
Elizabeth J. Owen *, Lisa Silbert, James D. Guest. (University of Miami, Miami,

Florida US).

INTRODUCTION: Spinal cord surgery carries risks of neurological injury. Neu-
roprotection is desirable, but no strategies have achieved broad clinical accep-
tance. Hypothermia, resulting from general anesthesia, is a readily achievable
neuroprotective strategy. Moderate hypothermia (32.5–35.5 C) carries smaller
clinical risks than deep hypothermia (<32.5C). We investigated the association
between incidental moderate hypothermia and complications in a review of
50 adults undergoing complex spinal procedures.

METHODS: Surgical procedures included: 41 cervical, 6 thoracic, 1 occipito-
cervical, 1 cervico-thoracic, and 1 thoraco-lumbar procedure. Systemic hypother-

mia followed induction of anesthesia, esophageal or bladder temperature was

monitored.

We studied time-temperature (T/T) curves to derive mean temperature, nadir
temperature, time course of hypothermia, and the hypothermic integral (dose)
in contrast to the threshold isotherm area under the curve (AUC)—actual T/T AUC. Patients
with and without complications were compared for age, body size, anesthetic du-
estration, and temperature measure.

RESULTS: Complications (14) included: one accidental durotomy, one hemor-

rhage, two wound infections, one collapse of a vertebral adjacent to a fusion,
one SVT, one brief intraoperative asystole, one episode of postoperative pulmonary edema,
one episode of delirium tremens, one postoperative death (DVT), two transient radiculopathies, and two cases of transient long-tract dys-

function.

Comorbidities were not significantly linked to complications. Patients with
(n = 14) and without (n = 36) complications were compared. P-values were sig-
nificant for anesthetic duration (0.007), blood loss (0.005), and hypothermic in-
TEGRAL (0.004). Neither mean nor nadir temperatures were statistically

associated with complications. Anesthetic duration and blood loss were linked (r = 0.62).
T/T profiles showed no uniform pattern.

CONCLUSION: Anesthesia duration was linked to complications and blood loss.
Regarding hypothermia, neither mean nor nadir temperatures were linked to
complications, but the "dose" of hypothermia was linked. Extended exposure to moderate hypothermia (>5 hours) is associated with increased risks.

Meaningful neuroprotective use of hypothermia during spinal surgery will re-

quire more precise delineation of the exposure.

P351.
CHRONIC CENTRAL PAIN IS ATTENUATED BY EXOGENOUS
LEUKEMIA INHIBITORY FACTOR (LIF) AFTER SPINAL CORD
INJURY (SCI).
K.M. Johnson*, B.C. Hains; D.J. McAdoo; C.E. Hulsebosch. (University of
Texas Medical Branch, Galveston, TX US).

After SCI, chronic central pain (CCP) develops in a majority of patients.
We wished to test if the CCP following SCI may be reduced by intracranial
injection of LIF, a key modulator of neuropathic pain that is thought to play a
anti-inflammatory role. We used a rodent model of SCI, unilateral hemisection at T13, and tested for the development of mechani-
cal and thermal allodynia. Male Sprague-Dawley rats (225–250 gm) were anes-

thetized, and at the time of spinal hemisection, 100 mg of LIF in 1.0 ml

of artificial cerebral spinal fluid (ACSF, pH=7.4) was injected 1 mm rostral

and 1 mm caudal to the spinal hemisection. LIF treated hemisection rats (n = 8)
were compared to vehicle treated hemisection rats (n = 8) by comparing
post-surgical behavior at 30 days with pre-surgical behavior to test if LIF

treatment attenuated mechanical and thermal allodynia. Treatment with LIF

produced statistically significant attenuation of both mechanical and ther-

mal allodynia. Thus, we hypothesize that central sensitization in CCP is at-
tenuated by LIF. Specifically, we propose that LIF treatment inhibits the

inactivation of neurotransmitters and lymphocytes both of which produce charknokine,
cytokines and other factors, including NGF, known to produce sensitization.
P353.
TRANSPLANT-MEDIATED REMYELINATION AND LOCOMOTOR RECOVERY OF THE MHV MODEL OF MULTIPLE SCLE
ROSIS
Denervating diseases such as multiple sclerosis (MS) are characterized by recurrent episodes of local denervation and progressive neurological impairment. To address the complex and reactive CNS environment of a neurodegenerative disease state, we explored the remyelinating capability of early neural progenitor cells following transplantation into lesions in the denervating mouse model of MHV infection. Stiriated stem cells were isolated from postnatal day 1 mice and grown on a nonadherent substrate in defined media with EGF, then differentiated into oligodendrocytes and astrocytes, but not neurons. These findings indicate that this stem cell preparation results in the restriction of cells to a glial lineage. Seven day-old uninjured, BrdU-labeled floating neurons were implanted into the throracic spinal cord of actively denervating MHV mice. After 21 days, alternating ~1 mm blocks of the spinal cords were plastic embedded and frozen sectioned. Behavioral analysis showed locomotor recovery starting at two weeks after the transplantation. In the non-transplanted animals, numerous denervated axons were present among vacuoles, myelin debris, activated macrophages, lymphocytes and necrotic cells. In contrast, transplanted animals showed large areas of remyelinated axons. The transplanted BrdU labeled cells were present after 21 days. These studies confirm that remyelination can take place during pathogenesis and indicate that transplanted glial-committed progenitors can be a source of extensive remyelination of regions of demyelination in the MHV model of multiple sclerosis. The success of remyelination following transplantation into this inflammatory environment is of central importance in approaches that aim to enhance remyelination in MS lesions. Supported by: Reeve-Irvine Research Center.

P354.
THE NEURONAL-SPECIFIC RNA BINDING PROTEIN HUD IS UP-REGULATED AND COLocalIZED WITH GAP-43 mRNA IN THE FACIAL NUCLEUS OF THE MOUSE DURING REGENERATION.
Kim D. Anderson* and Oswald Siewert. (University of California, Irvine, Irvine, CA US).
The Growth-Associated Protein, GAP-43, is highly expressed during development of the nervous system and is re-expressed during regeneration of the peripheral nervous system (PNS). In vitro, the GAP-43 mRNA is highly labile and its half-life is regulated post-transcriptionally, in part, by the neuronal-specific RNA-binding protein Hud. In vivo, however, the regulation of GAP-43 mRNA is poorly understood. To begin to analyze the molecules involved in the in vivo regulation of GAP-43 mRNA, Hud protein expression was examined following a PNS lesion in which successful regeneration occurs. C57Bl/6 mice were given a unilateral crush injury of the facial nerve and were allowed to survive for various time intervals. In situ hybridization studies revealed high levels of GAP-43 mRNA in the ipsilateral facial nucleus one week following injury. At the same time, immunohistochemistry demonstrated Hud protein to be colocalized with GAP-43 mRNA in the motor neurons of the facial nucleus ipsilateral to the injury. Neither Hud protein nor GAP-43 mRNA were detectable in the contralateral facial nucleus. These results suggest that Hud protein may play a role in the in vivo regulation of GAP-43 mRNA and may account for the prolonged expression of GAP-43 following a PNS lesion during which successful regeneration does occur. Supported by the NIH NS-41170 (KDA) and NIH NS-32720 (OS).

P355.
THE ROLE OF OSCPCLAUDIN-11 IN OLIGODENDROCYTE PROGENITOR CELL PLATING AND REMYELINATION FOLLOWING DEMYELINATION OF THE ADULT SPINAL CORD.
Oligodendrocyte-specific protein (OsgClausdin-11) is a major protein of CNS myelin, forming tight junctions within myelin sheaths. OSCPclaudin-11 is involved in membrane interactions with the extracellular matrix and appears to modulate proliferation and migration of oligodendrocytes, a process essential for myelination and repair. Using an anti-NG2 antibody to identify oligodendrocyte progenitors (OPs), we have previously shown an acute increase in the number of NG2+ cells in normal white matter surrounding a region of antibody-induced demyelination in the adult rat spinal cord. These NG2+ cells incorporated BrdU, illustrating a local proliferation of cells. An absence of NG2+ or BrdU+ cells 2 weeks after demyelination suggested these cells had migrated into the demyelinated area to become remyelinating oligodendrocytes. In the present study, 16% of the NG2+ cells surrounding a region of demyelination in the adult spinal cords of OSCPclaudin-11 homozygous knockout mice were still BrdU+ 2 weeks after demyelination, suggesting restricted migration of OPs into the demyelinated area. No NG2+/BrdU+ cells were seen in normal white matter of wild type mice. In addition, the total number of BrdU+ cells in the dorsal column of knockout mice was 40% higher compared to wild type mice, supporting a role for OSCPclaudin-11 in proliferation. We examined the extent of NG2 co-localization with PDXGR, another marker for OPs involved in proliferative ability. More NG2+/PDXGR+ cells were seen in normal white matter surrounding a region of demyelination in OSCPclaudin-11 knockout mouse, further suggesting limited migration of OPs into the demyelinated area. These data indicate that OSCPclaudin-11 knockout mice display restricted migration of proliferating OPs into a region of demyelination, in support of a role for OSCPclaudin-11 in migration. Current investigations are underway to assess the extent of remyelination in both knockout and wild-type mice. These studies were support by Multiple Sclerosis Society of Canada and NIH Neurol Repair Training Grant.

P356.
LOCOMOTOR TRAINING IN A RODENT MODEL OF INCOMPLETE SPINAL CORD INJURY.
Locomotor recovery was assessed in 14 female adult Long-Evans rats with incomplete thoracic spinal cord injury (SCI, T10 moderate contusion) with (n = 9) and without (n = 5) quadrupedal treadmill step training (15 minutes/day, 5 days/week, starting 1 week post-injury for 12 weeks) using 2-D and 3-D kinematics of gait and the BBB locomotor score. Mean hindlimb stance width (SW), eversion angle (EA), and stride length/velocity (SLV) were obtained from 4-5 passes of overground walking in a track pre-injury and every week post-injury if the animal could plantar step. 3-D analysis determined hip, knee, ankle, shoulder and elbow angles for all four limbs during 4-20 cycles of treadmill locomotion. The effects of training, the BBB score (<14 (1.67 ± 0.15 (SEM) or ≥14 (16.69 ± 0.28)), and time post-injury were examined (5-factor, repeated measures, p < 0.05). For all SCI rats the SW, EA, and SLV were significantly greater pre-injury. Also, they were significantly different between the training groups or based on the BBB score. EA of the trained group for the first 4 weeks post-injury was larger than that for the last 5 weeks and at all times smaller than that for the untrained group. For BBB>14, both SW and EA were significantly smaller in the trained group. 3-D analysis indicated that 13 weeks post injury the untrained group had a significantly increased ankle extension and range (124 ± 7.0% vs. 164.7 ± 11.0% with a loss of double burst pattern) and reduced knee extension, knee range and elbow range (81.0 ± 4.2% vs. 50.3 ± 6.0% vs. 61.9 ± 15.4%). Preliminary analyses of trained rats (n = 3) indicated only a reduced knee range (49.0 ± 3.6%). These quantitative kinematic indices indicate that injury alters hindlimb as well as forelimb function. They suggest that the degree of injury influences the kinematic impairments and the effects of treadmill training on locomotor recovery. (Support: KSCIRIC-59A and HD-40355).
P357. GENETICALLY TARGETED ASTROCYTE SCAR ABATION RESULTS IN LIMITED, LOCAL GROWTH OF CORPUSCULAR TRACT AXONS AFTER SPINAL CORD INJURY.
J.R. Lomova-Kafkurs, (UCLA, Huntington Beach, CA US).

Genetically targeted astrocyte scar ablation results in limited, local growth of corpusceral tract axons after spinal cord injury. J.R. Lomova-Kafkurs, J. Hermann, N.B. Doan and M.V. Sofroniew. Department of Neurobiology and Brain Research Institute, UCLA, Los Angeles CA 90095-1763.

After spinal cord injury (SCI), scar tissue formed by reactive astrocytes is thought to prevent axon regeneration. We used a genetic targeting strategy to ablate reactive astrocytes after SCI. Transgenic mice that express herpes simplex virus thymidine kinase (HSV-TK) from the mouse glial fibrillary acidic protein (GFAP) promoter were given the antiviral agent ganciclovir (GCV). Transgenic and non-transgenic mice received a bilateral lesion of the corpusceral tract (CST) at T9/10. Non-transgenic mice exhibited dense astrocyte scars. Transgenic mice given GCV exhibited substantial ablation of scar-forming astrocytes. Areas depleted of astrocytes exhibited a statistically significant 5-fold increase in the density of nerve fibers detected by immunohistochemistry of neurofilament M, suggesting the sprouting and growth of local nerve fibers. CST axons were assessed using biotinylated dextran amine (BDA) injected unilaterally into the motor cortex. In non-transgenic mice, many large BDA-labeled projection fibers were evident proximal to the glial scar and no labeled fibers were observed within or distal to the lesion. Transgenic mice given GCV had fewer retraction bulbs, and areas depleted of astrocytes exhibited many fine BDA-labeled fibers. In some cases, finely beaded and branched CST fibers grew across and beyond the lesion for a moderately long distance. Supported by Christopher Reeve Paralysis Foundation, NIH grant #NS07479, and CA State Roman Reed Initiative for SCI Research.

P358. HP184, A COMBINED SODIUM AND POTASSIUM CHANNEL BLOCKER, IMPROVES LOCOMOTOR SCENES 35 DAYS AFTER A MODERATE SPINAL CORD INJURY IN THE RAT.
Michel Rathborne, Shucai Jiang, Mohammad Khan, Yao Lu, Josef Buttigieg, David Lee, Jessee Horrey, Kristien Paulsenth, Rani Beni, Adrel Safdor, Sacie Wang, Jay Soudof, Dave Haneye, Margaret Pety and Craig P. Smith (Division of Neurology and Neuroscience, McMaster University, Health Sciences Centre, Hamilton, Ontario and Aventis Pharmaceuticals, Inc., Bridgewater, NJ USA).

There are currently no available therapies for restoring function to patients with chronic spinal cord injury (SCI), a population estimated at >250,000 in the USA. Recent literature suggests that clinically significant neuroregenerative improvements may be obtained with 4-AP (4-aminopyridine). However, therapeutic use of 4-AP may be limited by various side effects, which include restlessness, confusion, and infrequently reported findings of generalized tonic-clonic seizure. HP184 is an analog of 4-AP which is a voltage-dependent blocker of potassium currents in PC12 cells and a use- and frequency dependent blocker of sodium channels. This combination of activities allows high levels of HP184 to be administered without danger of convulsion. Consistent with other sodium channel blockers, HP184 also has neuroprotective properties, and pre-dosing with 10mg/kg, p.o., attenuates the reduction in infarct volume caused by permanent middle cerebral occlusion in mice. Also, we have observed efficacy in well established SCI. In spinal cord injured rats, HP184 significantly improves open field walking in longstanding (35 day) spinal cord injury of moderate intensity (1.5, 1.0 & 3 mg/kg, p.o).

P359. PRO-CYSTEINE COMPOUND (OTC) DECREASES THE NUMBER OF ACTIVATED MACROPHAGES/MICROGLIA FOLLOWING SPINAL CORD INJURY.
Kanencnic HP, Kelly M, Danann A, Schäfle E, Griebel RW, Paterson PG, Jurrinkl BHI. (University of Saskatchewan, Saskatoon, SK, CA).

In this experiments we have examined the effect of L-2-oxo-thiazolidine-4-carboxylate (OTC) administration, which promotes glutathione synthesis, on inflammatory responses, especially accumulation of activated macrophages/microglia, and myeloperoxidase activity within first 24-hr following severe clip-induced spinal cord injury. The spatial localization and morphology of activated macrophages/microglia were described quantitatively at 24-hr after injury in the injured segment and segments directly adjacent, rostral and caudal, by using immunohistochemistry. OTC at 12 mmol/kg initially followed every 12 hr with 4 mmol/kg was administered intraperitoneally.

Administration of OTC significantly reduced the number of ED1-positive cells epidurally, intra- and periventricularly within necrotic tissue at the epicenter of the lesion. Myelin was severely vacuolated not only at the site of injury but also rostral and caudal to the damage after the first 24-hr. Longitudinal sections through central canal showed a decrease in the number of activated macrophages/microglia by ~30% in the gray matter at the site of injury following OTC treatment. Saline-treated animals have higher accumulation of ED1-positive cells in the gray matter up to 3 mm rostral and caudal to the site of injury. We also analyzed myeloperoxidase (MPO) activity, an enzyme predominantly located in neutrophils, and found that OTC administration significantly decreased (p < 0.0001) MPO activity in two groups, in male rats 12-hr and in female rats 24-hr following injury.

In conclusion, our data suggest that OTC administration decreases neutrophils and activation of microglia and/or extravasation of monocytes and prevents much of the secondary damage following spinal cord injury. Supported by the Christopher Reeve Paralysis Foundation and H. Kanencnic holds an HSURC Saskatchewan Post-Doctoral Fellowship.

P360. THE ADMINISTRATION OF VARIOUS DOSES OF L-2 OXOTHIAZOLIDINE CARBONATE TO PROMOTE RECOVERY FROM NEUROTTRAUMA.
Kelly MEB, Griebel RW, Kanencnic H, Schäfle E, Paterson P, and Jurrinkl BHI. (University of Saskatchewan, Saskatoon, Saskatchewan, Canada).

Background: Decreasing oxidative stress by maintaining tissue glutathione following spinal cord injury improves functional outcome in a rat model. We are analyzing the dose response to various levels of L-2 oxothiazolidine-4-carboxylate (OTC) after rat spinal cord injury.

Methods: An extradural aneurysm clip with a calibrated force of 50 grams was applied to the rat spinal cord at the T6 level. Intraperitoneal administration of OTC was performed. The functional recovery of the rats was assessed for six weeks after receiving either 1) saline, 2) 2, 3) 10 mmol/kg OTC 30 minutes after injury and then every 12 hr for 5 days, 1) 30 mmol/kg OTC for one dose 30 minutes after injury and 10 mmol OTC/kg 30 minutes after injury followed by 1 mmol/kg bolus at 12 hours. Functional recovery was assessed using standard techniques BBB behavioral scoring. Results: A statistically significant improvement in functional recovery (36% of animals walked) was seen in the animals receiving 1 mmol/kg OTC and 4 mmol/kg OTC for five days. Only 25% of animals receiving the 30 minute bolus of 10 mmol/kg OTC and 10 mmol/kg OTC with a 12 hour 1 mmol/kg bolus recovered 10 on the BBB score (walking ability). Not one of the saline vehicle-treated animals ever achieved a BBB score greater than 9.

Conclusions: Spinal cord injury results in a significant increase in oxidative stress at and distant to the site of injury. The administration of OTC in various doses significantly improves functional recovery in a rat model over saline controls. Research supported by the Christopher Reeve Paralysis Foundation.
P361. CEREBRAL METABOLIC AND BLOOD FLOW DIFFERENCES BETWEEN TRAUMATIC HEAD INJURED PATIENTS: INFLUENCE OF COCAINE


Purpose: Cocaine use can be high in patients with traumatic brain injury (TBI). Recent studies with dogs have shown that cerebral blood flow (CBF) responsiveness to carbon dioxide may be affected by cocaine. The purpose of this study was to determine if CBF and metabolism differences exist between patients who did or did not test positive for cocaine on admission to the hospital following severe TBI.

Methods: We studied prospectively, 23 age-matched, consented head injured patients (median admission GCS = 7, M:F 15:8, mean age 27.8 ± 2.9, mean days studied 4 ± 5). Seven patients tested positive for cocaine upon admission. Arterial and jugular venous samples were collected during daily 133Xenon CBF (ml/100g/min) studies and were analyzed for oxygen and carbon dioxide and cerebral metabolic rates calculated. Additionally, microdialysis catheters were placed in 5 of the cocaine positive patients and 9 of the cocaine negative patients.

Results: Following injury, CBF was higher in cocaine positive patients mean CBF 41.4 versus 37.9, p = 0.22. Additionally, CBF in the gray matter (GIS) was significantly greater in the cocaine positive patients 66.6 versus 53.6, p = 0.03. Arterial CO2 levels correlated more strongly with CBF but not GIS in cocaine positive patients: CBF r = 0.65, p < 0.001 versus r = 0.43, p = 0.06; GIS r = 0.48, p = 0.005 versus r = 0.52, p = 0.001.

CMRO2 was significantly less in cocaine positive versus cocaine negative patients, 1.4 versus 1.2 ml/100g/min, p = 0.04, respectively. Microdialysis showed that cocaine positive patients had significantly lower levels of glucose (mmol), 0.61 versus 1.05, p = 0.009, lactate (mmol) 0.32 versus 0.62, p = 0.05, and pyruvate (mmol) 2.19 versus 3.73, p = 0.008.

Summary: This study shows that TBI patients who tested positive for cocaine exhibit unique cerebral blood flow and metabolism characteristics compared to age and injury severity-matched cocaine negative patients. Thus drug use may affect hemodynamic and metabolic responses to TBI and potentially responses to therapy.

P362. ACUTE METABOLIC DEVIATION FROM NORMAL PREDICTS LONG TERM OUTCOME AFTER TBI


Object: Metabolic dysfunction after TBI is a complex phenomenon; available data are characterized by uneven numbers of studies at varying collection times. The purpose of this study was to develop a novel statistical methodology to quantitatively model metabolic abnormality after moderate or severe TBI and relate these measurements to 6 month Glasgow Outcome Scale.

Methods: Serial assessments of cerebral metabolic rates for glucose (AVDglu and CMRglu), oxygen (AVDO2 and CMR(O2)) and lactate (AVDLac and CMRLac) were performed using a modified Kety-Schmidt method, with bedside 133Xenon CBF. Forty-two patients, (mean age 37 ± 17 years, median GCS 6, 71% male), were studied from post-injury days 0 to 5. Indices of metabolic deviation from normal were derived using a demographically similar sample of 28 healthy volunteers (mean age 34 ± 8 years, 70% male). For each metabolic study in the trauma cohort database, a multivariate Mahalanobis distance from the normal was computed. This metric, takes into account correlation of measurements in the normal metabolic state, and thus quantification of abnormality examines both the magnitude of deviations of individual components from normal and the extent of pairwise "uncoupling".

Results: A three component abnormality measure using CBF, AVD(O2), and AVDLac was strongly associated with 6 month GOS (p < 0.001) and with survival (p < 0.002). The patients with GOS of 1 (death) had the highest degree of abnormality and those with GOS of 5 (good recovery) had the lowest. These associations remained strong after controlling for known prognostic factors such as GCS, pupillary status, CT score, CPP and ICP (p < 0.01 for both).

Conclusions: During the first 5 days after moderate or severe TBI, the combined degree of abnormality in CBF, AVD(O2), and AVDLac is strongly associated with poor longterm outcome. The deviation from normal methodology identifies multivariate measures of metabolic dysfunction that are predictive of outcome.

P363. EXTRACELLULAR CALCIUM FLUCTUATIONS AFFECT VASCULAR TONE IN ISOLATED RAT MIDDLE CEREBRAL ARTERIES


Rationale: Following traumatic brain injury extracellular levels of calcium (Ca(2+)) have been shown to fluctuate in both humans and rats. The purpose of this study was to determine if minor fluctuations of extracellular calcium affect vascular tone in isolated rat middle cerebral arteries (MCA).

Methods: MCA were isolated from male Sprague-Dawley rats (250-300g) and mounted in an isolated vessel chamber (Living Systems, Burlington, VT) which was perfused with phosphate buffered saline, temperature 37°C, pH 7.39, aerated with 20% oxygen, 5% carbon dioxide, and balance nitrogen. Vaselidmeter was measured by a video dimension analyzer and data stored in a data acquisition program. Extracellular Ca(2+) concentrations were modified from normal concentrations of 1.6 mM, to a minimum of 0.6 mM and a maximum of 3.1 mM. Pressure within the vessel was maintained at 60 mm Hg. Results: Altering extracellular Ca(2+) caused the MCA to dilate at low concentrations and constrict at higher concentrations. At 0.6 mM calcium the arteries dilated to 123 ± 8% of the 1.6mM concentration, n = 7, p < 0.05, ANOVA. Extracellular Ca(2+) at 3.1 mM caused the MCA diameter to constrict to 78 ± 5% of the baseline diameter, n = 7, p < 0.05 ANOVA. Both vasodilatory and vasoconstrictive effects of Ca(2+) were inhibited by the L-type calcium channel antagonist verapamil at 10-6 and 10-5 M, but not at 10-7 M. However, the N-type calcium channel antagonist omega-conotoxin did not affect the Ca(2+) mediated vascular responses.

Summary: Minor alterations in extracellular calcium affect vascular tone in an isolated MCA preparation. Following traumatic brain injury, extracellular calcium concentration may change due to excitatory amino acid activation, adenine nucleotides, depolymerization, and other pathophysiological events. Thus, the hemodynamic status of the cerebral vasculature may be modulated by and fluctuate along with brain extracellular calcium concentration.

P364. CYCLOSPORIN A DOES NOT AMELIORATE THE ANAEROBIC GLYCOLYSIS RESPONSE TO VIBRISSA MOTOR CORTEX STIMULATION FOLLOWING TRAUMATIC BRAIN INJURY


We have demonstrated that the traumatically injured brain can respond metabolically to vibrissa motor cortex stimulation. This study examines the neurochemical response to stimulation following lateral fluid percussion injury (LFPI). Adult male Sprague-Dawley rats were studied at 1d (n = 7) and 7d (n = 8) following LFPI or sham injury (n = 5) conducted under isoflurane anesthesia. A microdialysis probe was placed 2 mm from the stimulating electrode in vibrissa motor cortex (ipsilateral to LFPI). Stimulation (100-200 mA, 0.3 Hz, 40-60 mV) elicited a vibrissa response and a corresponding increase in both extracellular glucose (10%) and lactate (23%) concentrations in the sham-injured group. In both LFPI groups, stimulation elicited significantly greater increases in extracellular lactate (55-63%) and a significant decrease in extracellular glucose (2-17%). These results suggest that the injured brain relies on anaerobic glycolysis to fulfill the increased energy demands of stimulation. In an attempt to improve oxidative metabolism, cyclosporin A (CsA: 20 mg/kg, i.p.) was administered at 15 minutes and 1d following LFPI. CsA in these stimulated animals did not improve the anaerobic glycolysis response. In contrast, FK506 (1 mg/kg, i.p., 15 min and 1d) reduced both metabolic and neurochemical responses to stimulation. In determining the consequence of this secondary energy demand, we noted that CsA increased the number of Fluoro-Jade positive neurons in a defined area of posterior, medial cortex seen with stimulation following injury whereas FK506 had no such effect. This CsA-enhanced Fluoro-Jade response was reduced with co-administration of PBN (100 mg/kg, i.p., 15 min), suggesting a role of oxygen free radicals. These results suggest that CsA does not ameliorate the anaerobic glycolysis response to stimulation at 1d following LFPI and in fact shows a trend toward increased neuronal degeneration. (NS3038. UCLA Brain Injury Research Center).
P365. OXYGEN, GLUCOSE AND LACTATE METABOLISM AS PREDICTORS OF OUTCOME AFTER TRAUMATIC BRAIN INJURY

Objective: Although acute metabolic dysfunction is presumed to play a fundamental role in the pathophysiology of TBI, the impact of such metabolic derangements on long-term outcome has not been well delineated. The purpose of this prospective study was to determine if the degree of abnormal oxygen, glucose and lactate metabolism were predictive of 6 month global outcome after moderate or severe TBI, relative to other prognostic factors.

Methods: Serial measurements of the cerebral metabolic rates for glucose (AVDglu and CMRglu), oxygen (AVDVO2 and CMRVO2) and lactate (AVDLac and CMRLac) were performed using a modified Kety-Schmidt method with bedside 133Xenon CBF. Forty-two patients, (mean age 37 ± 17 years, median GCS 6, 71% male), were studied from post-injury days 0 to 5.

Results: Six months post-injury Glasgow Outcome Scale was most strongly associated with cerebral metabolic rate of oxygen (CMRO2, p < 0.0001) and lactate (p < 0.005) and post-resuscitation pupillary status (p < 0.01), other important factors were patient age (p < 0.05) and % time CPP < 60 mmHg (p < 0.05). Patient survival versus death was most strongly associated with CMRVO2 (p < 0.0005), post-resuscitation GCS (p < 0.02), pupillary status (p < 0.02), mean ICP (p < 0.01), mean CPP (p < 0.005) and % time CPP < 60 mmHg (p < 0.001). The stronger predictors to outcome remained strong even after controlling for known prognostic factors such as GCS, pupillary status, CT score, CPP and ICP.

Conclusions: During the first 5 days after moderate or severe head injury, reduced CMRO2 and CBF are strongly associated with poor long-term outcome. Overall, CMRO2 appears to be a key predictor of global neurological outcome. Whether manipulations to decrease metabolic demands or alternatively to increase metabolic capacity will ultimately improve neurological recovery warrants further study. Support: NIH/NINDS, Grant # NS30308.

P366. PERICONTUSIONAL TISSUE DISPLAYS VULNERABILITY TO REDUCTION IN CEREBRAL PERFUSION PRESSURE WITHOUT MICRODIALYSIS EVIDENCE OF ISCHEMIA

Intracerebral hematoma may induce a state of metabolic dysfunction in surrounding brain tissue. Spontaneous reduction in cerebral perfusion pressure (CPP), may induce further damage in vulnerable tissue. We hypothesized that spontaneous changes in CPP elicit ischemic neurochemical changes in both perihematoma tissue and in minimally injured white matter (MIWM).

Intracerebral microdialysis was performed in 13 consecutive patients with traumatic intracerebral hematoma (ICH). MD catheters were placed into MIWM adjacent to the ventriculostomy in all patients and a second MD catheter adjacent to perihematoma tissue in 7 patients. Hourly values of glucose, glutamate, lactate and pyruvate concentrations were measured and correlated with hourly CPP, ICP and SJVO2 values. In comparison with MIWM, perihematoma values of glucose were lower and glutamate, lactate and lactate/glucose (L/G) was higher than MIWM during normal CPP. In MIWM mean glucose concentrations were lower with CPP < 70 mm Hg (0.78 vs 0.93 mM, p < 0.05). However, mean glucose concentrations in perihematoma tissue decreased to a greater extent during reduced CPP (0.63 vs 0.40 mM, p < 0.001). In both MIWM and pericontusional tissue mean lactate concentrations were higher with CPP < 70 mm Hg (1.2 vs 0.8 mM p < 0.01 and 1.3 vs 0.9 mM, p < 0.01). However, the L/G ratio did not increase when CPP < 70 mm Hg in either tissue type (p < 0.4).

Despite this, pericontusional tissue displayed increase in glutamate (5.8 vs 6.8 mM, p < 0.03) with a reduction in CPP, whereas MIWM did not. We conclude that under conditions of normal CPP baseline differences in brain neurochemistry exist between pericontusional and MIWM tissue. However, during reduction in CPP < 70 mmHg, perihematoma tissue demonstrates a more dramatic reduction in glucose and increase in glutamate with an "ischemic" increase in L/G ratio. (Support: NINDS 30306, NS02389).

P367. SIMULTANEOUS QUANTITATIVE MEASUREMENTS OF GLUCOSE METABOLISM, CEREBRAL BLOOD FLOW AND ADENOSINE TRIPHOSPHATE (ATP) LEVELS FOLLOWING TRAUMATIC BRAIN INJURY and
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Traumatic brain injury (TBI) causes an acute uncoupling of glucose metabolism and CBF, which can profoundly affect neuronal cell viability. We have developed a quantifiable technique to simultaneously measure regional cerebral glucose metabolism (CMRglc), CBF and the resulting tissue ATP levels in rats. Four male Sprague-Dawley rats were urethane-anaesthetized (1.6g/kg) and cannulated for double-label autorigraphy using 18F-fluoro-deoxyglucose (FDG) and 14C-indocyanine (IAP) and secured in a 5 kW microwave (Thermex). FDG bolus (1mCi) was injected intravenously and timed samples were collected through the catheterized femoral artery for 30 min. Immediately after the FDG study, IAP (33mCi) was infused to O2 levels, and timed arterial blood drops were collected onto filter paper and assessed for radioactivity. Animals were sacrificed by microwave irradiation (6 sec, 1.8kw). Brains were frozen and coronal sections (20μm) were processed immediately for autoradiography and ATP quantification. Autoradiographic sections were dried onto coverslips and exposed to BioMax film for 75 min to obtain the FDG image. Two days later, sections were re-exposed to film for 1 day with 14C-standards (Amersham) to obtain the CBF image. For ATP analysis, 20μm tissue sections were mounted onto subbed slides and reacted to 80μm sections of frozen Luciferin-luciferase enzymatic solution. The slides were reacted for 30 s and blueluminescence images were compared with Fluor-S Multihorn (Bio-Rad) and quantified using the ATP standard curve (R2 = 0.99). Regional ATP levels were obtained from the standard curve and expressed as μmol/g ATP. FDG-based CMRglc and IAP-based CBF were determined by the operational equations of Sokoloff (1977) and Sakurada (1976), respectively. The mean ± SEM rates for control cortical cases were 1.43 ± 0.19 μmol/g ATP, 69.83 ± 2.68 μmol/100g/min CMRglc and 77.03 ± 2.54 μL/100g/min rCBF. This method of quantifying ATP, CMRglc and rCBF rates from the same region is a potentially powerful tool in understanding the events following TBI.

P368. DO NEURONS UTILIZE ALTERNATIVE FUELS ACUTELY FOLLOWING HUMAN TRAUMATIC BRAIN INJURY?
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We studied gray-white (GM-WM) metabolic differences of glucose and oxygen utilization in acute-injured TBI patients using positron emission tomography (PET). The objective was to determine whether there was evidence of abnormal cellular compartmentalization of energy metabolism and/or alternative fuel utilization.

Methods: 8 adult TBI patients and 11 age-matched healthy volunteers were studied. Each subject underwent quantitative FDG, (O-15)H2O, and (O-15)H2O PET studies and a coincident 3-D SPGR MRI. Parameter images of cerebral metabolic rate of glucose (CMRglc, μM/100g/min) and CMRVO2 (μM/100g/min) were generated. Restricted masks delineating cortical GM and WM were extracted using a MRI-based segmentation technique (JCBFM 2001:21,5572) and applied to the parametric PET images. The area-weighted-average values of CMRglc and CMRVO2 for the cortical GM and WM regions were calculated. The metabolic ratio (oxygen to glucose use ratio, OCR, in μM O2/μM glc) was calculated for GM and WM. A two-tailed t test was used for statistical analysis.

Results: Statistical analysis confirmed the following findings: (1) TBI patients show a selective depression of CMRglc in GM compared to controls (16.8 ± 3.0 vs. 23.7 ± 5.0; p < 0.005). GM-WM ratio of CMRglc was significantly lower following TBI (-17.4 ± 2.04 vs. 23.6 ± 0.29; p < 0.0001); (2) the percent reduction in oxygen utilization was similar in GM (110 ± 35 vs. 140 ± 33; p > 0.05) and WM (42 ± 6 vs 55 ± 15; p > 0.01) following TBI. Compared to normals, OCR in GM (6.6 vs. 5.9) and WM (4.6 vs. 5.4) yielded a significantly higher GM-WM ratio in TBI patients (1.47 ± 0.26 vs. 1.12 ± 0.12, p < 0.01).

Conclusion: TBI causes discordant changes of CMRVO2 and CMRglc in GM but not in WM. The selective increase of OCR in GM, where most neuronal and glial bodies are, suggests an altered metabolic state specifically neurons following TBI. The high GM OCR value (≥6.0) further suggests that alternative substrates are used in GM after TBI. Supported by NINDS 30308 and DE FC0387-ER60615.
P369.

MICROARRAY GENE EXPRESSION ANALYSIS OF POSTNATAL DAY 19 RAT CORTEX AFTER LATERAL FLUID FUSION INJURY
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Traumatic brain injury (TBI) triggers an interconnected cascade of pathophysiological changes. Following TBI to the immature brain, these effects are superimposed upon a growing and developing substrate. To better understand the complex patterns of gene expression after developmental brain trauma, we used microarray analysis of total parietal cortex RNA collected 4 hours (n = 4) and 24 hours (n = 4) after mild-moderate lateral fluid fusion injury (FFI) in the postnatal day 19 rat pup. Injury severities were similar in 4h and 24h animals (apex 23.5 ± 4.5 vs. 22.3 ± 2.6, respectively; unresponsiveness to toe pinch 76 ± 11.3 vs 70.3 ± 5.3, respectively). Comparisons were made with age and time-matched sham (n = 4 per point time). RNA was labeled and hybridized to Rat Genome Arrays (Affymetrix). Gene expression changes occurring in 75% of the comparisons between sham and injured were considered significant if the average fold-induction was >2. Using these criteria, 11 genes were significantly altered at 4h post-injury and 25 genes at 24h. At each time point, approximately 70% of these genes were upregulated and 30% downregulated. Several transcription factors (NAC-1, NCGF-1) and signal transduction molecules (tyrosine phosphatase, CaM kinase) were reduced after developmental FFI. Genes induced after FPI included those coding for metabolic enzymes (acyl CoA hydrolase, UDP-glucuronosyltransferase), neurotransmitter receptors (mGluR6), transcription factors (immediate-early serum responsive JE) and glial proteins (GFAP, vimentin, s100). These results demonstrate involvement of diverse molecular pathways following traumatic brain injury. This work provides a starting point for better understanding the unique vulnerabilty of the developing brain to traumatic injury. Support: NS30308, NS37965, NS22454 and UCLA Brain Injury Research Center.

P370.

AGE-RELATED MORPHOLOGIC CHANGES FOLLOWING TRAUMATIC BRAIN INJURY
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Abundant clinical data indicates increased morbidity and mortality following traumatic brain injury (TBI) in aged individuals. Little is currently known about the cellular substrates underlying this adverse response in the aged nervous system. The present study was designed to help characterize possible age-related morphologic response to TBI using a rodent model. Male Fisher 344 rats (5, 12, and 24 mos) were subjected to either a mild, moderate, or severe cortical injury using a lateral controlled cortical impact model of TBI. The animals were anesthetized with isoflurane and killed at 7 days post injury for assessment of cortical tissue sparing using unbiased stereology. We found an age-related and injury severity related difference in the magnitude of cortical sparing. The greatest sparing at each level of injury severity was observed in young animals while the aged animals showed the least amount of tissue sparing. Following a mild injury, young animals show almost no adverse consequence to the injury while aged rats have significantly less tissue sparing. At the moderate and severe injury levels, clear differences could be observed even in the 12 mos subjects compared to 3 mos subjects. Within each age group, there was a marked decline in tissue sparing dependent upon injury severity, the least amount of sparing observed with the most severe injury. The mortality rates among the different age groups were injury sensitive. The aged rats demonstrated the highest mortality rates similar to that observed in the clinical data. These results support the feasibility of using aged F344 rats to study age-related changes following TBI. Supported by NIH NS39828 and KSHIRT #9-20.

P371.

IN VIVO APPLICATION OF INOS ANTISENSE OLIGONUCLEOTIDES EXACERBATES HYPOPERFUSION AND UPREGULATES ENDOTHELIN-1 EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY.
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Nitric oxide (NO, a vasodilator) and endothelin (ET-1, a powerful vasoconstrictor) participate in the regulation of brain's microcirculation influencing each other's expression and synthesis. Following injury to the brain, NO is formed largely from the inducible form of nitric oxide synthase (iNOS). We used the Marmarou's model of traumatic brain injury (TBI) to study the cerebral blood flow and expression (mRNA) of ET-1 in rats that were pretreated with antisense iNOS oligodeoxynucleotides (ODN). Spengle-Dawley male rats were sacrificed 4, 24 and 48 h after TBI. Intracerebroventricular application of iNOS ODNs resulted in reduced synthesis of iNOS as detected by Western analysis. The cerebral blood flow (measured by laser Doppler flowmetry), generally decreased after TBI, was further reduced in the treated animals and remained at low levels up to 48 h post TBI. The expression of ET-1 (detected by in situ hybridization in cortex and hippocampus) was increased 2-3 fold following TBI alone and this increase reached 5-6 fold in animals pretreated with antisense iNOS ODNs. The results suggest that NO generated by iNOS, but not by other isoforms of the enzyme, suppresses ET-1 production and that a decrease of NO results in upregulation of ET-1 via transcriptional and translational mechanisms. Increased availability of ET-1 at the vascular bed and the neuropil may contribute to the altered microvascular reactivity and reduced perfusion of the brain following TBI. Supported by: NIH Grant NS398560

P372.

MICROARRAY ANALYSIS OF MICROGLOBULIN ACTIVATED BY SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.
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The emerging field of genomics provides potential for new insight into the pathophysiology of traumatic brain injury, by determining differences in gene expression in normal vs. injured brain. However, examination of expression differences in brain tissue does not provide a profile of genetic changes in the many individual cell types in the brain. Using an in vitro model of traumatic brain injury, we have previously identified several alterations in microglial calcium signaling and chemotaxis. Here, we examine selected gene expression profiles in resting and activated microglia. MG were isolated from 7-10 day old mixed brain cell cultures from neonatal rats. Resting MG were maintained in astrocyte-conditioned medium. Activated MG were prepared by a 24 hr exposure to medium conditioned by traumatically injured astrocytes for 3 hr, using an in vitro model for traumatic injury. cDNA was prepared from resting and activated microglia, followed by hybridization to selected expression arrays. Activated microglia displayed a dramatic increase in osteonectin (SPARC, secreted protein acidic rich in cysteine), an important component of the extracellular matrix that regulates cell motility. HNK-1 sulfotransferase was also increased, as well as mitochondrial encoded CoA hydrolase and the endoplasmic reticulum protein ERP29. Interestingly, expression of lysosome and the ferritin-H subunit were decreased. Alterations in these genes represent, in part, the early response of microglia to soluble factors released by injured astrocytes through the 3 hr post-injury period, and may be important in initiation of the inflammatory component of trauma. Supported by NS40490.
P373. APOLIPOPROTEIN E EPSILON 4 IN PEDIATRIC TRAUMATIC BRAIN INJURY: PHASE I—DIFFICULTIES IN OBTAINING APPROVALS AND PATIENT ENROLLMENT

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Identification of children with high risk of neurologic sequelae following traumatic brain injury (TBI) would allow more focused treatment. We hypothesized that children with an allele of apolipoprotein E epsilon 4 (apoE 4) are predisposed to poor neurological and functional outcomes after TBI, indicated by a higher pediatric cerebral performance category (PCPC) score. Our goal was to conduct a retrospective cohort study of children admitted to Children’s Hospital of Wisconsin from 1/1995 to 5/31/01 with a diagnosis of TBI.

Obtaining IRB and informed consent approvals caused a 1-year delay, illustrating the difficulties associated in dealing with pediatric patients. The IRB had initial concerns about the adequacy of consent, the propriety of genetic testing in children and confidentiality.

Following IRB approval, a chart review obtained demographic and clinical variables associated with outcome after TBI. Discharge and follow up pediatric overall performance category (POPC) and PCPC scores were obtained. An interview with the family was conducted to assess current status at follow-up. DNA was isolated from blood or buccal swab samples for standardization apoE4 genotyping. We found that either blood or buccal swab samples were sufficient to provide DNA for genotyping and hypothesized that patient enrollment is more easily accomplished in children when there is no blood draw. A total of 37 children were enrolled, ages 1 month to 16 years. Median Glasgow coma scale score (GCS) was 12 (range 4-15) and median pediatric performance score (PCPS) was 4 (0-25). Chi square analysis showed significant associations between admission GCS and PRISM with both discharge and follow-up PCPC scores.

P375. THE POTENTIAL ROLE OF THE CHEMOKINES MCP-1 AND IL-8 AS WELL AS ICAM-1 IN TRAUMATIC BRAIN INJURY

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Chemokines and adhesion molecules are required to orchestrate cerebral accumulation of leukocytes after traumatic brain injury (TBI) in particular with focal lesions. Monocyte chemotactic protein (MCP)-1 and Interleukin (IL)-8 as well as the intercellular adhesion molecule (ICAM)-1 were measured by ELISA in ventricular cerebrospinal fluid (CSF) of patients with severe TBI for up to 14 days after trauma. For comparison, the same factors were detected either in brain homogenates by ELISA or in tissue sections by immunohistochemistry of rats subjected to diffuse impact-acceleration TBI.

In all TBI patients, increased levels of MCP-1, IL-8 and ICAM-1 were detected as compared to control CSF. The mean MCP-1 CSF levels were highest at the day of admission and declined thereafter, whereas IL- 8 and ICAM-1 concentrations remained elevated during the whole study period. Interestingly, IL-8 levels in patients presenting with focal brain injuries (NEM, NEML according to the Marshall Score) were significantly higher than in patients with diffuse injury (DI, DI III) (p < 0.05; Student’s t-test). Impact-acceleration brain injury in rats resulted in early upregulation of MCP-1 concentrations between 4 and 16 hours and a relatively late ICAM-1 overexpression between 1 and 4 days post-injury as compared to sham operated control animals. In contrast, concentrations of the chemotactic factor macrophage inflammatory protein (MIP)-2 (the rodent analogue of macrophage chemoattractant protein-2) did not significantly exceed the constitutive levels detected in control brains, corroborating the findings of absence of neutrophil infiltration in this model as well as the similarity of low IL-8 levels in patients with diffuse vs. patients with focal brain injury.

These data provide evidence that in the impact-acceleration model, the ongoing inflammatory response is comparable to that seen in patients with diffuse axonal injury. The selective induction of inflammatory mediators present in focal vs. diffuse TBI suggests the existence of distinct immunomodulatory pathways for each type of TBI.

P374. THE ROLE OF CEREBRAL INFLAMMATION AFTER TRAUMATIC BRAIN INJURY—A CONCEPT REVISTED

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Cerebral inflammation begins immediately after traumatic brain injury (TBI) and is orchestrated by a large variety of inflammatory mediators secreted by activated cells of the immune and the nervous system. Propagation of immunoinflammation through the brain parenchyma also affects the healthy tissue surrounding the lesion, possibly causing alteration of the homestasis of larger regions of the brain. Despite the extensive knowledge acquired in recent years on the role of cerebral inflammation after TBI, it still remains to clarify to what extent inflammation contributes to the progressive loss of neuronal cells, a process which persists for long time after the traumatic event.

Although inflammation has been considered as a potentially harmful cascade due to its ability to induce several neurotoxic molecules, increased blood-brain barrier permeability, cerebral accumulation of leukocytes, and neuronal cell death, evidence gained in the last years and the concept of neuroinflammation, making it essential for the mechanisms of tissue repair. This dual function is suggested by both in vitro studies and animal models of TBI, which provide evidence for the induction of neutrophilic factors by cells stimulated with cytokines, as well as for specifically blocking cytokine action in animals resulting in improved neurological outcome after TBI. However, experiments with cytokine gene knockout mice have added further controversial significance to neuroinflammation rendering it as a temporarily bifunctional event. The distinction between the acute and the delayed phase of inflammation seems to be crucial for its beneficial or deleterious effects. Despite the controversial action displayed by inflammation, it appears clearly that the experimental setting chosen is crucial to address any scientific issue related to inflammation as often distinct approaches - molecular cytokine blockade versus cytokine-gene knockout mice - can lead to apparently opposite results. Therefore, caution is required when interpreting such data.

P376. INTRACRANIAL PRESSURE DYNAMICS: CHANGES OF BANDWIDTH AS AN INDICATOR OF CEREBROVASCULAR TENSION

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The transmission bandwidth (BW) of arterial blood pressure (ABP) to intracranial pressure (ICP) was examined as a means of bedside monitoring of the state of cerebrovascular tension of patients with severe head-injury. Changes of experimental values of BW, relative arteriolar resistance and intracranial compliance were obtained from a piglet model equipped with a cranial window during induction of asphyxia, hypercapnia, and hypoxia. Comparisons of experimental BW values to simulated changes of BW produced by a mathematical model of ICP dynamics were used to evaluate the hypothesis that during active cerebrovascular tension changes of BW are inversely related to changes of CPP. Induction of asphyxia produces BW changes characterized by an initial active cerebrovascular tension phase followed by a passive cerebrovascular tension phase. During the active tension phase the correlation between BW and CPP was inversely correlated to 1.41*10.5*x*8.32 (r = 0.98, p < 0.005, n = 45). During passive tension the relationship was correlated to 10.12*x*9.22 (r = 0.94, p < 0.005, n = 18). One hour later during reventilation both BW and CPP increased. Furthermore, the observed 2 Hz increase of BW was predicted by regression relationship between BW and CPP for passive tension. Hypercapnic and hypoxic challenge produced changes of experimental BW that were matched with BW simulations of the mathematical model designed to depict active tension. Relationships between values of BW and relative average cerebral arteriolar resistance and intracranial compliance were inverse and strongly correlated (r > 0.80) to a regression of x*8(a), where the parameter a ranged from 0.832 to 1.145. These preliminary experimental and theoretical results support the stated hypothesis.
P377. THE ABBREVIATED INJURY SCALE IS A NEGLECTED TOOL IN TRAUMATIC BRAIN INJURY RESEARCH
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Background: The Abbreviated Injury Scale (AIS) is an anatomically based, consensus derived, global severity scoring system that classifies each injury in every body region according to its relative importance on a six point ordinal scale. It has gone through several revisions since its origin in 1976 and over the course of time, it has become the most widely used injury severity scoring system in the world. Despite its uniform acceptance as the gold standard for the determination of severity of injury by most trauma centers and national transportation agencies worldwide, the AIS has been little used in neurotrauma circles.

Methods: The AIS dictionary was reviewed, evaluated and the reasons that the AIS has not become popular in neurotrauma circles were documented after interviewing more than 100 investigators. The structure and utility of the AIS are demonstrated. The AIS and the GCS were then individually and correlationally evaluated from a data set of 56,000 head injured patients.

Results and Discussion: There are several sections of the AIS involving head injury that describe injuries to the scalp, cranial nerves, skull and brain. Brain injuries are described in three redundant schemes involving anatomic injury, length of unconsciousness and level of consciousness. The AIS differs from physiological injury severity tools such as the Glasgow Coma Score and is complementary to the GCS so that the use of both systems will increase the precision of injury severity and injury outcome predictions. Correlations between the AIS and the GCS are demonstrated to be significant (p < 0.01) and the cross-product of GCS and AIS show a stronger relation to early death than does either tool individually.

Conclusion: This is an opportune time for the neurotrauma community to evaluate the AIS since it is now being revised and input from the neurotrauma community would be welcome.

P378. CORTICAL COMPACTION INJURY IN TRANSGENIC MICE TO STUDY THE ROLES OF REACTIVE ASTROCYTES

Astrocytes respond to CNS injury by hypertrophy, altered gene expression and proliferation, a process commonly referred to as 'reactive astrocytosis'. Both beneficial and detrimental effects in the response to injury have been attributed to reactive astrocytes; their roles are incompletely understood. Transgenic technology provides various means of manipulating specific cell types and dissecting the functions of specific molecules. We are developing various transgenic models to study the roles of reactive astrocytes and the specific molecules that they produce in traumatic brain injury (TBI). These transgenic models include (i) the selective ablation of reactive astrocytes in adult mice expressing GFAP-HSV-TK, and (ii) the knockout of genes encoding specific molecules synthesized by reactive astrocytes in adult mice using the Cre-loxP and tetracycline regulatable systems. As a model of TBI in mice we have chosen cortical compaction injury (CCI). Here we will report on the characterization of CCI and initial studies in non-transgenic and transgenic mice of the C57Bl6 background strain. Quantitative morphometric analyses will include lesion size and responses of specific cell types identified by immunohistochemistry after transgenically targeted ablation of reactive astrocytes and TBI. (Supported by UCLA BIRC and NIH NS42039)

P379. THE EFFECT OF POST-INJURY IRRADIATION ON NEURAL STEM CELL PROLIFERATION AND RECOVERY OF FUNCTION
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Neural stem cells are considered by many to be the 'magic bullet' for neurologic disorders. Several studies with Parkinson's disease and dysmyelinating disorders have demonstrated a functional benefit with exogenously supplied neural stem cells. It has also been shown that treatments such as enriched environment, running, estrogens and anti-depressants cause increased neurogenesis, while stress, glucocorticoids and opiates cause a reduction. Furthermore, many brain insults such as ischemia, status epilepticus and traumatic brain injury (TBI) have been shown to result in an increased proliferation and neuronal differentiation of endogenous neural stem cells. However, no functional benefit of the increased neurogenesis following traumatic brain injury has been demonstrated. To examine the role of neural stem cells, we used irradiation, to kill the proliferating cells (putative neural stem/progenitor cells). Half of the animals were irradiated at 12 Gy 24 hrs following TBI. Animals were then evaluated both histologically and for cognitive deficits in the Morris water maze at varying times after TBI. We observed a decrease in number of proliferating cells due to the irradiation, as expected. At 15 and 30 days after TBI, there was a significant difference in cognitive deficits between injured irradiated and injured non-irradiated animals. By 60 days the TBI non-irradiated animals displayed some recovery of cognitive deficits, however the TBI-irradiated animals were still impaired compared to sham-irradiated animals. We conclude that the increased neurogenesis observed following TBI plays a role in long-term cognitive recovery. Supported by NS-12587-26 and the Reynolds and Lind Lawrence Foundations.

P380. EFFECTIVENESS OF SEATBELTS TO PREVENT HEAD INJURY IN LATERAL VERSUS FRONTAL MOTOR VEHICLE IMPACTS
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Motor vehicle crashes remain one of the highest sources of traumatic brain injury. The object of this study was to evaluate the efficacy of seatbelt restraints to prevent head injury after frontal versus side impact motor vehicle accidents. The US Dept of Transportation National Automotive Sampling System (NASS) database files from 1993-2000 were evaluated for drivers and right front seat occupants in frontal [principal direction of force (PDOF) 11-1 o'clock] and near side (PDOF 8-10 o'clock or 2-4 o'clock) impacts. Head injury was graded using the Abbreviated Injury Scale (AIS). From the weighted data set there were 1,296,356 near side impact occupants (88.7% were belted) and 10,803,453 frontal impact occupants (86.0% were belted). Frontal impact resulted in a head injury (AIS 1-6) in 3.0% of occupants (9.6% of unbelted and 2.0% of belted occupants). Near side impact resulted in head injury in 6.8% of occupants (11.5% of unbelted and 6.2% of belted occupants). The pattern of injury was similar for moderate/severe head injury (AIS 3-6) with near side impact resulting in 2.4% injuries when unbelted and 0.3% when belted versus frontal impact causing 2.7% injuries unbelted and 0.9% belted. Of all occupants with a head injury, 17.4% of side impact occupants suffered a moderate/severe head injury compared to 19.3% of frontal impact occupants.

These data suggest that belted occupants have a 3-fold increased risk of suffering a head injury from a near side impact compared to a frontal impact. This trend is seen at all severities of head injury. While unbelted occupants have a higher rate of head injury independent of the PDOF the variance is less than that seen in belted occupants.
P381.
REGIONAL AND TEMPORAL PROFILE OF MITOTICALLY ACTIVE CELLS THROUGHOUT THE TRAUMATIZED BRAIN FOLLOWING TRAUMATIC BRAIN INJURY
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Following a variety of acute CNS injuries, a massive proliferation of stem/progenitor cells occurs. This study characterized cellular response of moderate traumatic brain injury (TBI) using indicators of cellular proliferation and mitotic division. Male Sprague-Dawley rats underwent fluid-percussion brain injury. The population of dividing cells was identified with bromodeoxyuridine (BrdU). Animals were sacrificed at 3 hr (n = 3), 1(n = 5), 2(n = 5), 3(n = 5), 5(n = 5), and 14 days (n = 5) after injury. Quantitative analyses of dividing cells were performed by non-biased cell counting in the ipsilateral and contralateral cortices and hippocampus. BrdU+ cells were observed in all sections ipsilateral and contralateral to the injured hemisphere at various times after TBI. In the cerebral cortex, the number of BrdU+ stained cells (mean ± SD x 104) increased in a time-dependent fashion between 3 hrs (8.1 ± 2.9) and 3 days (146.6 ± 17.3, p < 0.001), after which, the number of cells remained elevated up to 14 days (144.5 ± 55.4). Contralaterally, a gradual increase in BrdU+ cells between 3 hours (6.2 ± 2.9) and 14 days (77.3 ± 53.7, p < 0.001) was observed. In the hippocampus, there was an increase in the number of BrdU+ cells between 3 hours (6.3 ± 4.0) and 3 days (54.1 ± 11.3, p < 0.001), after which it remained elevated up to 14 days (41.6 ± 16.7). On the contralateral side, a gradual increase in BrdU+ cells, between 3 hours (5.5 ± 1.8) and 14 days (28.5 ± 17.2, p = 0.008) was observed. There was a statistically significant difference (p < 0.001) between ipsilateral and contralateral sides at all time points except 3 hours. Within the subventricular zone, there was a significant increase in BrdU+ cells at all time points investigated. A large number of double-labeled cells were stained positively for GFAP, with smaller numbers being positive for neuN. These results demonstrate a time-dependent increase in the number of proliferating cells following moderate TBI and provide baseline data for future studies assessing the effects of growth factor treatment on cellular proliferation and differentiation. NS30291.

P382.
THERAPEUTIC HYPOTERMIA PRESERVES ANTIOXIDANT DEFENSES AFTER TRAUMATIC BRAIN INJURY IN INFANTS AND CHILDREN
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A wealth of experimental and clinical data support a contribution of oxidative stress to secondary damage after traumatic brain injury (TBI). Hypothermia has been shown to decrease endogenous antioxidant consumption and lipid peroxidation after experimental brain injury (2,3). We hypothesized that therapeutic hypothermia attenuates oxidative damage as assessed by markers of lipid peroxidation, protein oxidation, and antioxidant status (glutathione and total antioxidant reserve [AOR]) in cerebrospinal fluid (CSF) after severe TBI in infants and children. We compared the effects of moderate hypothermia (32–33°C) vs normothermia in 15 patients with severe TBI (GCS score <8). Patients were treated in a single center in a multi-center randomized controlled trial of hypothermia in pediatric TBI. The general paradigm for patients treated with hypothermia (n = 9) involved cooling to target within ~6h for 48h and then re-warming. Protein thiols and glutathione (fluorescence assay), AOR (chemiluminescence assay), and F2-isoprostane (ELISA) were assessed in ventricular CSF samples (n = 49) on day 1-3 after injury. Protein oxidation was attenuated by hypothermia vs normothermia (p < 0.05, d=1-2). CSF levels of glutathione were higher on d3 in hypothermic vs normothermic patients (p < 0.05). AOR was higher in hypothermic vs normothermic patients (p < 0.05, d=1). F2-isoprostane levels were ~4-fold higher in normothermic vs hypothermic patients (p < 0.06, d1). To our knowledge this is the first study assessing the effect of hypothermia on oxidative stress after severe TBI in infants and children. We report dramatic protection by hypothermia across a broad spectrum of markers of oxidative stress. Our data also demonstrate that CSF represents a valuable tool for monitoring treatment effects on oxidative stress after TBI. (1) Buyar et al, 2002 (2) Kamberb et al, 1994 (3) Lei et al, 1994, SUPPORT; Eric Bundy Memorial Fund, Charles Schertz Fellowship grant, NS 34884, Lacerdal Foundation, Children’s Hospital of Pittsburgh GCR.

P383.
SHORT-TERM EFFICACY OF THE TREATMENT OF BRAIN TRAUMA MAY NOT TRANSLETE INTO LONG-TERM IMPROVEMENTS
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We have previously found that treatment of brain-injured rats with MgSO4 or the non-competitive NMDA-receptor antagonist, NPS-1506, improved cognitive and histopathologic outcome by one week following trauma. To determine the persistence of this efficacy, we evaluated the effects of both treatments at 4 months following injury. Male Sprague-Dawley rats (350-400g) were subjected to parafascicular fluid percussion injury (2.5±0.5 atm) and were given injections of NPS-1506 (1.15 mg/kg, i.p., n = 12) 5 min and 4 hr post-injury, MgSO4 (125 mmol, i.v., n = 12) 15 min post-injury, or vehicle (saline, 2 ml/kg, n = 9). Sham animals received identical surgery without injury, and received saline (2 ml/kg, n = 10). Four months following injury, animals were evaluated for learning ability using a water maze paradigm. Briefly, animals were trained over 3d (6 trials/day) to locate a submerged platform. Escape times (latency) were averaged over the final 6 trials. Following behavior testing, animals were sacrificed and perfused with 4% paraformaldehyde. To assess the extent of cortical tissue loss, coronal sections between -5.8 and -6.04 bregma were stained with hematoxylin and eosin and analyzed using NIH-imaging software. Using the contralateral hemisphere as a control, the percentage of tissue loss in the ipsilateral hemisphere was calculated. We found long-term learning dysfunction (3-10% increased latency times) in the vehicle-treated injured compared to sham animals (p < 0.001). However, no improvements in latency were found for the MgSO4 or NPS-1506-treated injured animals. Likewise, while injury in vehicle treated animals resulted in a 30% loss of tissue in the ipsilateral hemisphere, this atrophy was not reduced in the drug-treated animals. These results suggest that acute treatment with MgSO4 and NPS-1506 may not convey long-lasting efficacy following injury. Long-term supplemental therapies may have to be considered to combat progressive neurodegeneration induced by brain trauma. Supported by NIH grants AG 21527, NS38104, and NS08803.

P384.
CALPAIN MEDIATED SPECTRIN BREAKDOWN PRODUCTS IN THE CEREBROSPINAL FLUID OF SEVERELY HEAD INJURED PATIENTS
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Calcium-induced, calpain-mediated proteolytic processes are considered key players in brain and spinal cord injury. Recent observations have suggested that therapeutic interventions, limiting the formation of calpain-mediated spectrin breakdown products (SBDP), are associated with improved outcomes in experimental TBI.

The present study was initiated to determine if SBDP accumulation can be detected in the cerebrospinal fluid (CSF) of severely head injured patients, providing a potential diagnostic and perhaps, prognostic tool for monitoring the course and severity of TBI.

Ventricular CSF was obtained from 9 severely head-injured patients (Glasgow Coma Scale <9) as part of an established protocol for raised intracranial pressure (>20mmHg). CSF samples of 4 patients treated for acute hydrocephalus associated with subarachnoid hemorrhage constituted the control group. The presence of SBDP were evaluated via Western blots using a monoclonal antibody capable of detecting intact non-erythroid alpha-l spectrin (280 kD) as well as its 150, 145 and 120KD cleavage fragments.

All severely head-injured patients displayed calpain-mediated SBDP 150/145 accumulation. Elevated protein levels were most striking in those samples taken at 2-5 days post-injury, with the most elevated levels associated with the least favorable outcomes. In three of the controls, a less pronounced SBDP-accumulation was also detected, a finding most likely associated with periventricular white matter injury due to hydrocephalus.

These results indicate that high SBDP-levels are detectable in ventricular CSF following severe TBI, and in some cases of acute hydrocephalus. Our observations suggest that detection of SBDP-levels may provide useful information on the severity of proteolytic processes set in motion by brain/waite matter injury of various origins. Further, they may serve as a useful tool in the assessment of injury severity and the prediction of outcomes.

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P385.  
TOPIRAMATE ATTENUATES TRAUMATIC BRAIN INJURY-INDUCED NEUROMOTOR DEFICITS IN RATS  
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The present study evaluated the ability of topiramate, an antiepileptic drug, to reduce edema formation and cognitive and motor deficits following lateral fluid percussion (FP) brain injury in rats. Anesthetized adult rats were subjected to lateral FP brain injury (n = 60) or left uninjured (n = 47, sham surgery). At 30 min post-injury, animals were randomized to receive either topiramate (30 mg/kg, i.p.; injured n = 35, sham n = 21) or sterile water (injured n = 25, sham n = 26) followed by administration of topiramate (30 mg/kg, p.o.) or vehicle at 8, 22 and 32 hrs postinjury. Following cognitive evaluation in the Morris water maze at 48h, a subset of animals was sacrificed for brain water content evaluation. All other animals received motor function testing at 48h, 1, 2, 3, and 4 wks post-injury using a 28-point neuroscore test, followed by cognitive testing at 1 month. Injured animals showed significant edema formation, cognitive and motor deficits when compared to uninjured animals. However, no differences in brain water content or cognitive function were detectable between drug and vehicle treated animals. Topiramate significantly attenuated motor dysfunction in injured animals in the neuroscore at 4 weeks postinjury (p < 0.05) and the rotating pole test at 1 and 4 weeks postinjury (p < 0.05) when compared to vehicle treated brain injured rats, suggesting a potentially beneficial effect of topiramate in improving motor function following TBI. Supported by J & J Pharm. R & D, LLC, NIH NS 08803, NS 40978, & GM 34569.

P386.  
ADENOSINE A2A RECEPTOR KNOCKOUT MICE ARE NEUROPROTECTED AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY  

The adenosine 2A (A2a) receptor exhibits multiple roles in the CNS--both detrimental (direct and indirect excitotoxic actions) and beneficial (blood flow promotion and anti-inflammatory effects). In models of stroke and Parkinson's disease, A2a knockout (ko) mice and animals treated with A2a receptor antagonists are neuroprotected. 1. We hypothesized that A2a receptor knockout mice would be neuroprotected after experimental traumatic brain injury (TBI) produced by controlled cortical impact (CCI). A2a ko and wildtype (wt) littermates (n = 13), genotypes confirmed by southern blot and immunohistochemistry, were injured at 15 wks of age. CCI was performed at a velocity of 5 m/s, a depth of 1.2 mm. Brain temperature was controlled at 37°C. At 24 h, mice were perfused with 4% paraformaldehyde and brain tissue was paraffin embedded for sectioning. Sections were stained with H&E and hippocampal neuronal counts (40X) were performed in CA1, CA2 and CA3 regions. In CA1, the A2a ko was markedly protected vs wt (91.75 ± 17.53 vs 35.20 ± 16.72 cells, respectively, mean ± SEM, p < 0.05). A 2-fold trend towards neuroprotection was also seen in CA3 (but not in CA2) for ko vs wt. We conclude that neuroprotection in A2a ko mice in the CCI model is consistent with recent reports in stroke and Parkinson's disease, suggesting a key pro-excitotoxic regulatory role for the A2a receptor. Our findings support the need to evaluate A2a receptor antagonists as a possible therapy in experimental and clinical TBI. 1Chen et al, 1999, 2Chen et al, 2001. SUPPORT: NS38037, NS 30381, HD 06866

P387.  
THE THERAPEUTIC EFFICACY OF THE 5-HT1A RECEPTOR AGONIST 8-OH-DPAT IN TRAUMATICALLY BRAIN-INJURED RATS IS NOT MEDIATED BY CONCOMITANT HYPOTHERMIA  
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We recently demonstrated beneficial effects on cognitive and histological outcome after a single acute dose (0.5 mg/kg, i.p.) of the serotonin (5-HT1A receptor agonist 8 hydroxy-2-di-n-propylamino)tetralin (8-OH-DPAT), which induces mild hypothermia transiently (Dixon et al., J Neurotrauma, 18:1172, 2001). Thus, to determine if the beneficial effects observed were mediated by hypothermia, we conducted an experiment identical to the previous, but included a group of 8-OH-DPAT-treated rats that were actively kept normothermic (37 ± 0.5°C) with a heating lamp. Briefly, thirty-nine isoflurane-anesthetized rats underwent a controlled cortical impact (2.7 mm deformation) or sham injury and then were randomly assigned to one of five groups (Sham/VEH n = 5, Sham/0.5 mg/kg 8-OH-DPAT n = 5, TBI/VEH n = 9, TBI/8-OH-DPAT Normothermic n = 10, TBI/8-OH-DPAT Hypothermic n = 10). 8-OH-DPAT or VEH was administered (i.p.) 15 min after TBI or sham injury. Cognitive performance was assessed in the Morris water maze on post-operative days 14-18. Both 8-OH-DPAT-treated groups attenuated cognitive impairment after TBI vs. VEH (p < 0.05). No significant differences were observed between the normothermic and hypothermic DPAT-treated groups, despite a rapid (15 min after injection), mild (34.4-34.9°C), and transient hypothermic effect (1 hr) in the latter group. These data confirm that systemic administration of the 5-HT1A receptor agonist 8-OH-DPAT provides cognitive protection after moderate TBI. The data also suggest that the beneficial effect is not mediated by concomitant hypothermia. 5-HT1A receptor agonists may be a novel alternative therapeutic strategy after TBI in humans. Supported by the Walter L. Copeland Fund (D 2001-0288) of The Pittsburgh Foundation (AEK) and NS33150 and NS40125 (CED).

P388.  
TEMPORAL AND REGIONAL ALTERATIONS IN ENDOGENOUS GDNF EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY  

Glial cell line-derived neurotrophic factor (GDNF) is an important regulator of neuronal development and exhibits neuroprotective activity in several models of CNS injury. However, to date, there has been no assessment of endogenous GDNF expression following traumatic brain injury (TBI). In the current experiment, the temporal and regional endogenous expression of GDNF was examined following lateral fluid percussion (FP) brain injury (2.7–2.8 atm, n = 4) anesthetized adult rats using enzyme-linked immunosorbant assay (ELISA). Control rats received neither surgery nor injury (n=6, animals, n=3). Twenty-four hours following injury, the animals were sacrificed and the injured brain hemispheres were dissected into cortical region. Cortical regions from brain-injured animals demonstrated a significant increase in GDNF protein expression at 24 h postinjury (23.9 ± 6.4 pg/mg protein) compared to naive cortical regions (9.3 ± 3.6 pg/mg protein) (p < 0.05). These alterations in GDNF protein expression may be involved in modulating the neuronal response after brain injury. Supported by, in part, by NIH NS 40478, NS 08803, GM3640 and a Veteran Administration-DOD Consortium Merit Review Grant.
P389.
DOWNREGULATION OF MATRIX METALLOPROTEINASE-9 AND ATTENUATION OF EDEMA VIA INHIBITION OF ERK MAP KINASE
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As a family of extracellular proteases, matrix metalloproteases (MMPs) are capable of degrading or modifying almost all components of the extracellular matrix and it may be involved in the pathophysiology of acute brain injury. However, the regulatory mechanisms involved in vivo remain unclear. We previously reported that autocrine protein kinase (MAPK) was upregulated after traumatic brain injury (TBI) and intraventricular administration of MAPK/ERK kinase (MEK) inhibitor decreased lesion volume after injury. In this study, we focus on a MAPK pathway that may trigger MMP-9 and MAPK inhibitors. We examined whether inhibition of the extracellular signal-regulated kinase (ERK) would attenuate MMP-9 levels, reduce blood-brain barrier damage, and attenuate edema after trauma induced by controlled cortical impact in mouse brain. A rapid upregulation of phospho-ERK occurred immediately after trauma. Double-labelled immunohistochemistry showed that ERK activation occurred primarily in neuronal cells in traumatized cortex. Treatment with U0126, MEK inhibitor effectively prevented the activation of ERK and reduced the trauma-induced upregulation in MMP-9. Correspondingly, U0126 attenuated the degradation of the tight junction protein ZO-1, which is an MMP-9 substrate, and significantly attenuated tissue edema. At 7 days after trauma, traumatic lesion volumes were significantly reduced by U0126 compared with saline-treated controls. These data indicate that the ERK MAPK pathway triggers the upregulation in MMP-9 after trauma, and further suggest that examination of signaling mechanisms that regulate deleterious MMP-9 activity may reveal new therapeutic opportunities for traumatic brain injury.

P390.
TREATMENT WITH THE IRREVERSIBLE, CELL PERMEABLE CASPASE-9 INHIBITOR ILL PROTECTS PROTECTION AGAINST CA1 TRAUMATIC NEURONAL INJURY IN THE HIPPOCAMPAL SLICE
Caspase-9 holds a key position in the initiation of programmed cell death. Caspase-9 is also released from mitochondria with brain injury, and is activated with binding to Apaf-1 and dATP. Activated Caspase-9 then cleaves other Caspases further downstream, including Caspase-3, -6 and -7. Due to the critical role of Caspase 9 in programmed cell death pathways, we hypothesized that inhibitors of Caspase 9 would be neuroprotective against CA1 traumatic neuronal injury. Therefore, we investigated whether treatment with the cell permeable irreversible Caspase-9 inhibitor III, Ac-LEHD-CMK, would preserve CA1 evoked response in hippocampal slices following fluid percussion trauma. Recovery was assessed one hour after trauma. Trauma induced rapid loss of CA1 orthodromic and antidromic PS response. After recovery for 60 min, CA1 orthodromic and antidromic population spike (PS) response regained only a mean 11% ± 2 and 15% ± 2 of initial amplitude. In contrast, treatment with 5 µM Ac-LEHD-CMK initiated within one min after trauma, improved recovery of CA1 orthodromic and antidromic PS response to 93% ± 5 and 94% ± 4 of initial amplitude (p < 0.05). Long-term- potentiation (LTP) was completely lost following trauma, while treatment with Ac-LEHD-CMK preserved LTP. With this inhibitor, tetanus produced a mean increase in CA1 orthodromic PS amplitude to 127% ± 2, similar to LTP induced in sham slices, which showed an increase of 126% ± 3. These findings suggest that caspase-9 mediated-effects play an important role in injury to CA1 neurons from trauma. Supported by the VA Research Service and the UCLA Brain Injury Research Center.

P391.
THE EFFECTS OF MEK INHIBITOR U0126 FOLLOWING TRAUMATIC BRAIN INJURY IN RATS
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(Indroduction) Biochemical cascades underlying posttraumatic neuronal degeneration have not been fully elucidated. We recently demonstrated that traumatic brain injury (TBI) induces ERK-phosphorylation in the rat brain. In this study, we investigate the role of ERK-phosphorylation using MEK1/2 inhibitors U0126 in histopathological changes and motor function after TBI. (Material & Methods) Adult male Sprague-Dawley rats (300-400 g) were subjected to lateral fluid percussion injury of moderate severity (1.5-4 atm). U0126 (100, 200, or 400 µg/kg) or vehicle (DMSO) was injected into the femoral vein 15 min before injury. Sham control animals were subjected to all the same procedures except for actual insult. Serial coronal sections (3-5µm-thick) were counterstained with hematoxylin and eosin. Severity of neuronal damage in CA3 subfield was evaluated by the number of survived or damaged neurons 72 hrs after TBI. Contusional brain volume 72 hrs after TBI was measured using an image analysis system by summing the contusional brain areas. Evaluation of brain atrophy 3 wks after TBI was calculated using the same formula. In addition, we assessed the motor function using a beam-balance task and a beam-walking task. (Results) The present results indicate that intravenous administration of the U0126 promote the CA3 neuronal survival and reduce the contusional volume 72 hrs after TBI. U0126 also improve the brain atrophic changes 3 wks after TBI. In addition, U0126 make a significant recovery of the motor function 3, 4, 5 days after TBI compared to that in vehicle groups. (Conclusion) Inhibition of the ERK-phosphorylation ameliorates the neurologic and histopathological outcome following TBI in rats. These findings suggest that the activation of the ERK cascade has deleterious effects on the damaged neurons following TBI.

P392.
CHANGES IN DARPP-32 PROTEIN EXPRESSION FOLLOWING CONTROLLED CORTICAL IMPACT IN RATS
Margaret S. Wilson, Youming Li*, X. Mo and C. Edward Dixon (Department of Neurosurgery, University of Pittsburgh, PA USA)
DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, is a cytosolic protein abundant in medium-sized spiny neurons in the neostriatum. When phosphorylated by cAMP-dependent protein kinase (PKA), DARPP-32 is a potent inhibitor of protein phosphatase-1, thereby regulating a large array of downstream effectors. DARPP-32 plays an integral role in signal transduction of dopaminergic neurons; regulators of its phosphorylation include dopamine, glutamate, GABA, and adenosine. To determine whether levels of DARPP-32 are altered following traumatic brain injury (TBI), Western blot analysis and immunohistochemistry were performed to compare DARPP-32 protein expression in the striatum of rats that received controlled cortical impact (CCI) injury versus sham-injured controls. Twenty-four hours after CCI (27 mm impact at 4 m/s), adult male rats (n = 4 injured, 4 sham) were anesthetized and decapitated, and the ipsilateral and contralateral striatum processed for Western blot analysis. Protein expression was measured using a primary antibody against DARPP-32, generously donated by Dr. Paul Greengard. Semiquantitative densitometry revealed an injury-induced decrease in DARPP-32 protein expression in both ipsilateral (28%) and contralateral (43%) hemispheres. Immunohistochemistry for DARPP-32 suggests the decrease persists up to 7 days post-injury. Qualitative analysis indicates a lack of apparent change in the number of DARPP-32 immunopositive cells, suggesting the decrease in protein expression is not due to neuronal cell death in the striatum. The wide range of signaling cascades that include DARPP-32 suggests a reduction may influence multiple facets of the injury process. Behaviors that are mediated by DARPP-32 include motor function, learning and memory, and motivation. Further insight into injury-induced changes in DARPP-32 protein expression and phosphorylation may further the development of novel treatments for TBI patients. (Supported by NS-33150)
P393. SYNAPTONAL Dopamine UPTAKE IN Rat STRIATUM FOLLOWING CONTROLLED CORTICAL IMPACT
Margaret S. Wilson*, X. Ma, Ian J. Reynolds and C. Edward Dixon. (Departments of Neurosurgery and Pharmacology, University of Pittsburgh, Pittsburgh, PA US)

Functional deficits following traumatic brain injury (TBI) are associated with alterations in markers of dopaminergic neurotransmission. Using the controlled cortical impact (CCI) model of TBI in rats, our lab previously demonstrated decreased dopamine (DA) D2 receptor protein in striatum (Yan et al., 1999), increased tyrosine hydroxylase protein in frontal cortex (Yan et al., 2001), and decreased dopamine transporter (DAT) protein in the striatum (preliminary data) 4, 4, and 2 weeks after injury, respectively. DAT plays a critical role in maintaining DA homeostasis. To assess the effects of TBI on the functional integrity of DAT, we investigated synaptosomal DA uptake in the striatum, where levels of DAT expression are highest.

Fifteen days after lateral CCI (2.7 mm impact at 4 m/s) or sham injury, adult male rats (n = 4 injured, 4 sham) were decapitated and striatal tissue rapidly dissected. Synaptosomal preparations from the ipsilateral and contralateral hemispheres were incubated at 37°C with 20 nM [3H]DA and varying concentrations of unlabeled DA in the presence or absence of 5µM uptake inhibitor, mazindol. DA uptake in injured-ipsilateral, injured-contralateral, sham-ipsilateral and sham-contralateral tissue was compared using repeated-measures ANOVA. No significant difference in synaptosomal uptake was found between groups 15 days after CCI. Our data suggest that striatal DAT is capable of normal function 15 days after CCI. However, it is unclear whether neurons in the injured striatum can properly regulate the activity of DAT. The lack of change in DAT kinetic activity in the injured striatum is surprising in light of our previous finding of decreased DAT protein by western blotting. Future studies will examine DA uptake at additional timepoints in the striatum and other brain regions, and assess other aspects of DA neurotransmission after TBI. (Supported by NS-33150)

P394. MORPHOLOGICAL, DYNAMIC AND CYTOSKELETAL PROPERTIES UNDERLYING NEURITE DEVELOPMENT AND AXONAL SPIRITING FOLLOWING LOCALISED TRANSECTION OF CORTICAL AXONS IN VITRO
Jyoti A Chaukernere* and James C Vickers (NeuroRepair Group, University of Tasmania, Hobart, Tasmania, AU).

Accumulating evidence demonstrates that mature central neurites retain the capacity to mount a regenerative attempt in response to physical injury when provided with a facilitative environment. Cytoskeletal recovery and remodelling may be crucial to this regenerative capacity. We utilised an in vitro model of neuronal trauma to explore whether the cytoskeletal alterations associated with post-injury axonal sprouting correlate with the sequence of cytoskeletal changes underlying neurite development. Neocortical cultures were generated using E18 rats. Neurites were dissociated and grown in Neurobasal medium containing B27 supplement. Under these conditions neuronal cell bodies formed aggregates that became progressively interconnected by neurite bundles and latissi. which were transacted under microscope guidance. Neurons were examined during development at 3 days in vitro (DIV) and at various time points following axotomy at 21DIV. Time-lapse imaging demonstrated that the post-injury response is highly dynamic, progressing through an initial phase of axonal retraction, followed by substantial axonal sprouting into injury sites within 4-6 hours following transection. Analogous to developing neurites, post-injury sprouts were highly motile and consisted of a slender shaft and an expanded growth cone-like end structure. AlexaFluorTM 488 Phalloidin and immuno-labelling (utilising antibodies specific for cytoskeletal and other proteins) demonstrated that filamentous-actin was the predominant cytoskeletal constituent in the extreme distal tips of sprouts, whereas beta-tubulin and tau were localised to sprout shafts and proximal regions of putative growth cones. The neurorlament triplet was, however, restricted to sprout shafts. A similar distribution of these cytoskeletal proteins was present in developing neurites at 3 DIV. Interestingly, a greater proportion of sprouts were immuno-reactive for beta-filamentin and tau than for neurofilament triplet proteins. The morphology, cytoskeletal constitution and dynamic properties of sprouts emerging from transected axons at 21DIV closely resembled those of developing neurites, indicating that post-injury axonal sprouting recapitulates these aspects of initial neurite development.

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P395. CLINICAL BIOMECHANICS OF PENETRATING BRAIN TRAUMA
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INTRODUCTION: Since 1992 gunshot wounds are the leading cause of brain injury death in the US. To investigate primary and secondary wound profiles in the brain due to penetrating head trauma, prospective clinical study, experiments, and computer-driven finite element model (FEM) were used.

METHODS: Patient CT/MRI scans were evaluated. Psychosocial measures and neurological status determined outcome. Images were transferred to software to create three-dimensional surface models of the primary and secondary wound tracks from which volumetric data were determined. A three-dimensional FEM of the brain and skull was constructed to reproduce penetrating trauma. It was validated with human tissue simulati experiments. Projectiles were discharged at 700 meters/second into 30-gm gelatin blocks (ex vivo models). Projectile paths were reconstructed with a digital camera at 9,000–18,000 frames/second. Wounding deformations quantified as a function of time validated the FEM. The computer model was exercised with varying projectile geometries (flat-face, pointed-face) and velocities to reproduce the damage profile seen in the CT/MRI.

RESULTS AND DISCUSSION: Time-varying intracranial deformations demonstrated the progression of the projectile into the skull and brain. Similar skull penetrations occurred with both projectiles. However, the extent of brain damage was greater with the flat-face projectile that produced transient, secondary waves with larger area/volume. Furthermore, the brain tissue sustained these waves for 1-2 milliseconds, approximately twice the duration of the pointed projectile. A significant loss of penetrating velocity occurred with the flat-face projectile. The winding capacity of the other projectile was low. These results indicate that kinetic energy from the flat-face projectile is transmitted to brain tissue not only in the region immediately surrounding the path of the projectile but also in regions away from its path. The geometry of the projectile is an important determinant in the extent of brain trauma in regions away from the primary penetration path.

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P396. LOCALISATION OF ALPHA-SYNUCLEIN FOLLOWING AXONAL TRANSECTION: IMPLICATIONS FOR REGROWTH AND REPAIR
Marion C Quirby*, Wei-Ping Gui†, Adrian K West, James C Vickers (NeuroRepair Group, University of Tasmania, Hobart, Tasmania, and *Department of Human Physiology, Flinders University, Adelaide, Australia).

Alpha-synuclein is highly enriched in presynaptic terminals, and abnormal processing of alpha-synuclein has been demonstrated in several human neurodegenerative diseases. Following head trauma, alpha-synuclein also accumulates in axonal swellings, but its relationship with the plastic changes associated with axonal regrowth and repair has not been examined. We investigated the localisation of alpha-synuclein in an in vitro model of axonal transection that results in regenerative sprouting. Primary rat neocortical neuronal cultures were established using E18 embryos from Hooded Wistar rats. Neurons were grown on coverslips in Neurobasal medium (GIBCO) containing B27 supplement. At 21 days in vitro, axonal branches were transected using a gonioscope knife. Cells were fixed and 24 hours post-injury immunostained for alpha-synuclein in combination with markers for phosphorylated neurofilaments, synaptophysin, microtubule- and growth-associated proteins. At 4 hours PI, alpha-synuclein accumulated diffusely as enlarged puncta in transected axons. In axons that resisted to injury by forming neurofilament ring- and bulb-like structures, high concentrations of alpha-synuclein were observed in the centre of these structures. By 24 hours PI, there was a decrease in alpha-synuclein accumulation at transaction sites, but ring- and bulb-like structures remained positive for alpha-synuclein. In post-traumatic sprouting axons at 24 hours PI, alpha-synuclein immunoreactive puncta were concentrated in the most proximal region of growth cone-like structures, with little labelling in the distal tips as revealed by GAP-43. Our findings suggest that alpha-synuclein may be actively involved in axonal injury and repair.

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P397.
REACTIVE AND REGENERATIVE NEURONAL CYTOSKELETAL ALTERATIONS FOLLOWING ACUTE LOCALIZED INJURY TO THE RAT NEOCORTEX.
Jumet C. Vickers*1, Jyoti A. Chaukkreeve and Sandrine Chapon. (NeuroRepair Group, University of Tasmania, Hobart, Tasmania AU).

We investigated the alterations in neuronal cytoskeletal proteins associated with localized brain injury. The neocortex of anaesthetized adult Wistar rats was injured by insertion of a 25 gauge blunt needle for 10 minutes. Animals were re-anesthetized and perfused transcardially with 4% paraformaldehyde at 1, 4, 7 and 14 days post-injury (PI). Multiple immunohistochemical labelling with antibodies to the neurofilament (NF) triplet, alpha-internexin, neuron-specific beta-tubulin, cytochrome C, GFAP and ferritin was performed. Needle injury resulted in a cavity surrounded by necrotic tissue (1 day PI) followed by significant wound healing (4-7 days PI) characterised by the development of central microglial/macrophage mass and peripherally located reactive astrocytes. By 14 days PI, a neuropil of generally normal appearance surrounded by a narrow microglial remnant. At 1 day PI, the needle tract was surrounded by abnormal neurites variably immunoreactive for beta-tubulin, cytochrome C, NF triplet and alpha-internexin, the latter intermediate filaments also localized to bulb- or ring-like structures. Surrounding nerve cell bodies had fragmented NF labelling as well as a high degree of cytochrome C labelling. By 4-7 days PI, substantial neurite sprouting beyond the zone of astrocyte proliferation and into the central mass of ferritin labelled cells had occurred, with more sprouting neurites labelled for beta-tubulin labelling than the NF proteins. Real-time PCR also demonstrated variable regulation of cytoskeletal gene expression following injury. In addition, beta-tubulin labelled cells were present within the central cellular mass at 7 days PI. Thus, cytoskeletal proteins are variably involved in the reactive and regenerative response of cortical neurones to injury. Furthermore, some neurones have motile properties that may contribute to remodelling of brain architecture.

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P398.
LONG-TERM ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE AND PRESENLIN-1, AND CASPASE-3 IN DAMAGED AXONS FOLLOWING BRAIN TRAUMA.
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Plaques composed of amyloid-beta (A-beta) have been found within days following a single incident of brain trauma in humans, similar to the hallmark plaque pathology of Alzheimer’s disease (AD). We have recently found that a potential source of this amyloid-beta is in long-term accumulations in damaged axons following incidental brain injury in the pig. Here, we used the same model to evaluate long-term changes in A-beta accumulation and potential mechanisms that could lead to its production. Brain injury was induced via nonimpact head rotational acceleration of 1100° over 20 ms in the coronal plane. Injured pigs were sacrificed at 3 days (n = 3), 7 days (n = 3) and 6 months (n = 3) post-injury. Two non-injured animals served as controls. Immunohistochemistry and Western blot analysis were performed on brain sections and tissues using antibodies specific for amyloid precursor protein (APP), A-beta, BACE, presenilin-1 (PS-1), caspase-3, and caspase-mediated cleavage of APP. Surprisingly, substantial co-accumulation for all of these factors was found in swollen axons at all timepoints, including up to 6 months following injury. Western blot analysis of tissue from injured brains confirmed a substantial increase in the protein levels of these factors, particularly in the white matter. Although in AD, A-beta is thought to be primarily produced via transthyretin cleavage of APP by BACE and PS-1 our data suggests that following trauma these factors, as well as caspase activity may produce A-beta within the axonal membrane compartment. Supported by NIH grants AG21327, NS38104 and NS08803.

P399.
DELAYED DISRUPTION IN AXONAL TRANSPORT FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS

Retrograde degeneration may contribute to delayed cell death in areas remote from the contusion following lateral fluid percussion(FP) brain injury. We examined the integrity of axonal projections to the injured cortex using the retrogradely transported neuronal tracer, Fluoro-Gold. Adult male Sprague-Dawley rats were anesthetized (sodium pentobarbital, 60 mg/kg, ip.) and subjected to lateral FP brain injury. Rats were then injected with 1ml of 2% Fluoro-Gold into the ipsilateral parietal cortex(AP -4.3, ML 5.5, DV 2.0) at 6 hours (n = 6) or 24 hours (n = 5) post-injury or immediately after sham injury(n = 6). At 1 week post-injection, frozen brain sections(40mm) were cut coronally from 1.6 to -6.3 mm bregma. In uninjured animals, numerous Fluoro-Gold positive neuronal cell bodies were observed in the bilateral parietal cortex and ipsilateral thalamus. In the group of animals injected at 6 hours post-injury, a modest reduction in the number of labelled neurones was observed in these areas, particularly in the contralateral cortex and ipsilateral thalamus. In contrast, Fluoro-Gold labelling was nearly absent in the contralateral cortex and ipsilateral thalamus in the group of animals injected 24 post-injury. These results suggest that there is a delayed, progressive impairment of retrograde axonal transport from the injured parietal cortex to the contralateral cortex and ipsilateral thalamus. Reduced axonal transport may be due to synaptic damage, cytoskeletal alterations, secondary axonopathy, or cell death of the projecting neuron.

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P400.
INCREASED APPARENT DIFFUSION COEFFICIENT IN NORMAL APPEARING WHITE MATTER
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Introduction: Diffuse brain injury (cerebral, ischemic, swelling) occurs following traumatic insult, causing persistent functional or psychological deficits, but may go undetected in conventional imaging. There is evidence of diffuse metabolic/structural abnormalities in normal appearing white matter (NAWM) of traumatic brain injury (TBI) patients, demonstrated by proton magnetic resonance spectroscopy (1). Laboratory models of concussion injury have demonstrated focal bicausal changes in apparent diffusion coefficient (ADC) with initial low converting to high values after 1 week (2). We propose that the ADC of NAWM is altered in humans following traumatic brain injury.

Methods: A common EPI sequence was used to obtain co-registered quantitative T1, T2 and ADC maps. An unsupervised clustering technique based on absolute T1 and T2 relaxation times was used to obtain reliable white matter regions of interest. ADC values for supra-verminal NAWM were obtained.

Subjects: 18 patients (mean age 36, range 17-63) admittted with a diagnosis of head injury (mean admission GCS 7.5, 9 mild, 6 moderate and 3 severe) were scanned an average of 7.5 days after injury (range 1-40). Results were compared with a control group (n = 12, av. age 35, 18-59). Results: The patient group showed a trend towards an increase in ADC in the NAWM reaching significance (p = 0.039 Mann-Whitney U) in superior slices corresponding to centrum semiovale (645 ± 42 ±10.6 mm^2), mean ± SD; control of 621 ± 30; p = 0.039 Mann-Whitney U), with no significant changes in absolute T1 or T2 relaxation times.

Conclusions: TBI patients at this time point have a diffuse increase in ADC in NAWM with a lack of concomitant rise in absolute T1 and T2 values suggesting a mechanism other than an increase in tissue water. Neuronal damage/injury or a disruption in tissue architecture may cause an increase in water mobility leading to a rise in ADC.

P401. TEMPORAL VULNERABILITY TO REPEETITIVE EXPERIMENTAL BRAIN INJURY: LONG TERM SEQUELAE OF MULTIPLE CONCUSSIONS.
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We evaluated the effects of the interval between two repetitive concussive brain injuries (CBI) on cognitive function and histological damage. Mice (n = 131) were anesthetized using isoflurane and subjected to sham-injury (n = 22), single CBI (n = 32) or repetitive CBI (n = 77) using a modified controlled cortical impact model. In the repetitive CBI group, the interval between the first and second concussion was either 3 days (n = 53), 5 days (n = 12) or 7 days (n = 12). Cognitive function was tested using the Morris Water Maze. Mice subjected to repetitive CBI 3 days apart exhibited greater dysfunction than either sham animals (p < 0.05) or than mice receiving a single injury (p < 0.01). These deficits were maintained when the injuries were 5 days apart (p < 0.05 compared to single CBI), but not 7 days apart, suggesting that there is a transient vulnerability of the brain during the first 3 days following a concussion. Contusions were not appreciated with the routine histological analysis, but scattered degenerating neurons, evidence of cytoskeletal damage and axonal injury were detected in the cortex, hippocampus, thalamus and hypothalamus in 72 hours to 4 weeks postinjury in all the brain-injured mice. These data suggest that CBI is associated with subcellular alterations and cell death as well as a transient vulnerability during which a second injury leads to behavioral dysfunction.

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P402. NET CONTRACTILE FORCES OF THE ACTOMYOSIN NETWORK POWER THE DELAYED ELASTIC RESPONSE OF THE AXONAL CYTOSKELETON FOLLOWING STRETCH INJURY.

Immediately following stretch injury in vitro, initially straight axons immediately exhibit several undulated regions along their length, slowly retracting to their initial shape over a period of 20 to 45 minutes. We have termed this response 'delayed elasticity'. We hypothesize the initial undulated appearance of the axons is due to local cytoskeletal damage, and the subsequent imbalance of motor protein forces acting upon the actin and microtubule networks in the local areas of disarray lead to a restoring force to power the retraction.

In this study, cultured rat cortical axons were exposed to a single, dynamic stretch of 65-110% that simulated mechanical injury. The mechanisms of the ensuing morphological recovery were examined by exposing the axons to actin depolymerizing agent latrunculin A, myosin inhibitor butanedione monoxime (BDM), microtubule depolymerizing agent colchicine, and microtubule polymerizing agent paclitaxel. The delayed elastic response was significantly inhibited in axons exposed latrunculin A, BDM, and paclitaxel, suggesting the contractile forces provided by the actomyosin network are important regulators for axonal shape following stretch injury. Delayed elasticity was not inhibited by colchicine, suggesting the microtubule network is not necessary to drive rapid axonal re-alignment after mechanical injury. Funding provided by NIH HD 41699 and PO1 NS 08803.

P403. THE INCREASED INTRACRANIAL PRESSURE AS AN IMPORANT NEGATIVE INDICATOR OF SEVERE HEAD INJURY OUTCOME.
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The main consequence of cerebral blood flow disturbances following severe head injury is the development of secondary mechanism of traumatic brain lesion. There is direct correlation between post injury cerebral blood flow impairment resulting in the increase of intracranial pressure (ICP), and brain tissue ischemia.

The aim of this study is to point out the importance of the increased ICP as main negative indicator of severe head injury outcome, as well as to stress the value of ICP monitoring in the management of such an injury.

During one-year period (January 2001–January 2002), 56 patients suffering severe head injury whose presenting Glasgow Coma Scale score (GCS) was less than 9, were treated at Division of Neurosurgery, Osijek University Hospital, Osijek, Croatia. External ventriculostomy and ICP monitoring was performed in 24 (66.7%) patients. Duration of ICP monitoring was between 2 and 8 days after admission, mean 5.3 days. Control group consisted 12 patients in whom ICP monitoring was not applied. All were admitted in Intensive Care Unit (ICU) and mechanically ventilated. Intracranial pressure was maintained below 25 mm Hg by moderate hyperventilation (pCO2 > 30 mm Hg), intermittent Mannitol infusion, and in the group of ICP monitored by ventricular drainage. Early mortality in the group of monitored patients was 29.1% (7/24), while it was 58.3% (7/12) in the control group. The data were statistically analyzed using chi-square test and nonparametric correlation tests. The significance was set at p < 0.05.

Correlation between the duration of the increased ICP in hours per day and low GCS in ICU discharge was noticed (p = 0.04).

Considering the results of this study, raised ICP is a strong negative indicator of outcome. Since early mortality in ICP monitored patients was significantly lower (p < 0.05) then in control group, ICP monitoring is well justified in the management of severe head injury.

P404. TRAUMATIC BRAIN INJURY IN HUMANS CAN INDUCE PROGRESSIVE CEREBRAL ATROPHY.

Atrophy of the brain is one of the most common radiologic findings in survivors of brain trauma. It is generally believed that this atrophy is a passive process, reflecting loss of nonviable tissue within a few months following injury. However, using animal models of brain trauma, others and we have found long-term neurodegenerative changes associated with remarkably progressive brain atrophy. Here, we examined serial images of brain trauma patients to evaluate potential progressive atrophic changes. To determine delayed atrophy, we limited our analysis to images taken no earlier than 3-4 months following injury to be compared with ones taken months to years later. We also limited our examination to easily seen changes. We excluded patients due to age (>50 years old at time of injury), history of alcoholism, evacuated hematomas, and multiple head injuries. In all, we evaluated 24 moderate to severe brain-injured patients (Glasgow Coma Scale score of 3-12), comparing serial cross-sectional imaging (computed tomography and magnetic resonance). Ten of the patients were noted to have moderate-to-severe progressive enlargement of the subarachnoid and ventricular spaces, indicative of diffuse parenchymal volume loss. An additional three patients developed mild diffuse sulcal dilatation. Focal, progressive malacic cavitations were noted in seven of the patients. Some of these changes were found to progress even years following injury. These data suggest that chronically progressive degenerative processes may be initiated by a single brain trauma event in humans. However, the incidence of progressive atrophy following trauma remains to be determined. Nonetheless, we may have to consider developing therapeutic strategies to ameliorate long-term neurodegeneration in brain-injured patients. Supported by NIH grants, AG12527, NS38104, and NS08803.
P405. CEREBRAL PERFUSION PRESSURE MANAGEMENT AS AN IMPORTANT FACTOR INFLUENCING THE OUTCOME OF SEVERE BRAIN INJURY

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Following severe brain injury, cerebral blood flow impairment occurs as a result of secondary mechanism of traumatic brain lesion. Cerebral perfusion pressure (CPP) decrease is the main cause of brain tissue ischemia after such an injury. Therefore, maintaining CPP values above critical level, as well as reducing intracranial pressure (ICP), is necessary to avoid ischemic brain lesion.

The purpose of this report is to stress the value of CPP management as an important factor that influence the outcome of brain injury.

Between January 2001 and January 2002, 36 patients who suffered severe brain injury were treated at Division of Neurosurgery, Osijek University Hospital, Osijek, Croatia. In all the admission Glasgow Coma Scale Score (GCS) was less than 9. Intracranial pressure monitoring was performed in 24 (66.7%) patients. Mean duration of ICP monitoring was 53.5 days. Cerebral perfusion pressure was individually calculated from the difference between mean arterial blood pressure and ICP. Control group consisted of 12 patients in whom ICP monitoring was not performed. Following admission and neuroradiologic diagnostics, as well as early surgery if necessary, all patients were admitted in Intensive Care Unit (ICU) and mechanically ventilated. Cerebral perfusion pressure was maintained above 70 mm Hg by moderate hyperventilation (pCO2 > 50 mm Hg), intermittent 20% Mannitol infusion, and in the group of ICP monitored by external ventricular drainage. Chi-square test and nonparametric correlation tests to get Spearman’s coefficient of correlation were used in statistical analysis with the significance set at p < 0.05.

The relationship between duration of the decreased CPP in hours per day and low GCS at ICU discharge was observed (p < 0.05). Early mortality rate in ICP monitored patients was significantly lower than in control group (p < 0.05).

Concerning the results of this report, CPP management is proved important factor influencing the outcome of severe brain injury.

P406. ASSOCIATION BETWEEN INTRAVASCULAR MICROTHROMBOSIS AND CEREBRAL ISCHEMIA IN TRAUMATIC BRAIN INJURY

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Ischemia is a prominent finding in traumatic brain injury (TBI). Some cases result from cerebral hemiation or brain compression by hematoma, others from hypotension or hypoxemia. However, over half of fatal TBI cases exhibit varying degrees of selective neuronal necrosis (SNN), the cause of which is unclear.

We reviewed samples of frontal and hippocampal cortical tissue from a cohort of 90 cases with fatal TBI. Tissue stained with hematoxylin and eosin (H&E) was reviewed and rated for severity of SNN. Since intravascular fibrin microthrombi may lyse within a few days of TBI, we restricted our analysis to subjects dying within 48 hours of injury. Medical records in all cases were reviewed to rule out severe or prolonged hypotension or hypoxemia. Eleven cases with severe or global SNN were compared to eleven cases in whom SNN was mild or absent. Slides adjacent to H&E sections were stained with immunofluorescent antibody to antithrombin III and reviewed for intravascular coagulation (IC). The number of microthrombi on each slide was counted by an investigator blinded to the H&E findings, and IC density calculated.

Intravascular microthrombi were noted in every section, excluding control (non-TBI) brain tissue. However, the density of IC varied with the degree of SNN. We found a highly significant difference in the mean IC density between cases with little or no SNN (2.5 ± 0.29/cm²), and cases of severe SNN (7.74 ± 1.10). These data support a strong link between IC and neuron death following brain trauma in humans and may have important implications for new therapeutic approaches. Supported by NIH grants, AG12527, NS38104 and NS08093.

P407. TRANSIENT HEMORRHAGIC HYPOTENSION DOES NOT AGGRAVATE BEHAVIORAL AND COGNITIVE DEFICITS IN BRAIN-INJURED RATS

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Traumatic brain injury is commonly associated with hemorrhage known to aggravate evolving brain damage. Blood loss exceeding 20% of total blood volume may compromise organ perfusion, oxygenation, and initiate a plethora of systemic and cerebral autodestructive cascades. We sought to determine the impact of a transient superimposed hemorrhagic hypotension (HH) on behavioral and cognitive deficits following fluid percussion injury (FPI) in rats.

After arterial and venous cannulation, pentobarbital-anesthetized rats were subjected to moderate-severe FPI (3.0 atm). Five minutes later, rats were randomized to controlled arterial HH (30% total blood volume; 30 minutes; n = 10) or sham-stabbed (n = 8) groups. Following HH, rats were fluid resuscitated with Lactated Ringer’s solution (3x shed blood volume) for 30 minutes. During HH rats were kept normothermic. MABP was recorded continuously and neurobehavioral (motor and cognitive) changes were evaluated up to 8 weeks.

The 50% reduction in MABP to 50–60 mmHg coincided with significant decreases in hemoglobin levels (15 ± 1 to 10 ± 1 g/dL; p < 0.05). Fluid resuscitation significantly increased MABP to pre-injury levels without, however, improving the hemoglobin count.

Significant motor and cognitive deficits following FPI (decreased composite neuroscore, beam balance score, and increased latencies in the Morris water maze) were not aggravated by the superimposed HH.

These preliminary data suggest that the chosen level of HH did not exacerbate FPI-induced motor and cognitive changes. Further studies are warranted to characterize the different independent variables (severity of brain injury, HH, and fluid resuscitation) more closely.

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P408. RISK FACTORS FOR DEVELOPMENT OF NEUROGENIC FEVER FOLLOWING TRAUMATIC BRAIN INJURY

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Neurogenic fever is a sequela of traumatic brain injury (TBI) and may be associated with poorer outcome. The purpose of this study was to identify factors associated with the development of neurogenic fever following severe TBI in adults. Charts of patients admitted from 1996–1999 with severe TBI at a large, urban Mid-Atlantic teaching hospital were retrospectively evaluated based on diagnostic criteria for each episode of hyperthermia to determine the diagnosis of neurogenic fever (N = 76). The incidence of neurogenic fever in this population was 11.8%. Data was collected regarding mechanism and area of injury, severity of injury, and demographic factors to determine potential predictors of neurogenic fever using logistic regression modeling. Diffuse axonal injury (Odds Ratio (OR) 9.06, 95% Confidence Interval (CI) 0.89, 20.7) and frontal lobe injury of any type (OR 6.68, 95% CI 1.1, 39.5) are independently predictive of an increased risk of development of neurogenic fever following severe TBI. The presence of a skull fracture and lower initial Glasgow Coma Score were individual predictors of development of neurogenic fever, but did not contribute to the final model. A set of predictor variables was identified to help clinicians target patients at high risk for development of neurogenic fever following severe TBI. Clinicians evaluating unexplained fever in TBI patients need to consider these factors during their diagnostic assessments.

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P409. REPEATED MILD INJURY CAUSES CUMULATIVE DAMAGE TO HIPPOCAMPAL CELLS

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Recent studies testing cognitive deficits in athletes and child abuse victims and in animals injured experimentally in vivo support the hypothesis that repeated mild traumatic brain injury (MTBI) may result in cumulative damage to cells of the brain. However, post-injury sequelae are difficult to address at the cellular level in vivo. Therefore, it is necessary to complement these studies with experiments conducted in vitro. In this report, the effects of single and repeated mild traumatic injury in vitro were investigated in cultured mouse hippocampal cells using a well-characterized model of stretch-induced injury. Cell damage was assessed using fluorescein diacetate (FDA) and propidium iodide (PI), and by the release of neuron specific enolase (NSE) and S-100β protein, two common clinical markers of CNS damage. Cultures received a second injury one hour after the initial insult. Repeated injury caused a slight increase in PI uptake versus single injury, primarily evident in the underlying glial layer. A reduction of neuronal phenotypes was also apparent 24 hr post-injury. In addition, the neurons of neurons that received repeated insults showed signs of damage not evident after single injury. Six hours post-injury, both NSE and S-100β levels were elevated after repeated injuries when compared to the single injury group. These results suggest that cells of the hippocampus may be susceptible to cumulative damage following repeated mild traumatic insults. Both glial cells and neurons appear to exhibit increased signs of damage after repetitive injury. The biochemical pathways of cellular degradation following repeated mild injuries may differ considerably from those that are activated by a single mild insult. Therefore, we hope to use this model in order to investigate secondary pathways of cellular damage after repeated mild traumatic injury and as a rapid and economical means of screening possibilities for treatment strategies for MTBI. Supported by NWO-MW, ALW, PIONIER.

P410. ACTIVATION OF GROUP II mGluRs DO NOT PREVENT TRAUMATIC OR ISCHEMIC INJURY OF WHITE MATTER

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Recent studies indicate that activation of group II mGluRs attenuates excitotoxicity. Here we examined the effects of selective agonists on traumatic as well as ischemic injury on spinal cord white matter in vitro. At concentrations shown to be selective for group II mGluR's.

A 30mm length of dorsal column was isolated from the spinal cord of adult rats, pinned in an in vitro recording chamber (37°C) and injured with 95% N2 and 5% CO2 or modified clip (2g closing force) for 15 sec. The functional integrity of the dorsal column was monitored electrophysiologically by quantitatively measuring the compound action potential (CAP).

The mean CAP decreased to 49.4 ± 2.6 % and 49.5 ± 5.7 % of control (p < 0.05) after trauma and hypoxia/ischemia injury, respectively. The selective group II agonist APDC agonist did not attenuate the posttraumatic and ischemic reduction of CAP amplitude. Whereas, blockade of group II mGluR receptors with LY 341495 and Rgpa resulted in significant improved recovery of compound action potentials (CAP) amplitude of controls (p < 0.05) after hypoxic/ischemic and traumatic injury to dorsal column white matter. Western blot analysis also identified the presence of mGluR II (231) positive immunoreactive of ~152 kD proteins in the spinal cord dorsal columns. The expression of protein is decreased after acute traumatic or hypoxic/ischemic injury of white matter. In conclusion, these data indicate that traumatic and ischemic injury induced acute activation of mGluRs II does not contributes to cellular pathophysiology of spinal cord dorsal columns in vitro.

P411. MEDIATORS OF PRECONDITIONING EFFECT IDENTIFIED BY MICROARRAY ANALYSIS OF RAT SPINAL CORD AFTER BRIEF ISCHEMIA

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A brief period of spinal cord ischemia protects neurons and preserves locomotor function after subsequent ischemic insult. Gene expression changes after preconditioning are likely to alter the response of the spinal cord to further injury. Using microarrays, we sought to identify gene expression patterns and neuroprotection-associated genes (NAGs) that mediate the preconditioning effect. Rats were exposed to 30 minutes of spinal ischemia by balloon occlusion of the descending aorta followed by 30 minutes to 48 hours of reperfusion. The mRNA levels of preconditioned lumbar spinal cords were compared to sham-injured controls in four replicates using microarrays containing 3,900 oligonucleotide probes. Cluster analysis reveals two discrete groups of significantly induced genes. The two most prominent K-means clusters each contain a stress-induced gene that has been associated with ischemic preconditioning in the heart and brain. The first group peaks at 30 minutes and includes several transcripts for inducible heat shock protein, hsp70. We validated mRNA levels by real-time PCR, with hsp70 levels reaching 40 times those of sham-injured controls. Immunostaining for HSP70 protein was observed by 6 hours and returned to baseline at 24 hours. A second group of genes shows increased expression in 6 and 12 hours after preconditioning and includes metallothionein-1 and -2. These results suggest a role for hsp70 and metallothionein, along with several other NAGs, in spinal cord neuroprotection.

W.M. Keck Center for Collaborative Neuroscience, Rutgers University, NJ, RPH, IBC, WY, Anesthesiology Research Laboratory, University of California, San Diego, CA (OK, MM) Center for Applied Genomics and PHRI, NJIT, NJ (DS, PT) Supported by: The SCI Project of the W M Keck Center, NJ Commission on Spinal Cord Research, NS32794

P412. ETHYL PYRUVATE IS PROTECTIVE AGAINST MILD AND SEVERE TRANSIENT GLOBAL ISCHEMIA IN GERBILS

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Background and Objective: Pyruvate has been shown to have protective effects in several ischemia models in different organs in both in vitro and in vivo. However, the mechanism(s) action need to be further elucidated. The purpose of this study is to test whether ethyl pyruvate, a derivat form of pyruvate, has protective effects against mild and severe transient forebrain ischemia.

Materials and Methods: Transient forebrain ischemia in adult male Mongolian gerbils was induced by the bilateral occlusion of common carotid arteries for 5 min (mild) or 10 min (severe). Ethyl pyruvate (500 mg/kg) or sodium chloride (104.5mg/kg) (isomolar) were injected at the time of occlusion. Seventy-two hours after occlusion, the animals were euthanized and their brains removed and frozen. The brains were sectioned at 10 um thickness using cryostat and stained with hematoxylin and eosin. The eosinophilic cells were counted and the data were analyzed using Statview v.5.

Results: The data show that ethyl pyruvate has neuroprotective effect against both mild (p = 0.0131) and severe global ischemia (p = 0.0013), respectively.

Conclusions: The data show that ethyl pyruvate is neuroprotective in global ischemia and could replace sodium pyruvate, which was abandoned as clinical therapeutic agent due to its instability.
P413. MILD HYPOTERMIA REDUCES ZINC TRANSLLOCATION, NEURONAL CELL DEATH, AND MORTALITY AFTER TRANSIENT GLOBAL ISCHEMIA IN MICE
Daisuke Tsukita*, Shuhuayi Hong, Takamasa Kayama, Phillip R. Weinstein, S. Scott Pantzer (Department of Neurological Surgery, University of California and VA Medical Center, San Francisco, CA; Department of Neurosurgery, Yamagata University School of Medicine, Yamagata, Japan).

The purpose of this study is to determine whether Zn2+ translocation associated with neuronal cell death occurs after transient global ischemia (TGI) in mice and to determine the effect of mild hypothermia on this reaction.

To validate the TGI model, carbon black injection and laser-Doppler flowmetry were compared in three strains of mice (C57BL/6, SV129 and HSP70 transgenic mice) to assess posterior communicating artery (PcomA) development and cortical perfusion. These results were then used to determine the optimal occlusion time (20 minutes) for C57BL/6 in TGI. Brain and rectal temperature measurements were compared to monitor hypothermia. Results of TGI were compared in normothermia (NT; 37°C) (n = 14) and mild hypothermia groups (HT; 33°C) (n = 14) by staining with Zn2+-specific fluorescent dye, N-(6-methoxy-2-quinoxalyl)-para-toluenesulfonamide (TSQ) and hematoxylin-eosin after 72 hours perfusion.

Zn2+ translocation observed in hippocampus CA1, CA2 and Hilus 72 hours after 20 minutes of TGI was significantly reduced by mild hypothermia. The number of degenerating neurons in the HT group was significantly less than in the NT group. Mild hypothermia reduced mortality significantly (7.1% in HT, 42.9% in NT).

Results suggest that mild hypothermia may reduce presynaptic Zn2+ release in hippocampal neurons from ischemic neurosis. Future studies may further elucidate mechanisms of Zn2+ induced ischemic injury.

P414. MLNS19 AND NEUROPROTECTION: CONTINUING THERAPEUTIC WINDOW STUDIES IN EXPERIMENTAL BRAIN ISCHEMIA INJURY AND COMPLETION OF A PHASE I SAFETY TRIALS IN HUMAN VOLUNTEERS
F. Tortella, A. Williams, J. Adams and P. Elliott (Walter Reed Army Institute of Research, Silver Spring, MD US).

At the 2000 INTS meeting, we reported our preclinical rat and early Phase I clinical results with the MLNS19 (formerly PS319). As documented in published reports, MLNS19 is a novel small molecule inhibitor of the 20S proteasome exhibiting neuroprotective properties in multiple rat models of cerebral ischemia, with efficacy established over a range of i.v. doses and distinguished by a substantial therapeutic window (TW). Since the failure of neuroprotection drug trials has been attributed, in part, to poorly established TWs, we have continued to evaluate the TW for MLNS19 in the rat MCAo model, as well as having now completed a Phase I i.v. safety trial in 67 individuals.

In the rat MCAo/72 h recovery model, significant reductions in brain infarction can be achieved with treatment delays of 10 h. MLNS19 (1.0 mg/kg, i.v.) neuroprotection correlated with 1) improved neurological function, 2) significant decreases in the number of neutrophils and macrophages present in ischemic brain regions, and 3) reductions in fragmented astrocyte throughout the core area of injury.

A Phase I double-blind, randomized, placebo-controlled trial investigating the safety, tolerability and pharmacodynamics of MLNS19 has now been completed in healthy, young male volunteers. MLNS19 was administered i.v., where 39 subjects received single doses of 0.012 mg/m2 (-1.6 mg/m2) and 28 subjects received doses of 0.5 mg/m2 -1.6 mg/m2 on three consecutive days. The drug was well tolerated; there was no clear treatment-emergent symptoms or abnormality of laboratory tests. Also, 20S proteasome activity in blood achieved the intended maximum target level of 70-80% inhibition, and was reproducible with repeated dosing. At Walter Reed, rat MCAo studies are continuing to further elucidate the cellular mechanism(s) of action of MLNS19, while Millennium and PAION Pharmaceuticals have recently joined forces to collaboratively advance the clinical development of MLNS19 to Phase II stroke trials.

P415. NEUROPROTECTIVE TREATMENT WITH THE SNAIL PEPTIDE CXG-1007 EFFECTIVELY REDUCES BOTH C-FOS GENE EXPRESSION AND APOPTOSIS IN A RAT MODEL OF FOCAL ISCHEMIA
Anthony J Williams* and Frank C Tortella (Walter Reed Army Institute of Research, Silver Spring, MD US).

The role of the immediate early gene (IEG) c-fos as either a neuroprotective or neurodegenerative promoter following cerebral ischemia has not been definitively determined to date. In this study, we have evaluated the up-regulation of c-fos mRNA and protein levels during the initial 24 h of transient middle cerebral artery occlusion (MCAo) in the rat and the effect of treatment with the novel NMDA receptor antagonist and neuroprotective agent CXG-1007. Treatment with CXG-1007 (0.5 nmol, i.c.v.) was given at 30 min post-MCAo. Brain tissue was collected at 1, 4, and 24 h post-injury and processed for quantitative RT-PCR and histological staining including TTC, TUNEL, and c-fos conjugation. CXG-1007 effectively reduced the presence of TUNEL-positive cells at 24 h, predominantly in cortical brain tissues. C-fos mRNA levels peaked at 1 h post-injury in both cortical and subcortical ischemic brain regions (30 fold increase), remained elevated at 4 h and returned to normal, pre-injury levels by 24 h post-injury. The increase in mRNA levels correlated to increased c-fos protein expression in the entire ipsilateral hemisphere at 1 h. Regions of necrosis at 4 h were void of c-fos immunoreactivity with continued upregulation in surrounding regions. At 24 h, no upregulation of c-fos protein was observed in the injured hemisphere except for sustained increases in the cingulate and parietal cortex of vehicle-treated rats. Post-injury treatment with CXG-1007 effectively reduced both mRNA and protein levels of c-fos at all time points examined. CXG-1007 treatment also reduced c-fos immunostaining in the cingulate cortex, a brain region which has previously been reported to be resistant to treatment with NMDA antagonists. This supports the hypothesis that c-fos may have neurodegenerative properties and that treatment with CXG-1007 is able to reduce the upregulation of c-fos and provide neuroprotective relief of cerebral ischemia.

P416. ETHYL PYRUVATE IS PROTECTIVE IN TRANSIENT FOCAL ISCHEMIA IN RATS
Daohong Yang*, Valerie Copper, Shuhuayi Hong, Angelo Zagna, S. Scott Pantzer (Department of Neurosurgery, University of California, San Francisco and Department of Neurology, Veterans Affairs Medical Center, San Francisco, CA USA).

Background and purpose: Sodium Pyruvate has proved to be neuroprotective against global ischemia in rats. Ethyl Pyruvate, with better stability than sodium pyruvate, is protective for the intestinal ischemia-reperfusion injury. However, whether ethyl pyruvate is protective in transient focal ischemia is not known.

Methods: Male Sprague-Dawley rats (250-300g) received 90 min middle cerebral artery occlusion (MCAo) by an intraluminal suture followed by 23 hrs reperfusion and were treated with Ethyl pyruvate (1000mg/kg, n = 5) or iso-osmolar NaCl (n = 4) infused intravenously at the beginning of ischemia, during ischemia and during reperfusion. TTC (2,3,5-triphenyl tetrazolium chloride) staining was used for measuring infarct size. Neurological deficits were measured 1h after surgery and before sacrifice.

Results: Neurological deficits were decreased 24hrs after surgery in the ethyl pyruvate group when compared to the NaCl group. The body weight loss in the ethyl pyruvate group was also less than that in the NaCl group. The total infarct volume in cortex and striatum following ethyl pyruvate therapy decreased almost 40% less than that in the NaCl-treated group.

Conclusion: Continuously infusion of ethyl pyruvate has the potential to protect against transient focal ischemia in rats.
P417. QUANTIFICATION OF NEURONAL DEGENERATION IN IN VIVO CLIP COMPRESSION MODELS OF SPINAL CORD INJURY USING FLUouro-JADE B
Karya J. Horan* and Michael G. Fellings. (Division of Neurosurgery, University of Toronto and Division of Cellular and Molecular Biology, Toronto Western Research Institute, Toronto, Ontario CA).

Fluoro-Jade B has been used as a fluorescent marker for neuronal degeneration in rat brain tissue. However, its effectiveness in spinal cord tissue has not yet been documented. The simple staining procedure makes it an attractive tool for examining neurodegeneration in central nervous system injury models. In this study we examine the effectiveness of Fluoro-Jade B as a marker of neuronal degeneration after acute spinal cord injury (SCI). In vivo spinal cord injuries in rat and mice were performed using a clip compression model of SCI at closing forces of 20g and 8.3g respectively. Rat spinal cord sections were stained with Fluoro-Jade B and double labeled with NeuN to determine cellular colocalization. Specificity of staining for dead or dying cells was determined using double labeling with Fluoro-Jade B and TUNEL. Results demonstrate that Fluoro-Jade B co-localizes with NeuN as well as TUNEL. Neuronal degeneration was quantified using Fluoro-Jade B staining in 1, 2, and 3 day injured mice (n=3/group). Data indicated a significantly larger number of Fluoro-Jade B labeled neurons 1 day (113.5 ± 18), 2 days (74.3 ± 4.2) and 3 days (61 ± 8.9) following injury compared with uninjured mice (5.3 ± 2.1). Spatial distribution of neuronal degeneration indicated a larger number of neurons labeled at the epicenter compared to the periphery of the injury. Fluoro-Jade B labeled neurons in mice with varying degrees of injury were also examined. Mice were injured at the T7 level using clips calibrated at 3.1g (mild injury), 8.3g (moderate injury) and 24.1g (severe injury). Preliminary results indicate a larger number of Fluoro-Jade B labeled neurons in mice with severe injury. Fluoro-Jade B is a quantifiable marker for neuronal degeneration in the in vivo mouse and rat SCI compression models that can be used as an efficient and reliable histological tool.

P418. INFLAMMATORY MECHANISMS REVEALED BY EXPRESSION PROFILING OF ACUTE SPINAL CORD INJURY
Jonathan Z. Pan; Patricia Soteropoulos; Peter Tolias; Ronald P. Hart. (Rutgers University, Newark, NJ US).

Inflammation likely contributes to secondary damage after spinal cord injury (SCI). An anti-inflammatory glucocorticoid, methylprednisolone (MP) is the standard therapy for acute SCI. Since other compounds are not as effective, we compared several drugs for their inhibition of inflammatory mechanisms following SCI. Using quantitative real-time PCR (Q-RT-PCR) and custom rat microarrays, we compared expression profiles following treatment with MP, acetaminophen, indomethacin, NS-398, IL-1ra and soluble TNF-RFc. We also correlated the established MASCIS impactor model with a cultured slice model of SCI. Adult spinal cord was dissected into 1 mm segments and incubated with or without drugs in serum-free medium for 4 hrs, then total cellular RNA was prepared. By Q-RT-PCR, IL-6 and TNFa mRNAs were reduced by acetaminophen and indomethacin, respectively, while IL-1b, TNFa, IL-6 and MIP-1a were all decreased following MP or combined IL-1ra and TNFR-Fc. There was no effect of these cytokine mRNAs. Clustering analysis of microarray results identified genes regulated by NS-398 differing from those affected by other drugs, which indicated distinct targets of cyclooxygenase II. Comparing data from slice cultures to the MASCIS model showed good correlation for most genes, but some differences were also noted. In summary, using microarray analysis, anti-inflammatory drugs with different mechanisms were distinguished by their gene expression profiles. Understanding inflammatory cascades initiated by SCI should allow design of improved therapies.
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P.Soteropoulos; P. Tolias (Center for Applied Genomics, PHRI, Newark, NJ, USA)
Supported by: SCI Project of the Keck Center, New Jersey Commission on Spinal Cord Research

P419. TREATMENT OF SPINAL CORD INJURY USING AN ANTIBODY TO THE LEUKOCYTE INTEGRIN ALPHA D/BETA 2 EFFECTIVELY PREVENTS THE EARLY INFLTRATION OF PHAGOCYTES AND MONOCYTES/MACROPHAGES INTO THE LESION

The inflammatory response following spinal cord injury (SCI) extends the damage caused by the initial physical insult, reducing the functional outcome. The alphaD/beta2 integrin, located primarily on the surface of neutrophils and monocytes/macrophages, plays a role in the migration of these cells to sites of tissue damage. Monoclonal antibodies (mAb) to the alphaD subunit of the alphaD/beta2 integrin inhibit the infiltration of monocytes/macrophages into the spinal cord following clip compression injury (CCI) in the rat. We determined the most effective monoclonal antibodies on the inflammatory response in the rat model by using immunocytochemistry to identify myeloperoxidase positive (MPO+) phagocytes (monocytes/macrophages and neutrophils) and ED-1+ monocytes/macrophages within the SCI at 8 hr, 18 hr, 72 hr and 7 days after severe CCI. Wistar rats were administered i.v. anti-alphaD or control mAb in one of three treatment regimens: 24 hr, 24hr/48 hr, or 24/hr/48/hr post-CCI, with the tissue processed at 72 hr post-CCI. Treatment with anti-alphaD mAb at 24/hr/48/hr post-CCI was the only regimen that effectively reduced the infiltration of MPO+ phagocytes (by 86%) and ED-1+ monocytes/macrophages (by 41%) into the lesion at 72 hr when compared to untreated SCI controls. The 24 hr regimen equally reduced ED-1+ monocytes/macrophages infiltration (by 42%); however, the 24 hr and 24/hr/48/hr regimens did not significantly reduce MPO+ phagocyte accumulation. Using the 24/hr/48/hr treatment regimen, anti-alphaD inhibited the infiltration of MPO+ phagocytes as early as 8 hr (by 31%) and continued to reduce them at 18 hr (by 57%), whereas a reduction in ED-1+ monocytes/macrophages at the SCI was not evident until 18 hr post-CCI (by 28%). By 7 days post-CCI, the accumulation of monocytes/macrophages in anti-alphaD and untreated SCI rats was qualitatively indistinguishable. Quantification is underway. Neurological outcomes using BBB open field locomotor skills are being assessed. The 24/hr/48/hr treatment regimen may therefore have more therapeutic potential, inhibiting only the early infiltration of phagocytes and monocytes/macrophages after SCI.
Supported by the Ontario Neurotrauma Foundation and ICOS Corporation.

P420. CHARACTERIZATION OF THE LYS-EGFP-KI TRANSGENIC MOUSE: A NOVEL SPINAL CORD INJURY MODEL

The inflammatory response produced following spinal cord injury (SCI) has been shown to promote either neuroprotection/regeneration or to extend the tissue damage. Two of the major inflammatory effectors involved are CNS-derived microglia and infiltrating systemically-derived monocyte/macrophages. These cell populations are distinct in the non-activated state but when activated become indistinguishable, making it difficult to determine their temporal roles in SCI. This prevents the accurate assessment of the role of each cell type in the inflammatory response to SCI. The Lys-EGFP-kI transgenic mouse, created by Faust et al (Blood, 96: 719, 2000), has the enhanced GFP gene inserted into its genome under the control of the lysosome M promoter that is specifically expressed in myelomonocytic cells including the monocyte/macrophage. This study examines the microglia in the Lys-EGFP-kI mouse at 3, 7, and 14 days post-SCI to determine if the microglia express GFP. If the microglia remain GFP- then this would allow for their distinction from the GFP+ monocyte/macrophages at the SCI. We confirmed that no GFP+ cells were present in uninjured spinal tissue and that the inflammatory response produced in the transgenic animals was normal in magnitude and location of infiltrating cells compared to the wild type parent strain. The presence of ramified microglia was confirmed in both the white and gray matter in the Lys-EGFP-kI mice by identification with an anti-CD11b antibody; however, no GFP+ ramified microglia were identified at 72 hr post-SCI. This finding supports the hypothesis that the microglia will initially be GFP- allowing for their distinction from systemically derived GFP+ M/F. Further confirmation using an anti-GFP antibody is currently underway. We are also examining the spinal tissue at 7 and 14 days post-SCI to determine if the activated microglia gain GFP expression when they lose their ramified structure.
Supported by Ontario Neurotrauma Foundation and ICOS Corporation.
P422.
T CELL-MEDIATED PROTECTIVE AUTOIMMUNITY AFTER SPINAL CORD INJURY AFFECTS IMMUNE-RELATED ACTIVITY OF MICROGLIA AND LEADS TO NEUROPROTECTION AND SPROUTING
Oleg Butovsky*, Elbid Hauben, and Michal Schwartz, (Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel).

Studies in our laboratory have shown that the spread of damage after central nervous system (CNS) injury can be reduced by post-traumatic passive transfer of T cells specific to myelin self-antigens, or by active vaccination. This phenomenon was defined by our group as "protective autoimmunity." Here we show that active or passive vaccination after spinal cord injury not only leads to protection of tissue but also results in extensive sprouting and limits cavitation. Electron microscopy and fluorescence immunohistochemistry, using antibodies to calcitonin gene-related peptide, 5-hydroxytryptamine, and GAP-43, revealed that post-traumatic vaccination not only rescues neurons and thus limits degeneration but also promotes massive growth of regenerating axons into the lesion site of the injured rat spinal cord. These findings suggest that T cells mediate both neuroprotection and sprouting by engaging in cross-talk with activated resident microglia/macrophages, thereby coordinating the cellular effects with the needs of the tissue. When spinal cord injury in rats was followed by passive T-cell vaccination, accumulation of T cells in the vicinity of the lesion site was accompanied by a dramatic increase in MHC-II and B7-2 expression on macrophages/microglia, possibly augmenting the ability of the microglia to support antigen-dependent T cell activation. Our in vitro studies showed that co-culture of microglia with autoreactive T cells resulted in up-regulation of the expression of MHC-II and B7-2 (both reminiscent of the activity of antigen-presenting cells) as well as increased expression of TNF-α and TNF-R1 and decreased expression of TNF-RII (possibly associated with termination of the microglial activity). We suggest that autoimmune T cells exert their protective effect through dialog with local microglia and invading macrophages, enabling these cells to express their beneficial activity, while at the same time imposing strict regulation on them to avoid their potentially detrimental long-term effects.

P423.
PROTEOMICS PROFILING AFTER SPINAL CORD INJURY: EARLY DETECTION OF A NEUROPROTECTION SIGNATURE
Jean-François Cornuel, Christelle Delalande, Pierre-Yves Simonin And Sophie Feldthuys*. (NEUROLAB, PARIS, FR).

The recent development of new proteomics tools allows a rapid analysis of protein regulation and led us to assess CNS disorders by a novel strategy. The present study reports the elaboration of proteomics fingerprints from our model of spinal cord injury (SCI) (C57BL/6 mice, 11 days post-SCI). Several groups of female SD rats were submitted, after SCI, to a neuroprotective treatment (NMDA-antagonist) with differences in effectiveness and compared to a non-treated group. Body fluids and spinal lysates were sampled on a daily basis or according to the stage of behavioral recovery. The protein expression profiles were generated by SELDI-TOF mass spectrometry, using several proteinchip arrays (SAX2, WCX2, IMAC3) to target different classes of proteins. SAX2, a strong action exchange array, provided different spectra depending on the pH and allowed the identification of a number of peaks of interest. The evolution of these protein expression profiles correlated to the behavioral recovery revealed several classes of relevant proteins. A first class demonstrated up-regulation in the TCP-treated group (2mg/kg) during the first week post-SCI. At least 4 proteins with a mass ranging from 11 to 46 kDa were expressed at greater levels than in the control group, in relation to the neuroprotection. A second class of proteins ranging from 8 to 16 kDa showed an early and transitory upregulation in absence of neuroprotection, specifically suggesting that these protein species might reflect several early drug targets. Correlated to the functional recovery, these kinetics protein expression profiles are providing effective prognostic tools as well as giving insights into the drug action mechanisms.

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P425.
GLUTAMATE KILLS OLIGODENDROCYTES IN THE RAT SPINAL CORD IN VIVO
Gyo-Ting Xu*, Michael G. Hughes, Zaining Ye, David J. McAdoo. (Univ. of Texas Med. Branch, Galveston, TX US).

Glutamic acid reaches toxic levels in the gray matter of the spinal cord following spinal cord injury (SCI). Whether it also does so in the white matter is also important to determine because damage to white matter causes much of the crippling that results from SCI and because oligodendrocytes bear AMPA glutamate receptors. Therefore we analyzed the level of glutamate released into the white matter of the spinal cord of the rat following SCI and then established whether that level of glutamate is toxic to oligodendrocytes in vivo in the spinal cord. Microdialysis sampling followed by HPLC analysis yielded a maximum estimated release of glutamate of 700 mM in the white matter following SCI. Glutamate was administered into the cord, oligodendrocytes were labeled with antibody CC1, photographed under a confocal/mage analysis system and counted in defined areas. Administration by microdialysis of the estimated concentration of glutamate released into the cord resulted in a 48 ± 4.5% (s.d.) decrease in the number of oligodendrocytes at 24 h versus an 11 ± 4% decrease when artificial cerebral spinal fluid was administered in the same fashion. Thus glutamate release following SCI appears to be toxic to white matter as well as gray matter, and likely contributes to the crippling that follows SCI.
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P426.
REGIONAL ENERGY METABOLISM FOLLOWING SHORT-TERM NEURAL STEM CELL TRANSPLANTATION INTO THE INJURED SPINAL CORD

Stem cells are shown to partly restore CNS function after transplantation into the injured CNS. Nothing is known about their influence on acute energy metabolism after spinal cord injury. The present study was designed to analyze regional changes in energy metabolism. Young adult mice were subjected to laminectomy with subsequent hemisection at the L3/L4 vertebral level. Immediately thereafter 2 µl of a suspension of the C17.2 neural stem cell line in phosphate buffered saline, pH 7.3 (PBS) were injected into the lesion site. PBS served as a vehicle control. After 4 and 24 h spinal cords were removed and ATP and glucose were analyzed by a bioluminescence approach in serial sections and compared to a laminectomized or hemisectioned vehicle control groups. The area of ATP decline was also determined morphometrically. At both time points ATP content of the hemisectioned group in the tissue segments adjacent to the lesion was increased and glucose content decreased when compared to the laminectomized control. At 24 h the area of ATP decline at the lesion site was significantly lower in the PBS group as compared to the hemisectioned or transplanted group. The decrease in glucose combined with an increased ATP in the adjacent segments may indicate that the tissue adjacent to the lesion responds with an increased use of glucose to support the tissue with sufficient ATP. The lower area of ATP decline 24 h after PBS administration suggests that PBS washes out toxic mediators, thus ameliorating hemisection-dependent secondary tissue damage.
Supported by Deutsche Forschungsgemeinschaft.

P427.
VACCINATION THERAPY IN RAT SPINAL CORD INJURY
Crista L. Adamson*, Ranini Varghese and Wise Young. (Rutgers University, Piscataway, New Jersey US).

Huang et al., demonstrated that vaccination therapy with homogenates of spinal cord in adult BALB/c mice results in extensive regeneration of large numbers of axons in the corticospinal tract (CST) following hemisection. The present study was carried out to determine whether therapeutic vaccinations promote axonal regeneration and functional recovery in a rat spinal cord contusion model.

Adult Long-Evans' rats (77 ± 3days) were injured using the MASCIS standard weight drop impactor (10g, 25mm). One group (n = 20) was vaccinated twice-weekly 3 weeks prior to contusion as well as 3 weeks post injury with spinal cord homogenate (SCH, n = 10) or liver homogenate (LH, n = 10). Second group, (n = 20) vaccination started the day of injury and continued for 3 weeks with SCH (n = 10) or LH (n = 10). The BBB scale was used to track the recovery of the rats for 3 months following injury. Blood was collected the day of injury, 6 weeks post, and at the time of sacrifice to determine the plasma IgG and IgM. Two weeks prior to sacrifice, biotinylated dextran amine (BDA) was injected bilaterally into the motor cortex. Upon sacrifice, perfused spinal cords were embedded in paraffin and processed to visualize BDA-labeled axons in the CST and spared fibers.

Both BBB and spared tissue analysis show no significant difference between LH and SCH groups irrespective of vaccination protocol. Histological examination of spinal cords did not show CST regeneration across the injury site; however there appears to be an increase in the density of axon sprouting in animals vaccinated before injury, (SCH and LH). Although our data thus far indicates that vaccination with SCH has no effect on axonal regeneration in rats, preliminary findings show no change in serum IgG suggesting a different vaccination protocol may be more effective in rats.

P428.
EFFECTS OF INOSINE ON RAT SPINAL CORD INJURY
Tatsuyoshi Ichikawa*, Castis Overk, Wise Young. (W. M. Keck Center for Collaborative Neuroscience, Rutgers University, Piscataway, New Jersey US).

The purine nucleoside inosine has been shown to have neuroprotective and neuroregenerative effects in cell culture. Benowitz et al (1999) reported inosine mediated sprouting after unilateral lesion of the corticospinal tract (CST) in rats. We therefore assessed the effects of inosine on the well-standardized rat contusion spinal cord injury model. Adult Long-Evans' rats (77 ± 3days) were injured using the MASCIS Impactor by dropping a 10g weight 25mm onto exposed T13 spinal cord. To assess possible neuroprotective effects, we gave half of the rats 10mg of saline or 10mg of inosine intrathecally at 30 minutes after injury. Rats were euthanized 6 hours or 24 hours after injury and we calculated lesion volumes from potassium concentration of spinal cord samples. To assess chronic effects of inosine we applied either PBS (n = 10) or 10mg inosine (n = 10) intrathecally at 0.5µl/hour for 2 weeks using an osmotic minipump. After evaluating the animals weekly for locomotor recovery using BBB score, we traced the CST by injecting biotinylated dextran amine (BDA) and stained for expression of BDA in the motor cortex at 6 weeks after injury. Two weeks later, we perfused the rats and examined the spinal cord for BDA labeled axons. Inosine did not significantly alter 6 or 24 hours spinal cord lesion volumes. However, rats treated for two weeks with inosine showed a slight but statistically significant (p < 0.05) improvement in BBB scores. Histological examination of spinal cords did not show CST regeneration across the injury site but suggested more CST sprouting in cord proximal to the impact site. We conclude that inosine is not neuroprotective but improves locomotor recovery and promotes axonal sprouting in the proximal cord.
P429. DEPLETION OF NORADRENERGIC FIBERS ATTENUATES HINDLIMB LOCOMOTOR RECOVERY FOLLOWING THORACIC CONDUCTION INJURY
M. Rachael Lovett, Darlene A. Burke, Y. Ping Zhang, Christine Nunn, Kim Fennessy and David S. K. Magnuson. (University of Louisville, Louisville, KY US).

Descending noradrenergic (NA) axons are involved in the modulation of spinal cord circuitry associated with locomotion. N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) has been shown to deplete central noradrenaline levels inducing degeneration of certain NA axons (Fritschy et al., 1989). At 50mg/Kg, DSP-4 is selectively toxic to neurons of the locus coeruleus and easily crosses the blood brain barrier. Furthermore, it can easily be administered by IP injection and does not affect hindlimb locomotion in uninjured rats. Although the effects of DSP-4 on behavior, electrophysiology, and even ischemic lesions have been examined, the effect of DSP-4 treatment on hindlimb locomotor recovery after a spinal cord conduction injury has not been documented. This study tests the hypothesis that DSP-4 treatment results in a 60% loss of spinal cord NA fibers (Fritschy et al., 1989) alters the recovery of hindlimb locomotor activity after a T9 12.5g cm conduction injury.

Three groups of three animals were used; T9 12.5g cm conduction injury treated with DSP-4 and uninjured controls treated with DSP-4. The animals were assessed weekly for 4 weeks using the BBB open-field locomotor score, a grid-walking task and SSEPs. Injured animals treated with DSP-4 had a mean BBB score of 12.3 ± 0.40 compared to 15.2 ± 1.5 for untreated animals (mean ± s.d.; p < 0.05, t-test). Uninjured, DSP-4 treated control animals had a mean BBB score of 20 ± 0.0. We conclude that DSP-4 treatment attenuates hindlimb locomotor recovery following thoracic conduction injury suggesting that the additional loss of noradrenergic fibers induced by DSP-4 treatment (beyond loss due to the conduction injury) restricts hindlimb locomotor recovery.

Supported by the Kentucky Spinal Cord and Head Injury Research Trust.


P430. TRANSPLANTATION OF OLFATORY ENSEATHING GLIA CELLS GENETICALLY MODIFIED TO SECRET THE NEUROTROPHINS BDNF AND NT-3 MEDIATES ENHANCED RECOVERY OF HIND LIMB FUNCTION IN RUBROSPINAL TRACT LESIONED RATS
Giles W. Planz1, Marc J. Raitenberg2, Gerard Boer2, Bas Blitz2, Joost Verhaagen2. (1Red’s Spinal Cord Research Laboratory, School of Anatomy, University of Western Australia, Perth, AU and 2Dept of Neuroregeneration, Netherlands Institute for Brain Research, Amsterdam, The Netherlands).

Transplantation of olfactory ensheathing glia (OEG) is a promising strategy to augment long-distance regeneration in spinal cord following injury. Genetic engineering of OEG to express additional neurotrophic genes may improve the growth-promoting properties of these cells yielding optimal "bridging" substrates for CNS regeneration. In the present study we have investigated the relevance of ex vivo adenoviral vector-mediated gene transfer to OEG in order to overexpress the neurotrophins BDNF and NT-3. Primary cultures of rat OEG infected with adenoviral vectors encoding BDNF and NT-3 express high levels of neurotrophin mRNA as detected by in situ hybridisation and ELISA techniques. Biological activity of transgenic BDNF and NT-3 was tested in a dorsal root ganglion (DRG) bioassay. Conditioned medium from Ad-BDNF or NT-3 infected OEG cultures induced a robust neurite outgrowth from embryonic DRG explants indicating transgenic proteins were biologically active. Following transplantation of transduced OEG into intact or C4 unilaterally hemisected dorsal spinal cord, high levels of transgene expression were observed. Transgene expression gradually declined between 7 and 30 days post implantation in lesioned spinal cord. Locomotion analysis during rope-walking, a test specifically developed for rubrospinal tract lesions, showed enhanced functional recovery of hind limb function in rats that received an implant of BDNF and NT-3 secreting OEG starting at 9 weeks post transplantation. Histological analysis of rubrospinal tract axons regeneration is currently in progress. Supported by: (NOW-GMW), NHMRC (Australia), NRP and ASRT.

P431. PROLONGED SPINAL CORD EDEMA IN ACUTE CERVICAL CORD INJURY
Isami Koyanagi*, Kiyohiro Hoshin, Hirokiyo Inamura, Kenji Matsumori (Department of Neurosurgery, Sapporo Medical University, Hokkaido Neurosurgical Memorial Hospital, Sapporo, Japan).

It has been known that spinal cord edema is one of major mechanisms causing secondary injuries of acutely traumatized spinal cord. Experimental studies using clinical investigations using MRI indicate that spinal cord edema occurs several hours after injury and lasts for several days to weeks. However, spinal cord edema more than several months is quite unusual in acute spinal cord injury. This paper describes two cases of acute cervical cord injury showing prolonged spinal cord edema after acute trauma. Case 1: This 68 year-old man became tetraplegic after fall from bicycle, and he eventually developed respiratory paralysis. The patient was referred to our hospital 84 days after trauma. MRI demonstrated marked cervical spinal stenosis with OPLL and an extensive intramedullary edema from the lower medulla to the upper thoracic level. Slight improvement of the level of sensory loss and decreased intramedullary edema were obtained after decompressive surgery, but there was no recovery of motor function. Case 2: This 31-year-old man became mildly tetraparetic after falling down on the floor during Judo exercise. Three days after trauma, he visited our patient clinic. MRI showed spinal cord edema and spinal canal stenosis at C3-4 and T1-2 levels. He was treated conservatively. Although his symptoms had improved several weeks after injury, MRI at 1 year revealed that spinal cord edema still existed. Disturbed circulation of cerebrospinal fluid (CSF) around the traumatized spinal cord and venous congestion will explain such a prolonged spinal cord edema. It is likely that alteration of intramedullary blood and CSF circulation underlies several types of posttraumatic progressive cystic myelopathy which should be intensively treated.

P432. HP184 IMPROVES LOCOMOTOR PERFORMANCE IN RATS WITH MILD ESTABLISHED SPINAL CORD COMPRESSION INJURY
Shucai Jiang*, Mohammad Imtiyat Khan, Jian Wang, Pamela Middemisit, Yukun Chen, Yao Lu, Kris Bieger, James Ramstrom, Craig F. Smith, Michel Rathborne. (Dept. of Medicine, Division of Neurology and Neuroscience, McMaster University, Hamilton, ON, Canada; Aventis Pharmaceuticals, Inc., Bridgewater, NJ, USA).

Demyelination of surviving axons after spinal injury causes axonal conduction block. Potassium channel blocking ameliorates this. Clinically, potassium channel blockade improves symptoms in spinal cord injury. HP184 is a voltage-dependent blocker of potassium currents in PC12 cells and a use- and frequency dependent blocker of sodium channels. This combination of activities allows high levels of HP184 to be administered without danger of convulsion in animals. To determine whether HP184 could improve motor function in rats with an established mild spinal cord compression injury (Gruner et al., Brain Res., 729-90-101, 1996), HP184 (3.10, 20 mg/kg) was administered daily by gavage from day 25-28 post-compression. All three groups of HP184 treated animals showed similar improvements in the open field walking task.
P433. 
SRC FAMILY KINASE INHIBITOR PPI IMPROVES MOTOR FUNCTION AFTER SPINAL CORD CONTUSION IN RATS.
Chihiro Akiyama*, Takomichi Yaguchi, Masami Nishio, Yoshiaki Fujinaka, Masaki Torisugi, Yoshikazu Nishiyama, Eiji Kohmura and Yoshiaki Yoshimura (Department of Neurosurgery, Osaka University Medical School, Suita, Osaka, Japan).

Following spinal cord injury, vascular permeability advances around the area of the injury and this causes secondary injury. The activation of Src participates in this phenomenon. We previously reported that Src family kinase inhibitor PPI reduced inflammatory response after spinal cord contusion. In the present study, we examine the effect of PPI in motor function with the slight spinal cord contusion model. Twenty-five female Wistar rats (body weight = 220g) were used in this study. Under general anesthesia the spinal cord was compressed for 5 seconds over the data with Bieler vessel clip, which has a squeezing power of 25g. The Src inhibitor PPI (1.5 mg/kg) was administrated intraperitoneally 10 minutes after compression (PPI group). The vehicle only was administrated to the control rats using the same method (control group). The motor function of hind limbs after surgery was evaluated in 7 scales (SD-6). At 3 days, 7 days and 14 days after surgery, the spinal cords were removed and the sagittal sections were obtained. The ranges of edema formation and inflammation in both groups was examined by using immunohistochemistry of anti-rat IgG and anti-ED-1 antibody. The motor function immediately after surgery was flaccid in both groups (S6). On 3 days after surgery, the gait on the knuckles was observed in PPI group (S3), but spasticity was observed in control group (S5). On 7 days after surgery, the ataxic gait was observed in PPI group (S2), although only the slight motion of knee was observed in control group (S4). The immunohistochemical analysis revealed that the ranges of edema formation and inflammation were also remarkably reduced in the PPI group. The motor function was significantly improved by administration of PPI. In the near future, we believe that Src family kinase inhibitor will be applicable to the treatment of spinal cord injury. The adverse effect should be investigated.

P434. EXPERIENCE OF ANTERIOR RECONSTRUCTION WITH KANEDA SR IN THE TREATMENT OF THORACOLUMBAR BURST FRACTURE
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Study design: A retrospective clinical study was performed in twenty-two patients with thoracolumbar burst fractures who underwent anterior decompression and reconstruction using Kaneda SR.

Objectives: To determine the effectiveness and safety of anterior decompression and reconstruction using Kaneda SR in the patients with thoracolumbar burst fractures.

Background of Data: The treatment of the thoracolumbar burst fractures using Kaneda SR has been reported with some variable results and complications. Additional data were needed. This report includes the result of our initial experience using Kaneda SR in the treatment of the thoracolumbar burst fractures.

Materials and Methods: Twenty-two consecutive patients with 23 thoracolumbar burst fractures who underwent anterior decompression and reconstruction using Kaneda SR were included in this study. The surgery was done with single-stage anterior decompression, strut grating or Hum's cage insertion, and Kaneda SR spinal instrumentation. Average follow-up period was 24 months (range, 14 months to 30 months).

Results: Mean age of the patients was 35.3 years old. The fractures were in the thoracolumbar junction (between T11 and L2) in 18 out of 23 fracture levels. Eighteen cases except 4 were associated with neurological deficit from cord and/or cauda equina injury. The majority of the patients with neurological deficit (17 out of 18 patients) were improved by at least one grade after the surgery, as measured with a modification of the grading scale of Frankel.

Average canal compromise was 42.2% preoperatively, and improved to 1.4% postoperatively. Average preoperative kyphotic angle was 15.8 degree and it was improved to 6.7 degree postoperatively, and this correction was lost approximately 2.2 degree in the follow up period. There was no screw fracture or hardware loosening.

Conclusion: The authors suggest that anterior decompression and reconstruction using Kaneda SR is an effective and safe method in the treatment of thoracolumbar burst fractures. Long term follow up study may be necessary.

P435. S-100BETA LEVELS AND MYELOPEROXIDASE ACTIVITY AFTER SPINAL CORD INJURY IN THE RAT.
E. Schlichter*, R.W. Griebel, H. Komence, V. M. Skihar, B.H.J. Jaarink. (Department of Anatomy & Cell Biology and Division of Neurosurgery, University of Saskatchewan, Saskatoon, CA).

Previously, we were able to demonstrate that administration of (Christopher Reeve quercetin dihydroxylidene contributed to recovery of motor function after spinal cord injury in an animal model. Here, we report on biochemical, histological and immunocytochemical changes observed within the first 24 h after spinal cord injury in the same model. Twenty-nine male and 20 female adult Wistar rats, 22 male and 16 female animals were submitted to mid-thoracic spinal cord compression injury (50 g calibrated aneurysm clip closed for 2 seconds). Animals received either 2 doses (12 h survival) or 3 doses (24 h survival) of 0.025 mmol/kg quercetin dihydroxy intraperitoneally, or weight adjusted doses of normal saline solution, starting 1 h after injury. Spectrophotometric analysis for myeloperoxidase (MPO) activity was performed for 20 male and 20 female animals. Analysis for S-100-beta protein was performed in the compressed spinal cord segment of 2 animals per group using an immunoluminometric assay (LiANAL® Surgtec®). Nine male animals were used for histological and immunocytochemical analysis.

MPO activity at the site of injury was significantly lower in female rats treated with quercetin as compared to saline controls (p < 0.0001). While a trend to lower MPO activity was also observed in male animals, there was no statistically significant difference between treated animals and saline controls. S-100beta levels, however, were higher in quercetin treated males compared to saline controls, with statistical significance at 12 h after injury (p < 0.05). No increased S-100beta levels were seen in quercetin-treated females within 24 h after injury. Supported by HSURC Saskatchewan and the Christopher Reeve Paralysis Foundation.

P436. EFFECTS OF HP184 ON C-FIBER MEDIATED HYPERREFLEXIVE BLADDER CONTRACTIONS INDUCED BY EITHER ACUTE SCI OR IRRITATION
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Normally, the bladder stores urine while the external urethral sphincter (EUS) is contracted. When the bladder switches to elimination, the EUS relaxes via an inhibitory supraspinal reflex. Following SCI and loss of supraspinal input, the EUS does not relax during bladder contractions and voiding does not occur. Moreover, normally silent C-fiber afferents become dominant in triggering reflex bladder contractions resulting in hypertensive bladder contractions, a condition that also occurs following bladder irritation. HP184 is a dual K⁺ and use-dependent Na⁺ channel blocker. We have tested HP184 in rats using a novel acute mild spinal crush (to 75% diameter for 15 sec at T9) model and following bladder irritation in normals (intravesical delivery of 10 mg/ml prostaglandin E2 followed by physiological urinary KCI (300mM) or Heloderma venom (100nM). Preliminary studies using mild acute SCI reveal that non-voiding, high frequency and high-pressure contractions of rat bladder are ameliorated by a single bolus dose of 10 mg/kg HP184 (iv). Furthermore, this same dose reverses the effects of bladder irritation caused by either Heloderma venom or 300nM KCI. These data strongly support the notion that HP184 can selectively restore descending spinal inhibitory input after acute injury or inhibit the pathway activity of C-fibers due to inflammatory agents.
P437.
MITOCHONDRIAL FUNCTION AS MEASURED BY REDOX POTENTIAL IS REDUCED FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY.
Wilson P. Daugherty, Dong Sun, M. Ross Bullock. (Medical College of Virginia/VCU, Richmond, VA USA).

Strong evidence suggests that aerobic respiration may be compromised following traumatic brain injury (TBI), and this may be in part due to mitochondrial dysfunction. Mitochondria affect aerobic respiration through oxidative phosphorylation which is coupled to the electron transport chain and uses the oxidation-reduction potential (redox) to generate ATP.

In order to test this hypothesis, we utilized Alamar Blue dye as an indicator of the redox potential to examine the effects of lateral fluid percussion injury (FFPI) and oxygen treatments on mitochondrial function. Rat cerebral cortex was taken and homogenized from animals treated with 30% O2, 100% O2, or hyperbaric O2 (100% O2 at 1.5 ATA) at 1 or 4 hours after lateral FFPI (~211 Atm) or sham injury. A symposial fraction was prepared to enrich for mitochondria.

Symposial were incubated with Alamar Blue dye and relative fluorescence was measured as an indicator of redox potential. In animals treated with 30% O2, the injured hemisphere showed a significant reduction in redox potential when compared to shams at both 1 and 4 hours after treatment. In animals treated with 100% O2 there was a significant reduction when comparing the injured hemispheres to sham animals at 1 hr but not at 4 hrs. Hyperbaric oxygen for 1 hr, significantly reduced redox potential in the injured hemisphere, compared to shams. Thus TBI appears to cause significant mitochondrial impairment, as measured through reductions in redox potential. Supported by NS-12587-26 and the R.W. Johnson Foundation.

P438.
GLYCOGEN LEVELS IN CORTEX AND HIPPOCAMPUS INCREASE 24 HOURS AFTER LATERAL FLUID-PERCUSSION BRAIN INJURY.
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Previous studies have demonstrated that traumatic brain injury (TBI) increases the vulnerability of the brain to an acute episode of hypoxia/ischemia. Recently, however, we demonstrated decreased vulnerability to forebrain ischemia 24 hr after TBI, similar to the phenomenon of ischemic tolerance. Glycogen is an important energy substrate, which, if elevated after TBI, may protect the brain against ischemia. The objective of the present study was to determine whether TBI elevates glycogen. TBI was evoked in Sprague-Dawley rats using lateral fluid-percussion. After recovery for 24 hr, the brain was frozen in situ with liquid nitrogen, and the brain was sampled bilaterally in 6 predetermined regions of cerebral cortex and in 2 regions of hippocampus for measurement of glycogen, using enzymatic fluorimetric methods.

The results indicated that in 5 of the 6 cortical regions analyzed, glycogen in the hemisphere ipsilateral to the KCl application was elevated 2.3-fold relative to that in the contralateral hemisphere (p < 0.05). In the 2 hippocampal regions, ipsilateral glycogen levels were 1.6-fold higher than those in the contralateral hippocampus (p < 0.05). Thus, the elevation in glycogen after TBI may contribute to the induction of tolerance to forebrain ischemia. Supported by NIH grant NS-05803 (FAW).

P439.
THE EFFECTS OF DELAYED BUT PROLONGED HYPOTHERMIA ON THE PIAL VASCULAR RESPONSE AFTER TRAUMATIC BRAIN INJURY IN RATS
Yuji Ueda, Eiichi Sawai, John P. Pavlides. (Medical College of Virginia Campus/VCU, Richmond, VA US).

Recently, in both the clinical and laboratory setting, the potential vascular protective effects of hypothermia have received attention in both stroke and traumatic brain injury (TBI). In laboratory studies of TBI, most have focused on the use of early hypothermic intervention, with little consideration of the potential efficacy of delayed but prolonged hypothermia, which would constitute a more clinically relevant paradigm. In the current investigation, we evaluated whether delayed but profound hypothermia after TBI protected the cerebral microcirculation. Male Sprague-Dawley rats were equipped with cranial windows for direct visualization of the pial arterial circulation and then subjected to impact acceleration brain injury, with the delayed (1h) induction of either 1h or 2h of hypothermia (32 degrees C) followed by the slow warming (32-37 degrees C; 90 min). Nonhypothermic animals served as controls. The pial arteriolar responses to acetylcholine (ACh) or hypercapnia were measured. Through this approach we found that both delayed hypothermia groups maintained normal arteriolar vascular responses in terms of ACh-dependent dilation and carbon dioxide reactivity, however, the prolonged hypothermic group showed more significant recovery. In contrast, arterioles subjected to TBI followed by normothermia demonstrated severely impaired vasoreactivity, with arteriolar dilation maintained throughout the duration of the study. The results of this study show that delayed but prolonged hypothermia attenuates the impaired vascular responsiveness seen after TBI, suggesting its potential clinical usefulness. This work was supported by NIH grants NS-20195 and T32 NS7288.

P440.
CHANGES IN CEREBRAL PERFUSION AND HIGH ENERGY-RELATED METABOLITES IN RESPONSE TO FLUID PErCUSSION INJURY
Paul Tongkai*, Paul C. Francoel, Jeremy Phelps, Robert J. Wienecke. (University of Oklahoma, HSC. Department of Neurosurgery, Oklahoma City, OK USA).

Introduction: The purpose of this study was to document changes in cerebral perfusion and high energy-related metabolites before, during, and after a fluid percussion injury. It is becoming clear that energy/metabolic state changes in the first few hours following injury may play a role in long-term outcome.

Methods: Following induction of anesthesia Sprague-Dawley rats were intubated and artificially ventilated. After placement of femoral arterial and venous catheters, burr holes were made in the skull for positioning of the fluid percussion mount, bilateral laser Doppler fibers, and a microdialysis probe. Arterial blood pressure, cerebral perfusion, and microdialysis samples were collected over a four hour period, from two hours pre-to two hours post-injury. Fluid percussion was administered at 2 atmospheres, a moderate injury.

Results: Immediately in response to the fluid percussion injury, cerebral perfusion decreased by 40% and slowly returned toward pre-injury levels over the two hour post period. All three metabolites increased in response to injury: hypoxanthine 4 fold (p < 0.001), inosine 2 fold (p < 0.02), and adenosine 7 fold (p > 0.01).

Conclusions: Early changes occur in both cerebral perfusion and energy metabolites in response to a moderate cerebral injury. These changes may be long lasting and contribute to the overall long-term outcome. Recognition of this early derangement of perfusion and energy state may be important in the overall therapeutic regimen.
P441. PERIVASCULAR NERVE DAMAGE IN THE CEREBRAL CIRCULATION FOLLOWING TRAUMATIC BRAIN INJURY
Yuji Ueda*, Susan A. Walker, Christina R. Marmarou, Richard H. Singleton and John T. Povlishock. (Medical College of Virginia Campus/VCU, Richmond, VA US).

It is well recognized that traumatic brain injury (TBI) causes alterations in the cerebral microcirculation ranging from abnormalities in cerebral dilatation to impaired reactivity to challenges such as altered carbon dioxide or acetylcholine application. Most have assumed that these impaired vascular responses were the result of endothelial and/or smooth muscle alteration, triggered by the traumatic event. No consideration, however, has been given to the possibility that the forces of injury may also damage the perivascular nerve network, thereby contributing to the observed abnormalities. To test this premise, we subjected rats to impact acceleration and sham injury. At 6hr, 24 hr or 7days post injury, the rats were re-anesthetized and transcardially perfused. Portions of the ventricles, basal and internal carotid system were removed and processed with antibodies targeting 5-hydroxytryptamine (5-HT) and the neuropeptide PGP-9.5. Lastly, the Fluoro-Jade procedure was employed to detect primary nerve fiber damage. Selected vessels were analyzed to determine the distribution of these markers and their overall density. Using the Fluoro-Jade marker for axonal degeneration, the perivascular nerve network showed no reactivity in either the sham or 6 hr animals; however, by 24 hr postinjury, Fluoro-Jade reactivity was noted in the perivascular regions. In concert with this marker of damage, antibodies targeting 5-HT accumulation and normal neuropeptide (PGP-9.5) distribution demonstrated intact and unaltered fiber populations in the sham and 6hr animals. By 24 hr postinjury, however, a significant reduction in the perivascular 5-HT accumulation occurred, together with a reduction in PGP-9.5 fiber staining. Collectively, these studies illustrate that within the cerebral circulation perivascular nerve fiber damage is a consistent feature of TBI. These studies suggest that neurogenic damage may be a contributor to some of the vascular abnormalities associated with TBI and obviously, this issue merits further consideration. Supported by NIH grants NS-20193 and T32 NS7288.

P442. HYPOTHERMIC CEREBROVASCULAR PROTECTION IS RELATED TO THE RATE OF POST HYPOTHERMIC REWARMING
Enoch P. Wei*, Yuji Ueda, Elichir Suemoto and John T. Povlishock. (Medical College of Virginia Campus/VCU, Richmond, VA US).

Recently, our labs, and others, have focused on the potential neuroprotective and cerebrovascular protective effects of hypothermia following traumatic brain injury. We have observed that the efficacy of posttraumatic hypothermia was related to the rate of rewarming after hypothermic intervention, with the finding that rapid rewarming exacerbated traumatically induced axonal injury and cerebrovascular dysfunction (J. Neurosurg 94:43-498, 2001). In the current communication, we revisit the use of hypothermia with varying degrees of rewarming to ascertain if, in the normal cerebral vasculature, varying rates of rewarming could differentially affect cerebrovascular responsiveness. To this end, we examined the effects of rewarming on the cerebral microcirculation in non-traumatized rats equipped with closed cranial windows. All animals were exposed to hypothermia of 32 degrees C for 1 hr duration, followed by either slow rewarming over a 90 min. period or rapid rewarming over a 20 min. period. Vasoreactivity to hypercapnia and ACh was assessed. Animals receiving hypothermia, followed by slow rewarming showed a restoration of normal vascular responsivity following rewarming. In contrast, the use of hypothermia followed by rapid rewarming elicited impaired cerebrovascular responses to ACh and arterial hypercapnia. Furthermore, hypothermia followed by fast rewarming impaired the dilator responses of sodium nitroprusside, a NO donor, and pinacidil, a KATP channel opener. These findings support the use of hypothermia followed by slow rewarming. They demonstrate that fast rewarming can trigger vascular abnormalities most likely through primary endothelial as well as vascular smooth muscle damage. Supported by NS 20193.

P443. STUDY OF MILD HYPOTHERMIA ON PBT02 AND BT PATIENTS WITH SEVERE HEAD INJURY
Professor Shuayan Yang. (Huamhu Hospital, Tianjin, Tianjin CN).

Objective To study the changes of partial pressure of brain tissue oxygen (PbtO2) and brain temperature (BT) in acute phase of patients with severe head injury, and effect of mild hypothermia on PbtO2 and BT.

Methods PbtO2 and BT of 33 patients with severe head injury were monitored, and hypothermia was induced within 20 hours of injury. Rewarming was began on 1-7 days (average 5.7 ± 4 hours) after the rectal temperature reached 31.5-34.9. The hypothermia was maintained within the range of normal value at 3days postinjury. BT was higher than RT in acute phase of patients with severe head injury. The difference between BT and RT significantly increased in mild hypothermia. Hyperventilation was used to increase the PbtO2. The average of PbtO2 was 25mmHg induced low PbtO2 since high ICP had been decreased.

Conclusion This study demonstrates that monitoring of PbtO2 and BT is a safe, reliable and sensitive diagnostic method to follow cerebral oxygenation. It might become an important tool in our treatment regime for acute patients of severe head injury requiring hypothermia and hyperventilation.

P444. APOPTOTIC CELL DEATH FOLLOWING IN VITRO TRAUMATIC INJURY IS INDEPENDENT OF CALCIUM INFLUX

Neuronal apoptosis is a common feature of traumatic brain injury in vivo. In this study we used an in vitro model to study the role of calcium in the induction of apoptosis following mechanical injury. Hippocampal neurons were plated onto a silicon wafer which was then injured with either mild strain (12-17%), severe strain (>50%) or severe strain at 10 days in vitro. Viability was assessed at 24 hours following injury. Fluorescence microscopy was used to detect live (Fura-2 positive), dead (propidium iodide positive, PI), and/or apoptotic cells. Apoptosis was detected using a fluorogenic substrate (FAM-DEV-D-FMK) for activated caspase-3.

In physiologic salt solution (PSS), both severe stretch and NMDA application resulted in significant cell death (PI positive, p < 0.001) compared to sham injured cultures. The extent of cell death after severe stretch injury was unaffected when either extracellular calcium was removed or when cultures were treated with 100 uM MK-801. As expected, treatments attenuated cell death following NMDA exposure (p < 0.001).

No significant increase in the number of apoptotic cells was observed following either stretch or NMDA in PSS when compared to uninjured cultures. Removal of calcium from the media resulted in increased apoptosis in both stretch injured and NMDA treated cultures (p < 0.001). Treatment with MK-801 did not affect the extent of apoptotic cell death after either mild stretch or NMDA application but did increase the number of apoptotic cells following severe stretch (p < 0.001, compared to uninjured cultures).

These data suggest that total cell death following mechanical injury is independent of NMDA activation. Using treatments to blunt the acute calcium transient resulted in an unmasking of a stretch activated apoptotic pathway that does not appear to depend on elevated cytosolic calcium.

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P445. REPEATED RAPID ACCELERATIONS PRODUCE INCREASED AXONAL INJURY IN THE IMMATURE BRAIN

Inflicted brain injury associated with widespread traumatic axonal injury (TAI) and subdural hematoma (SDH) is a leading cause of death in infants and children. A pediatric (3–5 day old, representing an infant < 3mo) anesthetized porcine model was used to study single (n = 5) and double (n = 6, injured approximately 30min apart) rapid (<20ms) nonimpact axial rotations of the head. Load level (peak velocity of 172 ± 17 for single, 135 ± 8 rad/s for double) was selected to induce a brief period of unconsciousness. Pinch reflex was absent for 1–20 min in all injured piglets. At 6h post-injury, animals were sacrificed and their brains perfusion fixed. Gross inspection showed SDH in frontal lobes and brainstem of 3 single injury brains, all double injured brains, and absent in uninjured controls (N = 3). Under light microscopy no subarachnoid hematoma (SAH) was observed in any brain. All double injured and single injured brains demonstrated TAI, defined as an accumulation of the 200kDa neurofilament protein in either contiguous axons or terminal bulbs. Nearly all TAI was observed in peripheral and central white matter tracts. Double injured piglets had significantly more injured foci (5.5 regions/brain) compared with single injury (0.8 regions/brain, p < 0.05), but the density of injured axons was not significantly different (2.2 ± 1.2 injured axons/mm2 in the double injured piglets versus 1.3 ± 1.3 axons/mm2 in the single). The data demonstrate that repeated, mild, nonimpact brain injuries have more widespread acute axonal injury compared with those experiencing a single event. Since inflicted head injuries may be single or multi-load events, these results have implications for the development of appropriate animal models to study inflicted brain injuries in children.

Support provided by NIH R01 NS 39679 and NIH R01 NS 541561.

P447. STEREOREAL COMPARISON OF REGIONAL HIPPOCAMPAL CELL LOSS IN INBRED MOUSE STRAINS FOLLOWING FLUID PERCUSSION INJURY
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Fluid percussion injury (FPI) causes hippocampus-dependent memory dysfunction in mice. Standard post-mortem histological analyses have reported hippocampal CA3 and hilar neuronal loss. We have employed design-based stereology combined with the optical volume fractionator to estimate quantitative neuronal loss within specific subregions of the hippocampus after lateral FPI in the mouse. Stereology is an unbiased, systematic random sampling procedure that combines cell numerical density estimates (from the optical dissector) with volume estimates (generated by point counting and the fractionator stereology method) to estimate the absolute cell number for hippocampal subregions: CA1, CA3, dentate gyrus, and hilus.

Anesthetized adult male C57BL/6 and C57BL/10 mice were randomly selected and subjected to either lateral FPI (1.1–1.4 atm) or surgery without injury. Mice were transcardially perfused 7–10 days post-injury, the brains removed, and post-fixed for 24 hours. Paraffin-processed mouse brains were embedded, sectioned exhaustively at 50 μm in the horizontal plane, and wet mounted on gelatin-subbed slides. Using the Olympus CAST system, optical dissector were systematically placed across every 3rd section containing the hippocampus and the volumes of hippocampal subregions were estimated by point counting on these same sections.

Our preliminary data indicate that (1) the number of CA1 pyramidal neurons in sham animals are similar between mouse strains, and (2) the ~17% reduction in the number of CA1 neurons one week following FPI in C57BL6 mice is exacerbated in the hyperexcitable C57BL10 strain (~40% reduction). The loss of CA1 neurons and the injured strain differences indicate cellular pathology throughout the hippocampus that may underlie hippocampus-dependent memory deficits. With these promising results, our attention is now focused on the CA3, dentate gyrus, and hilar subregions of the hippocampus.

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P446. NEURONAL LOSS FROM BRAIN NUCLEI AFTER HUMAN BLUNT HEAD-INJURY
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Quantitative evidence for loss of neurons after human blunt head-injury is lacking. The hypothesis that an unbiased quantitative analysis might provide such evidence was tested.

Material was obtained from the archive of the Department of Neuropathology, Southern General Hospital, Glasgow. That material (age range 18–64) consisted of 9 age and sex matched control patients with no history of head-injury in life, 4 severely head-injured (SHI) (GCS on admission less than 8) with survivals of 3–7 days, and 9 (SHI) patients with survivals between 8 and 395 days. Paraffin, coronal sections of the left and right thalamus and the left hippocampus were cut and stained using cresyl violet. The point counting technique was used to estimate the area of each brain nucleus and these were compared between patient groups. The size of pyramidal neuronal cell bodies in sub-fields of the hippocampus, diencephalal, lateral and ventral thalamic nuclei was measured and used to determine the number of neurons. A number of neurons within the brain regions of interest (vide supra) were counted in either non-head-injured control (n = 9) or severely head-injured patients (n = 13) (age range 18–64). The Student's t test was used for statistical analysis.

At 1 week survival there was loss of pyramidal neurons from hippocampal sub-fields CA1 (p = 0.012), CA3 (p = 0.003) and CA4 (p = 0.002) but not CA2. Further loss occurred at 6 months but only in sub-fields CA1 (p = 0.033) and CA4 (p = 0.013). No quantitative evidence for loss of neurons from any thalamic nucleus was obtained.

Loss of neurons after blunt head-injury occurred both with a different time scale and to a different extent in different brain nuclei. Stereology provided evidence for different levels of loss of neurons from different sub-fields of the hippocampus at a week after injury. But, in addition, provided novel data for loss of neurons from hippocampal sub-fields at longer post-traumatic survivals. However, stereology did not provide support for loss of neurons from thalamic nuclei after head-injury.

P448. AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS AND OXIDATION ARE NOT EVIDENT CHRONICALLY FOLLOWING EXPERIMENTAL BRAIN INJURY IN THE RAT
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The enduring cognitive and sensory-motor deficits that result from traumatic brain injury (TBI) are associated with metabolic stress and free radical cascades, which establish conditions that may promote mitochondrial DNA (mtDNA) deletion and oxidation, often observed as a consequence of normal aging. Without substantial mtDNA repair mechanisms, permanent alterations to essential mitochondrial enzymes could perpetuate post-injury pathologic cascades. To determine whether mitochondria from the injured cortex and hippocampus sustain mtDNA damage after TBI, we evaluated both deletion and oxidation of mtDNA following lateral fluid percussion TBI in the anesthetized adult Sprague-Dawley rat (4 mo) compared with uninjured adult and aged rats (n = 4/group). The presence of 4.8 KB comet mon deletion in mtDNA was assessed by conventional PCR to generate products representing total, non-deleted wild-type, and deleted mtDNA in homogenized tissue and isolated mitochondria 3 and 14 days following TBI. Total and wild type mtDNA amplification products were obtained from cortical and hippocampal tissue and mitochondria for all conditions. Although no mtDNA deletions were observed following experimental TBI, mtDNA deletion was detected in cortical tissue, but not isolated mitochondria, of naïve, aged (24 mo) Sprague-Dawley rats, suggesting that the isolation may excise mtDNA harboring mtDNA damage. Oxidative mtDNA damage in isolated mitochondria assayed by ELISA for 8-hydroxy-2-deoxyguanosine (8-OHdG) from cortical (0.50 ± 0.08 pg 8-OHdG/gmt mitochondria) and hippocampal (0.35 ± 0.02 pg 8-OHdG/gmt mitochondria) regions were unaffected by TBI. However, mitochondrial protein yields from injured and aged brains were comparable, and significantly smaller than unjured brain, suggesting some similar underlying pathology between TBI and aging.

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P449.  
LONG-TERM PRION PROTEIN ACCUMULATION IN DAMAGED AXONS FOLLOWING INERTIAL BRAIN INJURY IN THE PIG. 
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Observing that trauma elicits the accumulation of pathologic aggregates found in neurodegenerative diseases, we evaluated the possible accumulation of prion protein (PrP) in pig model of diffuse axonal injury (DAI). This investigation was initiated by the assumption that PrP transport in axons would be interrupted following brain trauma. Nine miniature young adult (6 months age) swine, weighing 20-26 kg, were used for this study. Six were subjected to head coronal plane rotational acceleration and the brains evaluated at 3 hours, 3 days, 7 days, and 6 months postinjury. Three animals were used as controls. Immunohistochemistry and western blot analysis was performed using anti-PrP monoclonal antibodies 3F4, P99/97.6.1, and polyclonal antibody CD230 To determine potential accumulation of an abnormal PrP isoform, PrPsc, sections and tissues were pretreated with 0.1% proteinase K. At all post-injury timepoints, we found extensive accumulation of prion protein in damaged axons in the brain injured pigs, including in the proteinase K treated sections. Additionally, we found a limited number of plaque-like profiles in the brain sections. No overt spongiform changes were found in the brains. On western blot, strongly immunoreactive bands were found with a mass of less than 30 kDa in proteinase K treated tissue at 3 hours, 3 days and 7 days following injury, which were slightly shifted down from faint bands found in the control animals. These data demonstrate a long-term process of PrP accumulation following brain trauma. Further, the data suggest that some of the accumulated PrP is an abnormal isoform, PrPsc, thought to be the key pathogenic agent in transmissible spongiform encephalopathies. However, it remains to be determined whether PrP accumulation plays a role in the progressive neurodegenerative changes observed following brain trauma. Supported by NIH grants, AG12227, NS 38104, and NS68033

P450.  
TRANSCRIPTIONALLY PROFILING THE EFFECTS OF CHRONIC METHYLPHENIDATE TREATMENT IN RATS AFTER TRAUMATIC BRAIN INJURY 
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Dopamine (DA) pathways have been implicated in cognitive deficits after traumatic brain injury (TBI). Clinical and laboratory studies have shown that post-traumatic cognitive deficits can be attenuated with DA agonists, including methylphenidate (MPD). While the beneficial effects of DA agonists have been attributed to increasing DA tone, we hypothesized that MPD’s effects were also associated with a unique profile of chronic gene expression. We examined the effects of daily MPD treatment on gene expression using microarray technology following TBI produced by controlled cortical impact injury (4 m/sec, 2.8 mm tissue deformation). Beginning one day after injury, rats were daily injected with either MPD (5 mg/kg, i.p., n = 3) or saline (n = 3). Sham rats (n = 3) were surgically prepared, but not injured. After 21 days, the rats were sacrificed and total RNA was extracted from a tissue region containing DAggressive cell bodies (bilateral substantia nigra and ventral tegmental area). Oligonucleotide expression arrays (Affymetrix neurobiology array) containing 1,322 mRNA sequences were used to determine the transcriptional profiles. Only mean expression level changes of 2-fold or more relative to sham controls are reported. The TBI+saline group produced 36 mRNA sequences that were upregulated and 42 sequences that were downregulated. The TBI+MPD group produced 34 mRNA sequences that were upregulated and 28 sequences that were downregulated. Of particular interest were treatment-associated changes in mRNA sequences involved in regulating neurotransmitter function. Approximately half of the mRNA expression changes in the TBI+MPD group were unique to the TBI+saline group. These changes in transcriptional profiles in the substantia nigra and VTM regions support the conclusion that there may be a transcriptional basis for the neuroprotective effects of chronic dopamine transporter (DAT) MPD therapy. Further gene profiling of experimentally effective treatments may help differentiate and refine specific therapies targeting clinical recovery of function after TBI.

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P451.  
ASSOCIATIONS BETWEEN DOPAMINE TRANSPORTER GENOTYPE AND CEREBRAL SPINAL FLUID DOPAMINE LEVELS AFTER SEVERE TRAUMATIC BRAIN INJURY 
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Dopamine pathways have been implicated in cognitive deficits after traumatic brain injury (TBI). While not associated with alterations in protein structure, the dopamine transporter (DAT) 3’ VNTR polymorphism has been associated with differences in DAT protein density and development of DA mediated pathophysiological conditions. Differential DAT expression presumably affects both presynaptic DA release, via reverse transport, and DA reuptake. Catecholamines, including DA and its metabolites, are subject to auto-oxidation, resulting in the formation of reactive oxygen species that can contribute to oxidative stress associated with secondary brain injury. Therefore, the purpose of this study was to determine the relationship between DAT genotype and cerebral spinal fluid (CSF) DA levels after severe TBI. We hypothesized that the DAT 10/10 genotype would be associated with lower CSF DA/Da metabolite concentrations after TBI. We evaluated 30 patients with severe TBI (GCS<8) admitted between 1995-1998 and determined DAT genotype for patients using previously banked samples of CSF. High performance liquid chromatography was used to determine post injury CSF levels of DA/Da metabolites. Mann Whitney-U analysis was used to determine differences between DAT genotype groups with post-injury average and maximum levels of DA and DA metabolites, including 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Seventeen patients had the DAT 10/10 genotype, and 13 patients had either the DAT 9/9 or 9/10 genotype. Results showed no differences between genotype groups for post-injury average or maximum CSF DA levels. However there were significant increases in maximum DOPAC (p = 0.023), HVA (p = 0.041), and DOPAC/Da ratios (p = 0.038) for the DAT 10/10 genotype group compared to the 9/9-9/10 group. Average DOPAC (p = 0.044) and DOPAC/Da ratios (p = 0.048) were also significantly higher for the DAT 10/10 group. These results indicate higher DA metabolism in patients with DAT 10/10 genotype, which may increase susceptibility to DA mediated oxidative injury after TBI. K08HD40833, R01NS40125, Pittsburgh Foundation

P452.  
DOPAMINE TRANSPORTER GENOTYPE IS ASSOCIATED WITH FUNCTIONAL AND NEUROPSYCHOLOGICAL OUTCOME FOLLOWING TRAUMATIC BRAIN INJURY 
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Alterations in dopamine (DA) pathways appear to impact cognition after traumatic brain injury (TBI). The dopamine transporter (DAT) 3’ VNTR polymorphism has been associated with DA system function and DA mediated cognitive disorders, with the DAT 10/10 genotype being implicated in attention deficit disorder. DAT regulation may have a role in DA mediated neurotoxicity acutely after TBI and play a compensatory role in improving DA neurotransmission chronically after TBI. Therefore, the objective of this study was to evaluate the relationship of DAT genotype to functional and neuropsychological outcome after severe TBI. DAT genotype was determined using previously banked cerebral spinal fluid samples from 36 patients with severe TBI. DAT 10/10 genotype was considered to be the risk genotype for poor outcome and neuropsychological function. DAT genotype was compared to Disability Rating Scale (DRS), Glasgow Outcome Scale (GOS), Wisconsin Card Sort Test (WCST), Trail Making Test, and WAIS-R Digit Span six months after injury. Fifty percent of the population had 10/10 DAT genotype. There were no significant differences with gender, age or injury severity between comparison groups. Results showed that people with the DAT 10/10 genotype had worse six month DRS scores (p = 0.024) and showed a trend to have worse GOS scores (p = 0.112). Patients with the DAT 10/10 genotype had fewer correct responses for digit span forward testing (p = 0.035), showed a trend to do worse with forward digit span (p = 0.085), and tended to be too cognitively impaired to count perseverative errors with WCST (p = 0.068). DAT genotype is associated with measures of functional and neuropsychological outcome after TBI. The results of this study suggest a role for DAT genotype in affecting cognition and outcome after TBI, and future work should focus on the role that DAT genotype may play in individual response to pharmacological and therapeutic interventions. K08HD40833, R01NS40125, NIDR#R01HP370013-00
P453. MICROGLIAL CHEMOTAXIS IS REGULATED BY ATP AND ADP RELEASED BY TRAUMATICALLY INJURED ASTROCYTES. C. Liang1, D. Bailey1, R. Fry, S. Merchant1, W. Wahl1, E. Ellis2, and B.A. Reigl11, 1Department of Central Florida, Dept. of Molecular, Biology, & Microbiology, Orlando, FL, 2Virginia Commonwealth Univ., Dep. of Pharmacology & Toxicology, Richmond, VA, USA.

Microglia (MG), the immune effector cells of the brain, are rapidly activated and recruited to the site of traumatic brain injury within hours of the initial insult, via chemotaxis. Recruitment of MG may contribute to neuronal damage through release of inflammatory mediators and secondary damage to uninjured cells. Therefore, control of chemotaxis may be a target for pharmacological intervention. Using Boyden-like chemotaxis chambers, we examined 2 types of MG, resting and activated. MG were isolated from 7-10 day old mixed brain cell cultures obtained from neonatal rats. Resting MG were maintained in astrocyte-conditioned medium. Activated MG were prepared by a 24 hr exposure to medium conditioned by traumatically injured astrocytes, using an in vitro model for traumatic injury. Adherence to collagen was decreased in activated MG, consistent with the enhanced motility of these cells. Medium conditioned by injured astrocytes was chemotactic for both resting and activated MG, suggesting that astrocytes release a soluble factor that induces chemotaxis. Glutamate (5-200 mM) was not chemotactic for resting or activated MG. Histamine was not active. The purinergic nucleotides ATP and ADP, but not UTP, were chemotactic for both resting and activated MG, suggesting that purinergic receptors are involved in chemotaxis. The chemotactic effects of medium conditioned by injured astrocytes was decreased by the purinergic receptor antagonist suramin, and pyrival-phosphoryl-2′-d-ribosephonic acid (PPADS). These results suggest that ATP and ADP released by injured astrocytes are involved in microglial recruitment to the site of traumatic injury. Supported by NS40490.

P454. MILD OR MODERATE TRAUMATIC BRAIN INJURY: BEHAVIORAL AND HISTOPATHOLOGICAL OUTCOMES IN MICE. K.J. Feek1; K.E. Saatman; R. Raghupathi. (Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA USA).

Traumatic injury may initiate specific cell death pathways dependent upon injury severity, thereby resulting in graded histopathological and behavioral deficits. The present study examined cognitive and motor function and histological damage associated with mild (0.5mm deformation; 5.0 m/sec) (n = 20) or moderate (1.0 mm deformation; 5.0 m/sec) (n = 20) CCI injury in anesthetized C57BL/6 mice. Memory function was assessed using the Morris water maze and gross motor function was assessed using a standardized battery of tests. Both mild and moderate injury produced significant memory impairment (p < 0.001) and motor deficits (p < 0.0005) compared to sham injury. In addition, animals subjected to moderate brain injury exhibited greater cognitive (p < 0.02) and motor (p < 0.01) dysfunction than mice subjected to mild injury. Brain-injured and sham-injured (n = 29) were sacrificed at 15min (n = 7), 4h (n = 8), 24h (n = 10), 2d (n = 10), 4d (n = 19), or 7d (n = 15) post-injury. All brain-injured mice exhibited cortical tissue tears at 15min, cell loss by 24h, and a pronounced cavity by 7d post-injury. The cavity associated with moderate injury extended through all six cortical cell layers. Moderate injury resulted in loss of Nissl stained neurons over a larger rostral-caudal and medial-lateral extent of cortex compared to mild injury. Tearing of the ipsilateral subcortical white matter was commonly observed after moderate injury. In the hippocampus, loss of pyramidal neurons in the CA3 and hilar neurons in the dentate gyrus was observed by 24h after moderate brain injury and by 4d after mild injury. Loss of pyramidal CA1 neurons and granule cells in the dentate gyrus was observed between 24h and 7d after moderate brain injury only. These findings illustrate that both behavioral deficits and histological alterations are dependent on injury severity.

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P455. A CONFOCAL MICROSCOPIC EXAMINATION OF THE EFFECTS OF STRAIN (STRETCH) ON CULTURED NEURONS AND ASTROCYTES. Judith M. Mair1, Karen A. Willoughby and Earl F. Ellis. (Department of Pharmacology and Toxicology, Medical College of Virginia Campus of Virginia Commonwealth University, Richmond, VA, USA).

We examined the time course of cytoskeletal and morphological damage to cultured neurons and astrocytes grown on a silastic membrane and subjected to rapid biaxial strain (stretch). Injury was assessed using immunocytochemistry for MAP-2, GFAP, and Bcl-2 and propidium iodide (PI) uptake. Neuronal plus glial cultures subjected to moderate (6.5 mm) or severe (7.5 mm) membrane displacement and stained for MAP-2 demonstrated tortuous and beaded neuronal processes in many neurons immediately following injury. With time, the soma and processes of some injured neurons were less intensely stained with MAP-2. At 24 and 48 hr, many neurons showed PI uptake and labeled poorly for MAP-2. 3D reconstruction of these cultures demonstrated that neuronal clusters sit upon a hill of astrocytic processes and 24-48 hr after injury, PI labeled nuclei are seen on top of the astrocytic processes surrounded by degraded MAP-2 labeled cytoskeletal elements. Pure astrocytes or astrocytes of mixed cultures showed similar patterns of GFAP expression and morphological changes with injury. As the cells were increasingly stretched (4.5-8.5 mm), the confluent astrocyte bed retracted and with time the astrocytic processes demonstrated stellate morphology, swelling and hypertrophy. Astrocytes demonstrating both PI uptake and GFAP staining were apparent at 15 min to 6 hr post-injury, but few astrocytes had PI uptake at 24-48 hr. In addition, BrDU labeling increased in injured astrocytes of pure and mixed cultures at 24 and 48 hr post-injury, potentially signaling enhanced glial proliferation induced by injury.

In conclusion, injury of neurons first shows damage to neuronal processes (beading and potential deafferentation) and, with time, degradation of the soma with a concurrent increase of PI uptake. Stretch injured astrocytes show the hallmarks of reactive gliosis—swelling, hypertrophy, increased GFAP immunofluorescence and glial proliferation. Supported by NS 27214.

P456. PROTEIN EXTRAVASATION, REACTIVE ASTROGLIOSIS, AND NEURONAL DAMAGE FOLLOWING MILD OR MODERATE TRAUMATIC BRAIN INJURY IN MICE. RL Pape1, KJ Feek1, JW Huh2, AK Clausen1, R Raghupathi1, KE Saatman1. (Neurosurgery, University of Pennsylvania; and Anesthesiology and Critical Care, The Children's Hospital of Philadelphia, Philadelphia, PA USA).

Spatiotemporal patterns and cellular mediators of posttraumatic neuronal or vascular damage may be related to injury severity. The patterns of vascular, glial and neuronal damage were evaluated in anesthetized C57BL/6 mice that were subjected to sham injury (n = 4), mild controlled cortical impact (CCI) injury (0.5mm depth at 5m/sec; n = 6) or moderate CCI injury (1.0mm depth at 5m/sec; n = 7). At 4hrs, 48hrs and 7days post-injury, 40µm coronal sections were examined immunohistochemically for IgG extravasation, glial fibrillary acidic protein (GFAP), and microtubule-associated protein 2 (MAP2). After mild or moderate injury, the ipsilateral parietal cortex and hippocampus exhibited intense IgG labeling at 4 and 48 hrs. Hippocampal IgG labeling at 7 days and acute thalamic IgG extravasation was observed only after moderate injury. Both mild and moderate injury resulted in hypertrophy of hippocampal astrocytes at 4 hrs. At 2 days, a marked increase in GFAP immunoreactivity was detected in the ipsilateral cortex and hippocampus at both severities; however, astroglia in the ipsilateral striatum and thalamus was pronounced only after moderate injury. By 7 days in both mild and moderate injury, the cortex, hippocampus, striatum and thalamus all exhibited increased GFAP immunolabelling in the ipsilateral hemisphere. Both mild and moderate injury produced dendritic disruption and MAP2 loss in the cortex, and marked decreases in immunolabelling of the CA2, CA3, and dentate hilar regions of the ipsilateral hippocampus at all time points. Profound loss of MAP2 labeling was consistently observed in the hippocampal CA1 region after moderate, but not mild, injury. These data suggest that both mild and moderate CCI brain injury produce cellular and vascular damage in the cortex. Damage was more prolonged and involved additional brain regions with increasing injury severity. (Supported by NIH NS08803 and NS41561)
P457. TRAUMATIC AXONAL INJURY DIFFERENTIALLY IMPAIRS FAST- VS. SLOW-CONDUCTING CORPUS CALLOSUM FIBERS. T.M. Reeves*, L.L. Phillips, J.T. Povlishock. (Department of Anatomy and Neurobiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, US).

Moderate closed head injury reliably elicits diffuse axonal injury. Recent findings have demonstrated the complex nature of this pathology, revealing a multiphasic sequence of perturbations to axonemal permeability, and to the structure and function of the axonal cytoskeleton and mitochondria. Evidence suggests subpopulations of axons may respond differently to injury, e.g., axonal swelling and neurofilament disruption do not always co-localize. Fiber size and degree of myelination are structural properties that may determine, in part, the specific injury response(s) of an axon. This study measured compound action potentials (CAPs) evoked through the corpus callosum fibers in brain slices from adult rats at 1 and 3 days following central fluid percussion injury (FPI), modifying a method of Baker et al. (2000).

The biphasic CAP waveform is comprised of an early waveform component (generated largely by 'fast' myelinated fibers) and a later component (largely 'slow' unmyelinated fibers). Recording at 23 degrees C increased the threshold latencies enabling reliable quantifications of the fast wave component, which was partially embedded in the stimulus artifact when recording at 36 degrees C. Injury effects at 3 days postinjury were not significantly different from 1 day effects. FPI reduced the maximal amplitude of the fast wave by an average of 33%, compared to sham rats, but the slow wave amplitude was reduced by 73%. The mean duration of the fast wave was increased by 34%, whereas durations for the slow wave were not altered by injury. These results suggest these two subpopulations of fibers were differentially recruited into the injury process, possibly due to distinct structural properties. Supported by NS020193.

P458. REGIONAL SPECIFIC ALTERATIONS IN NERVE GROWTH FACTOR (NGF) & NEUROTROPHIN-4/5 (NT-4/5) AFTER TRAUMATIC BRAIN INJURY IN RATS. N.C. Royo*, S. Shimizu1, K.E. Snodgrass1, T.K. McIntosh1,2. (Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA; 1Veterans Administration Medical Center, Philadelphia, PA USA).

Neurotrophins are required for the development, maintenance and regeneration of the central nervous system (CNS). These properties have stimulated an interest in these molecules as potential therapeutic agents for the treatment of brain injuries and neurodegenerative diseases. We evaluated the regional and temporal alterations in protein levels of NGF and NT-4/5, after moderate lateral fluid percussion traumatic brain injury (TBI) in anesthetized rats using an ELISA procedure, 3h, 24h, 72h and 1 week after surgery in the ipsilateral cortex and hippocampus of naive, sham and injured animals (n = 5-6 per time point and per group). NGF levels were significantly increased in the ipsilateral cortex compared to sham animals (comparison with a two-way ANOVA, injury effect P = 0.0051). No significant changes were observed in NGF levels in the hippocampus. NT-4/5 levels were significantly increased at 24h postinjury in the ipsilateral cortex compared to sham animals (mean ± S.E.M.: 1.8 ± 0.3 versus 0.9 ± 0.2 pg/mg of protein, P < 0.05) and 72h (2.3 ± 0.5 versus 0.7 ± 0.2 pg/mg of protein, P < 0.001) and had returned to baseline by 7 days post-injury. NT-4/5 was also significantly increased in the ipsilateral hippocampus of injured animals compared to sham animals (comparison with a two-way ANOVA, injury effect P = 0.0125).

These studies suggest that region-specific alterations occur in NGF and NT-4/5 in the acute period following TBI. As NGF has been shown to be neuroprotective in this model when administered during the first two weeks following TBI, we hypothesize that alterations in NGF levels following TBI may play an endogenous neuroprotective role. Whether this is true for NT-4/5 remains to be determined.

These studies were supported, in part, by NIH NS 40978, NS 08803, GM 34690 and a Veterans Administration-DOD Consortium Merit Review grant.

P459. NON-INVASIVE ASSESSMENT OF ICP FROM CEREBRAL BLOOD FLOW VELOCITY AND ARTERIAL BLOOD PRESSURE USING A FUZZY PATTERN CLASSIFICATION METHOD. B. Schmidt1,2, S.P. Buckl1,3, M. Pajk1,3, M. Czorny1,3, J.J. Schwartz2, J. Klinke1,3. (1Dept. of Neurology, Clinic for Neurology, Medical University of Vienna, Austria; 2Dept. of Systems Theory, Technical University, Chemnitz, Germany; 3Academic Neurosurgical Unit, Addenbrooke's Hospital, Cambridge, UK).

Objects: The authors previously introduced a method for a non-invasive assessment of ICP (nICP). The underlying mathematical model established a linear relationship between certain hemodynamic parameters (TCD characteristics) and the quotient between mean ICP and arterial blood pressure (ICP/APB). Some results suggested that this relationship might not be globally valid but might be influenced by additional parameters like, e.g., the patient's type of disease, the arterial CO2 pressure and the state of cerebral autoregulation (CA). In the current approach the formerly globally expressed relationship between TCD characteristics and the ICP/APB ratio was specifically modified to certain subgroups of patients in order to adapt the model to individual cases. Methods: In 113 traumatic brain injured patients (3-76 years of age, mean age: 31 ± 16 years) signal data of cerebral blood flow velocity (FBV), ABP and ICP was studied. TCD characteristics, calculated at several time points from FBV and ABP recordings, together with time corresponding ratios ICP/APB were sampled. CA was assessed by correlation of cerebral perfusion pressure and ABP. A method called Fuzzy Pattern Classification was used to identify substructures (classes) in the samples of TCD characteristics. On each of these classes a specific relationship between TCD characteristics and ICP/APB ratios was established. This construction facilitated the calculation of nICP as follows: Using FBV and ABP the TCD characteristics were computed and related to the matching classes. The estimator of ICP/APB was calculated and multiplied by ABP resulting in nICP. Results: Median error between ICP and nICP was 6.9 mm Hg in patients with impaired CA (N = 66) and 4.5 mm Hg in patients with preserved CA (N = 50). Plateau waves, B waves and long-term trends of ICP could be visibly assessed. Conclusions: The class structure of facilitates nICP assessment in heterogeneous patient groups. Moreover, its modular structure enables a stepwise extension of the target patient group, without affecting the current validity. The results encourage further investigations of Fuzzy Pattern classification method in view of nICP assessment.


On the basis of the contradiction between data on experimental head trauma showing oxidative stress-mediated cerebral tissue damage and failure of clinical trials using free radical scavenger drugs, we monitored the time-course changes of malondialdehyde (MDA), ascorbate and dephosphorylated ATP catabilites in cerebrospinal fluid (CSF) of traumatic brain-injured (TBI) comatose patients suffering from severe TBI (Glasgow Coma Scale on admission of 6 ± 1). First CSF sample was collected within 2.95 hours from trauma (SD = 1.98) and during the next 48 hours once every 6 hours. All samples were analyzed by an ion-pairing HPLC method for the simultaneous determination of MDA, ascorbic acid, oxyaurines and nucleoids. In comparison with values recorded in 10 herniated lumbar disk, non-cerebral control patients, CSF of TBI patients had high values (0.226 micromol/L; SD = 0.196) of MDA (undetectable in samples of control patients) and decreased ascorbate levels (96.25 micromol/L; SD = 31.74), already at the time of first withdrawal. MDA was almost constant in the next two withdrawals and tended to decrease thereafter, albeit after 48 hours from hospital admission (0.072 micromol/L, SD = 0.026) were still recorded. Ascorbate was normalized 42 hours after patient hospital admission. Evident changes in CSF values of ATP degradation products suggested neuronal energy metabolism derangement following TBI. These data demonstrate the early onset of oxidative stress in TBI patients, propose a valid explanation for the failure of clinical trials based on oxygen radical scavenger drug administration and suggest a possible rationale for testing the efficacy of lipid peroxidation "chain breakers" in future clinical trials.
Rapid Upregulation of Phosphorylated-ERK Suggests a Role for the Mitogen Activated Protein Kinase Pathway in Traumatic Brain Injury

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The mitogen activated protein kinases (MAPKs) cascades are well known membrane-to-nucleus signaling modules that have recently been implicated as mediators of cellular injury after cerebral ischemia and trauma. In this study, we investigated the involvement of the MAP kinase Erk, and the activated, phosphorylated form of Erk (p-Erk) in our controlled cortical contusion model of traumatic brain injury (TBI) in rats. Quantification of Erk and p-Erk in the contused cerebral cortex was made by western blot 10 min and 24 h after severe trauma. There was a strong increase in p-Erk 10 min after the injury. At 24 h after trauma, there was a marked accumulation of aggregated p-Erk in the lesioned tissue. The cellular identities of p-Erk expressing cells were studied by immunofluorescence double staining 24 h after trauma in formalin fixed frozen sections. At this time, numerous GFAP positive cells were double labeled with p-Erk, suggesting expressing astrocytes. Fewer cells were p-Erk expressing NeuN positive neurons. To investigate the potential connection between MEK inhibition and reactive oxygen species in this injury pathway we included animals treated with the MEK inhibitor U0126 and the free radical scavenger S-PBN, both with neuroprotective properties in TBI. Overall, the results implicate a significant role of the mitogen activated protein kinase Erk in the secondary injury cascade after traumatic brain injury.

Effect of Duration of Hypothermia Following Controlled Cortical Impact in Immature Rats


Rationale: Moderate hypothermia (HYPO) following experimental traumatic brain injury (TBI) has been shown to improve behavioral outcome in both adult and immature rats. Studies specifically addressing the effect of timing and duration of hypothermia on its efficacy in immature rats are lacking. The goal of this project was to begin to investigate the optimal timing and duration of therapeutic HYPO following TBI in immature rats.

Methods: Sprague-Dawley (postnatal day (PND) 7) rats were randomized to moderate HYPO 32-33OC applied for 1 h (target temperature achieved at time of injury) or for 4 h (delay to initiation of cooling for 15 min following CCI) vs. normothermia (NORM) 37OC (n = 10/treatment arm) and then injured using controlled cortical impact (CCI) (left, frontoparietal, 3mm ip, 4 nms, 1.75 mm deflection). To test functional outcome following injury and treatment, the Morris water maze (MWM) paradigm was used on post injury days (PND) 11-17.

Results: Following CCI treatment with HYPO for 1 h or 4 h significantly improved MWM performance as compared to NORM (p < 0.05). HYPO for 4 h additionally tended to improve MWM performance as compared to HYPO applied for 1 h though this difference was not statistically significant.

Conclusions: Moderate HYPO applied for 1 or 4 h after CCI in immature rats improved MWM performance as compared to NORM. In addition, HYPO applied for 4 h was still effective in improving functional outcome despite a 15 min delay in initiation following injury. The optimal timing and duration of HYPO after TBI in the immature rat needs to be further defined.

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Delayed Treatment with Aniracetam Improves Cognitive Recovery After Traumatic Brain Injury in Rats

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In contrast to the acute, excitotoxic processes that dominate immediately after traumatic brain injury (TBI), research into the chronic post-traumatic alterations of neuronal processes supports a suppressed or hypofunctional neuronal state. If a reduced level of neuronal activity is a long-term consequence of TBI that contributes to persistent impairment of function, then the chronic, post-injury enhancement of neuronal activity should improve recovery after TBI. The purpose of the present experiment was to test the effectiveness of aniracetam in reducing the cognitive deficits produced by TBI. Aniracetam acts through the allosteric potentiation of AMPA-specific glutamate receptors. The consequences of this drug are a reduction of glutamate receptor desensitization and potentiation of metabotropic glutamate activity. Beginning 24 h after midline fluid-percussion injury, either 25 (n = 9) or 50 mg/kg (n = 9) of aniracetam was administered daily for 15 days. On days 11-15 after TBI (n = 9) or sham injury (n = 10), rats were tested in the Morris water maze (MWM), and the latency to reach the goal platform was recorded. Results indicated that, compared to injured-unanesthetized rats, both the 25 and 50 mg/kg doses of aniracetam significantly improved MWM performance (p < 0.05). In fact, the MWM performance of injured, aniracetam-treated rats did not differ significantly from sham-injured rats. These results demonstrate the efficacy of using a positive modulator of AMPA receptor function as a delayed treatment for the cognitive impairments produced by TBI. These data also support the hypothesis that a depression in neuronal activity contributes to the chronic deficits produced by TBI. Supported by the Commonwealth Neurotrauma Initiative Fund.

Caspase Inhibition After Traumatic Brain Injury Alters Amyloid Precursor Protein and Amyloid-Beta Production in a Mouse Model of Alzheimer's Disease


Traumatic brain injury (TBI) is a risk factor for Alzheimer's disease (AD). Brains from head-injured patients have frequently shown AD-specific pathological changes, including overproduction of amyloid precursor protein (APP) and amyloid-beta (A-beta). Although the mechanism for this acute upregulation of amyloid in TBI is unknown, several in vitro studies have suggested that caspases, which are known to be activated after TBI, may be involved in APP processing. We examined the effects of caspase inhibition on hippocampal production and processing of APP at 24 and 48 hours after TBI in "humanized A-beta" mice. These gene-targeted animals contain the APP Swedish mutations and have had their A-beta sequence changed from rodent to human, allowing detection of human A-beta production in mouse brain. We found that hippocampal APP expression was altered at 24 and 48 hours after weight-drop TBI. Production of A-beta increased after TBI, although no A-beta deposits were detected. Immediate post-injury administration of a single i.p. dose of 100 μM BAF, a pan caspase inhibitor, reduced A-beta production in injured brain compared to sham and vehicle-treated mice. These data imply that APP processing acutely after TBI results in production of amyloidogenic fragments, and that a mechanism for this altered processing may be caspase-dependent. Thus, the neuroprotective role of caspase inhibition may be through decreased production of neurotoxic A-beta as well as through inhibition of apoptosis. Supported by AG05133 and NS03018.
P465. ATTENUATION OF OXIDATIVE STRESS AFTER ACUTE BROMOCRIPTINE TREATMENT IN TRAUMATICALLY BRAIN INJURED RATS
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Oxidative stress has been reported to be an important contributor to the secondary sequelae of traumatic brain injury (TBI). Therefore, pharmacotherapies that display antioxidant properties, such as the dopamine receptor (D2) agonist bromocriptine (BRO) may benefit outcome. We have recently reported that both acute and chronic BRO attenuates posttraumatic functional deficits (Massucci et al., 2001; Kline et al., 2002). In this study we examined the effects of acute BRO treatment on TBI-induced oxidative stress. Thirty-six dopamine-antagonist rats received BRO (5 mg/kg, i.p., Injury/BRO = 12; Sham/BRO = 6) or vehicle (Injury/VEH = 12, Sham/VEH = 6) 15 min prior to controlled cortical impact (2.7 mm impact at 4 m/s) or sham injury. At 1-hr post-surgery, rats were sacrificed and changes in lipid peroxidation, a major indicator of oxidative stress, was measured in the frontal cortex, striatum, and substantia nigra using thiobarbituric acid reactive substances (TBARS) assay. The data are expressed as nmol malondialdehyde per mg tissue ± SEM. TBARS was increased in all regions examined in the Injury/VEH group vs. sham. In contrast, no differences were observed between the Injury/BRO and sham groups. A trend toward decreased TBARS was observed in the striatum of the Injury/BRO vs. Injury/VEH groups (5.44 ± 0.44 vs. 5.60 ± 0.44) and substantia nigra (4.18 ± 0.35 vs. 7.76 ± 2.05) of the Injury/BRO vs. Injury/VEH groups, respectively. These findings suggest that BRO-induced oxidative stress in the striatum and substantia nigra is attenuated by acute BRO treatment, which may explain the functional benefit previously reported by our group. (Supported by NIH NS33150 and NS40125).

P466. INCREASED EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN RAT BRAIN AFTER TRAUMATIC BRAIN INJURY
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Glia cell line-derived neurotrophic factor (GDNF), a member of the TGF-beta superfamily, plays important roles not only for the differentiation of neurons during normal development but also for the survival and recovery of many populations of mature neurons. GDNF is a highly specific neurotrophic factor for dopaminergic neurons. It has been reported that GDNF has protective effects on various injuries for central and peripheral nervous systems in vitro and in vivo. However, the effect of traumatic brain injury (TBI) on the expression of GDNF is currently unknown. To determine if there is alteration in GDNF after TBI, we examined the effect of controlled cortical impact (CCI) injury on GDNF protein levels at 1 day and 7 days following injury by utilizing a commercially available antibody specific to GDNF. Rats were anesthetized and surgically prepared for CCI injury (4 m/sec, 2.7 mm) and sham surgery. Injured and sham animals (N = 4 per group) were sacrificed at 1 day and 7 days, respectively, and perfused with 4% paraformaldehyde. Coronal sections (35 mm thick) were cut through the hippocampus. An increased expression of GDNF protein was observed by immunohistochemistry in the hippocampus and the cortex in injured rats compared to sham controls. The increased expression of GDNF is more evidently observed in the ipsilateral hippocampus and the area around the contusion in the cortex. In the cortex, GDNF immunoactivity appeared greatest in cells with glial morphology. However, in the hippocampus, GDNF immunoactivity was greatest in neuron-like cells. These changes were observed at both 1 and 7 days postinjury. We speculate that the up-regulation of the GDNF protein may reflect its neurotrophic and neuroprotective effect on dopaminergic system response to the TBI insult. (Supported by grants: NIH-NS33150, NIH-NS20318, NIH-NS312296).

P467. EXPLORATORY STUDY OF ACUPUNCTURE TREATMENT ON TRAUMATIC BRAIN INJURY (TBI) IN RATS
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Traumatic brain injury (TBI) is the leading cause of death and injury-related disability among young adults, making it one of the most tragic and prevalent of all neurological disorders. Acupuncture is a component of the health care system of China that can be traced back for at least 2,500 years. The effectiveness of acupuncture has been scientifically investigated in both human clinical trials and animal studies for the past 30 years. Since acupuncture is known to possess many effects, such as analgesia, promotion of homeostasis, and changes in the microcirculatory network as well as improvement in brain circulation, we believe that it is logical to hypothesize that acupuncture treatment can reduce the pathological changes and will accelerate recovery of function following TBI in rat. To test this hypothesis, anesthetized male Sprague-Dawley rats were subjected to a controlled cortical impact (CCI) injury of moderate severity (4 m/sec, 2.7 mm deformation) and randomized into acupuncture treatment (N = 8) and control group (N = 8). Motor functions (beam balance and beam walking) were evaluated on post-operative days 1-5. Rats were sacrificed at 28 days after TBI. Electroacupuncture (EA) treatment (2 Hz, 20 minutes each of, 2 hours apart) on bilateral Zusanli (St 36) starting at 1 hour after TBI for six days significantly reduces the motor deficit after TBI compared to control group (P < 0.05). EA treatment for 21 days significantly reduced the contusion (lesion) volume and the hemispheric tissue loss after TBI compared to the control group (P < 0.05). The data demonstrate the beneficial effect of post-injury acupuncture treatment on motor function and histopathological deficits caused by TBI in rats. The potential for acupuncture is just beginning to be understood. Thus, further studies of acupuncture to find the optimal therapeutic parameters and their mechanisms are warranted. (Supported by grant NIH-NS40125).

P468. CHRONIC IMPAIRMENT OF EXTRACELLULAR K+ HOMEOSTASIS FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT.
Raimondo D’Ambrosio*, and David S Gordon. (Department of Neurological Surgery, University of Washington, Seattle, WA USA).

We have previously shown that, acutely following fluid percussion injury (FPI), rat hippocampal astrocytes are reactive, have decreased membrane potassium conductance that results in impaired extracellular K+ homeostasis which, in turn, contributes to abnormal neuronal excitability (1). However, it is not known how such acute impairment progresses overtime following injury. We have now assessed the efficiency of extracellular K+ homeostasis in rat hippocampal slices at subacute and chronic time points following moderate mild FPI. Moderate (3.54tn) mild FPI was induced. Hippocampal slices were obtained two days, two weeks or one month following FPI or sham operation. K+ selective microelectrodes were employed to measure K+ accumulation and evoked field potentials in CA3 stratum pyramidale during antidromic Schaffer collateral stimulation at 0.05Hz. We found that, during stimulation, baseline (K+;j0) was elevated at two days, two weeks and one month after injury. (K+;j0 was higher by 0.4 ± 0.04mM two days post-FPI (mean ± SD, n = 9; p < 0.01), and by 0.3 ± 0.04 mM two weeks post-FPI (n = 5; p < 0.01), and by 0.25 ± 0.02 mM one month post-FPI (n = 13); p < 0.01), in respect to K+ levels measured in similar manner in slices obtained from age-matched sham operated rats two days, two weeks or one month after surgery (n = 12, 6 and 8, respectively). We conclude that impaired extracellular K+ homeostasis persists at chronic time points following TBI, therefore contributing to chronic tissue hyperexcitability and to the likelihood of transition from interictal to ictal activity (2). (Supported by NIH, NS 40823 (7R).)


P469.
THE mGluR1 ANTAGONIST AIDA REDUCES POST-TRAUMATIC EMPTYING OF CALCIUM STORES IN NEURONS AND ASTROCYTES
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Using a cell culture model of strain (stetch) injury, we examined the effects of the mGluR1 antagonist (RS)-1-aminoindanoic-5,6dicarboxylic acid (AIDA) on intracellular Ca2+ stores in astrocytes and neurons using fura-2. We have previously shown that AIDA blocks post-traumatic increases in astrocyte IP3, which can stimulate Ca2+ release from Ca2+ stores (Floyd et al., J. Neurotrauma 16: 961, 1999). We have also reported that in both astrocytes (Rizgala et al., J. Neurochem 70: 2377, 1998) and neurons (Weber et al., Cell Calcium 26: 289, 1999) elevation of [Ca2+][5 by thapsigargin, which inhibits the sarcoplasmic-endoplasmic reticulum Ca2+ATPase and allows release of Ca2+ stores, is abolished 15 min post-injury. This implies injury-induced depletion of Ca2+ stores. In the current study both immediate and immediate post-injury treatment with AIDA reduced depletion of Ca2+ stores 15 min post-injury in astrocytes and neurons, suggesting store depletion via activation of mGluR1.

As previously reported, in injured neurons the initially abolished Ca2+ increase by thapsigargin returns within 3 hours and is potentiated at 3 hr post-injury (Weber et al., J. Biol. Chem. 276: 1800, 2001). Using Ca2+free medium has shown that the size of the neuronal Ca2+ stores is normal at 3 hr and that the enhanced response is due to extracellular Ca2+. The enhancement is also partially blocked by a store-operated channel inhibitor, SKF96365. In the current studies we found the enhanced neuronal response to thapsigargin at 3 hr was partially reduced by pre- or post-injury treatment with AIDA. In summary, our current findings implicate mGluR1 receptors in the initial post-traumatic depletion of astrocyte and neuronal Ca2+ stores and the delayed, potentiated capacitative Ca2+ influx in injured neurons. These findings provide insight to the cellular mechanisms by which AIDA produces its beneficial effect in vivo and in vitro experimental trauma.

P470.
DIFFERENTIAL EFFECTS OF ACUTE AND CHRONIC EXERCISE ON PLASTICITY-RELATED GENES IN THE RAT HIPPOCAMPUS REVEALED BY MICROARRAY
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Exercise has a healing potential in the injured brain, but the lack of knowledge on the molecular mechanisms involved has hampered the implementation of exercise as a therapeutic tool. A standing question for planning therapeutic applications is whether exercise provided for a short period of time can have the same benefit as long-term exercise. Hippocampal RNA from rats exposed to a running wheel for 3, 7, and 28 days were examined using a microarray with 1,176 cDNAs (Clontech). The expression of selected genes was quantified by Tagman RT-PCR or RNase protection assay. The largest upregulation was in genes involved with synaptic trafficking (synaptotagmin, and syntaxin), signal transduction pathways (Ca2+/calmodulin-dependent protein kinase II, CAM-KII; mitogen-activated/extracellular signal-regulated protein kinase, MAPK/ERK 1 and 2; protein kinase C, PKC-d) or transcription regulators (cyclic AMP response element binding protein, CREB). Genes associated with the glutamatergic system were upregulated (N-methyl-D-aspartate receptor: NMDAR-2A, NMDAR-2B, and excitatory amino acid carrier 1: EAAC1), while genes related to the GABA system were downregulated (GABA A receptor, glutamate decarboxylase GAD65). The temporal profile of gene expression seems to delineate a mechanism by which specific molecular pathways are activated along exercise performance. For example, brain-derived neurotrophic factor (BDNF) was the only trophic factor whose gene was consistently upregulated at all timepoints. These results, together with the fact that most of the genes upregulated have a recognized interaction with BDNF, suggest a central role for BDNF on the effects of exercise on brain plasticity. (Supported by NIH awards NS39878, NS39522, Alzheimer’s Association, and UCLA Brain Injury Res. Ctr.).

P471.
THE USAGE EFFICACY OF PROLONGED VENTRICULAR DRAINAGE AND APRICOT JUICE ON MANAGEMENT OF CNS INJURY
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The efficacy of using of prolonged ventricular drainage and apricot juice three groups (with 21 patients on each) were almost identical, in mise of clinical- has been studied on 63 patients (divided in to three groups), with hard CNS injury. All neurological characteristic and age-gender attitude.

The patients of first group took usual traditional treatment, where to the patients of second group in addition to the traditional treatment, was mounted the prolonged ventricular drainage through laid farse hole on Dandy drainage, which promote dosage effusion of liquor from stomach systems of brain in to the special container. The patients of third group, besides the traditional treatment and prolonged ventricular drainage, also took the natural apricot juice, the 80-100 ml/5-6 times a day, (using the stomach probe until the patient regains its consciousness), containing calcium and diuretic efficacy.

The evaluation the effect of realtional treatment was occurred by calculating the dynamics of clinical manifestations, sensation of liquor, the period of being at hospital door, mortality. Issue of trauma evaluated on Glasgow scale.

The positive neurological dynamics seen in first group for 1-12 days, in second and in third groups accordingly 9-10 and 8-9 days. The sensation of liquor composed accordingly—for 17-18, 12-13 and 10-11 days. Lasted period of being in hospital composed accordingly: 24.8, 22.9, and 21.3 days. The received trauma results improvement, on Glasgow scale were seen in 7 patients of first group, and 8 and 9 in second and third groups accordingly, moderate invalidization accordingly composed—7, 8, 9, 8, hard invalidization accordingly—1 patient on each, and mortality composed—4, 2, 2 accordingly.

Therefore, using the prolonged ventricular drainage and apricot juice in complex treatment of CNS injury promotes the results of treatment, decreasing the invalidization and mortality of patients.

P472.
A HIGH-FAT SUCROSE DIET (HFS) EXACERBATED TRAUMATIC BRAIN INJURY (TBI)-INDUCED IMPAIRMENTS IN COGNITION AND NEURAL PLASTICITY
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Traumatic brain injury (TBI) results in long-lasting functional impairments in cognitive function, but the molecular mechanisms remain unknown. Although TBI patients have elevated susceptibility to subsequent insults, the effects of nutritional factors on neural healing following TBI have not been experimentally scrutinized. We have recently reported that a HFS diet decreases brain-derived neurotrophic factor (BDNF) and its downstream effectors in the hippocampus, resulting in impairments in neuroplasticity and cognition. Based on the role of BDNF on neuroprotection and excitability, we have examined the neuroplasticity after TBI and the possibility that a HFS diet may reduce the capacity of the brain to react to injury by affecting BDNF-related neuroplasticity. Male Sprague-Dawley rats were maintained on HFS diet or a low-fat, complex-carbohydrate (LFCC) for 4 weeks before a fluid percussion injury (FPI) or sham surgery were performed. All rats were killed after one week. The mRNA levels of BDNF, synapsin I, and cyclic AMP-response element-binding protein (CREB) were determined in the hippocampus by Real-time quantitative RT-PCR. The cognitive function was assessed after surgery using a water maze. Results showed that (1) FPI decreased BDNF mRNA level in HFS-fed rats, but not in LFCC-fed rats; (2) FPI decreased synapsin I and CREB mRNA levels in both LFCC and HFS-fed rats with a stronger effect in HFS-fed rats; (3) FPI impaired cognitive function in both LFCC- and HFS-fed rats with a worse outcome in HFS-fed rats. Results showing that TBI induced impairments in cognition and neuroplasticity are exacerbated by HFS suggest that HFS decrease the capacity of the brain to compensate for traumatic injury. (Supported by NS39878, NS39522, Alzheimer’s Association, and UCLA Brain Injury Research Center).
P473. IS MINOCYCLINE REGULATING GLUTAMATE TOXICITY AFTER TRAUMATIC BRAIN INJURY? S. Bele*, A. Brausins (Department of Neurosurgery, University of Regensburg, Regensburg, Germany).

Objective: Former experiments proved a positive effect of minocycline after ischemia that led to reduced infarct sizes. We were interested if this holds also true after trauma and what possible mechanisms underlying a potential beneficial effect of minocycline after trauma.

Model: Severe tbi was induced in male Wistar rats using the controlled cortical impact device introduced by Dixon et al. with a velocity of 7 m/sec on a depth of 2 min. 12 h and 24 h after tbi minocycline was applied in a dosage of 90 mg/kg bw followed by 45 mg/kg bw twice daily for 1-4 days. Animals were then sacrificed and the brains processed for DNA-array analysis. Control animals received saline instead. Animals without surgery served as absolute controls.

Results: The DNA arrays demonstrated that 72 h and 96 h after the animal treatments with minocycline had different expressions of the brachyder channel antioxidant, which plays an important role in glutamate metabolism.

Discussion: Former experiments using minocycline after ischemia demonstrated beneficial effects on infarct volume. Our group also showed a significantly lower number of apoptotic neurons after tbi when using minocycline. It is known that minocycline has a direct effect on interleukine-1β-converting enzyme, which plays a role in apoptosis. Our results showed that minocycline may act beneficial after tbi by influencing the glutamate pathways.


Post-traumatic headache (PTH) is often reported following minor and moderate head injury. Sex, repeated head injury and skull fracture are the most relevant predictive features in epidemiological studies. PTH occurs more frequently after minor head injury than after severe brain injury and in patients with impaired cognitive functions in some studies, or in less cognitively impaired patients in others. Therefore, data on this issue are not conclusive. We evaluated the incidence of PTH after very severe traumatic brain injury and in cases of severe neurosurgical disorders were investigated. Some patients suffering from PTH of migraine type were studied by means of transcranial Doppler (TCD), and compared with patients suffering from idiopathic migraine.

The incidence of headache was reported in about 10% of patients, skull fracture or cranialitis, post-traumatic epilepsy and a good recovery of the cognitive functions being the most frequent features associated with the presence of headache. Tension-type headache was the commonest in these patients, whereas migraine occurred in the minority of the patients. All patients were affected by anxiety or depression.

The low frequency of headache following severe traumatic brain-injury may be secondary to the diffuse impairment of cerebral structures with a pivotal role in the affective component of pain. In fact, only patients with good cognitive recovery may develop PTH. The presence of anxiety and depression is possibly related to the awareness of disabilities due to trauma. Therefore, PTH may be considered as an adaptive disorder in which both altered biological and psychological features play a pathophysiological role.


Using a model of mild concussive TBI with secondary ischemia we have demonstrated posttraumatic brain ischemic hyperexcitability under normoglycemia. Under our experimental conditions, antecedent mild TBI changes the pathology of a 6 minute forebrain ischemic insult into the pathology of a 10 minute forebrain ischemic insult. Posttraumatic cerebral ischemia often occurs during a time of high serum catecholamines and hyperglycemia which exacerbates the response of the brain to primary or secondary cerebral ischemia. Under our experimental conditions (serum glucose >= 400 mg/dL), results in secondary seizures and widespread neuronal loss. Pretreatment with combined scopolamine and MK-801 reduces posttraumatic ischemic sensitivity in this model under fasted conditions and the purpose of the present study was to determine if comparable receptor blockade was also effective with hyperglycemic T+I.

Using a Wistar rat model of mild fluid percussion TBI with 6 min of immoded forebrain ischemia, we examined if the increased brain damage occurring with posttraumatic but preischemic serum hyperglycemia of > 400 mg/dL could be reduced with combined 1mg/kg scopolamine and 0.1mg/kg MK-801 antagonism given before the insults. Two groups of fasted rats (N = 10/group) were given 6 min forebrain ischemia preceded by null TBI 1 hr before. Thirty minutes before ischemia, rats received either Ip. glucose solution or saline. Hyperglycemic rats with T+I developed status epilepticus within 24 hr after combined injury with extensive intra- and extra-hippocampal damage. However, scopolamine and MK-801 pretreated hyperglycemic rats with T+I had a reduction in postischemic seizures and histopathological damage. These data suggest that TBI increases the sensitivity of the brain to hyperglycemic ischemia in part by excitatory neurotransmitter cascades. Supported by NS35365.
P477. BRAIN TISSUE PO2, INTRACRANIAL PRESSURE, ADENOSINE AND PURINE DEGRADATION PRODUCTS AFTER SEVERE HEAD INJURY IN ADULTS: A PRELIMINARY ANALYSIS


The usage of brain tissue oxygenation (Pto2) as an invasive monitoring tool in severe traumatic brain injury (TBI) has increased. Although ischemic threshold values have been determined for survival, correlations to physiological parameters have been limited2,3, and the relationship between Pto2 and purine degradation products (PDP) remain unclear. Continuous Pto2 monitoring was performed in adults (n = 6) with severe TBI (GCS<9), utilizing Liqui Probes placed within the non-lesioned white matter of the frontal lobe. Ventricular cerebrospinal fluid (CSF) samples (n = 97) were obtained every 4h for the first 24h and then every 6h for 4d. CSF adenosine, inosine, hypoxanthine, xanthine, lactate, and pyruvate rati were quantified by HPLC, and correlated to physiological parameters (mean arterial pressure, MAP), electrolytes (ETCO2), intracranial pressure (ICP), cerebral perfusion pressure (CPP), and rectal temperature (TEMP) at the time of the CSF sampling. Mean Pto2 was 32.7 ± 16.6 mmHg for all patients, significantly correlating with ICP (r = -0.51, p = 0.001), ETCO2 (r = -0.31, p = 0.02), and TEMP (r = 0.266, p = 0.015). Pto2 also correlated to PDP, xanthine (r = -0.36, p = 0.001), and indicators of anoxic metabolism, lactate (r = -0.344, p = 0.001), and ICP (r = -0.247, p = 0.022). Two patients exhibiting profound reductions in Pto2 (<10 mmHg), had dramatic concurrent increases in adenosine and hypoxanthine. Our data support the hypothesis that critical reductions in Pto2 are accompanied by energy failure, as reflected by concomitant increases in adenosine and PDP. J Crit Care Med 26(9):1576, 1998; Acta Neurochir 71:153, 1998; Neurosurg Rev 23(2):94, 2000. Support: NS 38087 and NS 30318.

P478. MULTICENTER STUDY OF CONTINUOUS VS INTERMITTENT CEREBROSPINAL FLUID DRAINAGE AFTER SEVERE TRAUMATIC BRAIN INJURY IN CHILDREN: EFFECT ON BIOCHEMICAL MARKERS

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Cerebrospinal fluid (CSF) drainage is routinely used in the treatment of severe traumatic brain injury (TBI)—either continuously or intermittently in response to increases in intracranial pressure (ICP). We have previously reported that levels of a variety of markers of injury or repair are increased in CSF after severe TBI (1). We hypothesized that these markers would be reduced in CSF drained continuously vs intermittently. We compared CSF levels of markers of neuronal injury (neuro specific enolase, (NSE), gial injury (S-100B), inflammation (interleukin-6 (IL-6)), and regenerative vessels endothelial growth factor (VEGFI) (ELISA) in 19 severely injured children whose CSF was drained continuously (n = 13) vs intermittently (n = 6) as part of standard care in two institutions. Mean level of each marker was nearly two-fold lower in CSF drained continuously vs intermittently, though only statistically significant (p < 0.05) for NSE. When controlled for all confounding variables, however, the difference between drainage methods was significant for all four markers. S-100B and VEGF were inversely associated with time post-trauma. Child abuse as a mechanism of injury was directly associated with level of NSE, and inversely associated with level of IL-6. We conclude that the method of CSF drainage greatly affects levels of CSF markers after TBI. The marked reduction in CSF levels of NSE in patients receiving continuous CSF drainage suggests the possibility that this method of drainage may more effectively reduce neuronal death. Alternatively, the difference may reflect increased clearance of CSF biochemical substances with continuous drainage. Correlation of CSF markers with physiologic (ICP) and clinical outcome is required. (1) Kochanek et al, Ped Crit Care Med, 2000. SUPPORT: HD400686, NS30318, NS38087, U. of Pgh CUR//DCCD, Eric Bundy Memorial Fund, Medic First Aid Foundation.

P479. DOWN REGULATION OF AQUAPORIN-4 IN AREA ADJACENT TO BRAIN INJURY IN A TRAUMATIC RAT BRAIN MODEL.


Aquaporin-4 (AQP4) plays a significant role in the regulation of brain water homeostasis. Several studies have shown that this molecule is up-regulated following non-traumatic brain injury. This study investigated the regulation of AQP4 following a focal cortical contusion injury in the rat. Twenty four hours post-injury, a marked increase in the expression of AQP4 was observed in the contusion cortex (craniectomy followed by contusion with a 1 mm diameter sphere using 800 g-cm force over ± 3 mm vertical depression). Five received a craniectomy with no trauma (sham Injury). Procedures were performed under sodium pentobarbital anesthesia with controlled body temperature. Animals were sacrificed at 4 and 24 hours. Brains were examined for water content by comparing the wet and dry weight of each hemisphere. AQP4 mRNA was measured by RT-PCR. AQP4 mRNA expression on the lesioned side compared to the control hemisphere was calculated for each animal at the injury site (parietal cortex), adjacent to the injury (occipital cortex) and distant from the injury (frontal pole cortex). Brain edema was significantly increased at the injury site, significantly decreased adjacent to the injury site in the occipital cortex, and not significantly different at a site well distant from the injury in the frontal pole. The magnitude of AQP4 mRNA up-regulation at the injured parietal cortex correlated (R2 = 0.74) with the degree of down-regulation in the adjacent occipital cortex. This study demonstrates (i) an up-regulation of AQP4 at the site of traumatic brain injury, and (ii) a down-regulation of this molecule adjacent to the site of injury. This down-regulation may be a protective mechanism reducing the development of brain edema following trauma.

P480. GENDER INFLUENCES ON CEREBROSPINAL FLUID PATHOPHYSIOLOGY AFTER TRAUMATIC BRAIN INJURY


Female sex hormones have been shown to affect some aspects of secondary traumatic brain injury (TBI) pathophysiology, including excitotoxicity, cerebral edema, and blood flow. As such, we investigated gender differences in TBI related excitotoxicity and ischemia by evaluating cerebral spinal fluid (CSF) levels of glutamate and lactate in a clinical population with severe TBI. We evaluated 123 patients (90 female, 33 male) with severe TBI (GCS≤5) admitted between 1995-1998. A prospective cohort model of this population was treated as a part of a randomized controlled clinical trial evaluating moderate hypothermia treatment (32-33°C for 48 hours). The remaining population, meeting clinical criteria, received 24 hours of hypothermia as standard treatment. Maximum CSF glutamate concentration and lactate/pyruvate ratio were determined at twelve-hour intervals for both 24 and 48 hours after injury. Repeated measures multivariate analysis, (adjusting for age, sex, time, injury severity and type, hypothermia status, and gender interactions) were used to determine the relationship of gender to CSF glutamate concentration, and lactate/pyruvate ratio. Results showed a significant gender effect on overall CSF glutamate production (p = 0.0023) and a significant interaction between glutamate, gender, and time (p = 0.0035) within the first 24 hours after injury. Females had lower levels of CSF glutamate compared to males, especially in the first twelve hours after injury (4.56 vs. 8.00 mmoles/l) of CSF glutamate to normal levels by 48 hours after injury. Additionally, there were significant gender differences in CSF lactate/pyruvate ratios (p = 0.0006) and a significant relationship between lactate/pyruvate, gender, and time (p = 0.0045) throughout the first 48 hours after injury. Females had lower lactate/pyruvate ratios than males, particularly within the first 12 hours after injury (24 vs 37). Hypothermia reduced glutamate levels in both males and females. These results signify the importance of studying how gender impacts TBI pathophysiology and efficacy with therapeutic interventions. R03HD41359-01, CCR310258-08.
P481. NEURONAL SURVIVAL AFTER CENTRAL NERVOUS SYSTEM INJURY REQUIRES AUTOIMMUNE T CELLS; TOLERANCE TO MYELIN ANTIGENS DIMINISHES NEUROPROTECTION
Jonathan Rijnjs*, Tal Mizrahi, Ehud Hauben and Michal Schwartz (The Weizmann Institute of Science, Mod'in, Israel, IL).

After trauma to the central nervous system (CNS), neurons that escaped the primary insult nevertheless undergo degeneration, spreading the area of damage beyond the epicenter. Immune activity (especially autoimmune activity) has traditionally been considered as detrimental for neuronal survival, but studies from our laboratory contradict this view. We show here that strains of rats and mice which are inherently resistant to secondary degeneration after CNS injury are endowed with an endogenous mechanism of neuroprotection, and that a key component of this mechanism is a population of autoimmune T cells. We further show that tolerance to myelin self-antigens, long thought to be preferred state for autoreactive cells, is to the individual’s disadvantage in coping with injury-induced stress. Neocortical immunization of rats with whole spinal cord homograft diminished the ability of the adult animal to respond to myelin immunization, and resulted in significantly worse recovery from severe optic nerve crush (assessed in terms of neocortical survival) or from spinal cord contusion (assessed by locomotor ability). We further show that CNS insult breaks down tolerance to CNS antigens and activates autoimmune T cells. The loss of neurons was greater in strains in which activation of autoreactivity is constitutionally delayed. Autoreactivity could be boosted by immunizing rats with the self-antigen or by depletion of their endogenous suppressor T cells (e.g. CD4+CD25+ regulatory T cells). These findings demonstrate that the autoimmune response to CNS injury, if well-controlled, is beneficial for neuronal survival after trauma. Boosting of this response by depletion of regulatory T cells or by safe immunization with altered self-peptides is likely to have clinical implications.

P482. MECHANISM OF PRO-REGENERATIVE VACCINE UNLIKELY TO INVOLVE ANTIBODIES AGAINST GROWTH-INHIBITORY PROTEINS
Benjamin E. C. L., Joanne Bertrand, Pauline Dergham and Lisa McKerracher (Department of Pathology and Cell Biology, Université de Montréal, Québec, Canada).

We have previously shown that a spinal cord homogenate (SCH) vaccine stimulates axon regeneration of adult rat retinal ganglion cells (RGCs) following optic nerve microcrush. This vaccine does not promote survival of injured RGCs. To examine if antibodies against growth inhibitors are important for RGC regeneration after vaccination, sera of vaccinated animals were tested by Western blot and ELISA against known growth inhibitory proteins. We were unable to detect serum antibodies to myelin-associated glycoprotein (MAG), Nogo-A, Nogo-66 receptor, or chondroitin sulfate proteoglycans (CSPG). However, antibodies to myelin basic protein, an abundant myelin protein, were detected. We also examined the ability of sera to override RGC growth inhibition on myelin or CSPG culture substrates. Preincubation of substrate with sera from SCH-vaccinated animals promoted growth on myelin but not on CSPG. Our results suggest that the growth promoting effect of the SCH vaccine is not mediated by antibody blocking of growth-inhibitory proteins, but by antibodies binding to major myelin proteins. Supported by the CIHR and the FRSQ.

P483. THE RISK OF BLADDER DENERVATION DURING ANTIREFLUX SURGERY: A RELIABLE NEUROPHYSIOLOGICAL MODEL
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Introduction and Objectives: Previous anatomical studies (Leistner et al. J. Urol. 2001) demonstrating the close relationship between uretero-vesical junction and urethral bladder innervation prompted us to develop a supporting animal model to demonstrate the risk of partial or complete neuronal damage caused by urethral bladder innervation during antireflux surgery.

Material and Methods: Laminectomy was performed on 5 male Göttinger minipigs and a modified Brinley electrode was implanted at S2 for sacral anterior root stimulation (SARS). The lower urinary tract was exposed by abdominal midline incision. The nerve bundle of the pelvic plexus was identified about 1 cm dorsal-medial to the uretero-vesical junction.

The bladder was filled with 150 cc NaCl solution and intravesical pressure monitored with an intravesical catheter. A blue longitudinal line down the middle of the bladder dome enabled exact visualisation of bladder contraction. Bilateral and unilateral SARS was performed, the nerve fibers of the pelvic plexus were either blocked by Xylocaine or cut under vision.

Results: Bilateral SARS evoked a bilateral detrusor muscle contraction with an intravesical pressure rise up to 39 cmH2O. Unilateral stimulation evoked an exclusively unilateral bladder contraction with maximum intravesical pressures of 13 to 14 cmH2O. Xylocaine injection at the uretero-vesical junction completely blocked the response to SARS. Neurotomy of the nerve bundles in close proximity to the ureter produced the same results. Unilateral neurotomy evoked unilateral detrusor decentralization at the site of nerve damage, whereas bilateral neurotomy evoked detrusor constrictivity. Successive cutting of the nerve fibers, starting 8 cm from the uretero-vesical junction and proceeding up to 1 cm away from it, led to a drop in detrusor pressure with SARS from 39 cmH2O to 12 and 0 cmH2O, depending on the closeness to the uretero-vesical junction.

Conclusions: Similar to human cadaver studies, the urinary bladder is innervated strictly unilaterally, the nerve supply emanating from the pelvic plexus runs in close proximity (dorsal-medial) to the uretero-vesical junction. Dissection away from the uretero-vesical antireflux surgery and/or pulling-stretching forces due to bleeding or anchor stitches (Vest) bear high risk of injury to the pelvic plexus which may result in uni- or bilateral detrusor decentralization.

P484. MECHANICALLY ELONGATED PNS AXONS SUSTAIN HIGH GROWTH RATES: IMPLICATIONS FOR NERVE REPAIR

Repair of nerve damage has traditionally relied on donor nerves and is limited by availability and associated donor site morbidity. As an alternative strategy, we propose that mechanically elongated axons grown in culture could be used to bridge even extensive nerve damage. Here, we evaluated the ability of rat dorsal root ganglion cells (DRGs) to rapidly grow under continuous mechanical tension. DRGs were plated on adjoining substrates and during a static growth period, axons cross the interface of the two substrates. Using a microstepper motor system, we then progressively separated the two substrates further apart from each other resulting in two populations of DRGs connected together via elongated fascicular axon tracts. Axon growth appears highly strain dependent early in the elongation phase due to the initially small axon lengths. As axons elongate, the rate is slowly increased based on a constant strain value until a maximal elongation rate is realized and growth can be maintained. We previously reported that CNS axons were limited to stretch induced growth of 1 mm/day. Here, we found that DRGs could be grown 6 mm/day reaching at least 3 cm in length. Immunocytochemical analysis revealed that elongated axons consistently expressed phosphorylated neurofilament, polymerized beta-tubulin and tau proteins. Despite this enormous growth rate, the expression of these cytoskeletal proteins in the area of elongation actually exceeded that found in adjacent non-stretched axons. These findings may represent a fundamental shift in our understanding of axonal cytoskeleton assembly during growth. We are currently using electron microscopic examination and proteomics to reveal more detail about cytoskeletal assembly during stretch growth. Furthermore, we are elongating DRG axons on substrates suitable for transplantation to repair peripheral nerve injuries. Supported by NIH grants, AG 21557 & NS 35104.
P485.
IMPLANTABLE NEUROCYBERNETIC INTERFACE WITH MECHANICALLY ELONGATED AXONS

Though once relegated to science fiction, neurocybernetic interfaces have now become a tangible strategy for restoring lost function following trauma. These interfaces must generally be non-invasive, yet integrate with the host neural network. Here, we used a new technique to mechanically elongate numerous axons interconnecting two populations of neurons to create such a device. Rat dorsal root ganglion cells (DRGs) were plated on a multi-electrode array (MEA) and an adjoining tubing substrate. The two populations of neurons were allowed to integrate, including the growth of axons across the interface of the two substrates. Using a microstepper motor system, we then progressively separated the two substrates further apart from each other resulting in two populations of cell bodies connected together via elongated fascicular axon tracts. Previously we found that sustained mechanical tension induced CNS axons to grow 1 cm in length over a period of 10 days. Here we elongated DRGs to lengths over 3 cm at a rate of 6 mm per day. Immunocytochemistry of these tracts revealed a normal appearing cytoskeleton, including expression of phosphorylated neurofilament, tau and tubulin proteins. The design of this interface allows for implant of neurons at one end into sensitive nervous tissue areas while MEA can be conveniently located for an electronic interface outside the body. We are currently evaluating electrophysiological interactions between the MEA and the elongated cultures. Supported by NIH grants, AG 21527 and NS 38104.

P486.
RESULTS OF PERIPHERAL NERVE RECONSTRUCTION BY AUTOGRaFT
Viktor Matejčik, M.D. (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician L. Déver Faculty Hospital, Bratislava, SK).

The purpose of this retrospective clinical study is to present the results achieved in microtechnique surgery performed during a 15-year-long period (1985–1999). By performing surgeries on 60 patients, 63 nerves were treated.

In 42 patients with injuries of peripheral nerves of upper extremities, 45 nerves were reconstructed by autografts. 14 patients were subjected to reconstructive surgery on peripheral nerves of lower extremities. In 4 patients we reconstructed the facial nerve by means of autograft. The analysis of surgical effects has been made in dependence on indicators as follows: period elapsed from injury to surgery, age of patient, nature of injury, length of autograft, location of injury, kind of nerve inflicted.

When assessing the results of reconstructive surgery of peripheral nerves of lower and upper extremities we observed a big difference on the behalf of upper extremities. High efficiency can be seen in tibial nerve surgeries of lower extremities. In general we achieved good results in facial nerve reconstructions.

The crucial factor that has an impact on the result of surgery is that of the time which elapsed from injury to reconstructive surgery. The factor is especially marked in younger patients.

P487.
OUR EXPERIENCES WITH SURGICAL TREATMENT OF INJURIES OF NERVS ISCHIADICUS
Viktor Matejčik (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician LDéver Faculty Hospital, Bratislava, SK).

This report presents the results of 44 surgical interventions performed in 44 patients during the period of 15 years, from 1983 to 1999. The report presents the basic lines of surgical treatment performed on a total number of 50 peripheral nerves of lower extremities—nerves ischias and its rami.

In the whole group of 44 patients, external neurolysis was performed in 23 individuals on 26 nerves. Remaining 21 patients were treated by reconstruction surgery that included 24 injured nerves. In this subgroup, suture of peripheral nerve was performed in 8 treatments on 9 nerves and neural graft was performed in 13 treatments of 15 nerves in cases of complete and persisting neurological deficit and in the absence of action potentials as revealed by EMG. Complete or severe motonic deficit and the absence of spontaneous recovery during the period of several months were the indications for the treatment. The analysis of the efficiency of surgical treatment was performed with respect to following parameters: period between the injury and operation, patient age, character of the injury, type of injured nerve, and type of surgical intervention.

The best results were obtained for external neurolysis which was applied in traumatic lesions of the least severity. The effective degree of recovery M3 was observed in 21 patients (91.3%). With respect to reconstruction surgery, more favourable results were obtained for treatments involving suture (in 6 patients, 76%) than for nerve grafts used for the treatment of the most severe injuries associated with a loss of nerve tissue. In the latter cases, improvement was observed after a delay and the extent of recovery did not always meet the expectations. The effective degree of recovery was observed in 4 patients (30.8%). Good and excellent results were typical for a, bifidals and they were not dependent of the type of surgical intervention, character and location of the injury, period from the injury or patient age.

Our results demonstrate that late and inappropriate treatment of injured peripheral nerves have severe and disturbing consequences for the patient. If a complete treatment of the injured nerve is not possible by the first contact physician, it should be performed in the shortest possible time by the specialist trained for microsurgical techniques of the treatment of peripheral nerves.

P488.
EXERCISE INCREASES THE REGENERATIVE POTENTIAL OF SENSORY NEURONS VIA NEUROTROPHINS
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Physical activity promotes functional recovery after spinal cord injury, but molecular mechanisms involved are still puzzling. We investigate mechanisms by which voluntary exercise helps the regenerative capacity of neurons in adult rats. Animals were exposed to exercise for 3 and 7 days and changes in mRNA levels in the DRG cells were analyzed in vitro by quantitative real-time PCR. DRG neurons from exercised rats showed increased axonal length, associated to increased levels of BDNF and NT-3 mRNAs, and a 2 fold increase in synapsin 1 mRNA (downstream effector of BDNF and NT-3 actions). In addition, GAP-43 mRNA levels showed a consistent increase in exercised rats. All changes were dependent on neurotrophins as injection of K252a in vivo blocked plastic changes. Lastly, to evaluate the effects of exercise on axonal regeneration, rats were exercise conditioned for 7 days and then sciatic nerve crush injury was performed. Two days later, the sciatic nerve was transected 0.5 cm distal to the crush site and regenerating axons were labeled by fluorgold (a retrograde axonal marker). The exercise-conditioned animals showed approximately 2 fold more fluorgold-labeled DRG cell bodies than sedentary animals, indicating an increased capacity for axonal regeneration in vivo. These findings indicate that physiological forms of activity, such as exercise, cause changes in expression of neurotrophins and this enhances the ability of these neurons to compensate for insults. (Supported by NS18978, NS39522, UCLA Brain Injury Research Center, and Roman Reed Awards).
P489. SURGICAL TREATMENT OF LESIONS OF NERVUS FACIALIS
Víktor Matejčík, M.D. (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician L. D'Éver Faculty Hospital, Bratislava, SK).

In this retrospective study, we present the results of 40 surgeries of 40 patients that within the period of 15 years, i.e. from 1985 to 1999 were provided the treatment of 40 lesions of n. facialis, historically treated as problematic in terms of successful healing. The work provides the fundamental lines of their surgical treatment.

From the total number of 40, external neurolysis was performed to 20 patients. The remaining 20 patients were provided with reconstruction surgeries of the injured nerves, while 8 surgeries were done by suture of peripheral nerve and 12 surgeries were performed by nerve graft, in cases of complete and persisting neurological deficit and absence of action potential at EMG. The mechanism of lesion included the damages of nerve from elongation, with or without fractures, "sharp" or "blunt" lesions, lesions of shooting, compressions and iatrogenic injuries. If the spontaneous adjustment did not occur within the period of 2-6 months after the lesion, the patients underwent surgery and with 27 of 40 an effective adjustment was achieved preventing the sagging of the foot trace and with 25 of 40 protective sensitivity appeared. We performed the analysis of the effectiveness of the surgical treatment depending on the following parameters: period of surgery from the lesion, patient's age, nature of lesion, degree of lesion, type of surgery intervention.

After neurolysis with 18 of 20 patients (90%) we achieved effective degree of adjustment in spite of heavy pre-surgical motor deficit. With 8 patients an "end to end" suture was performed and with 6 (75%) the degree of adjustment was 3 or higher. 12 patients repeated reconstruction surgeries with the help of nerve grafts, the length of grafts varied from 4 to 20 cm. The grafts were shorter than 5 cm with 2 patients, 1 with cut lesion and 1 patient with iatrogenic lesion. With both patients the function was adjusted to the degree M4. With 1 of 4 patients (20%) with the graft of 6 to 12 cm and with none of 6 with the grafts from 13 to 20 cm the adjustment of the degree 3 or higher was not achieved. In this case, however, we noticed partial adjustment of trophic and tonus, however at the absence of motor adjustment.

Similarly as with other nerve injuries, the perfect pre-surgery examination and timely surgery are needed for achieving optimum results. The excellent results of proximal injuries of n. facialis in comparison with more distant in the area of knee are worth noting.

P490. SURGICAL TREATMENT OF LESIONS OF NERVUS FACIALIS
Víktor Matejčík. (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician L. D'Éver Faculty Hospital, Bratislava, SK).

The study presents the results of reconstruction surgery of lesions on n. facialis performed in our clinic in the time period 1998-2000. Four patients were treated by anastomosis of n. facialis with n. hypoglossus (HFA), 1 patient by anastomosis of n. facialis with n. accessorius (AFA) while nervus facialis in 3 patients was reconstructed by neural graft. In the last group, neural graft in one patient originated from nervus auricularis magnus and in two patients from nervus suralis. All operations were performed under the microscope; HFA and AFA anastomoses were sewed without tension at perineuromatosis. The technique of separation of facial nerves did not differ from the separation of peripheral nerves in extremities. During neural graft surgery, the grafts were loosely deposited between two nerve endings in such a way that the graft overlapped the nerve endings by 1-5 mm (depending on transplant length). Fascicles or groups of fascicles were connected by value 8.0 sewing material.

The results were objectivized by a VI grade Brudny modification of House-Brackman classifications. I introduced originally for scaling of the outcome of hypoglossal-facial anastomoses. In this study, this classification has been used for the objectivization of all reconstruction microsurgical interventions of n. facialis. The results of neural graft treatments were superior to the results of cross anastomoses HFA or AFA. Grade II was achieved by all patients treated by neural graft. There were no symptoms of global hemiatrophy or atrophy of m. sternocleidomastoideus and m. trapezius in this group that were observed in patients treated by cross anastomosis with n. hypoglossus or n. accessorius. Minute synkinesis in the region of labial angle and chin occurred in the excited emotional state or during a long-lasting extensive speech. Improved mimics was apparent here compared to the group treated by HFA and AFA. Reconstruction surgery by HFA and AFA resulted in all cases in grade III of the scale. Synkineses in the region of lower eyelid were manifest in patients treated by HFA and they were even more pronounced in patients with AFA anastomosis. Major dizzinesses were not observed in any of reported treatments.

Compared to AFA anastomosis, HFA anastomoses resulted in improved mimics and synkineses present here were finer. We prefer HFA anastomosis also because the discomfort caused by atrophy of m. trapezius and m. sternocleidomastoideus was apparently more perceived by patient treated by AFA than the negative effects of hemiatrophy reported by patients treated by HFA.

P491. SPINAL CORD INJURY EXPRESSION PROFILING: FEATURES OF NEURALN DAMAGE ARE ASSOCIATED WITH CELL CYCLE PROGRESSION AND DEPRESSION OF NEUROGENESIS
Simone Di Giovanni, Susan Knoblach, Eric Hoffman, Alan I. Faden, (Georgetown University, Children's Medical Center, Washington DC US).

Spinal cord injury is a major cause of disability, and it is known that much of the functional deficit results from delayed cellular consequences of injury repair mechanisms. To define the temporal series of gene expression changes following a spinal cord injury, rats were subjected to a controlled impact injury at T8-T9 by weight drop (10gm. 17.5 mm). Rats were sacrificed at four time points (30 min, 4h, 24h and 7 days), with 4 to 6 individual rats spinal cords expression profiled at each time point (total 26 profiles) using the U34A Affymetrix genechip containing 8700 probe sets. Genes showing 40% or more "present" calls in 26 profiles by Affymetrix analysis were retained for further analysis (data scrubbing), and p values (>0.05) and fold changes (2-fold threshold) correlated, with temporal and functional clustering. Specific RNAs were verified by QMF-RT-PCR using fluorescent primers, protein level quantified by western blot and localized by immunocytochemistry. We found induction of DNA damage-inducible genes and genes favoring cell cycle progression at 4 and 24 hours after injury; at these times there was also depression of genes associated with neurogenesis. Changes in mRNA expression were associated with changes in respective proteins as shown by western blots and immunocytochemistry. Cell cycle and DNA damage related proteins were frequently localized in neurons showing signs of DNA damage and apoptotic features. We conclude that gene associated with DNA damage and progression of cell cycle were significantly upregulated in response to a low-modern level of spinal cord injury and may contribute to subsequent apoptosis. Such changes were temporally associated with suppression of genes implicated in neurogenesis.

P492. UPREGULATION OF EPHRIN LIGANDS AFTER SPINAL CORD INJURY
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Spinal cord injury (SCI) generates a cascade of events that lead to inhibition of axonal regeneration. The molecular and biochemical changes represent the presence of repulsive factors that may restrict or block neurite outgrowth after SCI. One class of factors with inhibitory activity for axonal outgrowth are the Eph receptors and their ligands, the ephrins. These molecules are involved in cell migration, axonal pathfinding, target recognition and synapse formation, by repulsive interactions after receptor-ligand binding. However, the pattern of expression and role of ephrins in adult injured spinal cord is unknown. Adult Sprague Dawley rats received a contusion to the thoracic vertebra T10 with the NYU Impactor device. Standardized semi-quantitative RT-PCR analysis of some ephrins genes were performed from injured and control spinal cord tissues. Our results indicate that the expression levels increased 7 days after SCI, that returned to basal level by day 14 in the case of ephrinB2 but remains elevated until day 28 for ephrinA1. Immunohistochemistry studies confirmed the upregulation data obtained at the mRNA level after SCI. The immunoreactivity was localized in the ventral region of the white matter. Ongoing studies will determine the phenotype of cells expressing the ligands and role of these molecules after spinal cord injury. The previous data suggest that ephrins may contribute in the non-permissive environment for axonal regeneration after spinal cord injury. This work was supported by NIH/NIHDS (NS 39405), RCMI (G12RR03051), PR-EPSCOR (EPS-9874782), KSCHIR Trust (8-29), Norton Healthcare, and MBRS SCORE (2 S06 6M8224).
P493.
METHYL PREDNISOLONE INHIBITS INTERLEUKIN-1 AND INTERLEUKIN-6 PRODUCTION IN THE SPINAL CORD FOLLOWING COMPRESSION INJURY IN THE RAT
Eugene S. Fu, M.D.*, Samuel Saporito, Ph.D., Jong J Kim, Ph.D. (University of South Florida, Tampa, FL, USA)

Introduction: Cytokine proteins are present in normal spinal cord tissue and their production is increased after tissue injury. We sought to evaluate the effect of methylprednisolone (MP) on the production of IL-1 and IL-6 protein in spinal cord tissue, following T7 compression spinal cord injury (SCI).

Methods: Sprague-Dawley rats were treated randomly with saline IM or methylprednisolone (30 mg/kg) IM. No lesions were produced in animals in the control groups (saline control, MP control). SCI was induced by extradural placement of a 55-g aneurysm clip at T7 for one minute. SCI animals were treated with saline or MP immediately after clip removal. Spinal cord sections at T7 were processed by enzyme-linked immunosorbent assays (ELISA) to measure IL-1 and IL-6 protein, expressed as mean ± sd.

Results: In the Saline and MP Control animals, the IL-1 levels were 37.72 ± 7.25 pg/mcg and 43.01 ± 14.36 pg/mcg, respectively. An increase in IL-1 was seen in the SCI + Saline animals (67.47 ± 19.99 pg/mcg, p < 0.02). Compared to SCI + Saline animals, those in the SCI + MP group had a decrease in IL-1 (32.65 ± 6.64 pg/mcg, p < 0.01). In the SCI Control and MP Control animals, the IL-6 levels were 20.01 ± 3.91 pg/mcg and 19.25 ± 4.10 pg/mcg, respectively. An increase in IL-6 was seen in the SCI + Saline animals (29.65 ± 5.46 pg/mcg, p < 0.01). Compared to SCI + Saline animals, animals in the SCI + MP group had a decrease in IL-6 (17.73 ± 5.93 pg/mcg, p < 0.01).

Conclusions: Spinal cord compression produced an increase in IL-1 and IL-6, which was inhibited by MP, confirming the anti-inflammatory role of MP. Further studies are warranted to discover if inhibiting cytokine production affects therapeutic benefit or renders spinal cord tissue more compatible to host neural cell transplantation.

P494.
VASOcular INDUCTION OF HEME OXYGENASE-1 AS A NOVEL APPROACH FOR STABILIZING BARRIER FUNCTION AFTER SPINAL CORD INJURY

Heme oxygenase (HO) catalyzes the breakdown of heme to carbon monoxide, iron and biliverdin. There is recent evidence that HO-1, the inducible HO, can alter vascular function as evidenced by its ability to attenuate inflammation, vasoconstriction and vascular proliferation. In this study we have developed a method for the selective induction of HO-1 in blood vessels and have used this approach to begin to identify the role of HO-1 in abnormal vascular induction of HO-1 stabilized the barrier after spinal cord injury. We found that HO-1 was specifically induced in spinal cord vasculature by systemic administration of stabilized hemin, as evidenced by both western immunoblots and immunocytochemistry. We next examined the extent to which induction of HO-1 prior to spinal cord injury influenced barrier permeability. 24 hours after systemic administration of either vehicle or stabilized hemin, adult, male mice were subjected to a moderate level of contusion injury. Luciferase, a marker of barrier permeability, was given intravenously 30 min prior to euthanized at 24 hours post injury. Luciferase was quantified in tissue prepared from the lesioned epicenter. There was a significant attenuation of barrier permeability to luciferase in the stabilized hemin as compared to the vehicle treated groups. These findings offer a novel role for HO-1 in limiting early vascular dysfunction after spinal cord injury.

Supported by NS39278 and NS 39647.

P495.
RECONSTRUCTION OF ANTERIOR COLUMN DEFICIT FOLLOWING CERVICAL SPINE INJURY USING THE HAMPS TITANIUM MASH CAGE
Never Esaiasnejad. (Neurosurgical Department of the Surgical Clinic KBC, Rijeka, HR)

Objectives: The optimal treatment of cervical fractures is still controversial. Although several techniques have been used to treat cervical spine injuries, the anterior cervical plate system combined with an artificial vertebral body implant is the surgical method to provide biomechanical stability in injured patients following partial or total corpectomy.

Methods: Clinical data, X-ray, CT and MRI scan records were reviewed retrospectively in 14 injured that were treated by vertebrectomy combined with titanium mesh cages and plate implants. These 11 males and 3 females of the mean age of 45 years (range 16-72) sustained cervical spine fractures in automobile accidents, by falling from one level to another and jumping in shallow sea.

The spine lesions were classified according to Maglery—5 patients had type A, 5 type B lesion. A preoperative and 12 months postoperative Frankel score and functional status were assessed. Preoperatively 6 patients had grade A, 2 grade B, 3 grade C, 1 grade D and 2 patients were neurologically intact.

Results: There was no neurological deterioration. Bone fragments were removed completely in all cases. Good spine realignment was achieved in 13 cases and in 1 patient spine deformation occurred—a kyphosis with an angle of 9 degrees.

Neurological status improved for one grade in one patient, two or more in 2 patients and remained unchanged in 11 patients.

Conclusion: Anterolateral approach can provide significant elimination of bone fragments, disc et haematoma. Good spine alignment and solid fusions are achieved by instrumentation—plating and Harms cages.

P496.
EFFECT OF 75NTR DELETION ON WHITE MATTER PROTECTION AFTER EXPERIMENTAL SPINAL CORD INJURY
W. Bradley Jacobs* and Michael G. Fehlings. (Toronto Western Research Institute, University of Toronto, Toronto, Ontario, Canada)

Background: Despite advances in medical and surgical care, spinal cord injury (SCI) remains a devastating event. Novel therapies are needed. Our laboratory has recently shown that members of the tumor necrosis factor receptor family death receptors (specifically, Fas and p75NTR) are temporally and spatially associated with post-SCI oligodendroglial apoptosis. While p75NTR is associated with post-SCI oligodendroglial apoptosis, little is presently known about the specific role of p75NTR in secondary injury. As such, we have started an investigation of the effect of p75NTR deletion on axonal integrity and white matter preservation after SCI.

Methods: To further delineate the role of p75NTR in post-SCI apoptosis we subjected mice null for p75NTR to extradural clip compression SCI at T6. One centimeter of spinal cord (centered at the injury epicenter) was extracted at 0 6 (uninjured), 1d, 3d, 7d, and 14d after injury (n = 3/time point), homogenized with protease inhibitors and used for western immunoblots. Immunoblots were probed with antisera to NF200, an axonal cytoskeletal protein whose degradation inversely correlates with axonal integrity and functional outcome after SCI.

Results: Preliminary results do not show a significant difference in the pattern of NF200 degradation between p75NTR null animals and strain-matched wild-type controls. However, our early results suggest that downregulation of p75NTR activity may not inhibit axonal degradation after SCI. This result needs to be further clarified by immunohistochemical analysis of axonal degeneration after SCI in p75NTR null animals. Further detailed investigation of the role of p75NTR in delayed cell death after SCI is warranted.

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P497.
ESTROUS CYCLE MEDIATED EFFECTS ON SPINAL CORD INJURY
Andrew S. Luciano, ( Rutgers University, River Edge, NJ US).

A recent study suggested that female rats have smaller spinal cord lesion volumes than males after a standardized weight-drop contusion injury. We hypothesize that these differences may be due to the neuroprotective effects of estrus that other scientists have reported. To test this hypothesis, we compared spinal cord lesion volumes in female rats during proestrus, estrus, and diestrus; comparisons were also made with male rats. Estrous levels should be highest during proestrus, intermediate during estrus, and lowest in diestrus and in male rats. If estrous accounts for the difference between male and female rats, we expect to see the smallest lesion volumes in female rats during proestrus and these lesion volumes should be significantly less than females in diestrus and male rats. We studied a total of 26 female (8 proestrus, 9 estrus, 9 diestrus) and 8 male Long-Evans hooded rats. The rats were all injured with a 10 gram weight dropped 25 mm onto the T13 spinal cord and euthanized at 6 hours after injury. The rats were 77 ± 3 days of age. Lesion volumes were calculated from the potassium concentrations at and around the injury site. In addition, we collected messenger RNA from the tissues for later analyses with large-scale microarray to identify genes that may be responsible for any neuroprotective effects. The results indicate that female rats undergoing proestrus have significantly smaller spinal cord lesion volumes, compared with rats undergoing diestrus and male rats. We are assessing gene expression of the rats at each of estrous stages to identify potential gene expression changes that may explain the neuroprotective effects.

Supported by SCI Project, Rutgers University Summer Undergraduate Research Fellowship, and Douglass College SUPER Project.

P498.
GLUTAMINE ADMINISTRATION HELPS TO MAINTAIN BASAL GLUTATHIONE CONCENTRATIONS IN RAT SPINAL CORDS FOLLOWING ACUTE INJURY.
S. T. Rigley*, H. Kameneic and BHI Juurlink. (University of Saskatchewan, Dept. Anatomy and Cell Biology, Saskatoon, SK Canada).

Glutathione (GSH) has been previously demonstrated to decrease oxidative stress resulting from spinal cord injury. Compounds designed to increase GSH production such as L-2-oxothiazolidine-4-carboxylate (OTC) have been shown to be neuroprotective following spinal cord injury. Glutamine supplementation of parental diets in rats has also demonstrated increased plasma GSH levels. This study was designed to examine the effect of administration of glutamine on GSH concentrations in spinal cord tissue. Wistar rats underwent surgeries in which a laminectomy was performed and a single aneurysm clamp used to pinch the spinal cord at the level of T6. In sham animals only a laminectomy was performed. Thirty minutes after surgical intervention 5mmol/kg glutamine or saline was administered into the peritoneal cavity, subsequently every 12 hours. Animals were sacrificed 24 hours after surgical intervention and C3, T3, T5, T6, T7, T9 and L4 spinal cord segments were collected. GSH was measured by HPLC. In the T5, T6 and T7 segments of spinal injured animals administered glutamine, GSH concentrations were similar to sham values and were significantly higher than those of the saline treated animals. GSH concentrations were not increased in glutamine treated sham animals over saline treated sham animals.

This was expected, as glutamine is known to be rate limiting only when GSH concentrations are decreased and in the presence of cysteine. These results suggest that glutamine is an effective agent for maintaining basal GSH concentrations and therefore decreasing oxidative stress after spinal cord trauma. This research is funded by the Christopher Reeves Foundation and S. T. Rigley holds a College of Medicine Scholarship.

P499.
NOVEL ROLE OF PROSTACYCLIN IN COMPRESSION-INDUCED SPINAL CORD INJURY IN RATS.
Yaji Taoka*, Kenji Okajima, Sinueke Katoch, Naosuke Yasui. (Dep. Orthopedics, The University of Tokushima, Tokushima, Tokushima, JA).

Although spinal cord injury (SCI) is a serious condition which produces lifelong disabilities, only limited therapeutic measures are currently available for its treatment. We previously reported that prostacyclin (PGI2) reduced the motor disturbances subjected to SCI in rats, probably by inhibiting the leukocyte accumulations at injury site and PGI2 could be a new therapeutic agent for patients with SCI (Taoka et al., J. Neurosurg. 1997). However, the precise mechanisms of PGI2 against SCI are not well known. The present study was conducted to clarify these mechanisms by using the compression-induced SCI model in rats. 6-keto-PGF1alpha level, a stable PGI2 metabolite, in injured spinal cord tissue significantly increased, peaking at 2 hr after the induction of SCI. Subcutaneous administration of indomethacin (IM), a non-selective cyclooxygenase inhibitor (5 mg/kg), completely inhibited this increase but significantly exacerbated the motor disturbances following SCI, and enhanced leukocyte accumulation as determined by myeloperoxidase (MPO) activity. TNF-alpha production which is a potent activator of leukocytes, and mRNA TNF-alpha expression at injury site in rats subjected to spinal cord trauma. Compression-induced motor disturbances were significantly reduced in animals given iloprost, a stable analog of PGI2 and in those with nitrogen mustard-induced leukocytopenia. Iloprost and leukocytopenia also significantly inhibited the IM-induced exacerbations of SCI. NS-398, a selective inhibitor of cyclooxygenase-2, did not attenuate the motor disturbances, and the increases in MPO activity, TNF-alpha production, and mRNA TNF-alpha expression induced by trauma. These observations indicate that the increase in trauma-induced PGI2 in spinal cord tissue, mainly mediated by cyclooxygenase-1, appears important in preventing the motor disturbances following SCI by inhibiting leukocytes activation.

P500.
LONGTERM RESULTS AFTER 36 MONTHS FOR CHRONIC BI-LATERAL NEUROMODULATION
Braun PM, Seif C., van der Horst C, Martinez Portilló F.J., Bannowsky A, Juennenmann KP. (University Hospital Kiel, 24105 Kiel, DE).

INTRODUCTION AND OBJECTIVES: Sacral root neuromodulation can be a beneficial treatment option in patients suffering from therapy-resistant detrusor instability or detrusor hypotonicity.

The implantable neuromodulation system as described by Tanagho and Schmidt enables unilateral sacral nerve stimulation. The electrode is inserted unilaterally into the sacral canal via the sacral foramen (S3). Reports have been made on sacral neuromodulation failures of up to 50% in patients undergoing this procedure.

We preferred bilateral electrode implantation and tailored laminectomy in order to achieve better effectiveness of the chronic sacral neuromodulation.

MATERIAL AND METHODS: After assessment of the beneficial effect by means of PNE test, 32 patients (18 with detrusor instability, 14 with hypocontractile detrusor) underwent tailored laminectomy for bilateral electrode placement. Minimally invasive laminectomy was performed. The electrodes were bilaterally positioned. Laminectomy allows optimum electrode placement and fixation.

RESULTS: In the patients with detrusor instability the incontinence episodes were reduced from 8.6 to 0.9 per day and the bladder capacity improved from 270 to 375 ml. In patients with hypocontractile detrusor, the residual initial urine level of 340 ml (170 to 480) dropped to 54 ml (40 to 66). Maximum detrusor pressure during miccition rose from initially 12 cmH2O (7 to 15) to 36 cmH2O (29 to 48). The average follow-up period was 36 months. There was no sign of deterioration in the effect of modulation in any of the patients.

CONCLUSIONS: Chronic sacral bilateral neuromodulation results in optimal long-term results in either hyper- or hypocontractile detrusors.
P501. THE INTRAVENOUS ADMINISTRATION OF AUTOLOGOUS BONE MARROW CELLS INTO THE RAT DEMYELINATED MODEL. M. Inoue1, O. Honma2, S. Itoh1, S. Oka1, K. Houskin1, K. Hashi, and J. D. Koestler1, 2. (1. Dept. of Neurosurgery, Sapporo Med. Sch., Sapporo, Japan; and 2. Dept. of Neurology, Yale Univ. Sch. of Medicine, New Haven, CT and VA Med Ctr., West Haven, CT, USA). The regenerative potential of the bone marrow cells was studied in the demyelinating model rat. Although both the focal injection and the intravenous administration of bone marrow cells isolated from bone marrow repaired the demyelinated spinal cord in the adult rats, the ideal protocol in terms of the administration method and the cell number remains unknown. This study was to examine how we should transplant the bone marrow cells and how many cells are required to establish the sufficient numbers of repopulated cells in the demyelinated spinal cords. A focal demyelinated lesion was created in the dorsal columns of the rat spinal cord using X-irradiation and ethidium bromide injection (EB-X). A suspension of bone marrow cells (1 × 10^2 – 1 × 10^5) collected from the same rat was directly transplanted into the middle of the EB-X-induced lesion or was injected into a femoral vein 3 days after the EB injection. Lesions were histologically examined 5 weeks after transplantation. Light microscopic examination revealed the demyelinated axons were extensively repaired by autologous bone marrow cells. The number of the repaired axons following bone marrow transplantation were in proportion to those of the transplanted cells. In addition, the effectiveness of the focal injection is 100 times more than the intravenous administration. These results demonstrate that the intravenous administration of the autologous bone marrow cells may be a better strategy for the injured CNS.

P502. TRANSPANTATION OF SCHWANN CELLS (WITH OR WITHOUT OLFATORY NEUROPATHY GLIA) AFTER SPINAL CORD INJURY (SCI): CAN PRETREATMENT WITH THE NEUROPROTECTIVE STRATEGY OF CO-ADMINISTERED MethylPREDnisolone and Interleukin-10 ENHANCE RECOVERY? Pearson, D.D.*, Marcello, A.E., Owings, M., Bodea, J.R., Wood, P.M. and Bunger, M.B.* (University of Miami, The Miami Project, Miami, Florida US). Methylprednisolone (MP) and interleukin-10 (IL-10) have been demonstrated to be protective when given acutely after spinal cord injury (SCI) and recent work from our laboratory has shown that a combination of these agents offers additive neuroprotection. The current study examined if acute neuroprotection with MP and IL-10 could increase the efficacy of Schwann cell (SC) grafts or combination grafts of SC plus olfactory ephrathine glia (OEG) transplanted 1 wk after moderate contusive injury. Efficacy of each strategy was determined by tracing of axonal regeneration, immunohistochemical analysis and behavioral testing (BBS score) 8 wk post-transplantation. Combination SC/OEG grafts displayed reduced astrogliosis (GFAP) and chondroitin sulfate proteoglycan expression (CSPG) in both the graft and host tissue compared to SC grafts. Furthermore, the presence of significant numbers of Rec21-positive cells and 5-HT fibers within and rostral to the SC/OEG grafts, but not the SC grafts, indicated that these grafts were better vascularized and supported the regrowth of brainstem neurons. Behaviorally, SC/OEG transplanted animals were significantly better than those transplanted with only SCs. The acute administration of MP and IL-10 after SCI, however, failed to enhance behavioral recovery in either transplantation paradigm and no benefit was observed in any of the histological parameters examined. The cell transplantation procedure could induce a second inflammatory response associated with the injection surgery or immune intolerance of the transplanted cells that damages any tissue that is spared from the acute neuroprotective strategy. Future combination strategies may require a less invasive transplantation procedure. A new administration of the protective compound during transplantation. (Supported by the NINDS09923, POINS3665 and The Miami Project)

P503. DIFFERENTIAL RESPONSES OF PAIN AND SENSORY ABNORMALITIES TO L.V. BARBITURATES AND LIDOCAINE IN SPINAL CORD INJURED PATIENTS. B. Bharatwaj*, A. Malis2, and A. Kraszewski2. (1. Pain and 2. Spinal Cord Injury Program, Krembil Neuroscience Center, Toronto Western Hospital, University of Toronto, Toronto, ON, CA). The incidence of pain reported after spinal cord injury (SCI) varies between 73-94%. In some studies chronic pain is more disabling than paralysis, bowel or bladder dysfunction. SCI pain may arise from multiple and possibly co-existent pathophysiological mechanisms of peripheral and/or central origin. SCI pain is difficult to treat and has been reported to respond fairly poorly to opioids, but much better to IV barbiturates. Cutaneous hyperesthesia responsive to barbiturates has been shown to be of central origin in other neuropathic pain patients. Spontaneous pain and sensory abnormality alterations were investigated via IV normal saline-controlled infusions of sodium amobarbital (SA), a medium action barbiturate, and lidocaine (L), a local anesthetic-type of drug, in 6 SCI patients (5 with thoracic and one with cervical spine lesions, 4 males, 2 females, mean age 35 yrs, mean pain duration 4.9 yrs). Spontaneous pain was reduced by 74% on average after SA infusion and 45% after L infusion, while sensory abnormalities were modified in 4/6 patients with SA and 1/6 patient with L infusions. The sensory abnormalities modified under the drugs consisted primarily of hyperesthesia to touch and pinprick, while dense hypesthesia (at and below the level of lesion) failed to change. The analgetic effect of IV SA is far superior to that obtained with IV L in SCI patients. The non-competitive NMDA receptor antagonistic action of SA may be responsible for the substantial alteration of cutaneous (centrally mediated) hyperesthesia.

P504. FUNCTIONAL RECOVERY AFTER MODERATE CONTUSIVE INJURY IN THE MOUSE: ROLE OF DHEA IN IMPROVING BLADDER FUNCTION. Pierre-Vyard Mure, Denise Inman, Nathalie A. Compagnone*. (Dept. of Neurological Surgery, University of California San Francisco, San Francisco, CA 94143). We have previously demonstrated that dehydroepiandrosterone (DHEA) promotes differentiation of motor neurons and facilitates axonal growth in the developing CNS. Most recently, we have shown that DHEA promotes functional recovery after spinal cord injury, as evidenced by improved locomotor performance, gait pattern and reduction of foot faults on an inclined ladder. The present study extends these previous observations by focusing on the role of DHEA in bladder function after spinal cord injury. Whereas DHEA treated animals developed a functional bladder by 10 days post injury, bladders of vehicle treated animals remained dysynergic for an extended period of time. DHEA-treated animals exhibited a significantly smaller bladder, coincident with a significant reduction in urine volume, as compared to vehicle-treated animals. Interruption of controlled voiding of the bladder in response to atraumatic neuropathy has been shown to induce distension of the bladder wall that is correlated to a change in the extracellular matrix (ECM) composition of the layer muscularis of the detrusor. We found that spinal cord injury also increases the ratio of collagen type III to collagen type I in the layer muscularis. Moreover, this ratio in the spinal cord injured, DHEA treated group was similar to that of the intact spinal animal. We next examined the relationship between previous measures of functional recovery and controlled voiding using cluster analysis. We found that early recovery of controlled voiding is predictive of motor recovery. Together, these findings emphasize the unique and beneficial role that DHEA plays in restoration of function after spinal cord injury. This work was supported by the Charitable Columbia foundation, the Roman Reed Program and NIH grant NS41998.
TARGETING THE RHO SIGNALING PATHWAY TO PROMOTE REPAIR AFTER SPINAL CORD INJURY
Pauline Derham*, Catherine Dubreuil, Matthew Winton, Benjamin Eliezac and Lisa McKerracher. (Département de Pathologie et Biologie Cellulaire, Université de Montréal, Montréal, Québec, Canada).

The activation state of Rho is an important determinant of axon growth and regeneration in neurons. We have investigated the use of antagonists to Rho or Rho associated kinase (ROK) to overcome growth inhibition and promote axon regeneration after spinal cord injury (SCI). In primary culture, inactivation of either Rho with C3 or ROK with Y-27632 promoted neurite growth on inhibitory myelin or chondroitin-sulfate proteoglycan substrates. To examine how the environment influences Rho activation states, we have isolated active Rho in tissue homogenates by pull down assay. Inhibitory substrates activated Rho when cells were plated in culture. In vivo, SCI activated Rho in homogenates of spinal cord. The increased Rho activation was blocked by treatment with the Rho antagonist C3-05. To examine the use of Rho or ROK antagonists after SCI, a lesion was made in mouse spinal cord at T7–T8, and the antagonists were applied in a fibrin gel. Three weeks to three months post lesion, the corticospinal tract (CST) of injured mice was immunologically labelled with WGA-HRP, and axon regeneration was detected in longitudinal cresyl violet sections of the spinal cord. Animals treated with either Rho or ROK antagonists showed long distance regeneration. Untreated animals showed retraction of CST axons from the lesion site. Functional recovery was scored by the BBB open field test. Treated animals showed a remarkable 24 hr recovery and continued to recover over next month with BBB scores significantly higher than untreated mice. Examination of the histology at 24 hours showed fewer Tunel-labeled cells after Rho inactivation. These results demonstrate that Rho is abnormally activated after spinal cord injury, and that inactivation of Rho is both protective and promotes axon regeneration. Supported by the Canadian Institutes of Health Research.

DIFFERENTIAL TEMPORAL EXPRESSION OF MATRIX METALLOPROTEINASES DURING WOUND HEALING IN A MURINE MODEL OF SPINAL CORD INJURY

We have previously shown that Matrix Metalloproteinase-9 (MMP-9) promotes the infiltration of neutrophils after spinal cord injury and that attenuation of neutrophil infiltration by blockade of MMPs promotes locomotor recovery and preservation of white matter. MMP-9 and other MMPs are integral to angiogenesis and thus may also be critical during wound healing in the injured spinal cord. We therefore examined the extent to which MMP-9 and MMP-2 are altered in the acutely injured cord (1 day post injury), during revascularization (7–14 days post injury), and after re-establishment of the blood-spinal cord barrier (26 days post injury). Gelatinolytic activity, defined by in situ zymography, was restricted to meninges and blood vessels in shams and identified in glia and macrophages at all time points after spinal cord injury. In addition, unusually large diameter blood vessels expressed gelatinase activity at 7 to 28 days post-injury. The active form of MMP-9, identified by gelatin zymography, was most prominent at 1 day and returned to control values by 14 days post injury. In contrast, the active form of MMP-2 activity was not identified until 7 days post injury; activity declined thereafter, but remained elevated over sham controls for the duration of the study. These findings suggest that although MMP-9 and -2 exhibit overlapping expression during revascularization, the former is primarily associated with acute injury responses and the latter with wound healing. Supported by NS39278 and NS39847.

THE EFFECT OF THE L-TYPE CALCIUM CHANNEL AGONIST, BAYK8644, ON REGENERATION OF CULTURED RAT SYMPATHETIC NEURONS
Iris Kulhati, B.Sc., M.Sc.*, and Charles H. Tutor, M.D., Ph.D., FRSC. (Toronto Western Research Institute, Toronto, Ontario CA).

Purpose: To investigate the effect of the L-type Ca2+ channel agonist, BayK8644, on the regeneration of transected rat sympathetic neurites in an in vitro model of spinal cord injury.

Hypothesis: BayK8644 enhances regeneration of cultured rat sympathetic neurites. Methods: Sympathetic neurites were harvested from the superior cervical ganglia of neonate rats. Injury of 14-day-old neurites was made with a microstriped rubber imperator. Various concentrations of BayK8644 were administered 20 min. pre-neuritotomy (1, 2.5, 5, 15, and 30 micromolar). The 30 micromolar solution was also administered 5 min. post-neuritotomy. Regeneration was assessed by measuring neurite density and length at 2 and 24 hr. post-neuritotomy using BIOQUANT imaging software.

Results: The 1 micromolar solution of BayK8644 enhanced neurite length at 2 and 24 hr. post-neuritotomy (p = 0.026 at 2 hr; p < 0.001 at 24 hr). Conversely, the 2.5 micromolar solution reduced neurite density and length at 24 hr. post-neuritotomy (p = 0.009 for density; p < 0.001 for length). The 5 micromolar solution reduced neurite density and length at 2 and 24 hr. post-neuritotomy (p = 0.005 for density at 2 hr; p < 0.001 for density at 24 hr, and length at 2 and 24 hr). The 15 micromolar solution reduced neurite length at 2 and 24 hr. post-neuritotomy (p = 0.002). Similarly, the 30 micromolar solution reduced neurite density and length at 2 and 24 hr. post-neuritotomy (p < 0.001). The 30 micromolar solution administered 5 min. post-neuritotomy reduced neurite density and length at 2 and 24 hr. post-neuritotomy (p < 0.001). The 30 micromolar solution administered 5 min. post-neuritotomy reduced neurite density and length at 2 and 24 hr. post-neuritotomy (p = 0.005 for density at 2 hr; p < 0.001 for density at 24 hr, and length at 2 and 24 hr). (0.005 for density at 2 hr; p < 0.001 for density at 24 hr, and length at 2 and 24 hr).

Conclusions: The differential effect of BayK8644 on neurite regeneration suggests that increasing Ca2+ influx through L-type Ca2+ channels enhances regeneration, but excessive influx inhibits regeneration. Further experiments are required to establish the effects of L-type agonists on neurite regeneration.

IDENTIFICATION OF PROLIFERATING EPENDYMAL CELLS IN THE RAT SPINAL CORD FOLLOWING TRAUMA

In lower vertebrates such as amphibians and lizards, the ependyma of the spinal cord plays a significant role in neuronal regeneration. After spinal cord injury (SCI), the ependymal cells rapidly proliferate, migrate, and differentiate to regenerate the cord. In adult mammals, limited proliferative activity has been reported in the normal ependymal canal. However, after SCI, ependymal cells become activated with specific characteristics of precursor cells, as we have previously shown by an increased bromodeoxyuridine (BrDU) labeling index and immunoreactivity to nestin, a marker for neural precursor cells. The purpose of this study is to specifically label the ependymal cells and compare their proliferative capacity following spinal cord trauma of varying severity. We have examined one minute clip compression injuries of mild (2.4g) and moderate (20g) severity at T8 level, and lateral stab wounds which do not disrupt the central canal. To label the ependymal layer along the neuraxis, a 0.2% (w/v) solution of DiI in dimethylsulfoxide was stereotactically injected into the lateral ventricle 24 hours prior to injury. We found that intraventricular injection of the lipophilic DiI specifically labeled the ependymal cells of the spinal canal, as shown by fluorescence and DiI fluorescence conversion with diaminobenzidine. Double-labeling with nestin demonstrated DiI labeled, nestin positive ependymal cells after spinal cord injury. We are currently investigating the proliferative capacity of ependymal cells following varying degrees of spinal cord trauma. Double-labeling for BrDU and DiI will be used to identify proliferating and migrating ependymal cells, and cell-type specific markers will be used to identify resulting progeny.
P509.
AXONAL PRESERVATION WITHIN DESCENDING VASOMOTOR PATHWAYS AND CARDIOVASCULAR CONTROL AFTER SPINAL CORD INJURY
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The association of cardiovascular abnormalities in individuals with traumatic spinal cord injury (SCI) and the severity of demyelination and axonal preservation was investigated. We focused on two areas of descending vasomotor pathways (DVP) in animals and humans which have been described in dorsolateral aspect of the lateral horn (Area I), and dorsal portion of the lateral funiculus (Area II). Data were analyzed using ANOVA and Student t-Test. Five individuals (1M, 3F; age 31 to 67 y; mean of 51.4 y) with well-documented cardiovascular abnormalities and neurological outcomes of SCI were analyzed. All subjects had cervical SCI (ASIA A 3, B 1, C 1). The mean survival in the post-injury period was 11.6 mos. (3.3 to 36 mos.). Severe hypotension immediately after SCI was observed in 3 individuals. Two of these subjects also developed autonomic dysreflexia. The histopathological findings from these individuals were compared to findings from 2 individuals with no cardiovascular abnormalities. There were no significant differences in the severity of demyelination (demyelinated areas: 24.7% vs. 9.3%; P = 0.2) between two groups. The number of axons within area I & II of DVP in individuals with cardiovascular dysfunctions was significant lower than subjects without cardiovascular abnormalities (P < 0.001). Moreover, area I in these individuals also had fewer axons compared to area II (P < 0.001). These data suggest that severity of destruction of DVP could contribute to cardiovascular abnormalities after SCI. (Supported: Christopher Reeve Paralysis Foundation; Heart & Stroke Foundation of Ontario).

P510.
ECG FINDINGS IN ACUTE SPINAL CORD INJURY IN HUMANS
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(Spinal Program & 1Dept. of Cardiology, Toronto Western Hospital, University of Toronto, Toronto, ON, CA).

Significant impairment of hemodynamic parameters occurs after acute spinal cord injury (SCI). However, the relationship between ECG changes the level and severity of injury is poorly understood. We conducted an analysis of ECG recordings of 17 consecutive patients (F 4, M 13; age 31-83 y.o.) in order to examine the incidence of abnormalities in heart rate and ECG in individuals with acute SCI. ECG data (atrial (APC) and ventricular (VPC) premature contractions; changes in T, ST, Q waves; ventricular tachycardia (VT), atrial fibrillation (AFIB), and supraventricular tachycardia (SVT)) were compared at day 1 and day 7 after SCI. Based on the level and severity of SCI subjects were divided into three groups: group 1 (n = 6)—high cervical injuries. ASIA A-B; group 2 (n = 5)—high cervical injuries. ASIA C-D; group 3 (n = 5)—low lumbar injuries. ASIA A-D. Abnormalities in heart rate and ECG were predominantly observed in high cervical injuries (group 1): APC—16% (day 1); ST changes—50% (day 1) and 25% (day 7); T wave changes—16% (day 1) and 50% (day 7); AFIB—25% (day 7). This represents a significant clinical concern during acute phase of SCI, the correlation between the severity of injury of descending cardiovascular pathways and changes in heart rate and ECG are under investigation. (Supported by Christopher Reeve Paralysis Foundation; Heart & Stroke Foundation of Ontario).

P511.
CERVICAL SPINAL INJURY WITH ESOPHAGEAL RUPTURE: REPORT OF TWO CASES
Ming-Yung Liu, Yung-Hsiao Chiang, Guan-Juh Chen. (Tri-Service General Hospital, Taipei, Taiwan).

Two consecutive cases of cervical spinal injury complicated with esophageal rupture were encountered recently. Two cases were all dislocated burst fracture, one at C5-6, another C6-7. The C5-6 patient was operated in Thailand and transferred to us 11 days later. The C6-7 patient was operated by us. The operation was anterior disectomy, open reduction, corpectomy, autogenous bone graft and plate instrumentation. The presenting symptoms and signs were dysphagia, delayed subcutaneous emphysema and wound infection. In a period of 10 years retrospective study of surgical treatment of cervical spinal injury in our hospital, we found 46 cases of fracture dislocation, 35 burst fracture, 28 dislocation and 12 compression fracture. No esophageal rupture was found until recently. Both were proved by endoscopy 15 and 17 days after surgery respectively. Esophagogram could not be performed due to dysphagia. Preoperative endoscopic examination sometimes cannot find the rupture in acute stage but should be done when patients have dysphagia or subcutaneous emphysema.

P512.
HP184 IS NEUROPROTECTIVE AND IMPROVES Locomotor FUNCTION AFTER MODERATE ACUTE SPINAL CRUSH INJURY IN RATS
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After central nervous system injury, neuronal ATP production is reduced, thereby compromising the maintenance of the sodium (Na+) gradient across the cell membrane. Thus, reduction of energy demand by down-modulation of voltage-gated Na+ channels has been tested as a rational strategy for neuroprotection. HP184 (Aventis) is a voltage-dependent blocker of potassium currents in PC12 cells and a use- and frequency-dependent blocker of sodium channels. To test whether HP 184 is neuroprotective in acute spinal cord injury, rat spinal cords were compressed to a moderate level (Gruner et al., Brain Res., 729:90-101, 1996). Within 15 minutes of crush (day 1), rats in HP 184 designated groups received ip injections or po gavage of 20, 10, 5 or vehicle. This administration was repeated on days 2 and 3. Methylprednisolone (MPS), on the other hand, was administered at 60 mg/kg ip at 15 minutes, and at 30 mg/kg ip at 2 hours, 4 hours, and 6 hours on day 1 after crush. This MPS dosing schedule has been described as optimal in the literature, and mirrors the dosing performed in humans. HP184 significantly improved locomotor function in open field walking task: hind limb placement and foot orientation testing as compared to vehicle control, as did the MPS treatment. Behaviour was evaluated on days 1, 2, 3, 7, 10, 14, 21 and 28. Most of the functional improvement occurred within the first 24 hours, consistent with a neuroprotective effect of HP184.
P513.
INFLUENCE OF CRANIOPLASTY ON CEREBRAL BLOOD FLOW AND CARDIAC FUNCTION
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Objective: Cranioplasty is usually performed for aesthetic, protective, and patient comfort reasons. However, there are many theories suggesting that an underlying physiological alteration may occur that may require the correction of the bone defect. The objective of this study is to prove that cranioplasty improve the cerebral blood flow by decrease the cardiac after-load. Methods: Twenty-two patients who had taken bone removal to prevent uncontrollable intracranial hypertension were included in this study. Arterial flow velocities were checked in MCA and ICA by TCD, and cardiac function was checked by echocardiogram. And the last ten patients. cerebral blood flow was measured perfusion CT. Results: The blood flow velocity of the lesion side was decreased from 48.8 ± 16.0 to 36.4 ± 14.2 cm/sec at the MCA and from 32.9 ± 8.9 to 28.1 ± 7.5 cm/sec at the ICA (P < 0.05). And the opposite side it was decreased from 56.7 ± 14.5 to 40.6 ± 14.2 cm/sec at the MCA and from 32.7 ± 7.6 to 26.7 ± 6.4 cm/sec (P < 0.05) at the ICA. On the cardiac function evaluation by measuring the stroke volume, was increased from 66.1 ± 18.6 to 73.4 ± 18.8 ml/Kg (P < 0.05). Cerebral blood flow evaluated by perfusion CT shows increased values, from 30.2 ± 9.3 to 45.4 ± 12.1 ml/100g/min. on lesion side and from 29.9 ± 6.8 to 44.7 ± 12.7 ml/100g/min. on opposite side (P < 0.05). But there was no correlation between the changes of blood flow velocity and the stroke volume changes (P > 0.05). Conclusion: We can conclude that there was no change at atmospheric pressure after cranioplasty, lessened the peripheral resistance and this can increase the cerebral blood flow and stroke volume without alteration of the systemic blood pressure. It appeared that skull bone defect be repaired as soon as possible, because cranioplasty has not only aesthetic or protective but also systemic therapeutic effect. (This study was supported by a grant of the Korea Health 21 R&D Project. Ministry of Health & Welfare. Republic of Korea.HMP-00-CN-01-0018).

P514.
VOLUMETRIC PROTON MR SPECTROSCOPY OF MILD TRAUMATIC BRAIN INJURY
Grant E. Gauger*, Vasanratn Govindraju, Andreas Ebel, Geoffrey T. Manley and Andrew A. Maudsley (Departments of Neurolurgy and Radiology, UCSF, San Francisco, MR Unit, SfVAMC, and SFB1HC).

Introduction: Proton MRS can assess biochemical changes of traumatic brain injury (TBI). Several single volume spectroscopy studies have evaluated metabolite levels at a few specific locations within the brain [1-2]. In this study, we used a volumetric proton MR spectroscopic imaging (MRSI) method to observe distributions of N-acetyl aspartate (NAA), choline (Cho), and creatine (Cr) providing biochemical information from the entire brain. Methods Twelve subjects (6 patients with Glasgow Coma Score (GCS) 14 and 6 controls) were studied at 1.5T: between 2 and 24 days after injury. A 70 mm TE 3D spin-echo excitation sequence was used [3], with 1.15 cm3 nominal voxel. Ratios of NAA, Cr and Cho across 25 different regions in 5 MRSI slices were obtained using an automated spectral analysis method [4]. Student’s one tail t-test was used. Results and Discussion: Our volumetric method allowed at least five contiguous 15 mm-MRSI slices to be obtained within the brain. Some regions were excluded because of strong B0 inhomogeneities. In one patient, NAA loss was seen in the immediate neighborhood of regions of MRI-observed injury. No evidence of increased lactate was seen. Metabolite ratios calculated from 25 locations in five slices showed no statistically significant difference between patients and controls. References 1. Ronn B.D. et al. J. Magn. Reson. Imag., 8 829. 1998 2. Brooks W.M., et al. J. Neurotrauma. 17 639. 2000. 3. Ebel A. et al. Magn. Reson. Med.. 46 1072. 2001. 4. Soher B.J. et al. Magn. Reson. Med.. 40 822. 1998. Acknowledgments This study was supported by PHS grants AG12119 and NS38029.

P515.
BRAIN TISSUE OXYGENATION MEASUREMENT IN SWINE UNDER DIFFERENT CO2 AND BLOOD PRESSURE LEVELS

Introduction: Brain tissue oxygen tension (Pto2) measurements are being used increasingly in the treatment of traumatic brain injury (TBI). Pto2 is known to be responsive to changes in mean arterial pressure (MAP), arterial CO2 (PatCO2) and inspired oxygen concentration (FIO2). Because Pto2 decreases with poor outcome, increasing the FIO2 to improve Pto2 has been proposed as a therapeutic intervention. However, the physiologic and metabolic consequences of this are not fully understood. We examined the effects of increasing FIO2 in uninjured swine brain at various levels of PatCO2 and MAP. Methods: Pto2, MAP, cerebral blood flow (CBF), and blood gases were monitored in swine (n = 6). FIO2 was adjusted to obtain Pao2 of 100, 300, and 500 mm Hg at each PatCO2 level of 25, 40, and 60 mm Hg at a constant MAP (85-90 mm Hg). The FIO2/PatCO2 “ramp” was repeated for MAP of 40, 60 and 150 mm Hg and a constant PatCO2 (38-42 mm Hg). Results: With MAP and PatCO2 at normal levels, increasing the PatCO2 from 100 to 500 mm Hg increased Pto2 from 19 ± 7 mm Hg to 44 ± 18 mm Hg. At a PatCO2 of 500 mm Hg, Pto2 decreased during hypocapnia (PatCO2 25) and hypotension (MAP 40) to 33 ± 15 mm Hg and 11 ± 9 mm Hg respectively. Elevations in Pto2 occurred during hypocapnia (PatCO2 60, Pto2 82 ± 57 mm Hg) and hypertension (MAP 150, Pto2 82 ± 21 mm Hg). Pto2 also increased during mild hypotension (MAP 60, Pto2 73 ± 16 mm Hg). Conclusion: As anticipated, the reaction of Pto2 to increased PatCO2 levels is altered at different blood pressure and arterial CO2 levels. The unexpected finding of increased reactivity during mild hypotension indicates a complex relationship between oxygen carrying capacity and flow. It also underscores the importance of further elucidating this relationship prior to implementing increased FIO2 as a therapeutic intervention for patients with TBI.

P516.
FUNCTIONAL MAGNETIC RESONANCE IMAGING AFTER ACUTE MILD TRAUMATIC BRAIN INJURY
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In the context of a large-scale study of physical and neurocognitive recovery from concussion in high school football, functional magnetic resonance imaging (fMRI) was used to study 15 players with Grade 2 (n = 10) or 3 (n = 5) (American Academy of Neurology guidelines) concussions within 12-18 hours of injury. Teams matched to the injured players on a pre-season neurocognitive measure also underwent fMRI studies. Whole brain echoplanar imaging was used to investigate changes in blood oxygenation level dependent (BOLD) signal changes during two fMRI protocols: (1) A verbal working memory task (Stenberg paradigm) was used to investigate the effect of memory load on brain activation. Use of similar tasks in functional imaging studies have shown a pattern of increased activation in mild traumatic brain injury, presumably due to recruitment of increased attentional resources for task performance in the injured compared to the control participants. (2) Coherence in spontaneous low frequency BOLD signals between a region of interest in the right and left hemisphere during rest was studied as a measure of interhemispheric connectivity. The region of interest in each hemisphere was defined functionally during a separate fMRI protocol using finger movement as the activation protocol. Imaging findings showed a similar pattern of functional activity in the injured and control participants in both the working memory and resting scan protocols. These findings were consistent with neuropsychological testing completed at the same time interval, which also failed to show differences between injured and uninjured players. Despite the suggestion of quick cognitive recovery, injured players continued to report postconcussive symptoms for approximately one week after injury. Overall these findings suggest that individuals with Grade 2 and mild Grade 3 concussions recover neurocognitive capabilities and related neuropathological function (measured with fMRI) relatively quickly following injury, and that this recovery precedes recovery of postconcussive symptomatic complaints.
P517. PRIMARY AND DELAYED ISCHEMIA IN SEVERE TRAUMATIC BRAIN INJURY
Hovhannes M. Manvelyan*, Roumen V. Fanafestian. (Yerevan State Medical University, Yerevan, Armenia).

The frightening growth of patients with severe forms of Traumatic Brain Injury (TBI) demands more investigations for understanding of the pathophysiology of that dangerous disease. For the in-time detection and continuous monitoring of Cerebral Blood Flow Velocity (CBFV) infringements in 78 patients with severe forms of TBI we used the method of Transcranial Ultrasonic Dopplerography (TCD). For further analysis we put the records of CBFV quantitative characteristics in M1 MCA and Basilar Artery. The TCD recordings of CBFV were performed starting first hours of TBI onset and were continued during next 14th–17th days to reveal any disturbances of flow velocity if they were present. Our investigations reveal succeeding each other similar processes of cerebral hyperperfusion (expressed decrease of CBFV), hyperperemia (sufficient increase of CBFV), and cerebral vasospasm development in all patients with severe forms of TBI. The hyperperfusion stage lasts during first hours and day after TBI, and leads to very dangerous low cerebral circulation (mean speed in M1 MCA was decreased about twice. equal to 30–40 cm/sec). We find the straight correlation of the CBFV drop level and risks of brain ischemia development. On days 2nd–3rd there was develop hyperperemia. The increase of CBFV in two times means the expressed narrowing of the diameter of the artery, and indicates vasospasm, and can leads to secondary brain tissue ischemia on days 6th–9th due to insufficient blood supply. The TCD signs of vasospasm were detected on days 4th–5th meanwhile the clinical signs usually develop during subsequent second-third days. So, first days and beginning of the second week of TBI onset are the most dangerous days of cerebral ischemia development and sufficient exacerbation of the disease. The mechanisms of ischemia are different but clinical signs are similar and demand steadfast observation and applicable treatment.

P518. DIFFERENTIAL ALTERATIONS OF VASCULAR REACTIVITY FOLLOWING TRAUMATIC BRAIN INJURY
Andreas Merke*, Lothar Schilling (Department of Neurosurgery, University Hospital Mannheim, University of Heidelberg, Germany).

Objective: Alterations of cerebral microcirculation may contribute to the development of secondary tissue damage after traumatic brain injury (TBI). The pursuit of the study was to characterize alterations of cerebrovascular reactivity following experimental TBI. Methods: In male SD rats anesthetized with chloralhydrate (360 mg/100 g i.p.) global TBI was induced by weight drop (lesion parameters: weight: 350g; height: 28.5cm). After 24h or 48h rats were killed and ring segments prepared from the basilar artery (BA) and the middle cerebral artery (MCA) for measurement of isometric force. Vasoreactive stimuli included i) contraction by isocapnic in 124 mM K+-Kreb's solution or endothelin (ET)-1, ii) endothelium-dependent nitric oxide (NO)-mediated relaxation by acetylcholine (ACH BA), bradykinin (BK, MCA) and the selective ETB-receptor agonist, sarafotoxin-6c (S6c) and iii) endothelium-independent relaxation by sodium nitroprusside (SNP) and 8-bromoguanosine cyclic monophosphate (cGMP). Results: Contraction due to 124 mM K+-Kreb's (reference contraction) was markedly decreased after trauma (MCA/BA: control 2.1 ± 0.8 / 5.1 ± 2.0; 48h after TBI 1.3 ± 0.4 / 3.2 ± 1.9; *p < 0.001) as were contractions upon serotonin (5-HT, BA) and the thromboxane mimetic U46619 (MCA). Relaxation upon ACh and BK was significantly enhanced whereas endothelium-independent relaxation induced by SNP and cGMP were not significantly altered. However, ET-1 induced contraction was significantly enhanced after TBI (MCA/BA: % control: 104 ± 40 / 95 ± 17; TBI 166 ± 54 / 142 ± 52; *p < 0.05) while S6c induced relaxation was shifted to higher concentrations (MCA/BA: control 8.6 ± 0.7 / 10.0 ± 0.7; TBI 7.9 ± 0.4 / 8.2 ± 0.7; *p < 0.001). Conclusion: TBI results in differential alterations of cerebrovascular reactivity. Contraction to a variety of stimuli was decreased, while NO-mediated relaxation appeared preserved or even enhanced. In contrast ET-1 induced contraction was increased, probably due to a decrease of ETB-receptor mediated relaxation.

P519. CASPASE INHIBITION ATTENUATES MITOCHONDRIAL RELEASE OF CYTOCHROME C AND APOPTOSIS-INDUCING FACTOR AFTER TRAUMATIC BRAIN INJURY IN RATS.

The pathobiology of traumatic brain injury (TBI) includes activation of multiple cascades followed by cell death with a spectrum of apoptotic phenotypes. Recently, activated caspase-2 was shown to have the capacity to induce release of cytochrome c (cyt c) and apoptosis-inducing factor (AIF) from mitochondria, establishing itself as a direct effector of the mitochondrial apoptotic pathway and adding an additional level of regulation into the caspase cascade. We examined the biochemical, histopathologic, and functional outcome effects of the pan-caspase inhibitor benzamide (OMe)-fluoro-methylketone (BAF) after controlled cortical impact (CCI) with secondary insult in rats. First, rats were randomized to receive 0.1, 0.5, or 1.0 mol BAF or vehicle (DMSO) via single intraperitoneal hippocampal injection 1 min after CCI (n = 4-6/group). At 24 h, caspase-3 activity, TUNEL+ neurons, and hippocampal neuronal loss were reduced in a dose dependent manner. To define the effect of BAF on intracellular trafficking of apoptogenic factors, Western blots on subcellular protein fractions were performed using antibodies against cyt c, AIF, and caspase-2. 1 mol BAF reduced cytotoxic cyt c, nuclear AIF, and proteolysis of caspase-2 vs. vehicle. An outcome study testing the effect of hippocampal injection of 1 mol BAF with or without nerve growth factor (NGF, 12.5 mg/kg x 3 i.v. via osmotic pump) did not reveal differences in motor function (d 1–5). Morris water maze performance (d 14–20), hippocampal neuron survival, nor contusion volume (n = 9–11 injured group; n = 5/sham group). These data suggest that pan-caspase treatment with BAF reduces acute cell death by inhibiting mitochondrial release of cyt c and AIF; possibly by a mechanism involving caspase-2. This effect appears temporary; however, and supports further study using chronic administration of caspase inhibitors. Support: NS 35620 and 30318.

P520. TEMPORAL SEQUENCE OF POLY (ADP-RIBOSE) POLYMERASE EXPRESSION IN TRAUMATIC BRAIN INJURY IN HUMANS
Elgin Yap*, BT Ang, Joyce Lim, WS Fan, Haw Hoo (Acute Injuries Laboratory, Section of Neurotrauma, National Neuroscience Institute, Singapore).

Poly (ADP-ribose) polymerase (PARP) catalyses covalent post translational modification of nuclear proteins during an early apoptotic cellular response to DNA breakage. In acute brain injury however, there is over-activation of PARP which depletes its substrate nicotinamide adenine dinucleotide (NAD) and then adenosine triphosphate (ATP) storage, leading to cellular energy depletion and cell death. Additionally, PARP is a substrate for caspases in apoptosis. We sought to determine the temporal characteristics of PARP expression in human peri-contusional tissue. Of 19 patients who had surgery for traumatic contusions (Marshall's Class 5 on CT), 12 were suitable for analysis. Ethics approval was obtained from our local institutional ethics committee. These patients were managed according to a standard severe TBI protocol. Demographics, opening ICP, ICP at 12 and 24 hours and PARP expression characteristics were studied. PARP was expressed in 8 of 12 specimens (67%). Differences were noted with regards to the predominant site of PARP expression. In patients who were operated early (less than 4 hours after event), PARP expression was predominantly cytoplasmic versus patients operated late (>4 hours) which showed more nuclear expression (p < 0.05). There were no differences in demographics and ICP values in these 2 groups. It appears that there is a temporal difference in site of PARP expression. Early expression of PARP is more cytoplasmic versus late expression which tends to be predominantly nuclear. PARP cleavage products have been shown in vitro cellular assays to translocate from the nucleus to the cytoplasm during apoptosis. This may suggest that PARP may mediate cell death via apoptosis rather than necrosis in the initial period following brain injury.
**P521.** INCREASED PHOSPHORYLATION AND NUCLEAR TO CYTOSOLIC TRANSLLOCATION OF FORKHEAD TRANSCRIPTION FACTOR IN RAT CORTEX AND HIPPOCAMPUS AFTER TRAUMATIC BRAIN INJURY.

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The Protein kinase B (PKB) signaling pathway may play a critical role in the regulation of cell survival after traumatic brain injury (TBI) and cerebral ischemia. Forkhead transcription factor (FKHR) is one substrate of activated PKB, and is a positive regulator of Fox ligand gene expression. We previously showed increased phosphorylation of FKHR coincident with decreased Fox ligand in human brain after TBI. To investigate intracellular trafficking of FKHR in vivo, adult rats (n = 25) were subjected to controlled cortical impact (CCI) with imposed secondary hypoxic insult. At 2, 6, 24 and 72 hours after injury, animals were sacrificed and dorsal cortices and hippocampi were dissected. Naive rats were used as controls. Proteins from whole cell lysates, nuclear and cytosolic enriched fractions were prepared and Western blots using antibodies against PKB phosphorylated PKB (pPKB; Ser473). FKHR and phosphorylated FKHR (pFKHR) were performed. Total PKB levels from ipsilateral cortex and hippocampus were similar in controls and all timepoints after CCI. In contrast, pFKHR was increased after injury in both ipsilateral cortex and hippocampus vs. control. A significant amount of PKB was observed within the nuclear fractions from both cortex and hippocampus. Relative protein levels of FKHR were increased in the cytosolic fraction of both cortex and hippocampus as early as 2 hours after injury. There was a decrease in FKHR in cortical nuclear fractions but an increase in FKHR in hippocampal nuclear fractions after injury. Total pFKHR was increased in both cortex and hippocampus after injury vs. control. These preliminary results suggest that after TBI, the transcription factor FKHR may translocate from the cell nucleus to cytosol, were it has the potential to be phosphorylated by PKB, sequestering it in the cytosol and thereby reducing transcription of its target genes that include Fox ligand. Support: NS 38620 and 30318.

**P522.** AGE-DEPENDENT SUSCEPTIBILITY TO OXIDATIVE STRESS AFTER TRAUMATIC BRAIN INJURY IN THE DEVELOPING BRAIN

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Children less than 4 years have worse outcome after traumatic brain injury (TBI) compared to older children and adults. This increased susceptibility may in part be due to differences in the response to oxidative stress. We hypothesized that the immature brain does not have an adequate compensatory response to injury from oxidative stress. We examined glutathione peroxidase (GPx) activity in cortical and subcortical regions in postnatal day 21 (P21) and adult mouse brain following a controlled cortical impact. Brain dimensions were measured to adjust the parameters of the impact accordingly. No significant differences were found between P21 and adult brain in the dimensions studied. except that the cortical mantle of the P21 brain was thicker than adult (p = 0.01). Injury was assessed by measuring brain edema through changes in water content, and the response to oxidative challenge was identified by changes in GPx activity. The P21 brain exhibited more prominent edema compared to adult (p < 0.0001). GPx activity in the adult brain was increased at 3 hours (ANCOVA, p < 0.05 over the injured cortex) and remained high until 24 hours post-injury whereas there was no compensatory change in GPx activity in P21 brain, although absolute levels had reached adult levels developmentally. These findings support our hypothesis and illuminate the important role of oxidative stress after TBI in the developing brain. (This research supported by the UC Neurotrauma Program and NS 41256).

**P523.** PEDIATRIC CCI ALTERS THE PHOSPHORYLATION STATUS OF TWO KEY PROTEIN KINASES, p70S6K AND p90RSK


Previous data from our laboratory showed phosphorylation changes of protein kinase B (PKB) and two key protein synthesis initiation factors, eukaryotic initiation factor 4E (eIF-4E) and eukaryotic initiation factor 2alpha (eIF2a) after pediatric TBI suggesting an acute upregulation followed by a longer lasting downregulation of PKB dependent protein synthesis regulation activity. Two other serine/threonine kinases, p70S6K and p90RSK kinase, are also important in developmental protein synthesis control. p70S6K is a mitogen activated protein kinase that is activated by translation of p85 catalytic subunit. p90RSK is a protein kinase activated by MAPKs such as ERK1/2. When activated p70S6K and p90RSK in turn phosphorylates ribosomal S6 kinase, important in both the translation of S6 terminal oligopyrimidine tract (STOP) mRNA and cap-dependent (eIF4E pathway) mRNA resulting in selective expression of growth associated proteins such as ribosomal proteins and elongation factors. We evaluated the level and distribution of brain phospho-p70S6K and phospho-p90RSK activity in injured and sham PND 17 rats at 6, 24 or 72 hours after moderate (4 m/s 2.0 mm deflection) controlled cortical impact (CCI) using immunohistochemistry (n = 7 per group). Increases in the phosphorylation of p70S6K and p90RSK activity in CA1 pyramidal cell bodies only were found at 6 hours with increased activity at 24 and 72 hr in all pyramidal neuron sectors following CCI. Decreased phosphorylation (activation) of p70S6K and p90RSK should decrease both cap-dependent and 5’TOP translation at 1-3 days after CCI providing additional evidence that nutritive and trophic factor enhancement of protein synthesis should be useful after pediatric CCI for both normal developmental growth as well as injury repair. (Supported by NIH NS40049 and NS01809).

**P524.** DIFFERENTIAL EXPRESSION OF GENES RELATED TO CELLULAR SIGNALING, SYNAPTIC FUNCTIONING AND ION CHANNELS POST-INJURY: A COMPARISON OF MODERATE AND SEVERE INJURY EFFECT ON GENE EXPRESSION IN HIPPOCAMPUS

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Cognitive deficits following traumatic brain injury (TBI) are often related to the function of hippocampus. The systematic study of molecular events within this structure following traumatic brain injury (TBI) is essential for better understanding of neuropahtology after TBI. Lateral fluid percussion injury was administrated to adult rats and gene expression profiles in hippocampus of moderately (n = 12) and severely (n = 12) injured brains were examined using the microarray technique. Of ~8,700 genes and expressed sequence tags (ESTs) examined, 760 genes and ESTs showed dynamic changes (>2 fold) at either 30 min. 4 hr or 24 h following severe injury. while 342 genes and ESTs were altered after moderate injury. One fundamental difference in gene expression profiles found between moderate and severe injury was that majority of genes with altered expression were upregulated following severe injury while they were down-regulated following moderate injury. Genes that have altered expression participate in diverse cellular activities. Differential time courses were observed in expression of most genes between moderate and severe groups. Among the differentially expressing genes are those related to cellular signaling, synaptic functioning and ion channels. NS13036. NS12756. NS27544.
P525. GENE EXPRESSION PROFILING FOLLOWING TRAUMATIC BRAIN INJURY IN WILD TYPE AND ALZHEIMER TRANSGENIC MICE
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The nature and extent of recovery after traumatic brain injury (TBI) is heterogeneous and not fully explained by known demographic and injury prognostic factors. suggesting that additional factors such as genetic influences modulate the secondary injury/recovery pathways. We and others have shown that the e4 allele of the apolipoprotein E gene influences recovery following TBI and variation in other genes influential in neurogenesis, neurodegenerative or neural repair mechanisms will likely modify recovery. Changes in gene expression that occur as a response to TBI implicate those genes and their encoded proteins in the cell injury and/or recovery process and identify targets for possible therapeutic intervention. Experimental modeling of TBI in wild type and transgenic mouse models of human disease and neurodegeneration, provide an environment in which some of the confounding variables (e.g. injury severity, patient demographics) can be controlled and the influence of specific genes can be investigated. Using microarray technology we are generating age-, time- and genotype-dependent gene expression profiles in wild type mice and mouse models of Alzheimer’s disease, following TBI or sham-injury, and correlating these with measures of blood flow and behavior. In addition to genes which have already been reported to respond to TBI (e.g. those encoding heat shock proteins, neurotrophic factors, metalloproteinases) we have identified responses from genes encoding neurospecific peptides and proteins which implicate previously unreported cell pathways as important in the neurodegenerative and neuroregenerative sequelae following brain injury. Supported by: The Roskamp Foundation and the Center for Traumatic Brain Injury Studies.

P526. GENE EXPRESSION PROFILES FOLLOWING MODERATE AND SEVERE BRAIN INJURY IN RATS: IMPLICATIONS OF ENERGY SHORTAGE AND CELLULAR VULNERABILITY IMMEDIATELY AFTER INJURY.
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Immediately following traumatic brain injury (TBI), there is an activation of ion pumps resulting in an increase in glucose metabolism which appears responsible for the reduction in ATP content within injured tissue. In addition, levels and durations of ATP reduction were associated to injury severity. In this study, adult rats (n = 8) sustaining a moderate or severe lateral fluid percussion injury were used to assess how these acute physiological responses are related to injury-induced genetic alterations. Using oligonucleotide GeneChip array (Affymetrix), we screened for the gene expression profiles of injured rats within the ipsilateral cortex and hippocampus and compared them to that of sham-injured controls. Thirty minutes after injury, a large number of genes was induced in the severely injured group for both cortex and hippocampus. The number of induced genes was significantly reduced 30 min following moderate injury, compared to that of severely injured animals. Five probe sets each encoding either alpha or beta subunits of Na+, K+-ATPase were induced up to 7.5 fold in hippocampus following severe injury, along with genes encoding many other voltage-gated ion channel proteins. Only one probe set for the Na+, K+-ATPase was induced in cortex following severe injury. A few genes that fall into this category showed an attenuation expressed in moderately injured hippocampus. These findings suggest that the activation of ion pumps after severe injury is regulated at the level of mRNA expression and provide a potential guide to reduce the level of energy crisis by gene therapy. NS30508. NS37365. NS27544.

P527. MICROARRAY GENE EXPRESSION ANALYSIS OF POSTNATAL DAY 26 RAT CORTEX AFTER LATERAL FLUID Percussion INJURY
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Our laboratory has previously described the distinct physiological, metabolic, behavioral and plasticity responses of postnatal day (PND) 26–30 rats to traumatic brain injury. The current study was conducted to screen for changes in gene expression following TBI in "juniors." PND26 Sprague-Dawley rats were given either sham surgery (n = 8) or moderate fluid percussion injury (n = 8). At 4 and 24 hours after injury, animals were sacrificed. SI cortex was dissected and frozen. Total RNA was isolated, labeled and hybridized to Rat Genome Arrays (Affymetrix). Injury severities between 4 and 24 hr groups did not differ significantly as reflected by aplea (96 ± 43.9s and 113 ± 41.5s, respectively) or time to toe pinch response (229 ± 95.3s and 194 ± 52s, respectively). Overall, changes in gene expression at 4 hours reveal the fold change in expression in 52 genes with 15.4% showing downregulation and 84.6% upregulation. By 24 hours post injury >2-fold change in gene expression was detected in 16 genes (38.2% decrease. 61.8% increase). Genes related to metabolism (lactate dehydrogenase B; Hmocoa reductase) showed upregulation at 4 hours, but returned to sham levels by 24 hrs. There was an early induction of several transcription and stress-related genes (NF-kappaB; hp70, grp78), but glia-related gene (GFPV; vimentin; n100) were only induced at 24 hours. This approach will help to identify those genes that are involved in the pathophysiological response of the juvenile brain to TBI. NS30308. NS37365. NS27544 and the Lind Lawrence Foundation.

P528. CHARACTERISATION OF A HIGHLY ADAPTABLE, NEW MODEL OF DIFFUSE TRAUMATIC BRAIN INJURY IN RODENTS
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Diffuse injury is a major feature of clinical traumatic brain injury. Despite this, few experimental models of diffuse traumatic brain injury exist. Those that do are limited in terms of their suitability for use in smaller animals, be they simply using immature animals for developmental studies or using the model in a smaller animal species (1,2). We have therefore developed a highly controlled model of diffuse traumatic brain injury that offers tremendous utility in its application to rodents. An adaptation of the Marmarou impact-acceleration model (1), the injury device delivers a hydraulically controlled, high-velocity impact to a steel disc cemented onto the rodent skull. The distance the impactor travels after contacting the steel disc is under user control thus varying the injury severity. Force of impact is recorded on an oscilloscope. The head is decelerated after impact using a molded gel-filled base upon which the animals head is supported during injury. Using an 18 mm injury, we demonstrate that the model results in moderate to severe motor and cognitive deficits. Diffusion weighted magnetic resonance imaging shows edema development within the first few hours after trauma with a maximum at 24 h. Phosphorus magnetic resonance spectroscopy confirmed that there was no energy depletion or pH changes after trauma, although significant declines in brain free magnesium concentration were observed as has been described in other models of diffuse traumatic brain injury. The diffusion appearance of amyloid precursor protein, considered to be a marker of axonal injury, was apparent in immunohistochemistry studies. These results confirm that this new model produces biochemical and neurological changes consistent with the diffuse axonal injury produced in other models, but with the added utility of being adaptable to various rodent sizes. (1) Marmarou, A., Foda, M.A.A., Van den Brink, W., Campbell, J., Kita, H. and Demetriou, K. (1994) J. Neurosurg. 80: 20-30. (2) Smith, D.H., Chen, X.H., Xu, B.N., McIntosh, T.K., Gennarelli, T.A. and Meaney, D.F. (1997) J. Neuropath. Exp. Neurol. 56: 822-834.
EXTRA- AND INTRACRANIAL PRESSURE PULSES DURING FLUID PERCUSSION INJURY

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Fluid percussion injury (FPI) is one of the most commonly used animal models of traumatic brain injury (TBI) and is well characterized in many aspects. However, the pressure pulse delivered to the brain is often estimated from recordings of the extracranial pressure wave. Here, we sought to compare the extra- and intracranial pressure waves measured simultaneously during FPI. To measure the pressure delivered from the FPI device into the skull cavity we used thin, flexible optic pressure probes (Samba Sensors, Göteborg, Sweden) with a diameter of 0.4 mm inserted into either the ipsilateral or the contralateral lateral ventricle. The pressure in the FPI-device was measured in the nozzle. 65 mm from the exposed dura mater. Simultaneous measurement of the pressure pulses extracranially and intracranially was done using two Samba Sensor 3000 optic pressure monitors connected to a computer with recording software with a frequency of 500 Hz. The FPI-device was set to deliver a pulse with 2.8-3.0 atm pressure, resulting in a moderate to severe injury with an apnea duration of 15-20 s. The extracranial pressure pulse showed a steep incline with a mean peak value of 2.92 ± 0.15 atm, followed by a gradual return towards the baseline over the ensuing 1200 ms. The intracranial pressure pulse showed a similar curve pattern with a mean peak value of 2.77 ± 0.30 atm. We found no significant difference between the pressure pulse in either ventricle compared to the extracranial signal. This suggests that the transient pressure pulse during FPI is similar intracranially and extracranially.

CLINICORADIOLOGICAL CLASSIFICATION OF TRAUMATIC INTRACRANIAL HEMATOMAS (THI).

Dr. A.V. Luntzh, Dr. V.E. Bantsulevich, Dr. A.V. Korobtsov, Dr. V.G. Siaure (Medical University of Vladivostok, Vladivostok, RU).

Based on the clinical and CT data of 94 consecutive patients with traumatic intracranial hematomas the classification is offered. The classification takes into account the following criteria: 1. Location of THI: cortical-subcortical—settle down in white substance of hemispheres and gray cortex of a brain with (or without) diffusions in subdural space (19 %); subcortical—locate in the white substance of hemispheres of the brain (74 %); central—location medially of a putamen (7 %); the cerebellum (3 %). 2. Size of THI: small—the maximal diameter in most demonstrative scans on CT is equal or more than 1.5 cm and less than 3 cm (volume of the sphere is about 2-15 cm3) (18 %); average—the diameter is equal or more than 4.5 cm (15-45 cm3) (57 %); large—the diameter 4.5 cm or more (25 %). 3. Peculiarities of the formation THI: in the focus of contusions (88 %); without any signs of the contusions (12 %); 4. Time of formation THI: primary—are formed directly after a trauma (93 %); deferred—24 hours and later after a trauma (7 %). 5. Combination THI: single (58 %); multiple (19 %); with subdural or epidural hematomas (23 %); with the focus of contusions of a brain on a distance (34 %). 6. Type of clinical current THI: without a light interval (13 %); with the light interval (40 %); with the complete light interval (16 %); with gradual restoration of the consciousness after its primary loss (31 %).

RELATIONSHIP OF APPROPRIATE HISTOLOGICAL PROCESSING TECHNIQUES AND ACCURATE EVALUATION OF EXPERIMENTAL BRAIN INJURY

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Accurate assessment and quantitation of large brain lesions has proven difficult for a variety of reasons. Including the inability to accurately evaluate spatial and volumetric parameters. We propose a novel technique for obtaining consistent and reproducible paraffin-embedded histological sectioning and volumetric lesion analysis. Our technique has allowed for accurate quantitative and quantitative lesion assessment following clinical and experimental brain alterations such as trauma or therapeutic intervention. Nine Yorkshire domestic piglets, between the ages of 5 day old and 4 month old, underwent a staged cortical impact injury to the fronto-parietal cortex. The subjects were then perfusion fixed, and the brains removed and processed for routine histology. Following a specific trimming technique, we were able to accurately compute lesion volume analyses through the use of parallel sections and the use of the Analytical Imaging Station. Lesion quantifications were computed by expressing the area of the lesion as a ratio of the injured area divided by the uninjured area of the contralateral hemisphere. This method was then used to determine the average lesion size across 3 comparable serial sections throughout the injured zone. The method was within and between subjects of different ages and tissue equivalents. Using this protocol, edema, hemorrhage, symmetry and various Magnetic Resonance Image signal abnormalities can be defined and co-registered with other parameters and histology. Our methodology permits accurate and quantitative assessment and co-registration of histologic, radiologic and immunohistochemical images. It is now clear that the accurate co-registration of these parameters is one of the more important and efficient methods for understanding central nervous system (CNS) disease, injury and therapeutic mechanisms.

CLINICAL CRITERIA OF SORTING, PROGNOSIS OF OUTCOMES AND TREATMENT OF THE PATIENTS WITH A CRANIOCEREBRAL TRAUMA AT STAGES OF MEDICAL EVACUATION.

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This work is based on the results of the unified complex study of 561 patients with a serious cranio-cerebral trauma (CCT), verified by outcomes from neurosurgery clinics of 4 countries (Uzbekistan. Russia. Lithuania. Estonia) with use of the Moscow scale of a coma and mark estimation of a condition of the patients (Shukhovich A.R., 1986, Mamadaliev A.M. 1987, 1988) 175 patients were subjected to surgical treatment. By outcomes patients are distributed to four groups: lethal outcome -129; outcome with rasping neurologic infringements — 71, with the moderate neurologic transgressions—103 and with good restoration of function down to the compensated condition—108 patients. The data about 150 patients served for realization of “examination” on the computer. On the unified base of the clinical data on CCT is found out, that the scale of a coma, which used, is a simple and adequate method of estimation of a condition of conscious. The terminal coma determined on presence of a bilateral fixed mydriasis and a muscular atony. per all days after a trauma is absolutely difficult to predict. Among the patients acted in hospitals in a condition of a deep coma 2/3 of patients die, and in a condition of a moderate coma—only 1/3 die; and at acting in a condition of a moderate coma the different outcomes down to restoration of function of organism with indemnification of a condition are possible. At a deaferation and suoper the probability of favourable outcome sharply raises also this tendency is brightly shown at the fifth day after a trauma. The developed system of dynamic forecasting of outcomes among the patients with CCT for various parts of practical public health services represents a package of 15 prognostic tables realized in the computer. The system provides forecasting of lethal and favorable outcomes at the first day after a trauma with reliability on the average 83 %, in 5-7 days 95 %, differential forecasting of different categories of recoveries with reliability 80-87 % in the first day and 84 % in 5-7 day. At possible mass death is recommended to use the system of sorting, offered by us, rendering of a medical care and evacuation of the injured depends on the seriousness of CCT. We developed new ways of treatment (invention patents are obtained) Cranio-cerebral trauma using endolumbar injection of ozone and nootropics. autoplasty of defects of a skull using fan-shaped titanium device.
P533.
PRIMING EFFECT IN REPETITIVE CONCUSSION: PROLONGED PERSISTENCE OF SENSITIZATION TO REINJURY

A rat model of repeated concussion was developed to determine to what extent and for how long a first concussion sensitizes the brain for subsequent concussive injury. Methods: Thirty rats were subjected to impact-acceleration head injury calibrated to a righting time of 8-12 minutes. After the initial injury, separate groups were given a second, milder injury either 3, 5, or 7 weeks later, using 10% less weight. Sham-injured and single-injury animals were used as controls. Spatial learning and memory were measured using the Morris Water Maze test (6 trials/day for 3 days) starting 17 days after the final injury. Results: Sham and single injury groups had final goal latency times averaging 15 and 26 seconds, respectively. Animals subjected to a prior concussion all showed markedly more severe cognitive deficits from a second injury. The goal latency times of the double-injured animals were increased to 94 and 58 seconds with a 3 or 5 week inter-injury interval. With a 7 week inter-injury interval, goal latency was partially improved to 53 seconds. The learning curves demonstrated severe impairment in the double injury animals on all trials. Conclusion: The data of this study suggests that a first injury profoundly sensitizes an animal to severe cognitive impairment after a second injury, far worse than after a single injury alone. This sensitization effect partially dissipates with time, but still persists far longer than the observed cognitive effects of the first injury. The neurological basis for this sensitization, and whether it persists indefinitely or eventually clears needs to be further investigated. Awareness of this priming effect should enhance guidelines for management of concussive head injury in athletes. Supported by: NINDS.

P534.
ROLE OF MICRODIALYSIS IN CLINICAL TRIALS FOR SEVERE TBI
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Introduction: Over 200 Phase III drug trials have failed to show any clinical benefit in severe TBI. We have recently tested the role of microdialysis as a pharmacokinetic tool to improve clinical trials design. Material and Methods: 20 severe head injured patients (GCS<9) received topiramate, a drug that inhibits glutamate release. In order to study central nervous system (CNS) drug delivery and to relate the extracellular fluid (ECF) concentration to its "neuroprotective" effect topiramate and glutamate were simultaneously recovered from cerebral ECF, using a microdialysis probe. Results: Patients receiving 11.4mg/Kg of topiramate showed significantly higher levels of unbound drug in the brain compared to patients receiving 5.7mg/Kg (p<0.05), however; this did not double the steady state concentration (Css) suggesting an active transport mechanism across the BBB that may be partially exhausted. Doubling the dose of topiramate also resulted in a tenfold decrease in Emax for dialysate glutamate (p<0.05) and in a mean r-value for top/glut correlation of -0.5 (p<0.0001). Conclusions: Microdialysis gives valuable information on temporal pattern of drug penetration across the BBB in the "in vivo" injured brain. A "neuroprotective effect" may be inferred from the dose-dependent glutamate lowering effect of the drug. This type of pharmacokinetic-pharmacodynamic analysis may be a powerful tool in clinical trial design in the future.

P536.
PRELIMINARY EXPERIENCE WITH DECOMPRESSIVE VENTRICULOCECTOMY BY CONTINUOUS VENTRICULAR CEREBROSPINAL FLUID DRAINAGE IN POSTTRAUMATIC DIFFUSE BRAIN SWELLING.

In head injured patients, high intracranial pressure (ICP) due to brain swelling is the main factor that can lead to death itself and, together with Glasgow Coma Score, the main prognostic factor. Several ways of intracranial pressure management have been used in the treatment of these patients, from clinical measures in the ICU to large decompressive surgeries, which can evolve to uncal herniation at the opposite side or even the herniation of the sick brain parenchyma through out the craniotomy with venous obstruction and hemispheric venous infarct. The truth cause of brain swelling is yet poorly understood. Although the widespread theory of brain swelling as a increase in cerebral blood volume due to microvascular dilatation, having edema just a minor play in the genesis of High Intracranial pressure (HIP), Marmarou et al (2000) have proved through total brain water analysis that brain swelling genesis is mainly due brain edema and not due to an increase in brain vascular volume. Intracranial pressure monitoring with a ventricular catheter has showed to be very useful in head injured patients. Not only because of being a trustful way to intracranial pressure measurement but also to allow a quick way to intracranial pressure relief by cerebrospinal fluid (CSF) drainage. Continuous CSF drainage with a ventricular catheter leads to a pressure gradient which causes a change in CSF flux direction with the edematous parenchyma water being drained into the ventricle and so ICP relief, as it causes a continuous drainage of pro-inflammatory substances present in post-trauma CSF. In this work we report the initial experience in the use of Decompressive Ventriculostomy as treatment of HIP in 40 patients with severe head trauma, admitted at the neurosurgery emergency room of Sao Paulo’s Medicine college clinical Hospital, discussing the pathophysiology of brain swelling and proposing a new and useful management in these patients.

P535.
CNS PROTECTION BY ANTI-OXIDANTS: PROMISING APPROACHES FOR HEAD TRAUMA IN A RAT MODEL.
B.H. J. Jourdain, J. W. Griesel, H. Kamieniecki, E. Schultka, M. Zhao, G.-F. Tian, A.J. Baker. (Department of Anatomy & Cell Biology and Division of Neurosurgery, University of Saskatchewan, Saskatoon and St. Michael’s Hospital, Toronto, Canada).
P537. 
DELETERIOUS EFFECT OF SECONDARY INSULTS ADDED ON TRAUMATIZED ORGANOTYPIC CULTURE IS MORE PROMINENT IN MILD TO MODERATE THAN IN SEVERE DEGREES OF INJURY
Eun-Shuh Shin, Yoon-Kwan Park, Joo-Han Kim, Heung-Seob Chung, (Department of Neurosurgery, Korea University, Guro Hospital, Seoul, KR) 

The present study aimed to elucidate the effects of secondary injury mechanisms on the different severity of primary insults in traumatic brain injury (TBI). Cultures of organotypic rat hippocampal slice (OTC) were subjected to TBI by using a centrifugal system. To test the possibility that exposure of cultures to TBI decreased cell survival from following secondary insults, we deprived of serum from media or added of hydrogen peroxide after TBI. Cellular injury of OTC was evaluated by measuring the fluorescence of propidium iodide. A graded diffuse injury was observed all over the slice, depending on the level of force, from 30 x g to 3,000 x g. Hippocampal pyramidal cells of the CA1 region and granule cells of the upper limb of dentate gyrus demonstrated a selective vulnerability to secondary injury. The extent of secondary cell injury of sham-injured or in low centrifugal forces (100 x g) was significantly greater than in high forces (3,000 x g). Pretreatment with N(omega)-nitro-L-arginine methyl ester (L-NAME) (an inhibitor of nitric oxide synthase) or free radical scavengers reduced the extent of secondary cellular damage in low level of TBI. But (+)-MK-801 hydrogen malate (MK-801) (an N-methyl-D-aspartate antagonist) was without effect on the injury, regardless of the severity. In conclusion, the extent of cell death after secondary insults is strongly correlated with the amount of survival cells in culture system from primary TBI and no increased vulnerability of traumatized cells is found.

P538. 
SPECIFIC INHIBITION OF APOPTOSIS AFTER DIFFUSE BRAIN INJURY BY MODERATE POSTINJURY HYPOThERMIA
Professor Shuyuan Yang. (Huanhu Hospital, Tianjin, Tianjin CN) 

Object: This study explores variant processes of apoptosis after different diffuse brain injury and the inhibition of apoptosis by moderate postinjury hypothermia. Methods: Diffuse brain injury was induced by trauma device reported by Marmarou. Using a terminal deoxyadenosine transferase-mediated deoxyuridine 5’-triphosphate-biotin nick end labeling technique (TUNEL), the neuronal cells with DNA fragmentation in cortex and hippocampus regions of the brain of rats subjected to brain injury were detected. Using agarose gel electrophoresis, the internucleosomal fragments of DNA in apoptotic cells were examined. Using electron microscopy apoptotic morphological specialty were observed. Results: 1) TUNEL: Apoptotic cells were increased according to injury degree. Their numbers peaked at 48 hours and then declined afterwards. In the mild injury apoptosis was located in hippocampus CA2 and CA3. In the severe injury apoptosis increased evidently, located in all of hippocampus, frontal and parietal cerebral cortex. The hypothermia-treated rats had lower apoptosis indices; however, even at 24, 48 and 72 hours there were significantly fewer of these cells than not treated. 2) Electron microscopy: At 24 and 48 hours after injury, the cells were characterized by a round and shrunken morphology; the nuclei were round and condensed. At 48 hours the apoptotic cells is more than at 24 hours. The hypothermia-treated rats had some apoptotic cells, however, even at 24, 48 and 72 hours there were significantly fewer of these cells than not treated. Conclusions: We suggest that apoptotic cells occur in diffuse brain injury and apoptotic cells were increased according to injury degree. Moderate hypothermia showed specific effect an inhibition of apoptotic cell death after diffuse brain injury in rats.

P539. 
NEUROPROTECTIVE EFFECTS OF AMINOGLUANIDINE IN A RAT MODEL OF LATERAL FLUID-PERCUSIVE BRAIN INJURY
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The present study examined the effects of a selective inducible nitric oxide synthase (iNOS) inhibitor on neuronal cell survival and post-traumatic recovery in rats following a lateral fluid percussion injury. In which brief displacement and deformation of brain had resulted from the rapid epidural injection. Injury was induced by closed cranial cavity. Daily treatment of aminoguanidine (AG) at the dose of 100 mg/kg or normal saline was given intraperitoneally into rats starting 2 hours before or 30 minutes after the head injury. Animals were sacrificed at 24, 48 and 72 hours post-injury. Treatment with iNOS inhibitor AG significantly reduced lesion volumes in rats after fluid percussion, as evaluated by high-resolution magnetic resonance imaging (MRI). Immunohistochemical analysis showed a marked diminution of iNOS expression in macrophages at the injury site in cerebral cortex and cerebellar ventricles (ipsilateral to the injury in rats. In parallel with the appearance of iNOS positive macrophages, apoptotic neurons were observed in the ipsilateral cerebral cortex by in situ terminal deoxytransferase d-UTP nick-end labelling (TUNEL). In rats receiving prophylactic or post-injury treatment of AG, the number of degenerating neurons was markedly reduced in the cerebral cortex compared with those receiving saline injection. The location and extent of these pathologic changes correlated with MRI findings. The neuropathologic studies showed both total and ambulatory locomotor responses were reduced in rats subjected to the traumatic brain injury (TBI). Administration of AG significantly improved the locomotor performance. Present results showed that inhibition of iNOS synthesis by AG improved the histopathological outcomes. It is suggested that nitric oxide (NO) may be involved in neuronal apoptosis following TBI.

P540. 
DIFFERENTIAL RESPONSES BY AXONAL MICROTUBULES AND NEUROFILAMENTS FOLLOWING RE-WARMING AFTER TRAUMATIC AXONAL INJURY

Amelioration of axonal pathology after TAI is greatest with post-traumatic hypothermia followed by slow re-warming. But this has been shown only by a 1 hr of cooling using light microscopy. The present study tests the hypothesis that a longer period of cooling, followed by slow re-warming, provides for amelioration within the ultrastructure of the axonal cytoskeleton. Stretch-injury (load 180-210 g over 19-21ms) was induced in the right optic nerve of adult guinea pigs (weight 750 ± 35g). Animals were cooled to 32-32.5°C as rapidly as possible and maintained for 4 hrs. Rapidly re-warmed animals were returned to a core temperature of 38.5°C within 45min, slow re-warmed animals over 120 min. All animals were maintained at 38.5-38.5°C until cooling 4 hrs later. Stereological analysis of transverse thin sections of axons was undertaken. 

Results: At odds of Ranvier:  
• Slow re-warming provides protection against compaction of neurofilaments  
• Both fast and slow re-warming protect against loss of microtubules  
• At Intemodes 0.0-1.0 μm diameter  
• Slow re-warming protects against compaction of neurofilaments  
• Slow re-warming protects against loss of microtubules while fast re-warming does not  
• At Intemodes 1.0-1.5 μm diameter  
• Slow re-warming protects against compaction of neurofilaments  
• But neither slow or fast re-warming provides protection against loss of microtubules
• At Intemodes 1.5-2.0 μm diameter  
• Slow re-warming protects against compaction of neurofilaments  
• Slow re-warming protects against loss of microtubules while fast re-warming does not

Conclusions: This study provides qualitative evidence that 4 hrs post-traumatic mild hypothermia followed by slow re-warming provides protection against pathology for neurofilaments but only partial protection for microtubules.
P541. MODULATION OF HYPOTHERMIC DELAY, DURATION AND REWARMING RATES POSITIVELY INFLUENCE THE GENESIS OF TRAUMATIC AXONAL INJURY (TAI)

By Takeshi Nishiyama, H. Fujisawa, M. Suzuki, J. Pavlohezk, (Department of Neurosurgery, Yamaguchi University School of Medicine, Yamaguchi, Japan). (Department of Anatomy and Neurobiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, USA).

Recently, we have shown that posttraumatic hypothermia significantly reduces TAI in rats, suggesting that hypothermia is neuroprotective. In the clinical setting, however, reports of hypothermia's neuroprotection have been mixed, with a recent large clinical trial reporting no benefit. In these clinical studies, confounds existed in terms of passive versus active rewarming differing times of initiation and duration, and patient inclusion criteria. In this communication, we have revisited issues related to hypothermic initiation, duration and rate of rewarming in a rodent model of TAI. Male Sprague-Dawley rats were subjected to impact-acceleration injury. In Experiment 1, the temporo-parietal muscle and rectal temperature were maintained at 37°C in the normothermic group, while the hypothermic group employed a 1h period of hypothermia (32°C) induced 2h postinjury. In Experiment 2, the period of hypothermia induced 2h postinjury was prolonged to 2 h. In Experiment 3, post-hypothermic rewarming to normothermic levels was accomplished either over a 20-minute period (rapid rewarming group) or over a 90-minute period (slow rewarming group). Twenty-four hours postinjury the animals' brains were processed for visualization of amyloid precursor protein (APP), a marker of TAI. The number of APP-positive axonal profiles per mm² was calculated. In Experiment 1, hypothermia provided no protection. However, in Experiment 2, prolonged hypothermia (2h) significantly reduced the number of the APP profiles in comparison to the normothermic group. Further, in Experiment 3, the APP-positive axonal density in the slow rewarming group was significantly reduced in comparison to the rapid rewarming group. Collectively, the results of this and previous studies show that early initiation, prolonged duration and slow rewarming enhance the protective benefits of hypothermic intervention, at least within an animal model of TAI. Supported by NIH NS020193.

P542. ENHANCED GASTRIC TOLERANCE TO INDOMETHACIN FOLLOWING NEUROTRAUMA

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Recent studies suggest that indomethacin may be considered as one of the frontline agents for correction of intracranial pressure and cerebral perfusion pressure following head injuries. However, head injury as well as indomethacin have been shown to exert deleterious effect on gastric microcirculation which may lead to gastric and duodenal ulcers. The present investigation was undertaken to study the gastrointestinal tolerance in rats following concomitant exposure to indomethacin and concussive head injury (CHI). Three groups of Albino rats weighing 220 ± 10 g were fasted overnight before the head injury using a controlled cediral impact device. The rats in group 1 and 2 received 45mg/kg of indomethacin orally whereas animals in group 3 received water only. The CHI was produced in group 2 (30 minutes after indomethacin) and group 3. Six hours after the CHI, the rats were sacrificed under light ether anaesthesia, the stomachs were removed and opened along greater curvature and the gastric lesions were quantified. The stomachs were analysed for thioribarbituric acid reactive substances (as a marker of oxidative stress) and myeloperoxidase (indicator of neutrophil infiltration). There were no stomach ulcers in the rats exposed to CHI alone whereas, all the animals treated with indomethacin (with or without CHI) gastric lesions remained in the glandular stomach with a mean ulcer score of 18.3 ± 2.3. Only one out of six rats exposed to CHI plus indomethacin had minor ulcerative change (score 0.66 ± 0.66). Our results clearly suggest that the gastric toxicity of indomethacin is significantly reduced in the rats with CHI.

P543. THE ROLE OF ION TRANSPORTERS IN POSTTRAUMATIC CYTOTOXIC BRAIN EDEMA.

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The pathological increase in intracellular osmolality is known to be involved in the formation of cytotoxic edema following traumatic brain injury (TBI). We wanted to determine which transporter(s) could be targeted to decrease cytotoxic edema. We used severe (4 atm) midline FPI in the rat. Acute brain slices were obtained 2 days post-FPI or sham operation, and randomly assigned to a control chamber and a test chamber, where they were incubated for 2 hours in the specific drug(s). Water content was computed as 100% (Wt.Wt-Dry.Wt) / Wt.Wt. Furosemide (2.5mM), a non-selective blocker of Na+/K+/Cl- co-transporter, caused water content elevation by 0.46 ± 0.14% in control (n = 5, p < 0.01) and by 0.3 ± 0.26% in post-FPI slices (n = 6, p = 0.01). Its selective blocker, bumetanide (50mM), did not affect water content in control (p = 0.18, n = 8, p = 0.32) and in post-FPI slices (p = 0.07 ± 0.08, n = 5, p = 0.13). DIDS (200mM), a non-selective blocker of Na+/2HCO3- co-transporter, did not affect water content (p = 0.1 ± 0.15, n = 7, p = 0.09) and slightly decreased it in post-FPI slices (p = 0.23 ± 0.21%, n = 6, p = 0.04). STS (50mM), a non-selective blocker of Cl-/HCO3- and Na+/HCO3- co-transporters, did decrease the water content in control (p = 0.13 ± 0.05%, n = 4, p = 0.01) and in post-FPI slices (p = 0.17 ± 0.03%, n = 4, p = 0.01). Amiloride (100mM), a selective blocker of Na+/H+ exchanger, caused water content elevation by 1.16 ± 0.26% in control (p = 0.01) and by 1.24 ± 0.33% in post-FPI slices (n = 5, p = 0.01). These results suggest that 1) no major ion transporter can be singularly targeted to completely and significantly control posttraumatic cytotoxic edema; 2) furosemide causes edema in normal brain and worsens edema following FPI, although it has previously been found to reduce cytotoxic edema in brain slices following an acceleration/hypoxia model of TBI. The previously observed beneficial effect of furosemide may depend on the traumatic animal model used. Supported by NIH NS04823 to RD.

P544. MODULATION OF NEURONAL AND GLIAL GROUP 1 MGLURS PREVENTS STRESS-INDUCED ENHANCEMENT OF NMDA RECEPTOR CURRENT

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Neuronal stretching in culture has been used to model diffuse axonal injury caused by acute head trauma and activation of N-methyl-D-aspartate receptor (NMDAR) activity. Following mild stretch, cortical neurons plated upon a confluent layer of astrocytes (NG), exhibited both increased maximal current (INMDA) and reduction in the voltage-dependent Mg2+ block. In contrast, neurons grown without an astrocyte monolayer (P7) only exhibited increased INMDA. In NG, prior activation of either mGluR1 or mGluR5 decreased the stretch-induced enhancement of INMDA. Similarly, prior inhibition of mGluR5 limited the stretch-induced INMDA changes whereas inhibition of mGluR1 had no effect. In contrast, in P7 inhibition of mGluR1 and mGluR5 prior to injury limited the stretch-induced enhancement of INMDA, whereas prior activation of mGluR1 or mGluR5 did not diminish the stretch-induced reduction of the Mg2+ block. In contrast, inhibition of mGluR1 exacerbated the stretch-reduced Mg2+ block in NG, but prevented the stretch-reduced Mg2+ block in P7. Inhibition of mGluR5 limited the stretch-reduced Mg2+ block in both culture conditions. Severe stretch had no effect on INMDA or the Mg2+ block in either culture condition, despite a correlation between injury severity and the release of lactate dehydrogenase measured post injury. None of the mGluR compounds used had any direct effects upon the NMDA receptor. Combined, these data suggest that during mild stress-induced injury, both group 1 mGluRs mediate enhanced NMDAR activity. We conclude that both neuronal and glial group 1 mGluRs regulate NMDAR activity during mild stretch-injury by modulating both the Mg2+ block and INMDA.
P545. INJURY-INDUCED CHANGES IN NMDA RECEPTOR SUBUNIT CONTRIBUTE TO PROLONGED CALCIUM-45 ACCUMULATION IN INTACT CORTEX
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The NMDA receptor's (NMDAR) physiological properties are imparted by its NR2 subunit composition. Receptors with a lower NR2A:NR2B ratio are more sensitive to glutamate, conduct larger currents, and are open for a longer time. A potential mechanism of the prolonged calcium accumulation following lateral fluid percussion injury (LFP) is an injury-induced alteration of the NMDAR subunit composition. To characterize the NMDAR subunit composition following LFP, 5 sham-injured and 6 mild-moderate LFP-injured rats were studied at each of 4 time points (1, 2, 4, and 14 days post-injury). Qualitative western blotting performed on region of interest homogenates using antibodies to NR2A and NR2B showed that the greatest alteration in the NR2A:NR2B relative ratio occurred in the ipsilateral parietal (p < 0.05) and occipital cortices at 1 day (20.5-33.2% decrease) and 2 days (8.8-12.5% decrease) normalizing to sham levels by 4 days. To investigate if this injury-induced subunit composition alteration contributes to the post-traumatic accumulation of calcium, 30 rats were subjected to mild-moderate LFP and at 1 or 2 days were treated with either saline (1d n = 5, 2d n = 5), MK-801 (n = 5; 0.3 mg/kg i.p.; inhibits the NMDA-associated ion channel in a non subunit-specific manner), or ifenprodil (n = 5; 30 mg/kg i.p.; inhibits NR2B subunit-containing NMDARs) followed by injection of calcium-45(μCi/g i.v.). After a five-hour uptake. brains were processed for autoradiography and optical densitometry. In regions where LFP does not alter NR2A:NR2B (ipsilateral frontal cortex). NR2B-specific ifenprodil blocked 25.4-41.9% of the NMDAR-associated calcium-45. However, in regions with a reduced NR2A:NR2B (ipsilateral parietal and occipital cortices), ifenprodil blocked 74.8-83.7% of NMDAR-associated calcium-45 flux, demonstrating that the ifenprodil-sensitive proportion of calcium influx is increased in regions with a decreased NR2A:NR2B. These results suggest that LFP induces a regional acute change in NMDAR subunit composition that is associated with a change in receptor function. Supported by NS30308 & NS27544.

P546. ASSESSMENT OF AGRIN EXPRESSION DURING TRAUMA-INDUCED SYNAPTIC PLASTICITY
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Agrin, a heparin sulfated proteoglycan located in the synaptic basal lamina, is known for its regulatory role in the formation of the neuromuscular junction. Interestingly, agrin mRNA is widely distributed throughout the adult rat brain, and has been associated with synapse formation (O'Connor et al., 1994; 1995). For example, agrin levels increase just prior to synaptogenesis in cultured hippocampal neurons (Perreault, 1999) and are required for the development of normal synapses. By contrast, suppression of agrin results in abnormal synapse formation in vitro. Given that traumatic brain injury (TBI) profoundly affects synaptic activity and that CNS agrin levels may be regulated in an activity-dependent manner (Rupp et al., 1996), we have assessed agrin expression during postinjury intervals of synaptic recovery. The present study examined agrin mRNA expression induced by either unilateral entorhinal cortex (UEC) lesion or moderate central fluid percussion TBI. RNA was isolated from rat hippocampal tissue at 7 days after trauma and RT-PCR was performed using specific primer pairs designed to produce a 200 bp agrin fragment. Contralateral unilateral hippocampi served as UEC controls and paired Sham-injured rats as controls for the TBI animals. Results showed that a 200 bp DNA fragment was produced in all cases. Initial analysis of this PCR fragment showed a 49% increase ipsilateral to UEC lesions when compared with the contralateral side, and a 26% increase after TBI relative to Sham-injured controls. These observations suggest that agrin may play a role in trauma-induced synaptogenesis. Supported by NS 12587; NSO 7288-13.

P547. MITOCHONDRIAL GENE EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY: ANALYSIS OF THE ND4 SUBUNIT OF COMPLEX I
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We have recently shown that traumatic brain injury (TBI) increases expression of the mitochondrial gene cytochrome c oxidase II, a protein integral to cytochrome c binding at complex IV of the electron transport (ET) chain (Harris et al., 2001). Given that the mitochondrial genome (mtDNA) appears to be sensitive to pathology elicited by TBI, and that elevations in ET chain proteins are associated with neuroplasticity (Yang et al., 2001), we have probed for changes in other mtDNA-encoded ET enzymes during postinjury intervals exhibiting synaptic recovery. The present study reports our current results from RT-PCR analysis of the ND4 subunit of ET complex I in rats subjected to moderate central fluid percussion injury. RNA was isolated from hippocampal tissue at 7 days after injury and RT-PCR was performed using specific primer pairs designed to produce a 200 bp ND4 fragment. Sham-injured animals were run in parallel as controls. RT-PCR was optimized by varying the concentration of both the RNA and cDNA templates, primer pairs and dNTPs, as well as cycle number. Results showed that a 200 bp DNA fragment was produced in both TBI and Sham samples. However, no significant difference in level of PCR product was observed after injury. This fragment, when excised and sequenced, was 100% homologous with the selected segment of ND4 mtDNA. These results suggest that transcription of ND4 is not altered periods of recovery following TBI, and support the hypothesis that ET complex proteins may exhibit a differential vulnerability to the pathology induced by brain injury. Supported by NS12587.

P548. AGE-RELATED CELL PROLIFERATION IN THE RAT CNS FOLLOWING TRAUMATIC BRAIN INJURY
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It has long been known that juvenile mammals recover much more completely than adults after traumatic brain injury (TBI). We have previously shown that TBI is the form of a lateral fluid percussion injury (FPI), greatly enhanced the total number of proliferating cells in both the subventricular zone (SVZ) and the hippocampus in adult rats. We, therefore, hypothesized that differences in neurogenesis may exist between adult and juvenile rats that could account for the improved functional recovery of the CNS in juveniles after injury. To test this, both juvenile (P28) and adult rats were subjected to a moderate FPI or sham injury. Following this injury (2.7. or 14 days), each animal received three i.p. injections of BrdU (50mg/kg) and was sacrificed 24 hours after the last injection. Coronal sections of brain were then stained for BrdU and the total number of BrdU-positive cells quantified. Preliminary results showed that there was a significant increase (P < 0.01) in the number of BrdU positive cells in the CNS of juvenile rats as compared to adult rats. This enhanced proliferation was localized to regions of the subventricular zone, corpus callosum, striatum and septal nuclei and was significant for up to 7 days post injury. These results, together with ongoing experiments aimed at determining the cell types of proliferating cells in the CNS in both juvenile and adult animals following FPI, may explain the fundamental differences in recovery as it relates to age. Supported by the Virginia Neurotrauma Trust.
P549.
SEVERE HEAD INJURY MANAGEMENT IN LATVIA
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Since 1998 when Neurosurgery Clinic of P.Stradiņa Hospital became a part of the international collaboration project with Brain Trauma Foundation (BTF), New York, USA there is a gradual improvement in the management of severe head injury (SHI) patients. BTF Guidelines have been widely used in the treatment of neurotrauma patients in Latvian hospitals. Patients with Glasgow Coma Scale (GCS) 3–8 admitted in our hospital within 12 hours after trauma have been managed observing BTF Guidelines and entered into internet database. In 1999–2000 123 patients were entered in Traumatic Brain Injury Survey database (Intracranial pressure (ICP) monitored in 83% patients). In 2001–2002 51 patients were entered in Traumatic Brain Injury: Quality Assurance Program (ICP monitored in 88% patients). GCS was used to compare early outcomes (10–14 days after trauma) in 2001–2002 and 1999–2000. GCS 13–15 31% (36% in 1999–2000), GCS 9–12 12% (11.9%), GCS 6–8 18% (13%), GCS 3–5 22% (11%), death 17% (29%). During four years of collaboration the death rate after SHI has decreased by 12%. The BTF Guidelines use has helped Latvia to develop the Head Trauma System thus improving the care for SHI in Latvian hospitals that admits neurotrauma patients. ICP monitoring is the key of postsurgical management. It is routinely performed in patients with SHI.

P550.
TRAUMATIC SUBARACHNOID HEMORRHAGE, EVOLUTION AND PROGNOSIS.
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Introduction: In head injury patients (HI) the cranial tomography scan (CT) image can change along the time and thus, the initial prognosis could be modified. Objective: To know the evolution along the time of the CT image in HI patients and traumatic subarachnoid hemorrhage (tSH). To determine the prognosis value of tSH seen at initial CT and those of the worst evolutive tSH image. Material and Methods: We studied prospectively 370 head trauma patients admitted consecutively to Intensive Critical Unit in 7 universities hospitals in Catalonia between February 1998 and January 1999. A CT was performed at admission. 24 hours later and when it was considered as one of the best clinical practice. The presence, and evolution along the time of tSH was assessed according to the Fisher scale. Initial and worst grade of tSH for each case were evaluated. A multivariate analysis adjusted for Glasgow Coma Scale, pupillary reactivity and age was made in order to know the prognosis value of tSH. A chi-squared test and odds ratio (OR) with 95% confidence interval was made, in order to compare the hospital mortality calculated between the initial and the worst tSH degree. Results: 190 patients showed tSH Fisher I. 51 Fisher II. 45 Fisher III and 84 Fisher IV at admission. tSH worsened in 13 cases (3.5%). From tSH Fisher I 4 cases changed to Fisher II and 3 to Fisher IV. From tSH Fisher II 2 cases changed to Fisher IV. From tSH Fisher III 4 cases changed to Fisher IV. All Fisher IV were the worst injured patients. The data indicate that at admission was 15.5%, 19.6%, 37.7% and 29.7% (p < 0.005). If tSH was present at admission, a highest mortality was observed (OR 2.21). The hospital mortality according to the worst degree of tSH was 13.7%, 29.7%, 39.1% and 31.9% for each group (p < 0.005, OR 2.26). There was not differences statistically significant between the hospital mortality according to the tSH admission classification and the worst evolutive degree (p = ns). Multivariate analysis showed that tSH was an independent prognosis variable. Conclusions: The tSH at admission hardly worsened. The presence of tSH is an independent prognosis variable. The prognosis value of tSH can be calculated following the first CT scan.

P551.
NMDAR-PSD95 INTERACTION MEDIATES SECONDARY TRAUMATIC NEURONAL INJURY.
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Neuronal damage from mechanically-induced trauma is comprised of "primary" and "secondary" components. Primary injury is the physical damage leading to cell death arising as the direct consequence of mechanical tissue deformation. Secondary injury arises from subsequent events at the tissue, cell and molecular levels that are triggered by, but are distinct from, the primary injury. As primary injury, by its nature, is not treatable, research is focused on understanding and treating secondary events in order to improve outcomes for CNS injury patients. Because primary and secondary traumatic injuries are intimately related, the mechanisms of secondary injury are difficult to study in isolation. By inducing stretch to cultured cortical neurons grown on flexible membranes, we developed a model in which stretch has no deleterious effect on neuronal survival (no primary damage), but renders the cells more vulnerable to subsequent insults (secondary injury). Using this model, our observations indicate that secondary injury occurred by distinct signaling via the NMDA subtype of glutamate receptors, as insults with sub lethal concentrations of NMDA and L-glutamate, but not kainate or the Ca ionophore A23187 produced secondary neuronal damage in stretched cells. The vulnerability of neurons specifically to NMDA toxicity occurred without stretch causing increased presynaptic glutamate release, without increased synaptic activity, and without effects on NMDA receptor-mediated ionic currents. Thus, this increased vulnerability occurred due to mechanisms downstream from, but specifically associated with, NMDA receptors. We investigated the role of the cytoskeleton in mediating the increased vulnerability of stretched neurons to NMDA toxicity. Actin and microtubule depolymerization did not reduce the vulnerability of stretched neurons to NMDA. Disrupting the NR2b-PSD95 linkage results in an attenuation of the vulnerability of stretched neurons to NMDA. These data indicate that the neurons subjected to sublethal traumatic injury become more vulnerable to a glutamatergic insult via a NR2b-linked signaling pathway.

P552.
SYSTEMIC HEMORRHAGE AND THE TYPE OF RESUSCITATION IMPACTS HIPPOCAMPAL FUNCTION FOLLOWING BRAIN TRAUMA.
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Clinical databases of human traumatic brain injury point to an association between hypotension and poorer outcome. Traumatically brain injured patients frequently suffer hemorrhagic shock secondary to systemic injuries. However, there is controversy regarding the optimal fluid resuscitation and hemodynamic targets of multi-system trauma patients with hemorrhagic shock. Controlled under-resuscitation is now advocated by some in the pre-hospital phase. This study was designed to use the functional evaluation of a model of traumatic brain injury to demonstrate and quantify the impact of hemorrhage, with the view of more closely calibrating the nature and target of optimal hemodynamic resuscitation. Male sprague-Dawley rats weighing 200-250gm were anesthetized and subjected to moderate intensity (1.8-2.0 atm) lateral fluid percussion injury or sham procedure. Immediately following, the animals were phlebotomized 50% of their calculated blood volume. After 30 min two groups of animals were resuscitated to near pre-injury blood pressure with either the hemorrhaged volume of blood or three times this with normal saline. After 2 hours the animals were decapitated, and hippocampal slices were studied at 36.5°C. The evoked population spike (PS) was extracellularly recorded in the CA1 pyramidal cell layer after stimulation of the Schaffer collaterals and the amplitudes compared between groups. At the maximum stimulating current (1.5mA), the PS amplitudes of sham, injured only and injured plus hemorrhaged animals were significantly different from each other at (mV ± SD) 8.6 ± 2.26; 4.9 ± 1.46; and 3.30 ± 0.98. The PS amplitudes from animals that received blood and normal saline resuscitation were 5.5 ± 1.49 and 3.57 ± 1.59. Hemorrhage following traumatic brain injury exacerbates the immediate hippocampal functional deterioration. Early resuscitation with blood prevented this while hemorrhagic shock (tSH) to the resuscitation with normal saline did not. Both the presence of systemic hemorrhage and the type of resuscitation impacted hippocampal function following brain trauma.
P553.
EXPERIENCE OF DIAGNOSTICS AND TREATING OF A SEVERE TRAUMATIC BRAIN INJURY (STBI), COMBINED WITH OPENED DAMAGES OF A CHEST.
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(Vladivostok State Medical University, Vladivostok, RU).

Experience of diagnostics and treating of a severe traumatic brain injury (STBI), combined with opened damages of a chest. OBJECTIVE. Intention of this study was to analyse of results of surgical treatment of the patients with STBI in a combined to opened damages of chest and determination of rational surgical tactics. METHODS. 10 consecutive multiple traumatic patients with severe head injury were treated between 1996 and 2001. The age of the patients was from 20 till 60 years, all—males. At 4 patients the bullet wounds of a skull were combined with bullet wounds of a chest, at 5—STBI (3—closed, 2—opened) was combined with penetrating knife wound of a chest and abdomen. At 1 there was a combination opened STBI and thoracoabdominal wound as a result of fall from height of 12 meters. A traumatic and hemorrhagic shock III–IV of a degree is marked at 6 patients. Diagnostics of multiple injured TBI was spent based on study of the mechanism of a trauma, clinical symptoms, radiographic and ultrasonic analysis, tapping of cavities. RESULTS. Six patients had closed TBI at 4—penetrating. The extensive fractures of the cranial vault and/or skull bases are revealed at seven sufferers. The 6 patients had attributes of the compression of the brain. The penetrating damages of the chest were accompanied by a wound of palmar at 5 patients. Wound of heart—1, hemorrhagic moorthy of a various degree—9 and haemothorax—1. The medical measures were directed on early liquidation of the disturbance of the external respiration, hemorrhage and struggle with a shock caused thoracic component of a trauma: adequate anesthesia, automatic controlled breathing, fluid and replacement therapy, aggressive therapy. The 6 patients was re-animation at receipt to hospital, water seal drainage of chest—8 patients. 5 sufferers is executed emergency laparotomy. 1—thoracoepiphrenolaparotomy. 1—thoracoctomy. Neurosurgical operation was executed for 6 patients. Four (49%) sufferers died. CONCLUSION. The patients with severe head injury and open damages of a chest need active surgical and neurosurgical treatmant.

P554.
HEAD INJURED PATIENTS WHO TALK AND DETRIMENTAL: ANALYSIS OF 86 CASES REGISTERED ON THE JAPAN NEUROTRAUMA DATA BANK.
Tatsuro Kawamoto, Tetsuya Katayama, and Japan Neurotrauma Data Bank Committee. (Japanese Society of Neurotraumatology, Department of Neurological Surgery, Nihon University School of Medicine, Tokyo, JP).

[Introduction] In order to clarify the clinical profile of head-injured patients who talk and deteriorate into coma, we reviewed 721 patients with head injuries who were registered on the Japan Neurotrauma Data Bank (Japan Society of Neurotraumatology) from 1996 to 2000. [Results] Eighty-six patients (12%) talked prior to deterioration, and 81 deteriorated into coma (Glasgow Coma Scale (GCS) <= 8 p). In all cases, CT scans revealed development of focal lesion(s) with a mass effect and resultant midline shift. Forty-three patients (50%) had a subdural hematoma, 25 (29%) had a cerebral contusion/intracerebral hematoma, and 18 (21%) had an epidural hematoma. The Glasgow Outcome Scale was GR in 24 (24%), MD in 11 (13%), SD in 13 (15%), VS in 5 (6%), and D in 36 (42%). The latent periods to deterioration were <= 3 hours in 59 (72%), 3–6 hours in 12 (15%), and > 6 hours in 11 (13%), demonstrating a shorter latency than those reported in previous studies. Sixty-four patients (74%) underwent surgery, i.e. evacuation of hematoma, and/or contusion necrotomy. The predictors for a poor outcome were a low GCS following deterioration, subdural hematoma, and being an elderly patient. In contrast, GCS during lucid intervals, and the length of time until deterioration or until operative intervention did not influence the final result. [Conclusion] A majority of cases (87%) showed deterioration within 6 hours post trauma, caused by a progressive mass effect. Deterioration into a low GCS resulted in a poor outcome, so that early operative intervention is strongly recommended prior to the inevitable deterioration.

P555.
SURGICAL COMPLICATIONS OF DECOMPRESSIVE CRANIECTOMY FOR HEAD INJURY.
Craniotomies I.5, Gambini R, Damante R, Ravenna G, Moscato F, Trevigne MA, Vivona C. (Department of Neurosurgery, University Hospital, Verona, IT).

Background: although in the last decade many published papers showed a renewed interest for this ancient surgical procedure and a debate is still open. In our knowledge few authors mentioned about complications. Patients and methods: we reviewed and analyzed clinical and TC data of all comatose head injured patients admitted in our Department in the period 1996-2001 and operated on for decompressive craniectomy and checked any surgical complication also in the post discharge period till one year. Results: 35 patients were included , age ranged from 14 to 69 years with a mean age of 36.6. The initial mean Glasgow Coma Scale on admission was 5. Decompressive craniectomy was not performed routinely but on surgeron judgment instead of simple debridement or lobectomy also performed in our Institute. Bone was stored and frozen at -80. Within 12 hours 9 patients (26%) had epidural or subdural collection far from the craniectomy and 6 re-operated. Bone flap was reversed at variable time but 5 patients needed shunt after reverse for clinical and radiological signs of concomitant hydrocephalus and all showed a degree of clinical improvement. Discussion and conclusion: in our knowledge this is the first report of a group of surgical complications after decompressive craniectomy for head injury and this should be weighted in the decision-making process. Beneath the number of patients is small, our experience suggests that early CT after decompression is needed and time of reverse bone flap and its influence on cerebrospinal fluid dynamics(1) should be investigated. REFERENCES: (1) Cosynska and coll.: Post traumatic hydrocephalus: influence of craniectomy on the CSF circulation. Letter to the editor. J Neurol Neurosurg Psychiatr 2000;66:248-256.

P556.
EARLY EDema FORMATION IN CEREBRAL CONTUSION: ULTRA-early study (<24 hours post-trauma) with diffusion MRI and ADC MAPPING.
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In the previous studies, we have reported that heterogeneous mechanisms exist in early edema formation in cerebral contusion, and cytotoxic edema plays an important role within 48 h post-injury. It is remains unclear, however when edema begins to develop following injury. In order to determine the time course of edema development, diffusion imaging and ADC (apparent diffusion coefficient) mapping are performed in 10 patients within 24 hours post-trauma with cerebral contusion using a 1.0T echo planar MRI. Within 3 hours post-trauma, diffusion MRI showed no remarkable changes, and the ADC values were within normal limit (ADC ratio = 1.00±0.21, (mean±SD)). At 6 hours post-trauma, diffusion images demonstrated a low intensity core in the contusion proper and a high intensity rim in the peripheral area of contusion. The ADC value increased in the contusion proper (ADC ratio = 1.26±0.13) and decreased in the peripheral area (ADC ratio = 0.58±0.19). These findings indicated that early cellular swelling in the peripheral area of contusion begins within 6 hours following injury. This delayed occurrence of contusion-induced cellular swelling suggests that the CBF does not decrease to ischemic level immediately following injury.
P557.
DOES THE USE OF JUGULAR BULB OXYGEN SATURATION IMPROVE THE PROGNOSIS IN HEAD INJURED PATIENTS?


Introduction: To use jugular bulb oxygen saturation (SjO2) determination helps in the proper management of head injury (HI) patients. It is widely used and could have a beneficial effect on mortality, but its efficacy has not been proved. Objective: A: To identify among HI patients with intracranial pressure (ICP) monitored those where the SjO2 is more frequently used. B: To determine if the use of SjO2 improves hospital mortality. Material and Methods: Of the 370 HI patients admitted consecutively to ICUs in 7 teaching hospitals in Catalonia between February 1998 and January 1999, we studied the cohort of 184 cases who underwent intracranial pressure monitoring. The patients were managed according to each hospital clinical practice. Demographic data were collected and patients were classified according to Glasgow Coma Score on admission (GCS) and cranial tomography scan (CT) image using both the Traumatic Coma Data Bank (TCDB) and a morphological product classification. Variables related to hospital mortality were analysed. Univariate and multivariate analysis were performed in order to identify the groups of patients in which the SjO2 was used and its potential benefit. Results: The patients had mean age of 36.7±19 years, mean GCS 6.78±3.3 points and hospital mortality was 29.3% (54/184 cases). The SjO2 was used in 96 patients (52.2%), was more frequently used in younger patients (54 ± 18 vs 40 ± 20 years, p < 0.05), with lower GCS (6.5 ± 3.4 vs 7.3 ± 3.2 points, p < 0.05), in those with at least one episode of ICP > 25 mmHg during 10 minutes (72% vs 37%, p < 0.001) and in those with CT classification: TCDB III or IV versus TCDB I or II (65% vs 43%, p < 0.05). We did not find any significant differences in its use related to pupillary reactivity: subarachnoid hemorrhage, morphological lesion on CT or hospital. The bivariate analysis did not identify any group of patients in which the use of SjO2 reduced the hospital mortality. When logistic regression analysis adjusted for variables statistically significant related with hospital mortality (age, GCS, pupillary reactivity, ICP > 25 mmHg and CT lesion) was performed, the use of SjO2 did not show any benefit. Conclusions: In our area the SjO2 catheter is widely used. It is more frequently used in younger patients with lower GCS. Its use in clinical practice does not appear to provide benefits in relationship to hospital mortality.

P558.
DIFFUSE AXONAL INJURY FOLLOWING FLUID PERCUSSION TRAUMATIC BRAIN INJURY IN THE RAT: CHARACTERIZATION AND CORRELATION BETWEEN ELECTROPHYSIOLOGICAL AND HISTOLOGICAL FEATURES.

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Diffuse axonal injury (DAI) is associated with poor outcome following traumatic brain injury (TBI). While the temporal pattern of histopathological changes involved in posttraumatic axonal injury has been well described, there has been no clear correlation between these observations and the functional status of axons in cerebral white matter. We sought to quantify the functional derangement of axons in a major cerebral white matter tract following TBI and correlate this with histological and molecular markers of injury. Adult male rats underwent central fluid percussion-induced TBI. Compound action potentials (CAPs) were recorded in the corpus callosum at 3h, 1d, 3d, 7d, 2wks and 4wks following injury and compared to sham (n = 5/group). Brains were harvested in shams. 3d. 7d. 2wks and 4wks (n = 4/group), sectioned, and stained for APP, injured myelin and TUNEL, and positively stained cells in the corpus callosum were counted. Corpus callosal CAPs in sham, mild and moderate injury were 1.11 ± 0.10mV, 0.82 ± 0.11mV and 0.49 ± 0.08mV respectively. After moderate injury, the CAPs were 0.55 ± 0.08 at 3h, 0.61 ± 0.08 at 1d, 0.60 ± 0.09 at 3d, 0.93 ± 0.21 at 7d, 0.62 ± 0.21 at 2wks, and 0.59 ± 0.08 at 4wks. APP expression was significantly increased at 3d, 7d, 2wks and 4wks, peaking at 7d. Injured myelin staining was also increased at these time points with a peak at 4wks. TUNEL counts were significantly higher at 3d, 7d and 2wks, peaking at 7d. APP and injured myelin deposition was distributed caudally and laterally in the corpus callosum, while apoptotic cells were located in the central portion. This study is the first to report an evaluation of the degree and temporal pattern of axonal dysfunction following TBI. Reversibility was seen between 3d and 7d electrophysiologically but not histologically. This highlights the value and importance of combined functional and morphological approaches in the evaluation of experimental TBI, especially DAI.

P559.
DELAYED TRAUMATIC INTRACEREBRAL HEMATOMA AND COAGULOPATHY IN THE PATIENTS DIAGNOSED WITH A TRAUMATIC SUBARACHNOID HEMORRHAGE.

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The detection of delayed traumatic intracerebral hematoma (DTICH) has increased steadily with improved imaging. However, the pathogenesis of DTICH has not been clearly elucidated. It has long been recognized that a traumatic insult to brain tissue may result in substantive coagulation abnormalities. The present study was carried out in an attempt to find out the association of coagulopathy and the development of DTICH in patients diagnosed with a traumatic subarachnoid hemorrhage (TSAH). Sixty-three patients were diagnosed as having TSAH from the initial CT scans obtained within 2 hours after trauma. On admission, peripheral blood samples for coagulation studies, including platelet, thrombin time, prothrombin time, activated partial thromboplastin time, fibrinogen, serum fibrinogen degradation product (FDP) were taken within 6 hours after injury. All patients had subsequent CT scans performed within 24 hours of admission. Thirty (47.6%) of 63 patients exhibited radiological evidence of DTICH on their subsequent CT scans. There was a significant correlation between the increased value of serum FDP (>40 micrograms/ml) and the development of DTICH. We observed that the origin of the hematoma might be caused by those radiographically unidentifiable parenchymal lesions often found with TSAH on the initial CT scan. We conclude that a clotting study at the time of admission is of value in predicting the occurrence of DTICH associated with TSAH.

P560.
PRELIMINARY REPORT: TRAUMATIC COMA DATA BANK PROJECT IN JAPAN.

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An epidemiologic research was conducted on medical treatment for traumatic head injury in Japan from January, 1998 to June, 2000. Sudden death by accident* including severe head injury is the third major cause of death in our country. National Police Agency announced the numbers of death by traffic accident as 10,000 per year. According to Ministry of Health, Labour and Welfare, traffic death reaches 13,000. Severe head injury is supposed to be a majority of them. Unfortunately these statistics include little information from medical facilities and we don't have any comprehensive data that describes situation around traffic death or severe head injury from a medical point of view. Ten medical emergency centers took part in the traumatic coma data bank project. Patients with severe head injury were eligible for entry with a Glasgow Coma Scale (GCS) Score of 9 or less. Children under 6 years old were excluded. We made an original data sheet with 376 items containing information about characteristics of the injury, prehospital treatment, diagnosis, treatment and follow-up information concerning outcome. This time, we studied 721 cases, classified into two groups; 445 cases of motor vehicle accident and 276 cases of non-motor vehicle accident. While focal brain injuries occupy a majority in the non-motor vehicle accident group, diffuse brain injuries surpass in number in motor vehicle accident group. We are still in the preliminary stage for this clinical study. However, we hope that our project will explain in part the actual circumstances of severe head injuries in Japan and that our report will be a significant data to be used in international comparative survey.
P562. THE X-CHROMOSOME-LINKED INHIBITOR OF APOPTOSIS (XIAP) PREVENTS CELL DEATH IN THE 158N IMMORTALIZED OLIGODENDROGLIAL CELL LINE
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Apoptotic cell death is a fundamental biological process involved in the normal development of the nervous system. However, apoptosis contributes to cell death in certain neurodegenerative diseases as well as following traumatic spinal cord injury, head trauma, and ischemia/stroke. The apoptotic program is executed by caspases, which are regulated, in part, by the inhibitor of apoptosis (IAP) family of proteins. The X-chromosome-linked IAP (XIAP) has been reported to reduce cell death in response to a variety of apoptotic stimuli. In this study, we examined whether transient overexpression of XIAP protected an oligodendroglial cell line (158N) from apoptotic cell death induced by staurosporine (STS) or dopamine (DA) treatment. 158N cells were transfected with either pCMV-Myc-XIAP or pCMV-Myc plasmid. At 48 hr post-transfection, western blotting and immunocytochemical staining showed robust XIAP overexpression in pCMV-Myc-XIAP transfected cells relative to non-transfected or pCMV-Myc transfected cells. Subsequently, similar groups of 158N cells were treated with STS (100nM) or DA (300µM) and cell viability was determined using the MTT and Live/Dead assays. As expected, STS treatment for 4 hr or DA treatment overnight resulted in significant cell death in non-transfected and pCMV-Myc transfected cells. In contrast, there was significant survival of cells transfected with pCMV-Myc-XIAP. These results show that XIAP overexpression in vitro protects cells from apoptotic cell death and suggests a therapeutic role for XIAP overexpression on oligodendroglial survival following an insult in vivo. Sponsored by NS40015 and KSCHTD (JES).

P563. SECONDARY COMPLICATIONS IN ELDERLY INDIVIDUALS WITH ACUTE TRAUMATIC SPINAL CORD INJURY
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Relatively little has been reported regarding secondary complications in the elderly (age ≥60 y) after spinal cord injury (SCI). This study was undertaken to clarify this critical issue, given the increasing prevalence of SCI in the elderly. Data were analyzed using ANOVA. Student-t and Chi-square tests. Complications occurring in the acute stage of SCI (≥60 days) in 78 consecutive patients with traumatic SCI admitted during a three-year period were analyzed. There were 52 elderly (14 F; 18 M; mean age 74 ± 11 y), and 46 younger (9 F; 37 M; mean age 39 ± 11 y) individuals. The severity and level of SCI were similar in both groups (P = 0.21; P = 0.73). Medical comorbidities were more frequent among elderly patients (84.6% vs. 39.1%; P < 0.01). Secondary complications were also significantly higher in geriatric patients (56.3% vs. 23.9%; P = 0.01). There was a trend, which did not achieve significance, for increased mortality in the elderly (elderly: 12.5% vs. 2.2%; P = 0.15). The most common secondary complications in elderly individuals were: infection (56.3%); psychiatric disorders (28.1%) and cardiovascular disturbances (15.6%). Early autonomic dysreflexia (5%) was observed only in younger individuals. The geriatric population is more susceptible to secondary complications after acute SCI. Greater awareness by clinicians is essential to minimize secondary complications after SCI and to improve quality of life for elderly individuals (Supported: Heart & Stroke Foundation of Ontario).

P564. AGE-DEPENDENCY ON DEVELOPMENT OF NEUROPATHIC PAIN BEHAVIOR FOLLOWING SPINAL CORD INJURY IN RATS
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Spinal cord injury (SCI) often leads to chronic central pain (CCP) syndromes such as allodynia and hyperalgesia. Although several experimental animal models for CCP exist, little is known about the effect of age on the development of CCP following SCI. In this study, we evaluated behavioral outcomes to mechanical and thermal stimuli using three different ages of Sprague-Dawley rats following SCI. SCI was induced by hemisection of the spinal cord at T13. Behavioral outcomes were measured by paw withdrawal frequency (PWF) in response to 10 applications of mechanical stimuli (von Frey filament) and paw withdrawal latency (PWL) to radiant heat stimuli on both the forelimbs and hindlimbs. In forelimbs, young rats (164.6 ± 5.46 g. 40 days) displayed increased PWF to mechanical stimuli (4.17; 9.48 nN) compared to adult rats (273.2 ± 8.09 g. 60 days) on both sides, whereas old rats (546 ± 12 g. 12 months) did not change. In addition, PWL of young and adult rats significantly decreased on both sides whereas PWL of old rats did not change. In hindlimbs, PWF of young rats significantly increased on both sides whereas adult and old rats did not change. Also, young rats (ipsilateral) and young and adult rats (contralateral) displayed significantly decreased PWL, but old rats did not change on either side. These results indicate that younger rats developed more robust neuropathic behaviors than older rats, indicating that age selection is important in animal models of CCP syndromes following SCI.
P565.
GENECHIP ANALYSIS AFTER ANEURYSM CLIP-INDUCED SPINAL CORD INJURY IN MOUSE: A COMPREHENSIVE STUDY OF CHANGES IN EXPRESSION OF GLUTAMATE RECEPTOR-APOTOPSIS-ASSOCIATED GENES; AND GENES RELATED TO OXIDATIVE STRESS

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Spinal cord injury (SCI) still remains one of the most devastating forms of trauma. During the last decades new biological techniques have greatly extended our understanding of molecular events during SCI. Microarray DNA chips are the newest tools for comprehensive gene expression studies. Although genechips have been used for the spinal cord injury models in rats, currently there is no information available on the mouse model of SCI. Current observations in our laboratory have shown alteration in glutamate receptor expression after SCI. Spinal cord injury is also associated with apoptosis in neurons as well as glial cells. There are also some reports on involvement of oxidative stress induced damages after SCI. In present study we have used customized 15.5k mouse cDNA microarrays to address the differential gene expression in a model of moderate SCI injury. We have investigated the following objectives: 1) Differential expression of glutamate receptors after SCI, 2) Temporal expression pattern of apoptotic related genes, and 3) To seek whether the expression of oxidative stress related genes is altered during SCI. An 8.3 g clip was used to induce moderate SCI at T7 level. Specific cDNAs for Glu-R1-7, Kainate 1-2, NMDAR1, R2A, R2B, R2C and R3D and R3A as well as metabolic glutamate reductase were prepared using One-step RT-PCR. The cDNAs were then printed on 15.5k mouse microarrays. Total RNA was extracted at 1, 6, 24, 48 hrs and 1, 2, 6 and 7 weeks after SCI. Cy5 and Cy3 labeled cDNA probes were generated using indirect labeling method. The results are currently being quantified and will be presented at the NINTS meeting. This study is the first application of genechips in mouse model of SCI. Funded by CIHR (MT-14459).

P566.
ALtered DISTRIBUTION AND EXPRESSION OF KVL.1 AND KVL.2 K+ CHANNELS IN SPINAL CORD WHITE MATTER AFTER CLIP COMPRESSION SPINAL CORD INJURY: ACUTE AND CHRONIC IN VIVO STUDIES

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Following spinal cord injury (SCI) surviving white matter axons display axonal dysfunction due to demyelination, and show enhanced sensitivity to K+ channel blockers such as 4-aminopyridine (4-AP) and d- (+)-tubocurarine (d-ATX). Studies from our laboratory have shown that this abnormal axonal function is associated with increased expression and altered distribution of Shaker voltage-gated K+ channel subunits Kv1.1 and Kv1.2 on spinal cord axons after chronic SCI (Nashmi et al., 2000). In normal CNS axons Kv1.1 and Kv1.2 subunits are highly co-localized in the juxtaparanodal regions; whereas after SCI they acquire a dispersed immunostaining pattern along the axons. In this study, we have investigated Kv1.1 and Kv1.2 subunits expression in white matter at varying times after in vivo clip compression SCI at T7. Using western blot analysis, we found an increased expression of Kv1.1 and Kv1.2 between one to two weeks after injury. We also used confocal immunocytochemistry to study the distribution of Kv1.1 and Kv1.2 along the injured axons. In contrast to uninjured spinal axons with juxtaparanodal localization, Kv1.1 and Kv1.2 showed a markedly dispersed labeling along the internodes of injured axons. This redistribution of Kv1.1 and Kv1.2 occurs as early as 1 hr postinjury along some injured axons, and appeared to be more pronounced one week after injury and evolves over time. To seek for mechanisms involved in aberrant distribution of Kv1.1 and Kv1.2 after SCI, we examined the localization of Caspr (contact-associated protein) which is found in the paranodes and is believed to separate K+ and Na+ channels in myelinated axons. Our immunostaining indicated a more diffusely localization of Caspr along injured spinal cord axons. These results suggest that redistribution of Caspr may be associated with aberrant localization of K+ channel subunits Kv1.1 and Kv1.2 on spinal cord axons after SCI. Funded by CIHR (MT-14459).

P567.
INOS INHIBITION BY PHARMACOLOGICAL OR GENE THERAPEUTIC MEANS LEADS TO REDUCED BLOOD-BARRIER PERMEABILITY AND NEURONAL SURVIVAL AFTER SPINAL CORD INJURY (SCI).


INOS is a key mediator of inflammation. INOS is expressed during CNS injury and is responsible for the formation of high levels of nitric oxide, due to both a stable mRNA transcript and its calcium-independent activity. The production of highly reactive and cytotoxic O2 species e.g. peroxynitrite, in turn leads to tissue damage. We have used an acute administration of INOS antagonist olmesartan (ASO) 3h after moderate contusive injury and the pharmacological inhibitors; 1400W (two injections i.v., 3 and 9h) and aminoguanidine (two injections i.p., 0 and 6h), to decrease the number of INOS immunoreactive cells and INOS activity at the site of a spinal cord contusive injury. Both INOS ASO and pharmacological inhibition of INOS reduced the degree of blood-brain-barrier-disruption (measured by calculating the area of plasma leakage of rat immunoglobulin G), however, that mediated by INOS ASO inhibition was much more pronounced and was comparative to an increased ablation of INOS. Hypertrophic astrocytes and marked gliosis in the white matter were also present with INOS inhibition. Hypertrophic astrocytes may allow these cells to help repair vasculature integrity and aid in preventing leakage. We observed that only the dramatic inhibition of INOS by ASO was able to reduce neuronal necrosis in the dorsal horn compared to controls. Neuronal necrosis was detected in vivo by examining if cell membrane disruption had occurred using an intra sub-archnoid injection of propidium iodide before perfusion. Neuronal accumulation within the injury site was not significantly reduced by any of the treatments. These novel findings report that an inhibition of INOS acutely is beneficial in retarding SCI pathophysiological processes. (Supported by NIH NS38665 and The Miami Project).

P568.
NEUTROPHIL INFILTRATION AND HEME OXYGENASE-1 INDUCTION ARE EARLY PROGNOSTICATORS OF SPINAL CORD INJURY SEVERITY

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We have previously shown that tissue damage in the injured spinal cord is in part related to an early inflammatory response that involves neutrophil infiltration. activation of matrix metalloproteinase-9 (MMP-9), and induction of heme oxygenase-1 (HO-1). In this study, we subjected adult male mice to either a moderate or severe spinal cord contusion injury and determined the extent to which neutrophil infiltration, MMP-9 activity, and HO-1 induction reflect injury severity. To assess neutrophil infiltration, we developed a novel method for extraction and quantification of neutrophils in the spinal cord using flow cytometry. We found that neutrophil infiltration was significantly greater in the more severely injured group. In contrast, MMP-9 activity defined by gelatin zymography, was similar within each of the injury groups. HO-1 induction, as determined by immunocytochemistry, appeared more robust with a greater axial distribution in the more severely injured group. Since it is also a marker of oxidative stress, we next determined the extent to which HO-1 induction correlated with patterns of blood flow using a lectin perfusion technique. In regions of no flow HO-1 was not induced. whereas endothelial induction occurred in low flow regions, and glial/endothelial induction was present in regions of relatively normal flow. Thus, cell specific HO-1 induction correlated with flow. We conclude that neutrophil infiltration and HO-1 induction can be used as early indicators of spinal cord injury severity. Moreover, cell-specific induction of HO-1 may serve as an index for defining relative vulnerability of cells to changes in the local microenvironment. Supported by NS 39278, NS39847, and the Dana Foundation.
P569.
A NOVEL APPROACH TO THE ONSET-MECHANISM OF CERVICAL SPONDYLOTIC MYELOPATHY: COMPUTER SIMULATIONS BASED ON MECHANICAL FEATURES OF THE SPINAL CORD.
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In cervical spondyiotic myelopathy (CSM), there are four pathologic and clinical features without adequate explanations: 1) the spinal cord is highly tolerant towards slowly progressive chronic compression; 2) the lateral corticospinal tract is more severely damaged than other white matter tracts; 3) the anterior spinal artery is almost never occluded; 4) symptoms often show step-wise progression. We previously found that (a) gray matter is more rigid although more fragile than white matter (J Neurotrauma 2001), (b) continuous compression of the spinal cord results in a gradual decrease in cord stress; (c) cord stress increases proportional to the speed of compression. In the present study, the four features were examined in 3 computer simulation models based on a), b) and c) mechanical features. Three models were simulated in vitro. A) Acute compression. B) Chronic compression(CSM). C) Acute on chronic compression(CSM). In the acute model, damage occurred in the gray matter and then the lateral corticospinal tract. In the chronic CSM model, gradual compression caused a gradual decrease in stress, the anterior funiculus did not show damage, and the anterior spinal artery was not severely compressed. In the acute on chronic compression model, cord stress increased in the gray matter and then in the lateral corticospinal tract, belging of the ligamentum flavum repeatedly compressed the spinal cord. and CSM mechanical features were gradually aggravated. Thus, anterior and posterior acute compression easily damages the lateral corticospinal tract and the decreased cord stress could also be related to high tolerance towards against slowly progressive chronic compression and the plasticity of the spinal cord.

P570.
ELECTROPHYSIOLOGY AND FUNCTIONAL IMAGING OF MATURE GLIAL CELLS IN SITU USING RAT SPINAL CORD SLICES
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Electrophysiological studies of functional properties of glial cells in spinal cord white matter have been mostly confined to cell cultures or acute slices of immature spinal cord of experimental animals (mice, rats). We have developed a viable acute in vitro 240 um-thick longitudinal slice preparation of mature rat spinal cord with preserved white matter compound action potentials, that allows for visual identification and patch clamp recording of white matter glial cells within their natural networks. The cells are visualized within the slices using infrared differential interference contrast videomicroscopy and approached with recording electrodes under visual control. The advantage of using longitudinal slices over more commonly used transverse slices of spinal cord is preserving the integrity of local white matter glial-axonal networks which is especially important for oligodendrocytes which extend processes for distances up to 250 um along the axons. Oligodendrocytes are easily identifiable by their close association with axons, which is further confirmed following intracellular injection of fluorescent dyes Lucifer Yellow or Alexa 350 that revealed long processes closely associated with axons. Astrocytes are identified by their characteristic stellar morphology and GFAP-positive immunostaining. Both oligodendrocytes and astrocytes (membrane resting potentials ranging between -42 mV and -66 mV, n = 58 and between -37 mV and -71 mV, n = 35, respectively) showed characteristic non-linear current-voltage relationships and pronounced voltage-dependent potassium currents activated at membrane voltages positive than -40 mV. AMPA/kainate agonists applied by microsuperfusion activated CNQX-sensitive inward currents (at -70 mV) in both types of cells. Our data represent the first electrophysiological recordings from mature spinal cord white matter astrocytes and oligodendrocytes in situ. The longitudinal slice preparation is viable for up to 8 hours in vitro and can be successfully used for both electrophysiological and fluorescence imaging studies of glial-axonal interactions in normal and post-injured spinal cord. Supported by CIHR and ONF.

P571.
NEUROPROTECTION AFTER ACUTE SPINAL CORD INJURY BY INHIBITION OF THE FAS APOPTOTIC PATHWAY
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Background: Recent evidence from our laboratory has shown an association between FAS receptor expression and apoptosis following acute spinal cord injury (SCI) (Cash et al Neuroscience 2001). Hypothesis: Administration of soluble Fas receptor (sFasR) in vivo may reduce SCI. Methods: Experiment 1. Using an organotypic slice model of injury, cell death was quantified using propidium iodide and sytox green. Experiment 2. In vivo SCI was induced using a clip compression injury model. With a mini-osmotic pump, sFasR was introduced to the intrathecal space at the level of injury. Immunoabsorb were used to identify NF 200, and caspase-3 activation. Results: The in vivo model of injury has shown decreased cell death with the administration of sFasR. In vivo preliminary results indicate decreased caspase-3 activation. Conclusion: The Fas receptor appears to be a promising new neuroprotective target after SCI. Funding: Ontario Neurotrauma Foundation Studentship (AA); Christopher Reeve Paralysis Foundation Operating Grant, Ontario Ministry of Health Career Scientist Award and Krembil Chair in Neural Repair and Regeneration (MGF).

P572.
DEATH RECEPTOR EXPRESSION AFTER HUMAN CERVICAL SPINAL CORD INJURY: IMPLICATIONS FOR THE DEVELOPMENT OF NOVEL NEUROPROTECTIVE STRATEGIES.
W. Bradley Jacobs, Wen Ru Yu*, Michael G. Fehlings. (Toronto Western Research Institute, University of Toronto, Toronto, Ontario, Canada).

BACKGROUND: Despite recent advances, therapies for spinal cord injury (SCI) are minimally effective. Improved neuroprotective approaches are needed. Our laboratory has recently implicated the death receptors, Fas and p75NTR, as mediators of post-SCI apoptosis in experimental models. To determine if death receptor mediated apoptosis is relevant to human SCI, we have examined molecular mechanisms of cell death in injured human cervical spinal cord tissue. METHODS: Eight cases (2 females/6 males, mean age 53 years) with SCI (2 wks–2 yrs post-trauma) were examined. Using hematoxylin and eosin/huxol fast blue staining, the morphology of the injury epicenter and areas rostral and caudal to the lesion were assessed by light microscopy. Apoptotic cells were identified via TUNEL staining and activated caspase-3 immunohistochemistry. Death receptor expression was determined using antibodies against Fas and p75NTR. RESULTS: Apoptotic cell death in neurons and oligodendrocytes was a prominent feature of human SCI. An increase in TUNEL positive cells was noted as compared with age-matched controls (p < 0.05). Caspase-3 activation occurred after SCI. Fas and p75NTR death receptors were expressed in adult human spinal cord providing supportive evidence linking this mechanism with SCI. CONCLUSIONS: Our findings suggest that death receptor mediated apoptosis occurs after human SCI and that this mechanism is a clinically relevant target for neuroprotective strategies.
P573. EVALUATION OF THE NEUROPROTECTIVE EFFECTS OF THE SODIUM CHANNEL BLOCKER RILUZOLE AND METHYLPREDNISOLONE IN A NOVEL ORGANOTYPIC SLICE CULTURE MODEL OF SPINAL CORD INJURY

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Until recently, methylprednisolone (MPS) was thought to be the standard of care for the acute treatment of spinal cord injury (SCI). However, concerns regarding the modest clinical benefits of MPS have resulted in the downgrading of this treatment to that of an option based on newly disseminated guidelines from the American Association of Neurological Surgeons/Congress of Neurological Surgeons. Based on this, further work to develop alternative or complimentary neuroprotective approaches to MPS are urgently required. Previously, we have shown that acute administration of riluzole (RIL), a sodium channel blocker, can mitigate secondary tissue loss, preserve axonal integrity, and enhance locomotor recovery in rodents with severe cervical SCI. In order to assess the neuroprotective effectiveness of RIL alone or in combination with MPS compared to MPS alone we evaluated mean cell death counts of pyridium iodide (PI) labeled cells in an organotypic spinal cord slice injury model prepared from adult mice. Weight-drop injured slices were treated with 10 um of RIL, MPS or RIL+MPS. 15 minutes post injury. Fluorescent PI-labeled cells were imaged by confocal microscopy at 4, 24, and 48 hours after treatment. Mean PI cell counts from each treatment group were normalized against total cell death counts from corresponding treated slices to generate a percentage of cell death. Analysis by ANOVA indicated a significant effect of treatment (F: 3.59; p = 0.025) with RIL and RIL+MPS treated slices having a smaller percentage of labeled cells than those treated with MPS alone or those not treated with a drug. These findings suggest that RIL alone or in combination with MPS is an effective neuroprotective strategy for SCI. Further preclinical in vivo evaluation of the effective time window for RIL +/- MPS is warranted as a prelude to consideration for translation to clinical trials. Supported by the Ontario Neurotrauma Foundation.

P574. AMPA RECEPTORS AFFECT THE DEVELOPMENT OF CHRONIC TONIC PAIN (CPP) AFTER SPINAL CORD INJURY (SCI)

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SCI results in CPP development in most patients. Cyclothiazide (CTZ), a positive allosteric modulator of AMPA receptors, potentiates the AMPA receptor by blocking desensitization whereas NBQX is a competitive antagonist. NBQX but not CTZ was hypothesized to improve behavioral outcomes. return glutamate receptor (GluR) subunit expression to control values. and protect neurons from excitotoxicity by blocking actions excitatory amino acids (EAA). To evaluate the effects of agents on EAA release, samples were collected for HPLC analysis before, during and after SCI (injury to T10 with an NYU impactor; 12.5 mm drop. 10 gram rod of 2 mm diameter) with microdialysis fibers inserted into the cord. Injury was immediately followed by an epicenter injection of the agent. Neither NBQX (15 nmol) nor CTZ (7 nmol) significantly affected the release of EAA. However, injection of the same amounts of the agents altered behavior and protein expression. CTZ administration returned thermal forelimb withdrawal latencies to control at post contusion days (PCD) 7 and 14 (p < 0.05). NBQX also increased thermal forelimb withdrawal latencies, but much later (PCD 47 and 54. p < 0.05). Protein expression after SCI was evaluated by western blot analysis at PCD 7 and 28. NBQX but not CTZ returned the expression of Glur2 to near non-SCI levels. We propose that NBQX stabilizes the composition of the AMPA receptor to its usual form by retaining Glur2 expression near its control levels in the cord, thereby decreasing the calcium influx and reducing the debilitating effects seen in SCI.

P575. TRANSPLANTATION OF HUMAN OLFACTORY ENSEHENDING CELLS IN THE INJURED ADULT RAT SPINAL CORD


In the present study we investigate the ability of human olfactory ensheathing cells (hOECs) to facilitate axonal regeneration and behavioral recovery following moderate contusion injury in the adult rat. Human neuroepithelial tissue was obtained via biopsy, providing a source from which hOECs were then isolated, cultured, and amplified in vitro. Five to seven days following contusion injuries in adult female rats, single-cell hOEC suspensions were transplanted into the lesion site. The animals were monitored and behaviorally tested over a period of 6 weeks post-injury. Fluoro-ruby was then injected in the motor cortex to label the descending corticospinal tract (CST), while fluoro-emerald was injected in the sciatic nerve to visualize ascending tracts. Two weeks after labeling, the animals were perfused and the tissue analyzed for the spread of transplanted cells. The extent of axonal growth, the ability of transplanted cells to remyelinate, and the injury response of endogenous cells. Our data indicate that injured animals transplanted with hOECs exhibit anatomical and functional recovery. This work was supported by the Reeve-Irvine Research Center and the Roman Reed Fund. Dr. Vawter supported by William Lion Peltzner Foundation.
P577. THE SMALL GTPase RIT INCREASES AXONAL BRANCHING IN AN MEK-INDEPENDENT MANNER
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Successful neuronal regeneration following central nervous system (CNS) injury requires axonal sprouting, elongation, branching, and synaptogenesis. Important signaling molecules that regulate these events include the small nucleotide binding proteins of the Ras superfamily. Rit is a member of the Ras subfamily that regulates growth-stimulating pathways in NIH3T3 fibroblasts, where expression of a constitutively active mutant causes tumorigenic transformation without activating known MAP kinase cascades or PI3K/Akt pathways (Rusyn et al., 2000, Oncogene 19: 4685). Here we investigate the role that Rit plays in neuronal regeneration using the SH-SY5Y human neuroblastoma cell line. Adenoviral expression of a constitutively active Rit (Rit79) induces robust neurite outgrowth, determined by measuring neurite initiation, elongation, and branching. Using phospho-specific antibodies, we determined that Rit79L activates Erk1/2 but not Akt, whereas both proteins were activated by constitutively active Ras. Interestingly, the MEK inhibitor PD 98059 blocked Rit79L-mediated increases in neurite initiation but not branching. These data, obtained in human cells, support previous studies showing Rit activates ERK, but not Akt, in rat PC12 cells (Spencer et al., 2002, J. Biol. Chem. 277: 21060) and newly identify a prominent function for Rit in axonal branching. Importantly, our results may identify Rit as a target for therapies to enhance multiple aspects of neuronal regeneration including axonal sprouting, elongation, and branching following CNS injury. (Supported by grants from NIH (EY10545 and DA12791 to DMS) and KSCERT (#0-8, to DLH)).

P578. ROLE OF CIRCULATING IGF-I IN FUNCTIONAL RECOVERY FROM SPINAL CORD INJURY UNDER NORMAL AND ENRICHED
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Physical activity (e.g., voluntary wheel running or enriched environment (EE)) has been shown to enhance return of locomotor function in spinal cord injured rats. A possible mediator of these beneficial effects is circulating IGF-I as uptake of this growth factor across the blood-brain barrier increases during exercise. In this study, animals were subjected to a moderate (12.5 gms MASCIS) spinal cord contusion injury and return of locomotor function was scored using the BBB- and BBBsub-scores. ThoracoLumbar Height test, Gridwalk and CatWalk during the next 8 weeks. In the first experiment, IGF-I (14 ng/day for 28 days) was infused sc using osmotic minipumps. IGF-I treated animals regained better locomotor function than controls. In a second experiment, IGF-I antisem or pre-immunum (20% 6 ul/day for 28 days) was infused sc in animals housed in the EE or under standard conditions. EE housed animals recovered better locomotor function than control housed animals and under both housing conditions IGF-I antisem diminished the level reached, significantly so with EE housing. Antiserum treatment did not completely block the beneficial effects of EE housing. We conclude that circulating IGF-I mediates the protective effects of enhanced physical activity, but not the effects EE housing exerts on central pattern generator function.

P579. A NOVEL DELIVERY SYSTEM FOR TREATMENT OF SPINAL CORD INJURY
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A novel method of localized administration of therapeutic agents for spinal cord injury (SCI) is being investigated. The strategy consists of a polymeric drug solution that gets upon injection in the subarachnoid space (SAS). A spinal canal model was developed to test the safety of implanting the drug delivery system (DDS) in the SAS. In vitro results showed that intrathecal implantation of the DDS is safe. The release of bioactive EGF and FGF-2 from the DDS was monitored in vitro for 2 months. In vivo, the safety of the DDS was evaluated by injecting collagen or aCSF intrathecally in Sprague Dawley rats. Both injured and injured, by 20g cord clip compression. Histologic analysis of the uninjured and injured cords showed the presence of collagen in the SAS up to 56d. Injection of collagen or aCSF in uninjured animals did not cause inflammation or astrocitosis. In injured animals, astroplastic astrocytes were evident in both the collagen and aCSF groups, and the response to injury was not different between groups. There were no macrophages in uninjured animals. In the injured groups, collagen did not increase the macrophages compared to controls. BBB scores between groups in uninjured animals were similar, and injured animals injected with either collagen or aCSF also had similar BBB scores at 56d. Thus, an injectable DDS may allow drug delivery over a prolonged period of time and holds promise as a therapeutic strategy for treatment of SCI. Supported by: CIHR, Univ. of Toronto.

P580. BRAIN DERIVED NEUROTROPHIC FACTOR INFUSION INTO THE MOTOR CORTEX PROMOTES SPRouting OF IN'TACT CORTICOSPINAL FIBERS WITHIN THE CERVICAL SPINAL CORD
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After spinal cord injury, one approach to enhance functional recovery or compensation is to exploit intact axons, which extend past the point of injury, to sprout and connect to potential targets. We have previously shown that application of the neurotrophin Brain Derived Neurotrophic Factor (BDNF) to the sensorimotor cortex stimulates the expression of regeneration-associated genes (GAP-43, T-lilin). This treatment also results in enhanced sprouting of corticospinal fibers rostral to the site of thoracic injury (Hiebert et al., 2002). In the present study, we investigate, whether infusion of BDNF into intact sensorimotor cortex induces sprouting of intact corticospinal fibers into denervated cervical spinal cord. A left unilateral pyramidal lesion of the corticospinal tract was performed. BDNF was infused (500 ng/0.5 ml/hr) into the contralateral intact sensorimotor cortex for 14 days after injury. On day 28, the intact corticospinal tract was labeled with BDA, and the animals sacrificed on day 42. Digital images of the C5 spinal cord were taken from cross sections, and labeled axon profiles from the intact sensorimotor cortex were manually traced using Photoshop. The cumulative area covered by the digital pencil tracing was quantified. Results show a 3 fold increase in corticospinal axon profiles into the denervated half of the C6 spinal cord. Other levels of spinal cord are currently being analyzed. We are also studying the efficacy of a number of locomotor and precision tasks (i.e., gait analysis, swimming analysis, kinematic analysis, and food pellet reaching task) in our model of injury, as well as assessing functional compensation as a result of treatment. This project is supported by Rick Hansen Neurotrauma Initiative and BC Neurotrauma Fund.
P581.
AUTOLOGOUS ACTIVATED MACROPHAGE THERAPY SHOWS POTENTIAL AS A TREATMENT FOR ACUTE COMPLETE SPINAL CORD INJURY
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Introduction: Complete spinal cord injury is a devastating and until now, almost untreatable condition. Experiments showing CNS regeneration and partial functional recovery in rats (Raspalino et al. Nature Medicine 4:814-821. 1998) served as proof of principle. Safety and efficacy have been demonstrated in extensive preclinical experiments that continue to produce promising results. Methods: A phase 1 clinical trial is underway, involving 8 patients with acute complete spinal cord injury (ASIA A). The treatment consists of activating autologous macrophages derived from the patient's peripheral blood and administering them into the parenchyma of the spinal cord within 14 days of injury. The follow-up consists of neurological, electrophysiological, rehabilitation and quality of life assessments. The patient outcomes are compared to historical controls. Results: Eight patients have been followed for up to 30 months, depending on the date of treatment. In none of them short- or long-term side effects that could be linked to the experimental treatment were detected. Of these eight patients, the three patients that have been followed for more than six months exhibit partial recovery of sensory and motor function and have upgraded their ASIA classification from ASIA A to ASIA C. These clinical findings are also supported by electrophysiological examinations. Conclusions: Based on the interim results of this small sample, it is proposed that the Autologous Activated Macrophage Therapy can be an effective treatment for Complete Spinal Cord Injury.

P582.
APPLICATION OF ADENO-ASSOCIATED VIRUS EXPRESSING BRAIN DERIVED NEUROTROPHIC FACTOR TO RUBROSPINAL NEURONS AFTER ACUTE AND CHRONIC AXONOTOMY

Rat rubospinal neurons (RSN) undergo massive atrophy after cervical spinal cord axonotomy and this can be prevented by BDNF infusion into the vicinity of the red nucleus. This treatment was still effective when initiated one year after spinal cord injury. However, the infusion of BDNF produces significant inflammation and tissue damage. Therefore, we used a single micro-injection (glass capillary) of an adeno-associated-virus (AAV) expressing BDNF, driven by the chicken beta actin promoter, into the vicinity of the red nucleus at various times after a C34 hemisection (in male SD rats). We observed that mainly glial cells in and around the RN were infected. Tissue damage and inflammatory reaction to the needle was minimal. Group 1 received AAV-BDNF at the time of axotomy and showed a significant (p < 0.02 vs. control virus) prevention of RSN atrophy. The cell profile sizes were 91% of contralateral on day 14 and 73% on day 21 respectively. In contrast, the control-virus treated cells displayed sizes of 62% and 57% of contralateral by 14 and 21 days. Group 2 received the AAV-BDNF virus injection 2 weeks after axotomy and was analyzed on day 21. Their cell sizes were found to be 65% of contralateral, which is somewhat bigger than the values of the control group (56%), but did not reach statistical significance (p = 0.07). Group 3 was injected with AAV more than 6 months after axotomy and RSN size were found at 63 % of contralateral, which is not significantly (p = 0.16) bigger than in control-virus treated animals (56%). We are presently analyzing the expression of regeneration associated genes, such as GAP-43 and -tubulin-1. Our data indicate that treatment with AAV-BDNF effectivly prevents the atrophy of acutely injured RSN but has only small effects on the reversal of chronic atrophy. Supported by the BC-Neurotrauma Foundation.

P583.
RELATIONSHIP OF MITOCHONDRIAL DEPOLARIZATION TO GLIAL CELL DEATH AFTER IN VITRO NEUROTRAUMA
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Hypothesis: Recent evidence has shown that mitochondrial depolarization and dysfunction can precede neuronal cell death after toxic insults such as glutamate, oxygen glucose deprivation, and physical injury. Less certain is the role of mitochondrial depolarization in glial cell death in the spinal cord after injury. To address this issue we examined the mitochondrial potential in cells from spinal cord mixed glial/neuronal cultures that died after physical injury. Methods: Mixed glial/neuronal cultures were derived from 1 week old mouse pups. The cultures were then injured with a scratch from a pipette tip at 3-4 weeks after plating. Prior to injury the cells were loaded with the mitochondrial potential dye Rhodamine 123. The pre-injury and post-injury whole cell fluorescence levels were then measured using a 40X water immersion objective and epifluorescence microscopy. The maximum increase in fluorescence after injury was normalized and compared to uninjured cultures. Fluorescence levels of cells directly in the path of the pipette tip were not used due to concerns of dye leakage from ruptured cellular membranes. Cultures were also loaded throughout the experiment with propidium iodide to assess cell death. Results: The maximum change in fluorescence in the injured cells was found to be an 86.2% increase. The maximum change for uninjured cells over a similar time period was found to be an 11.0% increase. These results were found to be statistically significant. Studied are currently underway to examine the effect of blocking mitochondrial depolarization on preventing posttraumatic glial cell death. Conclusion: Spinal cord glial cells undergo significant mitochondrial depolarization prior to cell death. Given the evidence that glial-axonal signaling is critical to white matter integrity after SCI, these results could have important pathophysiological implications.

P584.
AMPA RECEPTOR EXPRESSION IN WHITE MATTER GLIA AND ASSOCIATION WITH APOPTOSIS AFTER ACUTE SPINAL CORD INJURY
Eugene Park. (Toronto Western Research Institute, Toronto, Ontario CA).

Increasing evidence suggest that AMPA receptors play a key role in mediating excitotoxic cell damage after acute spinal cord injury (SCI). In the present study we examined changes in AMPA receptor expression, the cellular distribution of these changes and the association between AMPA receptor expression and apoptosis after SCI. Immunohistochemistry and Western blot were used to examine the distribution of AMPA receptor in spinal cord white matter. A lack of GluR2 expression in white matter glia suggests that these cells are highly calcium permeable and susceptible to Ca2+-mediated secondary injury mechanisms. Quantification of AMPA receptor expressing cells in spinal cord white matter indicated a predominance of GluR3 expression in oligodendrocytes and proportionately greater GluR4 expression in astrocytes. A clip compression model of SCI was used to examine the changes in AMPA receptor expression in dorsal column white matter 1, 3, 7 and 14 days after injury. Quantitative analysis of GluR3 levels of expression indicate a significant decrease at 3 days post-injury compared to uninjured animals, followed by a recovery of expression by 2 weeks. GluR4 levels of expression followed a similar temporal pattern of expression. Gene expression analysis of GluR3 and GluR4 mRNA splice variants exhibited a similar temporal pattern of expression that correlated with protein expression of the respective subunits. Quantification of TUNEL positive cells expressing GluR3 and GluR4 subunits indicated a significant increase at 1,3 and 7 days after injury. A large decline in GluR3 expressing oligodendrocytes 3 days post-injury in association with TUNEL data suggest that this subunit may be involved with the early induction of apoptosis in white matter glia. An increase in GluR3 and GluR4 expression 7 and 14 days after injury correlates with the phenomenon of astrogliosis after CNS trauma. The effects of altered cell populations expressing AMPA receptors after SCI may have important implications with respect to plasticity and neurological recovery after SCI. Supported by CIHR/NCRRP.
P585. WHITE MATTER INJURY AND ACUTE INFLAMMATION IN ENDOTHELIN-1 INDUCED SPINAL CORD ISCHAEMIA
DJ Corkill & VH Perry. (CNS Inflammation Group, Southampton, UK).

Many spinal cord injury models rely on impact injury to stimulate the effects of contusion of the spinal cord in man. Although physical injury is often the initiating event in spinal cord injury, the resulting lesion may involve ischaemia, excitotoxicity and the acute inflammatory response. We have developed a model of focal ischaemia in the rat spinal cord, which allows us to dissect the mechanisms involved without the effects of direct trauma. Microinjection of endothelin-1 (250nl = 15pmol ET-1) into the spinal cord ventral grey matter results in an ischaemic lesion characterised by rapid (within 24 hours) neutrophil recruitment followed by a pronounced mononuclear phagocyte response at 3 days [1, 2]. APP positive axons and axonal end-bulbs are present in the white matter adjacent to the lesion site from 6 hours after microinjection. peaking at 24 hours. This suggests that the developing ischaemia in the ventral grey matter can have an effect on adjacent white matter structures. Longitudinal spinal sections show that the lesion develops rapidly rostrocaudally, so that motor neurons one vertebral level above or below the microinjection site are absent at 6 hours. Neutrophils and macrophages are also found at distances up to 7mm rostral or caudal to the microinjection site at later times. Laser-Doppler flowmetry has been used to observe the kinetics of the ET-1 induced ischaemia and the rostro-caudal extent of the hypoperfusion. These data may enable us to relate the extent of ischaemia to the observed white matter pathology. 1. Corkill. D.J., D.C. Anthony, and V.H. Perry. Contrasting inflammatory responses in endothelin-1 induced ischaemic lesions in rat brain and spinal cord. British Neuroscience Association Abstracts. 2001. 16: p P12.07. 2. Corkill, D.J. and V.H. Perry. A model to dissociate the ischaemic and mechanical components of spinal cord injury. Journal of Neuroimmunology. 2001. 118(1): p. 59.

P586. IN VIVO EVIDENCE OF MEMBRANE DAMAGE FOLLOWING COMPRESSION OF ADULT GUINEA PIG SPINAL CORD
Ryi Shi. (Purdue University, West Lafayette, IN US).

Loss of membrane integrity plays an important role in the pathogenesis of traumatic spinal cord and brain injury. It is well established in in vitro studies that controlled mechanical insults inflict membrane damage, which correlates with functional loss and cell survival. Much less evidence, however, has been demonstrated in vivo with similar injuries, where significant secondary degeneration occurs. Current study constitutes an attempt to document the dynamics of the loss of membrane integrity following controlled compression at 1 hour, 1 day, 3 days and 7 days post injury in a live animal model. Female adult guinea pigs were subjected to spinal cord compression with modified forceps, which crushed the cord from an original width of approximately 3.5 mm to 1.3 mm in a span of 2.3 mm along the longitudinal axis of the cord. Using an HRP-exclusion assay to examine the membrane integrity, we have found that membrane damage was evident 1 hour after the injury in the center, but not at 10 mm away from the compression area. However, at 3 or 7 days after compression, the membrane damage spreaded to outside of the original crushing site. Apparent tissue loss at gross level accompanied the membrane damage. In summary, membrane damage existed days after initial mechanical insults and spreaded to the neighboring uncrushed area. Therefore, the secondary tissue loss may be the result of retrograde degeneration of damaged axons and/or biochemical toxins released from the original site which can independently inflict further membrane damage.

P587. NEURALIZATION OF MULTI-POTENTIAL STEM CELLS ISOLATED
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The surprising possibility has recently emerged that some non-neural adult tissues may possess stem cells capable of neural differentiation. Here, we aimed to more clearly define the neurogenic potential of recently identified multi-potential Skin-derived Precursors (SKPs) (Toma et al., Nature Cell Biol 3:9. 778-784). SKPs produce floating clusters of cells that are isolated when trypsinized rodent skin cells are grown in the presence of FGF-2 and EGF. Following serum-based differentiation on laminin/poly-D-lysine-coated slides, a sub-population of SKPs migrates out from the clusters and displays multiple characteristics of neural precursors: (i) expression of the neuroepithelial stem cell marker, nestin, (ii) maintenance in an undifferentiated state with FGF2, and (ii) FGF-2 withdrawal-induced differentiation into neural or neural crest progeny, including neurons, glia, and smooth muscle cells. In short term cultures (<10 passages), extensive neuronal differentiation was evidenced by the expression of both early and late pan-neuronal genes (ie. bIII-tubulin and MAP2), as well as appropriate neuronal morphology. In longer term cultures (10-50 passages), neuronal differentiation could be enhanced by modulation of serum concentrations or supplementation with specific neurogenic factors, including BMPs and retinoic acid. Together, these data identify skin as an easily accessible source of potentially transplantable neural precursors.
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CURRENT CONTROVERSIES IN SHOCK AND RESUSCITATION

Michael Orlinsky, MD, FACEP, William Shoemaker, MD, FACS, Ernane D. Reis, MD, and Morris D. Kerstein, MD, FACS

To paraphrase Kirby in his introductory remarks at the Hyland Symposium in 1978, “There are some readers, no doubt, who feel the subject of colloid and crystalloid therapy . . . has been overly emphasized . . . and there can be little new information to add to an already voluminous body of literature.” Well, that subject and many other controversial issues have continued to be studied and written about, perhaps because the thirst for knowledge continues to grow as new technology leads to new findings, or perhaps what appeared initially to be simple questions were indeed far more complex than imagined. The aim of this article is not necessarily to resolve all the controversies, but rather to point out a select few, provide some understanding of current knowledge, and foster an ongoing interest by individual clinicians as to how best to care for their patients.

To better appreciate the issues, it is important first to emphasize some pathophysiologic concepts of hemorrhagic shock and see how they intertwine with newer, and particularly non-invasive, monitoring devices. Most health care providers, and no doubt many physicians, would define shock by the level of blood pressure and judge successful treatment on the reestablishment of a preconceived baseline value. This

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would be erroneous, not only because of all the false positive and false negative blood pressure values that can occur, but it would not really represent the problem, or the solution, of hemorrhagic shock. Indeed, a normal arterial blood pressure in the face of observed blood loss means that some organ somewhere is underperfused by vasoconstriction of its vascular bed. Oxygen is not stored in tissues, so underperfusion produces progressive hypoxia and proportional damage.

Shock is typically recognized by nonspecific signs and subjective symptoms such as cold clammy skin, pallor, weak thready pulse, unstable vital signs, cyanosis, mottled skin, restlessness, and an altered level of consciousness. Unfortunately, these findings are imprecise, subjective, and observer-dependent; they are secondary effects of acute circulatory failure, not the principal physiologic problem. The lack of objective criteria and the inability to quantify these signs and symptoms have been major problems for understanding the physiology and for the development of optimal therapeutic goals. Nevertheless, shock is first recognized, routinely diagnosed, and often managed by these symptoms. Monitoring is used to recognize circulatory problems, to describe temporal physiologic patterns, to reinforce clinical opinions with physiologic analyses, to evaluate effectiveness of alternative therapies, and to predict and improve outcome.

CONVENTIONAL MONITORING, PHYSIOLOGY, AND THERAPEUTIC APPROACH TO SHOCK

Monitoring is frequently used to measure and evaluate mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), hematocrit (Hct), urine output, and arterial oxygen tension (PaO₂). These traditionally monitored variables characterize circulatory failure, especially in advanced stages of shock. They are not measures of the adequacy of circulatory function or tissue perfusion in the early stages. They reflect secondary aspects of shock syndromes, not primary mechanisms. Moreover, the appearance of hypotension or other signs and symptoms of shock do not mark the beginning of circulatory failure, but rather represent the beginning of decompensation. Measurements begun after the appearance of hypotension reflect late effects, not early primary mechanisms. Hemodynamic and oxygen transport variables, not the secondary manifestations of the syndrome, should be used to evaluate circulatory function and shock.21, 196, 197

The current widely held therapeutic paradigm for hemorrhagic shock is to promptly give sufficient therapy to reduce the symptoms and normalize the vital signs, arterial blood gases, hematocrit, urine output, and other routine circulatory measures. The assumption is that normal values of these parameters are criteria of circulatory normalcy and that their attainment assures adequate resuscitation; however, the underlying low flow, inadequate tissue perfusion, and tissue hypoxia may remain unnoticed and untreated until organ failure appears. Blood
pressure early in the course of shock is not well correlated with blood flow. In a study of patients with multiple injuries and head trauma, Scalea et al. noted that despite being normotensive and neither tachycardic nor oliguric, 80% of the patients had evidence of inadequate tissue perfusion based on elevated lactate levels or decreased venous oxygen saturation.

An appropriate basic assumption is that low flow, poor tissue perfusion, shock, and other circulatory dysfunctions can be recognized early by objective noninvasive criteria, and that more promptly delivered therapy might be more efficacious. Noninvasively monitored data may be used to titrate early fluid therapy to achieve optimal physiologic criteria to prevent development of lethal organ failures.

**PATHOPHYSIOLOGY OF SHOCK**

Tissue injury, pain, fear, and hypovolemia activate the sympathoadrenal axis, releasing epinephrine and norepinephrine from the adrenal medullae and sympathetic effector neurons. Also, in response to hypovolemia, extracellular fluid is translocated from the interstitial space into the intravascular compartment. Continued stress and the sympathoadrenal response activate the hypothalamic-hypophyseal-adrenal axis, stimulating the adrenals to secrete cortisol, which increases cardiac output and in part mediates the post-traumatic hypermetabolic state. The hypermetabolic state, which requires increased blood flow, makes tissues more susceptible to local ischemic events.

Cardiopulmonary catecholamine effects immediately after trauma include increased blood pressure, heart rate, cardiac contractility, minute ventilation, and peripheral vasoconstriction. Although these adaptive effects are often beneficial, especially in minor insults, exaggerated but uneven peripheral vasoconstriction leads to maldistributed microcirculatory flow with localized areas of hypoperfusion and tissue hypoxemia. The hypoxic, acidic endothelium of poorly perfused capillaries activates macrophages and leukocytes and produces cytokines, platelet activating factor, eicosanoids, intravascular coagulation, and other immunomodulatory cascades. The activated macrophages and white blood cells produce oxygen-free radicals and local tissue destruction that mark the systemic inflammatory response syndrome (SIRS). With resuscitation and reperfusion of hypoxic capillaries, these activated cellular and immunomodulatory cascades are washed into the venous circulation and lead to SIRS, end-organ dysfunction, multiple organ failures, and death. Survivors have greater physiologic reserve capacity and the ability to generate increased flow and tissue perfusion needed to provide adequate tissue oxygenation in the presence of increased metabolic need. Differences between survivors' and nonsurvivors' hemodynamic patterns have motivated investigators to suggest aggressive fluid therapy titrated to reach optimal physiologic goals, defined by the survivors' patterns, as a strategy to improve patient outcome. Fluid therapy is titrated to main-
tain intravascular volume, improve tissue perfusion, and overcome regional circulatory deficiencies caused by uneven, maldistributed vasocostriction.

**Oxygen Transport as a Measure of Tissue Perfusion**

The common denominator in early shock is inadequate oxygen delivery (DO₂), needed to meet normal or increased metabolic activity as measured by oxygen consumption (VO₂). An oxygen debt is said to accrue when the actual oxygen consumed is less than that needed. (Recall that DO₂ is the amount of oxygen delivered to the tissues per minute, as described by the formula DO₂ = CO × CaO₂ × 10, where CO is the cardiac output, and CaO₂ is the arterial oxygen content. Also recall that VO₂ is the amount of oxygen consumed by tissues and is equal to the difference in O₂ delivered to tissues and the O₂ returning from tissues, denoted by VO₂ = CO × (CaO₂ − CmvO₂) × 10). Inadequate DO₂ is an early pathogenic event that precedes hypotension, limits metabolism, leads to oxygen debt, and increases mortality. Increased CI (cardiac index = CO/body surface area) and DO₂ correlate well with survival, but failure of the body to develop adequate DO₂ and VO₂ responses is highly correlated with death.²¹, ¹⁹⁶-¹⁹⁸, ²⁰⁷

Inadequate VO₂ may be produced by combinations of low blood flow from hemorrhagic shock, maldistribution of microcirculatory flow from uneven vasoconstriction, and increased metabolic needs from trauma. The physiologic problem is the imbalance between the supply (DO₂) and demand (VO₂) of oxygen. Oxygen debt from an inadequate VO₂ secondary to an inadequate compensatory DO₂ response is the common denominator of most shock syndromes and a major determinant of outcome. Reduced VO₂ and O₂ debts were greater in patients who died.¹⁹⁶ Survivors compensated for tissue hypoxia by increasing CI by way of their neuroadrenal stress response, leading to an increase in DO₂ and VO₂. (Further compensation is provided, if need be, by the tissues extracting more oxygen from the blood, lowering venous oxygen saturation. The limits of compensation are reached when the mixed venous oxygen saturation reaches about 50%. Beyond this point, anaerobic metabolism ensues, leading to a build up of lactate and base deficit.) Nonsurvivors, who poorly compensated for their VO₂ deficiencies, developed lethal multiple organ failures.²⁷, ¹⁹⁷, ¹⁹⁸ The survivors' patterns of CI, DO₂, and VO₂ are assumed to represent the effects of the initiating stress and the body's successful compensatory response. The nonsurvivors' patterns are assumed to reflect the effects of severe illnesses and the body's inadequate compensatory responses with subsequent decompensations.

The bulk movement of oxygen is a useful measure of tissue perfusion and is a measure of overall circulatory function. DO₂ reflects perfusion to peripheral tissues; its early increase compensates for earlier inadequate tissue oxygenation after trauma. The temporal patterns of
DO₂ and VO₂ changes are more informative than a single set of measurements, as sequential changes provide a history of physiologic events that lead to shock and subsequent organ failures.

The Hemostatic Plug

With vascular trauma there is another physiological aspect that assumes great importance. Active bleeding from an injured vessel may be uncontrollable without surgical hemostasis or may stop spontaneously by vessel retraction, vasoconstriction, tamponade, or intra- or extraluminal thrombus formation, buying precious time for eventual surgical correction. The hemostatic plug, which forms over several minutes, consists of platelet aggregates and fibrin mesh containing blood cells and other plasma components. According to La Place's law, Poiseuille's law, and the Bernoulli equation, factors that would tend to prevent plug formation and allow for continued blood loss or provide for leakage through and around the plug and allow for renewed bleeding include increased volume, increased blood pressure, vasodilation, and decreased blood viscosity secondary to hemodilution—all factors that are associated with fluid resuscitation. However, given that fluid resuscitation leads to increased oxygen delivery to the tissues, and that ischemia/reperfusion injury leading to SIRS and multiple organ failure may occur earlier than thought, the issue of immediate (prehospital) emergency department (ED) versus delayed (intraoperative) fluid resuscitation becomes important. The fact that multiple organ failure is the most common cause of late death after injury, and with the exception of immediate mortality resulting from neurologic lesions or exsanguination, is the main cause of posttraumatic death, underscores the significance of the issue.

SURVIVOR/NONSURVIVOR PATTERNS

Early Hemodynamic and Oxygen Transport Patterns in Postoperative Patients

High-risk surgical patients may be used as a model for other etiologic types of shock, because time relationships are precisely documented in the chart after elective surgery. Patterns of nonsurvivors of high-risk surgery consisted of reduced flow and oxygen transport in the intraoperative and immediate postoperative periods, while survivors had less intraoperative circulatory deficits and significantly increased flow and oxygen transport in the early postoperative period. Survivors, compared with nonsurvivors, had: greater increases in CI and flow-related variables with lower CVP and wedge pressures, less pulmonary vasoconstriction (lower PVR and MPAP), greater increases in DO₂ and VO₂ with lower oxygen extraction rates and normal blood
gases, greater hematocrit, blood volume, and red cell mass, and less pulmonary shunt (Qsp/Qt).21, 196, 197, 199 These postoperative survivor and nonsurvivor patterns were consistent despite a wide variety of surgical illness and operations. The assumption was that early changes statistically related to survival represented the effects of surgery plus adequate physiologic compensations and, therefore, these values could be used as therapeutic goals, while early changes related to death reflected the overwhelming effects of surgery plus inadequate bodily compensations. These values may be used as early warning criteria for impending death.

Prospective Clinical Trials of Supranormal CI and DO₂ as Therapeutic Goals

The hypothesis was tested that increased CI and DO₂ represent compensations that have survival value when DO₂ increases and there is concomitant improvement in VO₂ indicative of improved tissue oxygenation.14, 26, 27, 197, 247, 253 An early prospective clinical trial evaluated effectiveness of supranormal values as therapeutic goals to improve outcomes over a 7.5-year period. The initial clinical trial evaluated 252 high-risk surgical patients allocated to one of three services. Normal values were used as therapeutic goals for the control service and the supranormal values as goals for the protocol service. There were marked significant reductions in mortality in the protocol patients (19% versus 44%).2 Subsequently, a randomized control trial was performed that preoperatively allocated patients to one of three groups: CVP catheter group with normal values as goals; pulmonary artery (PA) catheter control group with normal values as goals, and a PA-protocol group with supranormal values as goals. The result showed no significant differences in outcome of the CVP group compared with the PA-control group. Both used normal values as goals. Thus, if the intent is only to maintain normal values, the PA catheter has no real advantage over the CVP catheter. By contrast, the PA-protocol group had significantly reduced mortality compared with the PA-control group (4% versus 33%, p <0.02), as well as fewer days on mechanical ventilation, fewer hospital days, fewer ICU days and reduced costs.197

Some recent reports have failed to confirm these observations. In an insightful meta-analysis, Boyd and Hayes28 showed no outcome improvement in eight randomized studies done several days postoperatively after organ failures had developed, but reduced mortality in eight prospective randomized studies done early (i.e., 8 to 12 hours postoperatively). Moreover, these trials showed improved survival, reduced organ failure, and lower costs when optimal values of CI, DO₂, and VO₂ were used as early goals. The concept of survivors’ supranormal values as optimal goals has been supported by a considerable number of studies. Edwards et al,67 Yu et al,253 and Boyd et al26–28 reported improved outcome with optimal values in postoperative patients; Scalea et al,185 Bishop et al,20 Pasquale et al,152 and Moore et al138 have reported
improved outcome with supranormal values in trauma patients, and Boyd et al.26–28 reported improved outcome in preoperatively randomized optimization of high-risk surgical patients; Edwards et al.187 and Tuchschiert et al.224 reported improved outcome in medical septic shock; the latter study was randomized. Creamer et al.185 demonstrated survival in 14 of 17 patients with cardiogenic shock after acute myocardial infarction when they were able to increase CI from 1.3 ± 0.5 to 2.6 ± 0.4 L/min · m². Edwards187 also showed improved outcome in cardiac patients. All had some variations in their protocols, but each used supranormal values as early goals.

Bishop et al.20 showed that severely traumatized patients resuscitated to optimal values in less than 24 hours after injury had an 18% mortality, while those who reached optimal goals in more than 24 hours or failed to reach them had 38% mortality. Other investigators have confirmed the increased CI, DO₂, and VO₂ in survivors of septic shock.165, 224

**Time Relationships in Shock**

When monitoring is started early, survivors start with low flow but promptly develop compensatory hyperdynamic states, while nonsurvivors continue with low or relatively normal flow and poor tissue perfusion/oxygenation. These lead to organ failure, capillary leak, and finally death.155 Time is the single most important outcome-related issue. When the aim in high-risk surgery was to achieve the optimal supranormal goals in the first 8 to 12 hours postoperatively, there was marked reduction in organ failure and mortality.16, 27, 187, 247; however, optimization of DO₂ and VO₂ in the late stage after organ failure occurred did not improve mortality.25 In Bishop’s50 study on trauma patients, those that were optimized when less than 24 hours had elapsed between the hospital admission and the time the goals were achieved, mortality was 18%; however when optimal goals were not achieved until after 24 hours after admission, mortality increased to 39%. In severe trauma patients, low incommensurate VO₂ responses to increased DO₂ in the first day after injury were associated with increased organ failure and mortality. By contrast, increased DO₂ associated with increased VO₂ favorably affected outcome. There was 92% survival when the optimal goals were achieved within 24 hours of ICU admission, but 93% mortality when achievement of the goals was delayed or not reached at all, and lactate levels did not return to satisfactory levels.20, 27, 197

**NONINVASIVE CARDIAC OUTPUT MONITORING**

The gold standard for evaluation of hemodynamic measurements has been by invasive PA balloon-tipped thermodilution (Swan-Ganz R) catheter. This technology is expensive, time consuming, and personnel intensive. Noninvasive alternatives to the thermodilution technique for
cardiac output estimation include thoracic electric bioimpedance, trans-thoracic (TTE) and transesophageal (TEE) Doppler echocardiography, the partial CO₂ rebreathing method, and the pulse contour method. Noninvasive monitoring allows calculation of the net cumulative deficits or excesses of each variable by integrating the area between continuously monitored values and normal or optimal values. The ideal cardiac output monitoring method is noninvasive, reproducible, inexpensive, continuously displayed, user friendly, in reasonable agreement with thermodilution, and acceptable to patients.

Thoracic Electric Bioimpedance Cardiac Output

In the impedance method, electrodes inject a small-amplitude (0.2–4.0 mA) alternating current at 40 to 100 kHz to produce an electrical field across the thorax from the base of the neck to the level of the xyphisternal junction. The electrical signals travel predominantly down the aorta, rather than through aerated alveoli. The changes in aortic flow throughout the cardiac cycle are correlated with changes in impedance (i.e., the apparent changes in resistance). Wang et al. developed and improved an impedance system that was marketed as the IQ system (Wantagh, Inc., Bristol, PA). They used noninvasive disposable prewired hydrogen electrodes positioned on the skin and three EKG leads placed across the precordium and left shoulder, and a 100 kHz, 4 mA alternating current passed through the patient’s thorax by the outer pairs of electrodes. The voltage was sensed by the inner pairs of electrodes.

Comparisons and Limitations of Bioimpedance and Thermodilution Measurements

Noninvasive monitoring compares favorably with invasive thermodilution catheter monitoring; however, there is appreciable disparity in the presence of pulmonary edema, advanced acute respiratory disease syndrome (ARDS), congestive heart failure (CHF), and late-stage septic shock with capillary leak. Nonetheless, the comparison is considered sufficiently accurate to be useful clinically for making therapeutic decisions in over 90% of acute critically ill patients. Trends of bioimpedance cardiac outputs closely track changes in thermodilution cardiac output method. The differences between thermodilution and impedance cardiac output estimations are more than offset by continuous on-line displays of data that allow instant recognition of abnormalities, calculation of the deficits of each monitored variable, and evaluation of therapeutic responses.

In all monitoring and imaging techniques, motion, anxiety, restlessness, shivering, agitation, and hyperventilation may interfere with measurements and increase physiologic responses; however, it is less important in emergency conditions to have the same accuracy required in
stable ICU conditions, since the patient’s own baseline measurements are often unknown, and optimal values for each patient may vary with comorbid conditions. In practice, 15% differences between invasive and noninvasive cardiac output estimations are acceptable when greater than 50% changes from the normal range are present. Thermodilution, however, also has appreciable inaccuracies in both high and low cardiac output ranges and especially when the patient has hypothermia, dysrhythmias, valsala effects, motion artifacts, shivering, anxiety, and errors from injectate temperature calibration.

MULTIPLE NONINVASIVE MONITORING SYSTEMS

Multiple noninvasive physiologic monitoring systems are feasible as the initial screening system or as the “front end” of invasive monitoring during the resuscitation of acutely ill patients shortly after ED admission. The temporal patterns of cardiac function, pulmonary function, and tissue perfusion obtained by noninvasive monitoring systems compare reasonably well with the patterns of invasive monitoring. These noninvasive monitoring systems display early small changes that can be treated before they progress to life-threatening proportions. Early deficits are easily and effectively corrected, while late effects of shock after organ failure has developed may be irreversible. These noninvasive monitoring systems have been clinically evaluated in widely varying circumstances in a large series of severely ill patients to identify clinical conditions where the impedance methodology is appropriate.200

The ED is the primary entry point into medical care for many acutely ill patients, and this early period provides a crucial opportunity for early assessment and rapid therapeutic interventions that may affect outcome. A major dilemma is that shock is easily diagnosed in late stages when therapy is ineffective, but early diagnosis is difficult because shock is first recognized by imprecise signs and subjective symptoms. Noninvasive monitoring of circulatory dysfunction is an alternative approach that allows very early application in the ED, operating room, and hospital floors. The continuous on-line graphic displays of data allow prompt recognition of circulatory abnormalities and early therapeutic intervention and titration of therapy to optimal physiologic goals in acutely ill emergency patients where time factors are crucial.200 Monitoring provides objective circulatory criteria that can replace clinical suspicion and guesswork with physiologic criteria related to outcome.

Clinical evaluations under worst case scenarios of emergency trauma cases in an inner city hospital have shown stable impedance signals and satisfactory agreement with simultaneous thermodilution cardiac output measurements. Simultaneous noninvasive measurements may be used to evaluate: cardiac function by blood pressure and noninvasive impedance, Doppler echo, or partial CO₂ rebreathing cardiac output systems; pulmonary functions by pulse oximetry estimation of arterial hemoglobin saturation; and tissue perfusion by Ptco₂, PtcO₂/
PaO₂ index, and PtcCO₂. These may be supplemented by invasively measured CI, DO₂, and VO₂ when available to validate noninvasive methods and to provide additional information such as wedge pressures. Baseline data sets, the low point (nadir) of the event, and the period of recovery immediately afterward describe the patterns of the interacting circulatory components: heart, lungs, and peripheral tissue perfusion/oxygenation functions. A microcomputer-based data acquisition system was developed to measure and record up to eight channels of data simultaneously and to display any two of them.  

Sequential Hemodynamic Patterns in Hemorrhagic Shock

Initial typical patterns of hemorrhagic shock as monitored noninvasively in the ED showed reduced flow (CI), MAP, and PtcO₂ with increased PtcCO₂; these effects were more pronounced in nonsurvivors. The initial SapO₂ and PaO₂ values were usually close to normal, while tissue perfusion, reflected by reduced PtcO₂ and increased PtcCO₂ rapidly declined with moderate degrees of hypovolemia. With severe hypovolemia, hyperpnea and tachypnea occurred usually with slightly reduced PaO₂ and pH values. With prolonged shock, poor tissue perfusion led to acidosis, base deficits, and increased lactate levels.

Rapid hemorrhage studied in the intensive care unit (ICU) by invasive pulmonary artery (PA) monitoring has demonstrated reduced MAP, CI, CVP, PAOP, stroke index, stroke work, mixed venous oxygen saturation (SvO₂), pH, hematocrit, DO₂, and VO₂ concomitant with increased systemic vascular resistance index (SVRI) and oxygen extraction ratio. The initial compensatory responses included increased heart rate, which increased CI by neural and neurohumoral mechanisms; increased SVRI, which tended to maintain arterial pressures in the face of decreasing flow; and increased oxygen extraction ratios, which improved tissue oxygenation when blood flow had been reduced.

With prolonged hemorrhage, the shock pattern showed greater reductions in hematocrit and lesser reductions in MAP, CI, DO₂, and VO₂. The reduction of VO₂ was lower quantitatively but more prolonged than that occurring after rapid losses of comparable quantities of blood. After bleeding was stopped and blood volume restored with appropriate fluids, the survivors' recovery pattern usually consisted of normal or elevated values for CI, DO₂, and VO₂. The smaller initial transient fall in cardiac index in survivors and the subsequent increase in flow and DO₂ were compensations to the initial low flow state. This is affected by the amount of injury, hypovolemia, preload therapy, and increased metabolic demand from the trauma itself. The combination of increased demand and abnormal tissue perfusion led to oxygen debt, multi-organ failure, and death.
Early Hemodynamic and Oxygen Transport Patterns
After Trauma

Evaluations of circulatory dysfunction are often essential for decisions to operate in patients with blunt trauma. Moreover the timing of surgical operations may depend on clinical evaluation of the circulation and the temporal progression of clinical signs and symptoms. In the standard Advanced Trauma Life Support (ATLS) course, the degree of shock and amount of blood loss are estimated by the systolic and diastolic blood pressure, pulse pressure, and chance observations such as pallor, cold clammy skin, pulse rate, capillary refill, temperature, respiratory rate, and mental status. Neither blood pressure nor other signs and symptoms, however, are well correlated with blood flow or outcome.106,206

Multiple noninvasive hemodynamic monitoring systems were used to prospectively evaluate circulatory patterns in 151 consecutively monitored severely injured patients beginning with admission to the ED in a university-run large urban county hospital. Noninvasive monitoring was feasible, easy-to-use, inexpensive, and safe during the resuscitation of emergency patients with severe trauma. Noninvasive systems provide continuously real-time displays of data from the ED to the operating room (OR), and to the ICU for early recognition of circulatory dysfunction in acute emergency conditions. The net cumulative deficit or excess of each monitored parameter was calculated by the area between normal values and the curve produced by the continuously monitored values for each variable in each patient. The deficits of cardiac, pulmonary, and tissue perfusion functions were analyzed in relation to survival by discriminant analysis and crossvalidated. The mean (±SEM) net cumulative excesses (+) or deficits (−) from normal in surviving versus nonsurviving patients, respectively, were: for cardiac index, +81 ± 52 versus −232 ± 136 L/m² (p<.037); for MAP, −10 ± 13 versus −57 ± 24 mm Hg, h (p<0.078); for arterial saturation, −1 ± 0.3 versus −8 ± 2.6%, h (p<.006); for tissue perfusion, +313 ± 88 vs. −793 ± 175 torr, h (p<.001). Discriminant analysis classified 95% of the survivors and 62.5% of the nonsurvivors shortly after the initial resuscitation. Survival was predicted by discriminant analysis of the net cumulative deficits of flow, arterial hypoxemia, and tissue perfusion, which were more pronounced in the nonsurvivors.202

In summary, the goals of multicomponent noninvasive monitoring systems are to obtain comparable data to that of invasive monitoring, but continuously and in real time.

The feasibility of a multicomponent noninvasive monitoring system was demonstrated in the immediate postadmission period. Impedance cardiography can be applied like ECG electrodes in the ER, OR, ICU, hospital floors, or in doctor’s offices and other prehospital settings. They are less labor-intensive, easier to operate, simpler, cheaper, and safer both on the patient and on the staff. The system allowed description of the time course of the major components of the circulation: total body blood flow reflecting cardiac function; arterial oxygenation by pulse
oximetry, reflecting pulmonary function; and PtcO₂ and PtcCO₂, reflecting tissue perfusion. The temporal patterns of these interacting noninvasive components were roughly comparable with the data obtained simultaneously with invasive monitoring.

Noninvasive systems may be used to characterize the physiology of surviving and nonsurviving patients beginning with ED admission. The data obtained with the three simultaneously monitored noninvasive systems—thoracic electric bioimpedance or other noninvasive measures of cardiac output, pulse oximetry, and transcutaneous O₂ tension instituted shortly after ED admission—were comparable with data from the invasive PA catheter when this became available. These three noninvasive systems provided evaluation of the cardiac, pulmonary, and tissue perfusion functions of the circulation.

When large volumes of fluids are required, CVP or PA catheters may be used to monitor venous pressures to avoid fluid overload. The noninvasive systems are less hazardous than invasive catheters for the patient in terms of catheter complications and for the staff in terms of exposure to hepatitis, HIV, and other infections. Continuous on-line noninvasively monitored data provide a means to calculate the net cumulative deficits or excess of each monitored variable. Discriminant analysis gives a more quantitative estimate of circulatory dysfunction and a powerful view of the rapidly changing circulatory dynamics, transcending the boundaries of old concepts of shock. Because noninvasive monitoring can provide the essential circulatory information in patients throughout the hospital and prehospital areas, the data may be used to describe survivor and nonsurvivor patterns, to predict outcome, to define therapeutic goals, to titrate therapy to achieve these goals, and eventually to improve outcome. This may change standard methods of managing acutely ill patients.

**FLUID THERAPY**

Based on previously discussed pathophysiologic concepts, it is apparent that the goals of therapy in vascular trauma are twofold: restore oxygen perfusion to the tissues and provide hemostasis through operative intervention. Oxygen perfusion is provided through proper ventilation, oxygenation, and fluid therapy.

**The Proper Amount and Timing**

Despite the aggressive approach to fluid resuscitation advocated by the Committee on Trauma of the American College of Surgeons, there is a history of wartime observation that urges caution. In 1983, Aprahamian et al looked at “scoop and run” patients versus those who were given prehospital care, including fluid resuscitation, by paramedics. Although there was little difference if the initial systolic pressure
was greater than 60 mmHg, fluids clearly increased survival from 15% to 60% in those patients with penetrating abdominal vascular trauma and systolic pressures less than 60 mmHg. On the other hand, a study of close to 7000 trauma patients did not show a survival benefit with early prehospital fluids, regardless of the injury severity score, and despite the fact that hypotension was associated with mortality. The question could then be asked if there was something good about no fluids, or was there something bad about using fluids. The issue was taken to the laboratory where, in an uncontrolled hemorrhage model in swine, Bickell et al showed that administering no fluids was better than rapidly administering lactated Ringer's (LR), which led to increased intraperitoneal hemorrhage and death. The problem was postulated to be a blow-out of the hemostatic plug caused by increased pressure and decreased blood viscosity. In an accompanying editorial, it was noted that despite the relatively enormous volume and rate given to the animals, too much fluid can be detrimental, and that surgical hemostasis is key for uncontrolled hemorrhage. In the same animal model, Bickell et al showed that hypertonic saline with dextran (HTS-D) led to more bleeding and death than no fluids, but LR was still worse. In similar animal model studies using normal saline (NS), controlled resuscitations at lower targeted pressures between aggressive fluid resuscitation and no resuscitation were investigated. Limited-to-moderate resuscitation was shown to be best. Dronen et al helped elucidate the problem further when they compared controlled to uncontrolled blood loss, with and without resuscitation. The worst survival was with no resuscitation in either model of blood loss, but they obtained 100% survival with fluids in the controlled model versus 22% with fluids in the uncontrolled model. Again, it was proposed that in an uncontrolled model of vascular injury, the hemostatic plug could be rendered ineffective, and increased volume could cause increased mortality.

Perhaps the most noted study in this controversy was done by Bickell's group in 1994. The study included 598 patients with penetrating torso injuries and systolic pressures less than 90 mmHg who received either standard prehospital/ED fluid resuscitation (immediate group) versus no fluids until they were in the operating room (delayed group). The delayed group had better survival, fewer complications, and shorter hospital stays. Again, the findings were attributed to accentuation of ongoing hemorrhage or hydraulic disruption of an effective thrombus, followed by a fatal secondary hemorrhage. The recommendation was to delay aggressive fluid resuscitation in hypotensive patients with penetrating torso injury until the time of operative intervention.

Studies that have occurred since Bickell's contribution basically arrive at the same conclusion, that moderate resuscitation is best for uncontrolled vascular injury, whether the end point is CI and oxygen delivery, the observation period is longer, the order of NS and blood is interchanged, the replacement ratio of crystalloid to blood loss is altered, regional blood flow in a venous uncontrolled hemorrhage.
model is looked at, crystalloids and hypertonic/collod solutions are used, or traumatic brain injury is present.

There are two additional points that should be mentioned. Firstly, although in Small's study the no resuscitation group eventually "caught up" to the favored moderate resuscitation group, the time spent in a hypoperfused state can be deleterious to the subsequent ischemia/reperfusion injury. Secondly, Owens brings up a good point that concerning HTS, it is the animal studies where there is a bleeding problem, not in the clinical studies.

Reduced morbidity and mortality might result if it could be determined who would benefit from early fluids and which patients would have increased bleeding and mortality. Concerning penetrating or vascular injury, the recommendation by Owens et al seems pertinent: "A limited prehospital resuscitation regimen in which fluid is administered judiciously to maintain a level of cardiovascular function, less than normal, but above the level of progressive circulatory shock might offer the optimal approach."

**Isotonic Crystalloids—Normal Saline or Lactated Ringers Solution**

Which crystalloid is preferred for patients in hemorrhagic shock centers on three issues: acidosis, survival, and compatibility with blood. Although a nonanion gap acidosis is associated with NS, it is not the hyperchloremia that accompanies excess saline that leads to acidosis. In fact, the so-called hyperchloremic acidosis is in reality a lactic acidosis caused by hypoperfusion. There is no apparent gap, because the severe depletion of serum albumin, an unmeasured anion, reduces the "normal" anion gap. It is not that NS causes the acidosis, but rather that LR improves it as the L-lactate isomer is metabolized by the liver and kidney to generate bicarbonate (HCO₃⁻) and provide a buffer. The concern that it takes several hours to metabolize the lactate because of decreased hepatic metabolism in the shock state was not born out in Healey's study when acidosis was improved despite large quantities of LR, leading to higher bicarbonate levels within 2 hours. Likewise, Coran et al showed that the low pH in hypovolemic shock went further down with NS, but the pH returned toward normal with LR. Indeed, both Cervera et al and Horton et al showed that adequate resuscitation returns the pH to normal regardless of which crystalloid is used. Additionally, lactate levels are not elevated with LR.

Two animal studies show an increased survival rate with LR. In one study, the nonsurvivors were considerably more acidotic; however, when Traverso et al compared NS with LR and Plasmalyte A (Baxter Edwards Critical-Care, Irvine, CA), both bicarbonate precursors, Plasmalyte had the highest mortality, despite having pH, HCO₃⁻, and base deficit levels equal to those of LR. Therefore, the acidosis may not be causative in the difference in mortality.

The issue of whether LR is compatible with blood may be the most
important. Storage of blood requires citrate-phosphate-dextrose (CPD) solution. The sodium citrate prevents blood from coagulating by chelating the calcium ion and disrupting the coagulation cascade. The theoretical problem with LR is that the calcium in LR will exceed the chelating capabilities of citrate in the stored blood and cause clot formation. Depending on the size of clot, infusion rates could be slower if clot blocks the blood filter or tubing, or clots could reach the circulation compromising the pulmonary capillaries. Lorenzo et al. studied whole blood and packed red blood cells (RBC) mixed with NS as control compared with LR with different amounts of calcium added. Despite a 1:1 ratio of blood to LR, there was no significant difference in infusion times, filter weight, or clot formation, except at the highest calcium level, which was considerably higher than the usual LR. In addition to the low concentration of calcium in LR, he felt the rapid rate of infusion, as would be used in emergent fluid resuscitation, prevented any clot formation. The recommendation from this study was to allow the use of LR in the rapid transfusion of packed RBCs. Cull et al. showed no difference between NS or LR mixed with PRBC at different flow rates with varying hematocrits; however, clotting did occur at a 1:1 ratio with LR, and at least a 2:1 blood to LR ratio was needed to avoid clotting. King et al. likewise saw clotting at less than a 2:1 ratio, but found no difference with NS or LR at a rate of 540 mL/hr. Ryden et al. felt that LR should not be used with blood, but they used slow infusion rates, and found that the slower the infusion and the warmer the ambient temperature, the more clots formed. It therefore appears that LR can be used with packed RBCs if the ratio of admixture is at least 1:1, preferably at 2:1, and the infusion rate is fast.

Although it appears that LR is the preferred choice of crystalloid, there is an exception, namely, traumatic brain injury (TBI). Although either crystalloid can be used as long as hypoosmolality does not develop, and rapid administration of large volumes are avoided, NS is preferred, because the 154 mmol/L of sodium is slightly hypertonic to the 130 mmol/L of sodium in LR. Lastly, the usual replacement ratio of 3:1 crystalloid to lost blood should probably be rethought as studies place the adequate ratio at 7:1 or 10:1 because of decreased colloid oncotic pressure secondary to decreased serum protein concentration from hemorrhage, capillary leaks, and crystalloid replacement.

**Hypertonic Saline With or Without Dextran**

Hypertonic saline (HTS) is usually supplied as 7.5% NaCl, often combined with a colloid, 6% dextran 70. Both in animal models and in clinical trials, it is most often used as an initial small volume bolus of 4 mL/kg or 250 mL. HTS works by shifting water into the plasma first from RBCs and the endothelium, then from the interstitial space and tissue cells. This leads to its two most important attributes: a rapid but transient increase in blood volume to support and improve hemody-
namic, and a hemodilution and endothelial cell shrinkage that decrease capillary hydraulic pressure and improve tissue perfusion.

There are many animal studies and clinical trials that show HTS and HTS-D to be effective at expanding plasma, raising blood pressure, improving cardiac output, lowering systemic and pulmonary vascular resistance, lowering subsequent fluid and blood requirements, and improving oxygen delivery. Adding dextran to HTS prolongs the circulatory effect. Improved regional tissue perfusion, decreased leucocyte-endothelial cell interaction, and decreased stickiness of leucocytes point to a potential decrease in ischemia/reperfusion injury and subsequent multiple organ failures. Adding dextran has been shown to increase regional blood flow.

Although some studies do not show any benefit to patient survival, other studies report an increase in predicted survival rates with HTS or an increased survival with HTS-D in patients destined for the operating room or having associated traumatic brain injury. Despite theoretical concerns, clinical use has shown HTS and HTS-D to be quite safe. Central pontine myelinolysis with HTS and bleeding, difficulty with cross-matching, or anaphylactoid reactions with dextran are rare to non-existent in the literature. Although animal studies have shown an exacerbation of bleeding in an uncontrolled hemorrhage model, this has not been a problem in patients.

Hypertonic saline with or without dextran seems effective without much downside in fluid resuscitation in hemorrhagic shock. Where small volume resuscitation is desirable, such as in the military or prehospital care setting, it certainly would represent a logical choice of therapy. Its hypertonic nature and small volume usage are particular advantages, in traumatic brain injury.

Hypertonic Saline in Hemorrhagic Shock and Traumatic Brain Injury

Head injury is the leading cause of traumatic death in the United States, and when combined with hypotension there is a doubling of mortality by creating a secondary ischemic injury. Thus, aggressive and rapid resuscitation of systemic hemodynamics seems appropriate. Because it is the osmolality, not the oncotic pressure, that affects water movement in the brain, and the blood brain barrier is essentially impervious to sodium, HTS would seem a logical choice of fluid. HTS-D was also shown to inhibit leucocyte margination in the cerebral microcirculation thereby attenuating an inflammatory response thought responsible for secondary brain injury. Additionally, concern still exists over excess volume resuscitation in traumatic brain injury, making HTS and its ability to provide small volume resuscitation a seemingly ideal choice for hypotension associated with hypovolemia. Several studies lend credence to the use of HTS in this subset of patients.

Several studies show that crystalloid infusion can be safely used in
TBI and that maintaining normovolemia with fluids does not lead to increased intracranial pressure (ICP). In a porcine model, however, Bourguignon et al. showed that LR decreased cerebral oxygen delivery and increased ICP. In studies where hypotension was combined with TBI, HTS and HTS-D improved cerebral parameters, however, in one investigation, LR resuscitation led to increased ICP. Patients with TBI without hypotension had their ICP successfully managed by adding 3% HTS or HTS-acetate to their treatment plan over several days. As the concentration of serum sodium increased, the ICP decreased. Importantly, in animal studies where hypotension occurred without TBI and hypertonic fluid and crystalloids were used to achieve resuscitation, good cerebral parameters occurred with the hypertonic fluid, but the crystalloids led to increased ICP. Even in comparison to mannitol, there are sufficient advantages such as avoidance of hypotension and renal failure that favor the use of HTS. Lastly, in an animal model, HTS offers similar advantages to improvement in microcirculatory flow of the spinal cord.

The Colloid Versus Crystalloid Controversy

Despite theoretical advantages for crystalloids such as the ability to replenish interstitial fluid loss, or for colloids such as the less likelihood of pulmonary edema, the practical situation is that the greatest difference between the two choices is the relatively exorbitant cost of the colloids. Because colloids have never proved superior to crystalloids for fluid resuscitation, the Consensus of the University Hospital Consortium favors crystalloid as the preferred fluid, except that colloids are appropriate to be used in conjunction with crystalloids when blood products are not immediately available when needed. Habits die hard, however, as Yim et al. discovered when their survey based on the guidelines showed that colloids were used more often than expected, and only 24% of colloid use was considered appropriate.

Over the years, there have been studies showing one type of fluid superior to the other. Hankeln et al. showed the colloid hetastarch to have better cardiopulmonary parameters than LR, or Moss et al. said that human serum albumin (HSA) performed as well as LR. The problem with these and many other studies was that the number of patients in each study was quite small. To demonstrate a 10% difference in treatment effect between crystalloid and colloid resuscitation, assuming a 15% baseline mortality, a two-tailed alpha of 0.5 and a beta of 0.20, a randomized clinical trial would need to involve almost 6000 patients. In large systematic reviews of all appropriate studies, there was no significant difference in overall mortality. Choi et al. found no difference in length of stay or development of pulmonary edema, but there was a trend toward better survival in trauma patients with crystalloid use. These systematic reviews recommend crystalloid use given no significant difference except the much higher price for the colloids.
There are two areas of animal research involving hydroxyethyl starch (HES), of importance to multisystem organ failure. First, several studies have shown that the iron-chelator desferoxamine can be combined with HES and can attenuate the iron-dependent generation of toxic oxygen-derived radicals during reperfusion of ischemic tissue. Second, an animal study showed that HES itself can reduce reperfusion injury through several possible mechanisms, including decreasing the oxidant-generating enzyme, xanthine oxidase.

The issue of pulmonary edema surfaces in most discussions on the fluid controversy. Rackow et al showed more clinical pulmonary edema and much lower colloid onotic pressures with NS compared with albumin, but Weaver et al demonstrated a greater need for ventilatory support and worse oxygenation with albumin. Other studies showed no differences in pulmonary functions, pulmonary failure, or lung water, despite the fact that crystalloids reduced the colloid oncotic pressure. Tranbaugh et al said that the oncotic pressure is only 25% as important as any changes in hydrostatic pressure, and Lowe et al said that although albumin crosses the capillary membrane, it is washed out of the pulmonary interstitium by the lymphatic system.

Lastly, albumin has been shown to have an increased risk of death in a systematic review of 30 studies with 1419 patients. Albumin has been shown to have a negative inotropic effect, reduced coagulation activity, and anticoagulant properties. It is recommended that the use of albumin be strictly reviewed and curtailed. Otherwise, the colloids, which include modified gelatins, dextrans, and etherified starches, are fairly equivalent, but in hemorrhagic shock clearly less preferred than crystalloids.

**Oxygen Carriers**

Given the values of moderate fluid resuscitation and small volume resuscitation described previously, coupled with HIV transmission in blood and the desire of the military for a rapidly available, easily storable resuscitation fluid, an oxygen carrier substitute fluid seems ideal. Unfortunately, early versions of such solutions had many problems such as a short half-life, high oxygen affinity, renal toxicity, vasopressor effects. It was noted, however, that altering the characteristics of acellular solutions could lead to profound physiologic differences. The goal of optimizing these properties to produce an efficacious product for shock resuscitation has led currently to nine products in various stages of development, hopefully some of which will be clinically available within a few years. There are three classes of oxygen carriers: hemoglobin-based, perfluorocarbons, and liposome-encapsulated.

Hemoglobin-based oxygen carriers are solutions of free hemoglobin that are modified to prevent dissociation of the hemoglobin tetramer into dimers, thereby preventing renal toxicity. Additional modifications
have led to different groups within this class. Surface-modified hemoglobin (e.g., pyredoxilated hemoglobin polyoxyethylene) is a conjugate of hemoglobin and larger molecules that will prolong intravascular retention (48 hours) and has retained a high oncotic pressure and viscosity, making them potent plasma expanders.\textsuperscript{249} While able to restore hemodynamics, studies have shown a concern with oxygen transport and mucosal ischemia.\textsuperscript{75, 148, 211} Intramolecular cross-linked hemoglobins (e.g., alpha-alpha Hb, DCLHb, HemAssist) include rHb1.1, manufactured using recombinant technology, providing for a reduced oxygen affinity and oxygen binding curve similar to normal human blood. Many studies show a restoration of mean arterial pressure, excellent tissue perfusion with uniform distribution, improvement in base deficit, and increased survival all equal or superior to other fluids.\textsuperscript{4} Serious side effects including marked vasoactive properties and increased mortality have led to failed phase III clinical trials however.\textsuperscript{81, 156, 157, 206, 249} Polymerized hemoglobins (e.g., PolyHeme [Northfield Laboratories, Chicago, IL], HemoPure [Biopure Corporation, Cambridge, MA], HBOC-201) have an oxygen affinity and oncotic pressure similar to those of human blood, increased molecular weight, leading to longer half-life, and few adverse effects in clinical trials.\textsuperscript{76, 77, 81, 99, 130, 249}

Perfluorocarbons are carbon-fluorine compounds that are completely inert but can dissolve large amounts of gases.\textsuperscript{210, 249} Because they are immiscible in water, they have to be emulsified. Instead of having a sigmoidal relationship like the modified hemoglobins and blood, po\textsubscript{2} and o\textsubscript{2} content have a linear relationship requiring a relatively high pa\textsubscript{o\textsubscript{2}} (Fi\textsubscript{o\textsubscript{2}}) to maximize oxygen transport. Nonetheless, perfluorocarbons exhibit excellent oxygen unloading.\textsuperscript{210, 213} Other advantages include being well tolerated with mild flu-like symptoms and transient thrombocytopenia, low cost, long shelf life (>1 year); however, they do not expand the intravascular compartment as do the modified hemoglobins and must be used in small volume because of potential reticuloendothelial system overload.\textsuperscript{34, 210, 249}

Liposome encapsulated hemoglobin (e.g., neo red cells [NRC]) offers the advantages of low viscosity and high oxygen transport ability,\textsuperscript{226} but the high cost and complexity of the manufacturing process and the potential for RES overload have prevented large-scale development.\textsuperscript{311, 226}

The main issue with this resuscitation modality is the vasoactivity caused by all the hemoglobin-based oxygen carriers, particularly with the intramolecular cross-linked hemoglobins. The predominant mechanism leading to vasoconstriction is the binding of hemoglobin to nitric oxide (NO), a key mediator responsible for the physiologic regulation of vasodilatory tone.\textsuperscript{146, 147, 155-157, 157} Other mechanisms that may play a role include endothelin release and increased sensitivity to adrenergic receptors. The question is whether vasoconstriction is a good or bad effect. Nolte et al\textsuperscript{146, 147} found that the vasoconstriction is short-lived (2 minutes) and is followed by a longer lasting alteration of vasomotion.

\textsuperscript{*References 48, 60, 119, 147, 155-157, 162, 190, 203, 229, 236
This modulation of vasomotion frequency and amplitude can interfere with microcirculatory flow distribution and velocity, leading to improved and homogeneous local tissue oxygen levels. Studies mentioned previously for the intramolecular cross-linked hemoglobins appear to support this contention. On the other hand, Spahn\textsuperscript{20} found that if the patient has a decreased cardiac contractility and a normal or high mean arterial pressure, this will lead to a decreased cardiac output. Although in a healthy patient who sustains massive hemorrhage, the volume replacement, added oxygen transport, and a certain degree of vasopressor support would be helpful, in penetrating trauma, too high a pressure might lead to increased bleeding. Spahn also said that the vasoconstriction does not lead to a uniform distribution of flow. Interestingly, there is question as to what extent vasoconstriction happens in humans, as it appears to some extent to be species specific\textsuperscript{47, 48}; however, Reah et al.\textsuperscript{147} have used cross-linked hemoglobin as a vasopressor in the medical ICU and have shown decreased norepinephrine requirements in septic shock and SIRS, although there was some decreased cardiac index and global oxygen delivery noted. Surface-modified hemoglobin has also been used with success in septic shock.\textsuperscript{249} Additionally, the vasoconstrictive actions have been attenuated by using hypertonic sodium acetate or NO inhalers along with oxygen carriers.\textsuperscript{156}

Recently, a new issue has been raised concerning mechanisms of oxygen transport. When modified hemoglobins are free in the plasma, they diffuse more readily in the tissue.\textsuperscript{240} This facilitated diffusion of HbO\textsubscript{2} may, through an autoregulation, cause vasoconstriction as the body attempts to regulate (decrease in this case) the amount of oxygen in the tissue. Resolving this issue is important to future development of oxygen carriers.\textsuperscript{240} If the primary mechanism of vasoconstriction is the binding of NO to heme, then to restrict that may prevent oxygen from combining, as the two molecules are similar in size. If the primary mechanism involves autoregulation, then decreasing facilitated diffusion by increasing the molecular size of the oxygen carrier, increasing solution viscosity, or altering oxygen affinity may be the answer. Another new direction involves combining superoxide dismutase and catalase to polyhemoglobin (polyhgb-SOD-catalase) to replace these enzymes normally present in RBCs. Without these enzymes, a worsened ischemia/reperfusion injury may occur; with these enzymes, there is an effective removal of oxygen radicals and peroxides, less iron release, and less methemoglobinemia.\textsuperscript{40}

In summary, it appears that the reality of providing a plasma expander with oxygen transport capability that will not require typing and cross-matching and be readily available, have a long shelf-life, not transmit disease, exhibit no antigenicity, and be free of serious side effects is in the near future.

**Allogenic Blood Transfusions**

It would seem logical, based on the pathophysiology of hemorrhagic shock, to replace blood loss with some form of blood product. Without
blood, the risk of morbidity and mortality from hypovolemia increases with duration and degree.\textsuperscript{128} Because humans are oxygen dependent, maintenance or restoration of oxygen delivery (DO\textsubscript{2}) to the tissues is of primary importance in dealing with blood loss, and should be the goal of RBC transfusion.\textsuperscript{78, 168} Recalling that DO\textsubscript{2} is proportional to cardiac output, hemoglobin, and oxygen saturation, it is reasonable to first increase oxygen delivery by providing the patient more oxygen and increasing the blood volume with crystalloid. Beyond a given limit for an individual patient, the addition of the oxygen carrying capacity of transfused RBCs or oxygen carriers will be needed to achieve a normal range DO\textsubscript{2}.\textsuperscript{78} It is not just the global DO\textsubscript{2} but the local DO\textsubscript{2} that is of concern. Indeed, a significant reduction in cardiac output leads to selective vasoconstriction, which may provide 100\% flow to a vital organ like the brain, but may restrict flow to 30\% at the gut level. As noted previously, the blood pressure and even the global DO\textsubscript{2} may be normal, but local areas may be quite underperfused with definite ischemic consequences, as Malone described from venous thrombosis and bronchopneumonia to ileus and wound dehiscence.\textsuperscript{128}

Countering the reasons to give blood, there are many reasons for caution, some known, and others less well known. Well-known problems with transfusion include acute and delayed hemolytic reactions and disease transmission. As Schreiber et al.\textsuperscript{169} pointed out, however, with modern screening techniques, the rates of infection transmission are low and essentially occur during window periods: HIV 1/493 000, HTLV 1/641 000, hepatitis C virus 1/103 000, and hepatitis B virus 1/63 000. With the use of more sensitive screening methods like p24 antigen, DNA, or RNA polymerase-chain-reaction (PCR) testing, the rates are expected to be even lower. The lesser known problems with blood transfusions are related to the fact that whole blood, and questionably to some extent other RBC prepared or stored products, cause immunosuppression.\textsuperscript{105, 104, 140, 150} Whole blood transfusions lead to post-operative infections,\textsuperscript{68, 91, 102, 105, 154, 143} but by eliminating leukocytes\textsuperscript{102, 103} or by using autologous blood,\textsuperscript{91, 134} the increase can be controlled. The immunosuppression caused by whole blood transfusions is also implicated in tumor recurrence\textsuperscript{143, 150} and an increased risk of first developing cancer.\textsuperscript{22} Perhaps most importantly for hemorrhagic shock, blood has been shown to be an independent risk factor in the development of multisystem organ failure,\textsuperscript{57, 66, 127, 183} as blood leads to an imbalance between proinflammatory and antiinflammatory mediators.\textsuperscript{7, 23, 104} As a result, some say to use blood fairly quickly\textsuperscript{128}; others are more cautious.\textsuperscript{168}

As expected, there is no definite agreed upon number or “trigger” when blood transfusion should be given. For years, a hemoglobin of 10 g/dL or hematocrit of 30\% (the “10/30 rule”) was considered the trigger, but in 1988, the National Institutes of Health Consensus Conference\textsuperscript{168} acknowledged that 7/21 would be more appropriate; however, clinical judgment should be the key ingredient along with the patient’s duration of anemia, extent of operation, existing blood volume, potential for massive blood loss, and preexisting comorbid conditions. Importantly, conference members felt, as did Greenburg,\textsuperscript{78} that certain laboratory
values should be considered that not surprisingly reflect oxygenation of the tissues: oxygen tension, oxygen extraction ratio, cardiac output, and lactate levels.

Given the potential problems with blood transfusions, future directions should include development of clinical monitors to better measure tissue perfusion, develop predictors to trigger transfusions, continue to modify and test oxygen carriers, and consider the use of autologous blood.

**Autologous (Shed) Blood Transfusion**

Autologous blood transfusions are relatively commonplace in many hospitals for a variety of elective operations and may represent an ideal fluid replacement in hemorrhagic shock caused by vascular trauma. Use of autologous blood would avoid the problems of transfusion reactions, disease transmission, and immunomodulation seen with allogenic blood.\(^{109, 121, 223, 227}\) It would provide a source of fresh blood that is rapidly available, normothermic, has physiologic oxygen affinity, and would be acceptable to Jehovah's Witnesses. The autologous blood used in trauma patients is not predonated, but rather it is shed blood immediately returned to the patient. This concept is somewhat controversial because of potential clotting mechanism abnormalities and the reinforcement of potentially contaminated blood.\(^{96}\) Likewise, it has not been used that frequently, because it is often difficult to know beforehand how much blood will be needed for a patient, or if there is bowel contamination that will discourage its use. Over the past several years many studies have helped put these problems in perspective and delineate the advantages and disadvantages of the two techniques to accomplish the transfusion: cell washing with centrifugation (CWC) and reinfusion after filtration (RAF).

Cell washing with centrifugation involves aspiration of shed blood through a machine that washes and centrifuges the blood to produce RBC concentrates with a hematocrit of 55% to 60%, and that is relatively free of plasma-free hemoglobin (free hemoglobin can precipitate in renal tubules leading to acute renal failure), procoagulants (can lead to DIC), bacteria, and malignant cells.\(^{121, 222}\) Unfortunately, platelets and plasma proteins also are essentially removed, which can lead to a dilutional coagulopathy. The washing process itself can lead to hemolysis (and increase plasma-free hemoglobin) and the “salvaged blood syndrome” because of retained platelet-leukocyte deposits on the centrifuge bowl that produce procoagulant leukotactic substances and a DIC picture. What washing accomplishes with free hemoglobin levels in a given patient is variable, up to 10-fold.\(^{199}\)

Although shed blood makes up only a percentage of total transfused blood in most patients, and it is difficult to determine which of the many causes of coagulopathies is responsible for bleeding in a given patient, Horst et al.\(^{96}\) found that 31% of patients had moderate-to-severe
prolongation of their PT and PTT, and the more shed blood received, the more abnormal the lab values. In the study, more than 15 units of intraoperative shed blood (220 mL per unit) led to coagulopathy, but with bowel injury, greater than 10 units was sufficient. Another study corroborated the amount at 3500 mL. Tawes et al. however, felt that despite some bleeding and minor clotting disorders, the DIC/ARDS problem was rare (0.05%) and usually resulted from other causes when it did occur. In fact, they felt most of the problems were technical and were caused by the dilutional effect of removing platelets and clotting factors during red cell washing, failure to properly wash out all particulate and soluble procoagulants, and inadvertent reinfusion of residual heparin. Use of fine screen filters, liberal heparinization, and high volume washing of RBCs was thought to eliminate the problem.

A second major concern is that plasma-free hemoglobin can lead to renal problems. It is assumed that the washing process removes free hemoglobin, and it is also known that free hemoglobin is found in banked blood up to 100 mg/dL without causing a problem, but Kodelle showed a correlation between total free hemoglobin and renal dysfunction (as defined as an increase in creatinine of 1 mg/dL over baseline) and felt that a free hemoglobin level should be determined beyond which shed blood may not be safe. Gocet et al showed that greater than five units of shed blood contributed to renal failure.

Using unwashed shed blood that is immediately reinfused through filtration (RAF) has advantages of a more efficient, more economical, more rapid, and less work-intensive set up. Whole blood is returned to the patient that still retains its platelets and proteins, but carries the concern of more procoagulant activity and free hemoglobin available to cause adverse reactions. Direct comparison studies of CWC versus RAF revealed that although free hemoglobin was higher in RAF, there were no renal consequences; that PT and PTT were elevated in both groups, but without coagulopathy, that platelets were decreased but without bleeding in either group, and that the lab abnormalities almost always returned to normal within 1 day. Importantly, a much higher percentage of shed blood was returned to the patient with RAF, and given the apparent safety, ease of use, and cost of operation, made the use of unwashed, directly reinfused blood more attractive than the expensive cell washed and centrifuged technique.

Two remaining issues needing further study include immunomodulatory effects and the use of shed blood from the abdominal cavity in trauma. There have been several studies that give conflicting results on whether washing affects inflammatory mediators, but one interesting study in guinea pigs showed that autologous blood transiently caused greater myocardial damage than dextran, independent of the hemorrhage per se, and related to activated leukocytes. The use of shed blood from the abdominal cavity is controversial. Some authors say to avoid; others report no major septic events. Smith et al found an association between shock, mortality, and contamination that was decreased by antibiotics, and Boudreaux et al stated that cell
wasting decreased, but did not eliminate bacteria. In Horst's study, although bowel injury led to a quicker DIC picture, there was no increased infection rate.

SEVERE SHOCK AND RESUSCITATION COMPLICATIONS

Survivors of vascular trauma associated with hypovolemic shock can develop severe dysfunction of organ systems and long-term sequelae. Some of these complications are direct consequences of end-organ ischemia; others are caused by resuscitative interventions. The most dreaded consequence of hypovolemic shock is brain damage; the most common is renal dysfunction. In severely injured patients, the leading cause of death is multiorgan failure, which occurs in at least 10% of patients with an Injury Severity Score (ISS) greater than 20. Resuscitation often requires massive transfusions of blood products, which may lead to fluid overload and electrolyte imbalance, pulmonary insufficiency, and coagulopathy. Development of multiorgan failure is influenced by severity, type, and distribution of injury, and duration of shock. Transfusions also contribute to a higher incidence of sepsis after trauma. When circulation is reestablished after either prolonged shock or repair of major vascular injuries, reperfusion-reoxygenation injury is another concern. Although almost any organ can be damaged permanently during shock and resuscitation, the main concern should be protecting the brain, heart, lungs, and kidneys; other organs rarely have dysfunction in the absence of damage to any of these four vital organs.

Critical in determining the prognosis of shock patients is the preexisting general health of the patient. Young and previously healthy patients have much more favorable outcomes than those with advanced age (“low functional reserve”), or comorbidities such as diabetes, atherosclerosis, renal insufficiency, or cirrhosis. Added risk can result from illicit drug or ethanol intoxication—present in a substantial proportion of trauma victims—that can impair both diagnosis and treatment, and alter the physiologic response to trauma.

Although early resuscitation has been shown to reduce multiorgan failure in extensive burns and other hypovolemic conditions, fluids should be administered in concert with hemostatic maneuvers in hemorrhagic shock. All sources of significant bleeding should be controlled before effective blood volume is reestablished. Early surgical intervention is most effective in preventing the potentially disastrous consequences of hemorrhagic shock.

Central Nervous System Damage

The normal brain can recover fully from severe hypoxia sustained for approximately 4 minutes, or even longer periods, especially if some
cerebral circulation is maintained. In the presence of associated conditions, however, permanent cerebral damage tends to occur after shorter periods of ischemia. Patients with atherosclerosis of the carotid, vertebrobasilar, or intracerebral arteries may develop focal deficits after otherwise successful resuscitation from shock. Experimental data support the concept that preexisting cerebrovascular disease influences the response to hemorrhagic shock, potentiating alterations of cerebral microcirculation and increasing vascular resistance. Similarly, ethanol intoxication can impair normal regulatory mechanisms and potentiate metabolic changes, thereby contributing to secondary brain injury during hypotension. Traumatic brain injury can be exacerbated by hypotension as a result of complex local phenomena that also includes loss of physiologic compensatory mechanisms.

To minimize secondary insult, management of patients with head trauma and hemorrhagic shock should ensure adequate cerebral perfusion by maintaining arterial pressure at a satisfactory level, which often requires continuous monitoring of both cerebral and arterial pressures. Hyperventilation is an effective means of reducing brain edema; however, prophylactic hyperventilation of patients with head injuries worsens outcome, presumably by exacerbating cerebral hypoxia. Studies in pigs have shown oxygen tension in the uninjured brain increases with hypoventilation and decreases with hyperventilation under continuous hemorrhage, suggesting hypercapnia may be beneficial in instances of cerebral hypoxia secondary to hemorrhagic shock. Data from experimental animal studies indicate controlled hypothermic cardiac arrest (core temperature of 10°C) can help preserve the viability of brain tissue during hemorrhagic shock. Although initial clinical studies showed induction of hypothermia to be beneficial in brain injury, a larger, multicenter trial demonstrated that hypothermia has no effect when compared with normothermia.

Severe hypoxia-ischemia may result in brain death, characterized by unresponsiveness, absence of respiration and all reflexes (including brainstem), and an isoelectric electroencephalogram. Lesser degrees of extensive bilateral cortical damage may lead to coma followed by a "vegetative state," characterized by open eyelids, Babinski sign, posturing, and periods of autonomic overactivity. Extensive multifocal or diffuse cortical infarcts are common histologic findings of anoxic-ischemic brain damage. "Watershed" infarcts can occur at territories between the major cerebral arteries (often involving the hippocampus) and can lead to persistent memory deficits and weakness of proximal muscle. Delayed postanoxic encephalopathy, progressive neurologic deterioration, coma, and death may occur rarely and are attributed to diffuse brain demyelination.

Spinal cord ischemia leading to paraplegia is caused most commonly by trauma or surgery of the thoracic aorta, but also has been reported rarely in association with acute hypotension and prolonged cardiopulmonary resuscitation. A case of occlusion of the anterior cervical spinal artery (with paralysis of the diaphragm) after cardiopul-
monary arrest has been reported. Nevertheless, in the trauma setting, mechanical compression of the spinal cord remains the major cause of paraplegia.

**Myocardial Ischemia and Heart Failure**

The heart can be resuscitated after 30 minutes of experimental isolation. Young and previously healthy patients rarely develop heart failure after trauma resuscitation, except in instances of prolonged shock or cardiac arrest. Therefore, unless cardiac disease is preexisting, heart failure is rarely the cause of persistent shock after trauma. Myocardial infarction is the most common cause of posttraumatic heart failure; other potential causes include preexisting cardiomyopathy (e.g., diabetic, viral), cardiac contusion, and tamponade. In multiorgan failure, the highest mortality rate occurs with a combination of respiratory and heart dysfunctions.

Heart failure—the inability of the heart to eject enough blood for the metabolic requirements of tissues—can be induced by a number of factors during hemorrhage and resuscitation. Although anemia should increase cardiac output, with hematocrits below 20%, oxygen delivery may decrease, as blood velocity exceeds the capacity for adequate exchange at the capillary level. Hyperkalemia, hypocalcemia, hypophosphatemia, and hypermagnesemia all can impair cardiac contractility and rhythm. Prolonged shock can allow platelet aggregates to form in the coronary microcirculation, contributing to myocardial ischemia and dysfunction. Resuscitation, particularly with blood products, can induce injury to the myocardium independent of the degree of hemorrhage.

Severe brain damage can contribute to myocardial depression and irreversible shock. Whereas mild hypoxia increases sympathetic tone and cardiac contractility, severe hypoxia impairs myocardial function; severe acidosis (pH <7.0) also impairs contractility. Cardiac failure after hemorrhage also has been attributed to "myocardial depressant factors," most likely derived from the pancreas in response to ischemia. Hypothermia (<35°C) is known to severely impair cardiac responsiveness. Patients with renal or liver failure are more susceptible to becoming fluid overloaded, which can precipitate heart failure. Vasopressors usually increase systemic vascular resistance, reducing the cardiac output, tissue perfusion, and oxygen delivery. Conversely, maneuvers that increase the preload and reduce afterload should help in achieving optimal myocardial oxygen consumption during resuscitation.

Persistent hypotension after trauma usually is due to an active bleeding source or inadequate fluid resuscitation. Given patients with coronary artery disease are at high risk of acute coronary events during hemorrhagic shock, persistent hypotension after adequate fluid resuscitation should raise suspicion of myocardial infarction, particularly in diabetic patients, those in coma, and those unable to speak because of
intubation or other factors. Decrease in variability of heart rate with respiratory cycles is a subtle sign of myocardial infarction.246

Lastly, the role of vasopressin in shock has been determined to be not only a potent splanchnic vasoconstrictor, but also a key humoral factor in the maintenance of post reinfusion blood pressure.92 Recently, it has been shown in an animal study that in hypovolemic cardiac arrest, it was vasopressin, not high-dose epinephrine, that resulted in sustained vital organ perfusion, less metabolic acidosis, and prolonged survival. Clinical evaluation of vasopressin during hypovolemic cardiac arrest may be warranted.235

Acute Renal Failure

As part of the response to hemorrhage and hypotension, blood flow to the kidneys—approximately 25% of the cardiac output—is reduced to help preserve effective blood volume. In addition, renin is released from the juxtaglomerular apparatus to cleave circulating α-2 globulin, generating angiotensin I, which, in turn, is transformed in the lungs (by angiotensin-converting enzyme) to angiotensin II, a potent vasoconstrictor. Hypotension and increased blood osmolality during shock also can activate the release of antidiuretic hormone by neurohypophysis. Loss of more than 15% of blood volume, however, usually exceeds the autoregulatory capacity of the kidneys. Renal blood flow is further reduced and redistributed; sodium and water retention mechanisms become impaired. If hypovolemia is profound or persistent, acute renal failure caused by acute tubular necrosis may ensue; however, with adequate resuscitation, the incidence of renal failure requiring dialysis should be less than 5%, even after severe trauma.126 Maintaining the urine output above 2 mL/hour without diuretics should protect the kidneys during shock resuscitation.126 The main factors predisposing to renal failure after trauma are advanced age, chronic hypertension, and preexisting renal insufficiency. Early renal failure after shock has been associated with myoglobinuria and hypotension during the resuscitation phase, whereas late renal failure correlates with development of sepsis and drug toxicity.126

Both ventilatory support and general anesthesia can impair autoregulatory renal responses and reduce renal perfusion. Nonsteroidal antiinflammatory drugs can aggravate renal vasoconstriction. Aortic cross clamping is a known risk factor for renal failure after trauma. Radiocontrast Iodinated contrast media used routinely may alter renal hemodynamics or cause direct tubular toxicity; therefore, except in rare situations, contrast studies should be done only when urine output and renal function tests are normal. Myoglobinuria and hemoglobinuria can cause renal tubular damage, especially in the presence of concomitant hypotension and acidosis. Prophylaxis and treatment of this complication are based on urine alkalization and maintaining high urine output through volume replacement and administration of sodium bicarbonate and
mannitol or loop diuretics. Myoglobin-induced renal failure is usually associated with crush, electrical, or reperfusion injury. Gastric stress ulcerations and platelet dysfunction also can occur as a result of renal failure.

Renal vasoconstriction subsides slowly after adequate resuscitation, and oliguria may persist despite normalization of blood volume. Diuretics should not be used under these circumstances. Low-dose dopamine has been advocated; however, a small randomized, double-blind, placebo-controlled trial showed low-dose dopamine offers no advantage to normovolemic patients after elective abdominal aortic surgery, patients with acute oliguric renal failure not included. Recent evidence suggests a potential for use of concomitant infusion of low-dose dopamine and norepinephrine. Anuria (urine volume <50 mL/day) after trauma is usually caused by mechanical obstruction. A third of critically ill patients may have glomerular or tubular damage despite normal routine renal function tests.

Hyperkalemia is the most common life-threatening result of renal failure; it requires immediate treatment, including potassium antagonist (10% calcium gluconate), correction of acidosis (sodium bicarbonate), stabilization of cardiac membranes (glucose-insulin infusion), and use of cation exchange resins (Kayexalate, Sanofi Winthrop, New York, NY).

Hemodialysis is indicated for fluid overload with pulmonary edema, refractory hyperkalemia, severe metabolic acidosis, and uremic pericarditis or encephalopathy. Dialysis may not be effective in correcting platelet dysfunction. Although controversial, early dialysis may benefit the hemodynamically stable patient with severe renal impairment by allowing adequate nutritional support and facilitating administration of fluids. Side effects of hemodialysis include hypoxemia, hypotension, bleeding, infection, and increased intracranial pressure. In addition, use of heparin during hemodialysis may increase the risk of bleeding in conditions such as pelvic fracture and retroperitoneal hematoma, even when protamine is administered into the inflow conduit. Hemodialysis without anticoagulation has the potential limitation of activating the coagulation system and causing greater fibrin deposition on dialyzer membranes.

Forms of dialysis used in critically ill patients include intermittent hemodialysis (IHD), continuous arteriovenous hemofiltration (CAVH), continuous venovenous hemofiltration (CVVH), continuous renal replacement therapy (CRRT), and slow low-efficient daily dialysis (SLEDD). CRRT and SLEDD appear to be advantageous over IHD because of hemodynamic stability, correction of hypervolemia, and better solute removal. A retrospective study of 100 patients showed that early institution of CCRT improves survival of trauma patients who develop acute renal failure. Although a relatively new method, SLEDD is less expensive than CRRT and allows better mobilization of the patient. Patients with acute renal failure treated with CAVH for a mean period of 10 days had a complication rate of 10%, mostly associated with the vascular access in the femoral artery. Although CVVH has
been associated with an increased likelihood of death, this appears to be related to severity of illness and not the treatment choice itself.\textsuperscript{191}

Early resuscitation can shift renal failure from the oliguric to the non-oliguric form. Non-oliguric renal failure (urine output >400 mL/day plus azotemia) is associated with a 20% mortality rate, whereas the oliguric form has a mortality rate of more than 50%.\textsuperscript{84} Although early fluid resuscitation may reduce the incidence of renal dysfunction and mortality in burn victims,\textsuperscript{82} achieving hemostasis should have the highest priority in hemorrhagic shock.\textsuperscript{19}

**Lung Injury**

Causes of pulmonary insufficiency (PCO\textsubscript{2} >45 mm Hg; PO\textsubscript{2} <60 mm Hg; normal or low pH) after trauma include atelectasis, lung contusion, pneumonia, thromboembolism (noted in approximately 14% of autopsies from trauma),\textsuperscript{19} and acute respiratory distress syndrome (ARDS).\textsuperscript{13} Respiratory failure is most common in patients with an ISS greater than 16, shock on admission, and age of more than 55 years. Head injury can contribute to respiratory failure because of increased risk of aspiration, hypoventilation, atelectasis, and need for ventilatory support (barotrauma). In addition, a form of “neurogenic” pulmonary edema has been described. Fat embolism should be suspected when cerebral and respiratory dysfunction, thrombocytopenia, and increased alveolo-arterial gradient are associated with pelvic or long-bone fractures.

Acute respiratory distress syndrome is characterized by hypoxemia resistant to increases in fraction of inspired oxygen (FiO\textsubscript{2}).\textsuperscript{13, 22} ARDS occurs most commonly in patients with peritonitis. The pathophysiology involves recruitment of leukocytes to capillary beds in the lungs. Subsequent local release of multiple inflammatory and vasoactive substances, including oxygen–free radicals, proteases (e.g., elastase, collagenase), arachidonic-acid metabolites that increase platelet aggregation, and complement activation, leads to increased capillary permeability, edema, further inflammation, and thrombotic occlusion of capillaries. Although shock itself can cause ischemia of pulmonary tissues, with impairment of ciliary activity and surfactant production, hemorrhage alone rarely causes ARDS, which usually requires an “inflammatory state.” Measurement of extravascular lung water in 16 severely traumatized patients did not correlate with hemorrhagic shock, massive transfusion, and crystalloid resuscitation; post-traumatic elevations in lung water, capillary hydrostatic pressure, and capillary permeability correlated with lung contusion or sepsis.\textsuperscript{219} Delayed-onset pulmonary insufficiency, however, has been demonstrated experimentally in non-human primates resuscitated from hemorrhagic shock.\textsuperscript{178} Although excess fluid aggravates ARDS, the role of fluid overload and massive transfusions as primary causes of pulmonary failure is controversial.

In one study, pulmonary complications were noted in approximately 10% of more than 3000 trauma victims.\textsuperscript{195, 45, 246} The lung is the most
frequent organ involved in multiorgan failure and is usually the first organ to fail after injury. Respiratory failure has the highest mortality rate compared with failure of other organ systems.

Use of extracorporeal life support (ECLS) has been advocated in adult trauma patients with multiple injuries and severe pulmonary failure. In 30 patients who had an estimated mortality risk greater than 80%, early institution of ECLS was associated with improved oxygen delivery, diminished ventilator-induced lung injury, and improved survival. Preclinical studies suggest resuscitation with hemoglobin solutions (pyridoxalated hemoglobin polyoxyethylene conjugates) can lead to vasoconstriction and elevated arterial and regional PCO₂.

The mortality rate of ARDS remains approximately 35% despite modern intensive care. Increased pulmonary hypertension indicates a poor prognosis in patients with trauma and respiratory failure.

Adrenal Insufficiency

Acute adrenal insufficiency secondary to bilateral adrenal hemorrhage or infarction has been described in trauma patients (adrenal apoplexy). Most cases of adrenal insufficiency after shock, however, are not associated with adrenal apoplexy. A state of "relative" adrenal failure may occur during phases of shock and resuscitation, particularly in elderly patients, because of the increased demand for adrenocortical hormones. Some patients may have subclinical adrenal hypofunction. In addition, it has been postulated that negative regulation of adrenal function may be caused by a sustained increase in plasma corticosterone levels because of decreased hepatic 11-HSD activity following trauma and severe hemorrhage.

Adrenal failure has been reported in 19% of critically ill patients who are hemodynamically unstable and on vasopressors. Adrenal hypofunction, as assessed by plasma cortisol, may occur in approximately 50% of critically ill patients. In unselected patients in the ICU, however, incidences as low as 1% have been reported. Therefore, screening may be indicated only for patients with prolonged stays in the ICU and ages of more than 55 years.

Mesenteric Ischemia

Nonocclusive mesenteric ischemia is a low-flow state that may result from hypovolemic hypotension in the trauma patient. Adequate cardiac resuscitation usually causes this entity to be extremely uncommon. Associated low-flow states may occur, however, and include cardiac failure, cardiac arrhythmia, myocardial infarction, shock, hypovolemia, aortic valvular insufficiency, and use of certain inotropic drugs. Vasoconstriction to such states is a compensatory mechanism designed to improve perfusion to vital tissues such as the brain, heart, and
kidneys. Vasoconstriction remains fixed until reversed by vasodilating drugs. Angiographic studies reflect spasm of segmental branches of the superior mesenteric artery; the spasm is diffuse, focal or concentric. Spastic areas are uniformly smooth. Clinical manifestations of this non-occlusive low-flow phenomena are the same as those mentioned when one discusses the response of the intestine to ischemia, no matter what mechanism. Trauma to the abdomen can result in direct injury to the stomach, duodenum, small bowel, and colon, resulting in hemorrhage or perforation. This setting, however, focuses on the sequelae of hypovolemic hypotension. Complications may present weeks to months following discharge in the patient who survives. The compound problem of a direct injury to the abdomen and GI track, with superimposed hypovolemic hypotension, presents a complex situation. Blunt trauma causes visceral or mesenteric injury and minor contusions. It may produce significant hematomas, or even perforations. Sometimes these injuries are severe enough to include mucosal injury, but the intact serosa prevents early peritoneal soiling. Given the compound problem of hypotension superimposed on abdominal trauma, the subsequent ischemic perforation will result in infection and fistula formation.

Nonoperative management of mesenteric insufficiency requires a diagnosis to determine whether visceral organ injury is present. Patients perceived to be stable undergo diagnostic tests, such as the computed tomography (CT) scan, for a definition of that injury. Differentiating between a mesenteric injury and bowel perforation can usually be ascertained by repeated physical examinations in the conscious patient. Peritoneal lavage may be optional. A negative celiotomy may occur, but it should be infrequent based on frequent clinical and CT examinations. Mesenteric ischemia with colitis is not an acute event, although it may occur during the ensuing 24 to 72 hours. The diagnosis is more often obtained through sigmoidoscopy. This procedure may be diagnostic in nearly 50% of the patients.

The trauma patient is not immune to non-trauma GI disorders, but that is not the thrust of this section. The issue of GI hemorrhage may be due originally to hypotension and hypovolemia with stress ulcerations or, subsequently, may cause hypotension hypovolemia with hematemeses and melena. Rarely, Dieulafoy’s syndrome may cause massive post-traumatic GI hemorrhage. The patient who is hypovolemic and, subsequently, continues to bleed from either an intraabdominal or extra-abdominal source will have some sequelae, such as ischemic colitis, following preresuscitation of hypotension and hypoperfusion. Bloody diarrhea may result. Exacerbation of preexisting peptic ulcer disease is possible, but hemorrhage of the stress ulceration following multorgan failure is more common, and occurs in the patient with a prolonged ICU course.

Acute gastric and/or duodenal ulceration may follow the stress of hypovolemic hypotension. Perforation may occur, but is less frequent than bleeding. In response to hypovolemic hypotension, the signs that may present include blood from the nasogastric tube, melena, and shock. As an extreme, one may see peritonitis, abdominal distension, and a
septic course. Diagnosis is usually through endoscopy, but for perforation and adverse events further in the GI tract, CT scan is appropriate.\textsuperscript{120} Hemorrhage from a duodenal stress ulceration is usually a later manifestation and not acute following hypovolemic hypotension; nevertheless, the treatment should involve H\textsubscript{2} blockers and antacids, gastric lavage, and others. The stress insult, hypotension, should be removed. The outcome of mesenteric ischemia is, at worst, perforation, but more likely a stricture of a segment of bowel. It does not necessarily require acute surgical intervention.

**Hepatic Dysfunction**

Hepatic dysfunction following major trauma is a relatively common adverse event. The epidemiologic setting of severe automobile accidents suggests this is a predisposing factor. Many accidents also are associated with alcohol and drug abuse; they have their own impact on direct trauma superimposed on the hypotension the body incurs. The primary adverse effect on the liver through trauma is shock and the need for anesthesia, massive blood transfusions, and prolonged operations. Among the clinical problems is the element of jaundice after the trauma. The jaundice one identifies is, more likely than not, caused by several issues: shock liver, benign postoperative cholestasis, hepatic venous congestion, increased pigment load, sepsis, drug-induced, and major hepatic resection.\textsuperscript{124}

The insult to the liver through hypovolemic hypotension and resultant hypoxia is secondary to hypotension, the most significant factor contributing to development of jaundice and liver dysfunction. Only occasionally does the investigation of posttraumatic jaundice lead to a single etiologic factor. More likely than not, multiple causes are listed as contributing to postoperative jaundice in the trauma patient with hypotension and hypovolemia.\textsuperscript{216} This patient usually has received multiple blood transfusions, prolonged operation and episodes of hypoxia. Shock alone could lead to hepatic mitochondrial swelling and centrilobular necrosis. In the typical patient, one sees the bilirubin as high as 5 to 20 mg percent within 1 week of the surgical intervention, or within 1 week of hypovolemic hypotension. The serum glutamic oxaloacetic transaminase (SGOT) may range from 100 to 500 U/mL, and the alkaline phosphatase may be elevated no less than two to three times normal. Treatment of the shock liver consists of supportive measures and avoidance of those drugs that adversely affect hepatic mechanisms.

A second type of liver injury associated with shock resuscitation, hypovolemic hypotension, and associated hypoxia is benign postoperative cholestasis. Patients with this condition have essentially the picture of obstructive liver chemistry with elevated bilirubins and markedly elevated alkaline phosphatase. The SGOT level is usually less than 200, and the majority of patients recover.

Another type of hypoxic hypovolemic liver damage is related to
prolonged venous congestion of the liver. Often referred to as ‘cardiac jaundice,’ it occurs with a pure cardiac defect that may be seen in the patient with hemorrhagic hypovolemia. One clearly sees the centrilobular necrosis and hemorrhage from elevated venous pressure and anoxia. Hypervolemia is a postresuscitation phase, and massive crystalloid infusion may contribute to the hepatic edema seen in this condition.4

In the immediate period following hypovolemic hypotension, one also can identify overt jaundice following a hemolytic reaction; however, this is uncommon without hepatic parenchymal dysfunction. The usual response to intravascular hemolysis is a decrease in haptoglobin level, the appearance of free hemoglobin in the blood and urine, and an increased unconjugated (indirect) bilirubin. The unconjugated bilirubin is strongly protein-bound and limited to the vascular space. It does not appear in the interstitial fluid and is not filtered by the kidney. Massive hemolysis associated with hypovolemic hypotension may saturate the reticuloendothelial system and bilirubin excretory system, allowing conjugated bilirubin to reflux into the blood. Conjugated bilirubin is more soluble and less strongly protein-bound than unconjugated bilirubin and, therefore, diffuses easily into the interstitial fluid and may appear in the urine. Posttraumatic hemolysis results from destruction of transfused red blood cells, which have a shortened lifespan. They also may occur because of adverse interdrug reactions, with hemolysis as a result. Among those drugs that precipitate hemolysis are aspirin, sulfonamides, or nitrofurantoin. The latter is particularly noticeable in glucose-6-phosphate-deficient patients. Major hepatic resections in the presence of hypovolemic hypotension and profound shock are associated with jaundice. The cellular mass in this case has been decreased and, therefore, the response of the liver—‘shock liver’—is more dramatic.

The manifestations of hypovolemic hypotension as regards hepatic dysfunction include: jaundice, encephalopathy, hepatomegaly, and acholic stools. The laboratory assessment should include enzymes, bilirubin, prothrombin time, ammonia, and assessment of albumin.

Management is one of resuscitation: volume resuscitation to restore hepatic viability, with adequate blood flow and metabolism. Ultimately, it will be necessary to determine whether any subsequent liver disorder has become hepatocellular or obstructive. Sepsis may follow the damage to the liver, and one has to be alert to this possibility. Subsequently, all medications and anesthetics must be identified carefully before they have an adverse impact on hepatic metabolism. One must address the role of hemolysis when jaundice appears, and whatever it takes for adequate oxygenation, including protection of pulmonary function, is necessary. The patient must be supported with adequate oxygen delivery through this adverse period.

Finally, hyperalimentation formulas have to be adjusted, depending on the hepatic metabolism and capacity of filtering. One must consider reducing aromatic amino acids and supplying extra arginine and branch-chain amino acids in patients with liver dysfunction. It is suggested that these alterations minimize adverse impact on liver function tests.
The management of hepatic encephalopathy requires eradicating blood from the GI tract if hemorrhage has been present, removing any drug that adversely affects the liver, and minimizing protein intake with the focus on branch-chain amino acids. To remove the nitrogenous load from the GI tract, one should consider enemas or cathartics. The use of neomycin may decrease urease-producing bacteria. Lactulose appears to minimize encephalopathy. Finally, one must again prevent a septic focus from occurring.

In summary, the principles of treatment for liver injury include control of bleeding, debridement of devitalized tissue, and establishment of appropriate drainage. The liver injury will further compound and compromise liver function in the patient with hypovolemic hypotension.

**Pancreatitis**

Pancreatitis is uncommon in the trauma patient, and is usually related to direct injury to the pancreas. During hypovolemic hypotension, however, the pancreas can be subjected to an ischemic event. Pancreatitis can then manifest with fever and/or an elevated white-blood-cell count, in addition to abdominal tenderness and ileus. Pleural effusions may occur.

The diagnosis is based on elevated serum amylase or elevated urine amylase. There also may be an elevated serum lipase. Abdominal x-rays suggest an ileus and a colon cut-off sign. CT will confirm an edematous pancreas. Treatment is nothing by mouth, nasogastric suction, and cardiovascular support with adequate resuscitation and intravascular volume. Future sequelae would require monitoring for abscess, pseudocyst, respiratory failure, hypocalcemia, and hemorrhage from an eroded artery. The effect of pancreatitis on the lung is well established, causing adult respiratory distress syndrome (ARDS) with destruction of surfactant by release of phospholipase A.

Ischemic pancreatitis is an extremely uncommon adverse event; when it occurs, the physician will be treating other problems in addition to the pancreatitis.

**Coagulopathy**

The hemostatic balance between coagulation and fibrinolysis requires adequate pH, temperature, and blood composition of cells and proteins. The observed decrease in levels of clotting factors early after severe hemorrhage is attributed to consumption and plasma dilution from resuscitation. Massive transfusions of blood, or colloid or crystalloid solutions, can lead to so-called "dilutional coagulopathy." During later phases, enhanced hepatic synthesis, appropriate replacement, and other factors contribute to restoration of clotting factors. Hypothermia and metabolic acidosis usually precede the development of coagulopa-
thy in severely injured patients requiring massive transfusion. Hypothermia-related coagulopathy requires both rewarming and clotting-factor replacement. A consumptive coagulopathy that develops within hours after blunt brain injury also has been described. Bleeding abnormalities in renal failure may be associated with suboptimal binding of the von Willebrand factor to platelet membranes, acquired storage-pool deficiency, and anemia. In management of uremic bleeding in the trauma patient, cryoprecipitate and desmopressin may be useful because of their short onset of action. Synthetic colloids, such as hydroxyethyl starch (HES), can be effective in restoring intravascular volume. Despite their antiplatelet properties, if used below upper-dose limits, these solutions can improve microcirculation and are safe for coagulation, reticuloendothelial, and renal functions.

In general, transfusion of more than 10 units of packed RBCs results in thrombocytopenia, low fibrinogen, and prolonged prothrombin time; more than 20 units cause coagulation defects in 70% of patients. Patients with ISS greater than 25 who receive 6 or more units of blood represent a high-risk group for development of multiple organ failure. Disseminated intravascular coagulation is often associated with multiple organ failure and the systemic inflammatory response syndrome. Staged laparotomy may be indicated in select patients with refractory coagulopathy after trauma.

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NEW APPROACHES TO TRAUMA MANAGEMENT USING SEVERITY OF ILLNESS AND OUTCOME PREDICTION BASED ON NONINVASIVE HEMODYNAMIC MONITORING

William C. Shoemaker, MD, FACS

Hemodynamic bedside monitoring by pulmonary artery catheterization (PAC) has been considered by many as the gold standard for critically ill patients, but its usefulness has been challenged, particularly in the late stages of illness after the onset of organ failures. Meta-analyses by Boyd and Hayes and Kern and Shoemaker showed no outcome improvements in seven randomized studies of patients who entered the ICU after organ failure or sepsis had occurred, but outcome was significantly improved in seven other randomized studies when PAC-directed therapy was given early or prophylactically. Because time may be important in the initial resuscitation and management of emergency patients, noninvasive monitoring is a useful alternative approach to identify and correct hemodynamic deficiencies at the earliest possible time. Previous studies have documented satisfactory correlation between thermodilution and bioimpedance cardiac output values for trauma patients in the emergency department (ED), surgical suite (OR), and under ICU conditions.

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In the present study, hemodynamic monitoring of severe trauma or high-risk surgical patients was reviewed, beginning in the ED, continuing in the radiology department, the OR, and then in the ICU. The author studied patients immediately after trauma or high-risk surgery because time factors are important and the early time course of circulatory events could be monitored.\textsuperscript{16, 17} Many other studies showed the importance of optimal goals in the early period after injury or surgery.\textsuperscript{4-6, 8, 14, 15, 20} Continuous visual displays of monitored data described rapidly changing patterns during unstable emergency conditions. Second, the author time-integrated the differences between the monitored curve and normal values or "optimal" goals derived from the patterns observed of previous series of survivors of acute severe trauma or operations. Third, the author then calculated the net cumulative excesses or deficits of each monitored variable for each patient and for the survivors and nonsurvivors.\textsuperscript{23} Fourth, the author reviewed studies of discriminant analysis to predict outcome.\textsuperscript{21} Finally, the author reviewed a stochastic control program that evaluates acute severely injured emergency patients throughout the course of acute illness and provides a decision support system.\textsuperscript{1-3, 19} Acute injury was studied because the onset of illness occurred immediately before admission, and the course of circulatory events could be monitored from the time of ED admission beginning in the ED and continuing until hemodynamic stability was achieved.

**MATERIALS AND METHODS**

**Noninvasive Hemodynamic Methods**

Noninvasive hemodynamic monitoring of acute trauma patients was able to quantify hemodynamic deficits at the earliest possible time. Noninvasive hemodynamic monitoring systems consisted of a bioimpedance method for estimating cardiac output, together with pulse oximetry to reflect pulmonary function, transcutaneous oxygen tension to reflect tissue perfusion, and blood pressure to reflect the overall circulatory status.\textsuperscript{20, 23-26} These continuously monitored noninvasive measurements were used to prospectively evaluate circulatory patterns in severely injured patients, beginning with admission to the ED in a university-run county hospital.

**Calculation of Net Cumulative Deficit of Hemodynamic Variables**

The area between the fluctuating monitored variables and either the normal values for blood pressure, $\text{Sao}_2$, and $\text{PtcO}_2/\text{FiO}_2$, or optimal value for cardiac index were calculated. This area was integrated over time to calculate the net cumulative amount of deficit or excess of each
monitored variable. The net cumulative deficit or excess for each variable was calculated in each individual patient and for survivor and nonsurvivor groups. For example, given a normal mean arterial pressure (MAP) of 85 mm Hg, in a patient whose MAP averaged 60 mm Hg for 2 hours before resuscitation, the calculated deficit is \((85-60) \times 2\) or \(-50\) mm Hg ⋅ h.\(^{21}\)

**Outcome Prediction by Discriminant Analysis**

There were significantly greater calculated deficits of cardiac index, pulse oximetry, and transcutaneous \(O_2\) in the nonsurvivors than in survivors during the period of monitoring (see Table 4). These three variables and the Glasgow Coma Scale (GCS) score, having moderate levels of significance with outcome, were selected for the stepwise discriminant analysis.\(^{21}\)

**Database for Stochastic Analysis and Control Program**

Databases for acutely injured patients have been developed to describe primary injuries, covariates, hemodynamic patterns by invasive and noninvasive methods, and outcomes, including survival or death, organ failure, other complications, hospital days, and ICU days. Noninvasive monitoring usually was begun in the ED, and the patient was followed to the OR, radiology department, and ICU. Invasive PAC was instituted when clinically indicated, usually after the patient arrived in the ICU. The time and place of monitoring, time of operations, times of ICU admission and discharge, and time of hospital discharge or death were recorded relative to time elapsed after admission.

These databases include the following 30 covariates:

- Age
- Gender
- Estimated blood loss in the preoperative, intraoperative, and postoperative periods
- Blunt truncal trauma
- Penetrating truncal trauma
- Nontruncal (extremity) injury
- Spinal cord injury
- Blunt cardiac injury
- Penetrating cardiac injury
- Pulmonary contusion
- Pelvic fracture
- Long bone fractures
- Head injury
- Brain death
Early stage (< 12 hours)
Middle stage (12-24 hours)
Late stage (> 24 hours) or after organ failure
Cardiac insufficiency (reduced cardiac reserve capacity determined by responses to standardized doses of transfusions, and fluid challenges)
Bacterial contamination, sepsis, or systemic immune response system
Respiratory dysfunction or failure immediately before the present acute illness
Preillness renal insufficiency or failure
Preillness hepatic failure
Nutritional insufficiency or failure
Uncontrolled diabetes
Preillness essential hypertension
Cardiac injury (blunt or penetrating)
Cardiac arrest
Pregnancy
GCS score
The injury severity score

Method for Stochastic Analysis Based on a Trauma Database

Bayard et al. developed a stochastic analysis and control program to determine individual patients' survival probabilities (SP), based on the patient's state and patients in the database with very similar states. The patient's "state" is defined by the primary diagnosis, 30 covariates, and hemodynamic variables. By "similar" is meant a group of patients, referred to as "nearest neighbors," with the same diagnosis who share the same set of specified covariates and have very similar hemodynamic patterns to the patient under study. Mathematically, the stochastic analysis is defined as policy iteration with respect to conventional therapeutic policy used for each patient as the database was developed. It is motivated by methods of machine learning and methods of dynamic programming for stochastic control. A therapeutic decision support program was designed to use the database of therapeutic responses to evaluate the relative effectiveness of various therapies by the responses of each therapy for the patient's nearest neighbors.

The state of the patient at any time is defined in terms of primary diagnosis, the covariates, and the hemodynamic measurements. A state vector, \( x(t) \), at time \( t \) is defined in terms of the various hemodynamic measurements, their derivatives, and their integrals. Assume that there are \( L \) different types of measurements taken on a given patient (e.g., cardiac index, blood pressure, pulse oximetry, and transcutaneous \( O_2 \) and \( CO_2 \) tensions). Specifically, for each measurement type, denoted as
$y$, define the state vector as a concatenation of the value $y$, its first and second derivatives $y', y''$, and its first integral $\int y \, dt$, as follows:

$$x(t) = \begin{bmatrix} y(t), y'(t), y''(t), \int_0^t y \, dt, \ldots, \ldots, y(t), y'(t), y''(t), \int_0^t y \, dt \end{bmatrix}^T$$

that is, for $L$ different measurement types there will be $4L$ states. In practice, the derivatives and integrals are approximated by finite differences and sums of the time-ordered data of the database.

RESULTS

Noninvasive Monitoring from the Time of Admission

The mean estimated blood loss, which reflects preoperative and intraoperative hemorrhage, measured at the end of surgery, was 2970 ± 3856 (SD) mL in survivors and 6263 ± 5540 mL in the nonsurvivors. In the present series, 22 patients had massive blood loss (> 5000 mL). Vigorous attempts were made to replace these losses at the time of surgery and in the immediate postoperative period.\textsuperscript{19, 21} Noninvasive monitoring systems were found to be feasible in acutely ill ED patients for early description of temporal hemodynamic patterns and to provide quantitative calculation of the total amount of deficit or excess accumulated by each monitored variable. Table 1 lists the mean values ± SEM of cardiac index, MAP, Sapo\textsubscript{2}, and PtcO\textsubscript{2}/FiO\textsubscript{2} for survivors and nonsurvivors averaged throughout the observation period. The CI, Sapo\textsubscript{2}, and PtcO\textsubscript{2}/FiO\textsubscript{2} values of the survivors were significantly greater than the values of those who died. Nonsurvivors’ Sapo\textsubscript{2} values were significantly lower than the survivors’ values, but these differences were not clinically important; when Sapo\textsubscript{2} reductions occurred, they were

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal or Optimal Value</th>
<th>Survivors (N=103) Mean ± SEM</th>
<th>Nonsurvivors (N=48) Mean ± SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI, L/min/m\textsuperscript{2}</td>
<td>4.0</td>
<td>4.14 ± 0.02</td>
<td>3.87 ± 0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>85</td>
<td>88 ± 0.37</td>
<td>80 ± 0.69</td>
<td>0.066</td>
</tr>
<tr>
<td>Sapo\textsubscript{2}, %</td>
<td>98</td>
<td>99 ± 0.05</td>
<td>96 ± 0.26</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PtcO\textsubscript{2}/FiO\textsubscript{2}, torr</td>
<td>200</td>
<td>206 ± 2.9</td>
<td>93 ± 2.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Normal values for MAP, Sapo\textsubscript{2}, and PtcO\textsubscript{2}/FiO\textsubscript{2}, or “optimal” value for cardiac index. Mean ± SEM for cardiac index (CI), mean arterial pressure (MAP), Sapo\textsubscript{2}, arterial hemoglobin saturation by pulse oximetry (Sapo\textsubscript{2}), and transcutaneous oxygen tension indexed to FiO\textsubscript{2}(PtcO\textsubscript{2}/FiO\textsubscript{2}) calculated for the monitored period, and P values for differences between survivors’ and nonsurvivors’ values. From Stoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 129:523–528, 2001; with permission.
rapidly corrected by intubation, mechanical ventilation, or increased FiO₂. MAP values of survivors tended to be higher than those of nonsurvivors (P = .066). Correlation between simultaneous thermodilution and bioimpedance cardiac output measurements in the present series was r = .91, r² = .83; bias and precision were −0.30 ± 1.10 L/min/m².²¹

**Net Cumulative Amount of Deficit or Excess in Monitored Variables**

The net cumulative deficits of flow and tissue perfusion measured during the initial resuscitation period were greater in nonsurvivors than in survivors; these differences were correlated with outcome. Flow calculations, measured as volume per unit of time, are L/min/m². When multiplied by monitored time in minutes this gives, as units, L/m² for cardiac index or L for cardiac output. The units for MAP, SapO₂, and PtcO₂/FiO₂ are mm Hg · h, % · h, and torr · h, respectively. For example, during the monitoring period, the survivors’ cardiac index averaged 81 L/m² more than the “optimal” 4.0 L/min/m², empirically determined from the plateau of high values of survivors within the first 24 hours of admission.⁵,⁶,¹⁶,¹⁷,²⁰,²¹ This was equivalent to 140 L of cardiac output per patient over the monitored period. During the monitoring period of those who died, the cardiac index averaged 232 L/m² less than optimal, and the cardiac output averaged 402 L per patient less than optimal. The difference between survivors and nonsurvivors was 542 L, using 4.0 L/min/m² as the therapeutic goal (Table 2).

**Outcome Prediction by Discriminant Analysis**

Based on the classification function generated for cardiac index, pulse oximetry, GCS, and transcutaneous O₂ variables, the discriminant

**Table 2. MEAN NET CUMULATIVE DEFICITS OR EXCESSES OF MONITORED VALUES OF SURVIVORS AND NONSURVIVORS THROUGHOUT THE PERIOD OF OBSERVATION**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors Mean</th>
<th>Survivors SEM</th>
<th>Nonsurvivors Mean</th>
<th>Nonsurvivors SEM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI, L/m²</td>
<td>+ 81</td>
<td>52</td>
<td>−232</td>
<td>138</td>
<td>&lt; 0.007</td>
</tr>
<tr>
<td>MAP, mm Hg · h</td>
<td>−10</td>
<td>13</td>
<td>−57</td>
<td>24</td>
<td>0.078</td>
</tr>
<tr>
<td>SapO₂, % · h</td>
<td>−1</td>
<td>0.3</td>
<td>−8</td>
<td>2.6</td>
<td>&lt; 0.006</td>
</tr>
<tr>
<td>PtcO₂/FiO₂ torr · h</td>
<td>+ 313</td>
<td>87</td>
<td>−793</td>
<td>175</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Net cumulative deficits or excesses expressed as mean values ± SEM for cardiac index (CI), arterial hemoglobin saturation by pulse oximetry (SapO₂), and transcutaneous oxygen tension indexed to FiO₂ (PtcO₂/FiO₂) for the monitored period; cumulative deficits are shown for mean arterial pressure (MAP); P values are for differences between survivors’ and nonsurvivors’ values. Note the pronounced differences in the net cumulative deficits and excesses of cardiac index and PtcO₂/FiO₂ of the survivors versus the nonsurvivors. From Shoemaker WC, Wu CC, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523–528, 2001; with permission.
Classification of survivors, Z > 2.36; where \( Z = 0.0011 (\text{cumulative PtcO}_2/\text{FiO}_2) + 0.3300 (\text{GCS}) + 0.0656 (\text{cumulative SapO}_2) + 0.0423 (\text{cumulative CI}) \). From Shoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523-528, 2001, with permission.

Outcome Prediction by Stochastic Analysis

A major assumption in the present approach is that circulatory deficiencies that ultimately lead to shock, organ failure, and death can be identified early by noninvasive monitoring, and the SP may be predicted by stochastic analysis of dynamic patterns. The SP is roughly equivalent to severity of illness. That is, the patient with a 90% likelihood of death is severely ill, whereas the patient with an estimated survival outcome of 90% may not be very ill. The proposed mathematical representation of the circulation defines the patient's state by specific diagnos-

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**Table 3. STEPWISE DISCRIMINANT ANALYSIS**

<table>
<thead>
<tr>
<th>Step Entered</th>
<th>Partial R²</th>
<th>Prob &gt; F</th>
<th>Cumulative R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cumulative PtcO₂/FiO₂</td>
<td>0.210</td>
<td>.0001</td>
<td>0.2099</td>
</tr>
<tr>
<td>2. GCS</td>
<td>0.188</td>
<td>.0001</td>
<td>0.3881</td>
</tr>
<tr>
<td>3. Cumulative SapO₂</td>
<td>0.053</td>
<td>.0047</td>
<td>0.3921</td>
</tr>
<tr>
<td>4. Cumulative CI</td>
<td>0.031</td>
<td>.0536</td>
<td>0.4107</td>
</tr>
</tbody>
</table>

---

**Table 4. CLASSIFICATION SUMMARY FOR THE SERIES (N = 151)**

<table>
<thead>
<tr>
<th>Actual Outcome</th>
<th>Predicted to Die</th>
<th>Predicted to Live</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (Row %)</td>
<td>N (Row %)</td>
<td>N (Col %)</td>
</tr>
<tr>
<td>Died</td>
<td>30 62.5</td>
<td>18 37.5</td>
<td>48 31.8</td>
</tr>
<tr>
<td>Lived</td>
<td>5 4.9</td>
<td>98 95.1</td>
<td>103 68.2</td>
</tr>
<tr>
<td>Total (%)</td>
<td>35 23.2</td>
<td>116 76.8</td>
<td>151 100.0</td>
</tr>
</tbody>
</table>

Misclassification: 23/151 = 15.2%

Classification summary of those predicted to live and those predicted to die, based on the net cumulative deficits of monitored variables. Row %, the percentage of patients in that row. Col %, the percentage of patients in that column. Note: 95% of those predicted to live after initial resuscitation did live, while 62% of those predicted to die did not survive. From Shoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523-528, 2001, with permission.
tic categories, covariates, hemodynamic variables, their derivatives, and their integrals in a multidimension grid. Since the database contains over 9000 time lines, each of which may represent a patient's state, there are many choices available for selection of the nearest neighbors. A patient's SP for a given state \( x \) is denoted by \( S(x) \), which is calculated by first extracting the 40 nearest neighbor states of patients having the same diagnosis and covariates as well as hemodynamic values that are closest to the given patients' values. The SP is then calculated as the fraction of these nearest neighbors who survived with this treatment. The SP also may serve as a measure of the patient's severity of illness.\(^{22}\) The average difference between a given patient's variables and the nearest neighbors' variable was between 0.1 and 0.2 of the SD. This indicates that, given a large database, one did not have to deviate far from the patient's state to find an adequate number of similar neighbors.

This approach was tested in the extenuating and sometimes chaotic circumstances of severely traumatized ED patients in a large inner city public hospital. Under these circumstances, the SP was found to closely track changes at each point during the initial observation period and the SP correctly predicted outcome in each patient before the end of the monitoring period, which was usually 12 to 24 hours after injury. Early clinical diagnosis and physiologic assessment are essential because they allow therapy to be initiated sooner, as earlier therapy may improve outcome in emergencies in which time is crucial.\(^{4,8,13,15-17,20,28}\) Moreover, the stochastic analysis and control program provided an independent mathematical tool to evaluate therapeutic responses objectively. The calculated SP of those who survived averages 75% or higher throughout the observation period. It was below 60% for nonsurvivors during the first 24-hour period of observation. Noninvasive systems in the early postadmission period provide continuously monitored, real-time displays of data from the ED to the OR, and to the ICU for early recognition of circulatory dysfunction in acute emergency trauma conditions. Initial studies showed that the differences between the measured SP values and the SP values predicted from the previous stage averaged \( 7 \pm 6\% \), indicating satisfactory consistency and reproducibility.\(^{19}\)

**Stochastic Control as a Decision Support System**

The stochastic analysis of dynamic patterns also may be used for optimal decision making with a feedback control that, on the average, has proven to work best for similar patients in similar conditions recorded in the database, that is, nearest neighbors.

**DISCUSSION**

The advantages of this noninvasive monitoring system include technical convenience and the continuous display of data, allowing for calculation of the amount of deficit or excess of each variable from the time-integrated area under the curve. The area under the curve gives
arithmetic solutions to replace subjective evaluation of irregular curves and provides estimates of cardiac, pulmonary, and tissue perfusion functions. The high early cardiac index values in survivors suggest that there may have been less hypovolemia or better physiologic compensations. This concept is reinforced by the greater PtCO\textsubscript{2}/FiO\textsubscript{2} net cumulative excesses, which suggest better tissue oxygenation in the survivors’ initial stages. These preliminary studies need to be independently evaluated in larger series of trauma patients.

The hypothesis underlying this approach is that circulatory deficiencies that ultimately lead to shock, organ failure, and death may be identified early by noninvasive monitoring, even in the extenuating circumstances of severely traumatized ED patients in a large inner city public hospital. Earlier diagnosis of circulatory deficits allows therapy to be initiated sooner; earlier therapy is likely to improve outcome in emergencies in which time is crucial. More importantly, noninvasive monitoring, which is easy, inexpensive, fast, safe, and sensitive,\textsuperscript{16, 17} provides similar information to that of the PAC, except for pulmonary artery occlusion pressures. Discriminant analysis and stochastic control programs provide mathematical basis for outcome prediction. Future prospective clinical trials at other institutions are needed to validate this approach and its cost-effectiveness. Because the essence of tissue perfusion is an adequate supply of oxygenated blood to the tissues, perfusion is inferred from the direct measurement of skin oxygenation using the Clark polarographic method for oxygen tension.\textsuperscript{20, 24, 26} Although the skin is not representative of all tissues, it is the largest organ and the first organ to be affected by the adrenomedullary stress response. PtCO\textsubscript{2} provides early warning in acutely ill emergency patients; it tracks VO\textsubscript{2} in acute clinical shock episodes and in the physiologic course of experimental hemorrhagic shock, as well as cardiac and respiratory failure, cardiac arrest, and CPR in acute surgical conditions; tissue perfusion was related to outcome.\textsuperscript{19-21, 25, 28}

In the present study, the author used discriminant analysis to analyze the data of variables with \( P \) values < .2 to limit the number of variables for analysis. Interrelated or poorly conditioned variables having a common term, such as the combination of cardiac index and oxygen delivery, were avoided to minimize statistical problems of discriminant analysis. This does not mean that the more conventional variables, such as tachycardia, hypotension, acidosis, skin color, lactate levels, or mental status, are not useful at times when they occur. Obviously, when they are abnormal, they are extremely useful and important; however, criteria of the present study focused on early noninvasive hemodynamic variables in the immediate postadmission period that most consistently separated survivors and nonsurvivors.

**SUMMARY**

The mathematical model satisfactorily predicted outcome in acute emergencies based on noninvasively monitored flow, pressure, pulse
oximetry, tissue perfusion values, and their cumulative deficits. A decision support system provided information on the relative effectiveness of various therapeutic modalities based on the responses of patients with very similar states.

The concept that hypovolemia and oxygen debt is an early primary problem that plays an important role in low flow and poor tissue perfusion states is supported by direct observation of massive hemorrhage, estimated blood loss of hemoperitoneum and hemothorax at the time of surgery, and prior studies in the literature that documented blood volume deficits in posttraumatic and postoperative patients who subsequently developed organ failures and death.18

References


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Meta-analysis of hemodynamic optimization in high-risk patients*

Jack W. Kern, PharmD; William C. Shoemaker, MD

**Objective:** The aim of this evidence-based report was to review pertinent randomized controlled studies that describe hemodynamic goals in acute, critically ill patients and to evaluate outcome of resuscitation therapy in association with physiologic, clinical, and therapeutic influences.

**Methods:** MEDLINE was the source of randomized controlled studies written in English. The inclusion criteria were acutely ill, high-risk elective surgery, trauma, and septic patients. The goals of therapy were to resuscitate to either normal or supranormal values; the latter were described as a cardiac index of \( \geq 4.5 \text{ L-min}^{-1}\text{-m}^{-2} \), pulmonary artery occlusion pressure of \( <18 \text{ mm Hg} \), oxygen delivery of \( >600 \text{ mL-min}^{-1}\text{-m}^{-2} \), and oxygen consumption of \( >170 \text{ mL-min}^{-1}\text{-m}^{-2} \). The outcome criterion was survival or death. We found 21 randomized clinical trials described in 20 articles. The studies were divided into groups based on the time that goals were implemented (i.e., “early,” 8 to 12 hrs postoperatively or before organ failure, vs. “late,” or after onset of organ failure) and the severity of illness, determined by the control group mortality as \( >20\% \) (12 studies) or \( <15\% \) (nine studies).

**Results:** In severely ill patients (control mortalities group \( >20\% \)), six studies had a 23% mortality difference (\( p < .05 \)) between the control and protocol groups with early optimization, but seven studies optimized after the development of organ failure did not have significantly improved mortality. Moreover, outcome was not significantly improved in less severely ill patients (control mortalities group \( <15\% \)) and normal values as goals or when therapy did not improve oxygen delivery.

**Conclusion:** Review of 21 randomized controlled trials with various approaches to treatment revealed statistically significant mortality reductions, with hemodynamic optimization, when patients with acute critical illness were treated early to achieve optimal goals before the development of organ failure, when there were control group mortalities of \( >20\% \) and when therapy produced differences in oxygen delivery between the control and protocol groups. (Crit Care Med 2002; 30:1686–1692)

**Key Words:** noninvasive hemodynamic monitoring; bioimpedance cardiac output; thermodilution cardiac output; pulse oximetry; transcutaneous oxygen and \( \text{CO}_2 \) monitoring; trauma; high-risk surgery; acute septic shock; therapeutic hemodynamic goals; organ failure

Goal-directed studies with the pulmonary artery catheter (PAC) are highly controversial. Many studies showed no advantage of the PAC in cardiac and other medical conditions or in postoperative patients admitted to the ICU after organ failures had developed (1–5). However, other investigators (6–21) reported that early, optimally increased cardiac index (CI) and oxygen delivery (\( \text{DO}_2 \)) \( <12 \text{ hrs after surgery or 24 hrs after trauma} \) were associated with improved survival. However, a evidence-based meta-analysis by Heyland et al. (22) showed that the attainment of supranormal hemodynamic goals did not significantly reduce mortality in critically ill patients. Recently, two consensus conferences also found insufficient evidence to fully determine whether PAC-guided therapy significantly alters outcome, but they did not consider time factors; by mixing early and late studies together, they concluded there were no significant differences in optimizing hemodynamic variables (23–25).

In an insightful meta-analysis, Boyd (18) found no outcome improvement in seven prospective randomized studies of patients who entered the ICU after organ failure or sepsis had occurred (4, 5, 9, 11, 25, 26), but they noted significant outcome improvement in six other randomized studies when PAC-directed therapy was given early or prophylactically, that is, before organ failure or sepsis occurred (6, 7, 12, 14, 16, 17). Two recent studies also showed improved outcome with early goal-directed therapy (19, 20), suggesting that early optimization of \( \text{DO}_2 \) and oxygen consumption values in high-risk surgical patients improves outcome. If, in some clinical circumstances, the hemodynamic values of survivors may be compensatory responses that have survival values, it is important to identify clinical conditions that may be appropriate for this type of goal-directed therapy. Second, it may be even more important to define therapeutic goals relative to the primary diagnosis and age; the presence of diabetes, hypertension, chronic cardiac and respiratory illnesses, and other co-morbid conditions; and the severity of illness, timing of therapy, dose ranges, and other limitations of this approach.

Evidence-based studies have become the standard for testing important therapeutic questions, but evaluation of a therapeutic intervention should be clearly related to the central scientific idea defined by the research plan. As a prerequisite for clinical trial evaluation, important aspects of experimental study designs should be considered, including: definition of diagnostic categories; timing and dose of the therapeutic modality being evaluated; the patients’ age, sex, and se-
verity of illness; the presence of significant co-morbid conditions; and the clinical setting (Shoemaker WC, Bayard DS, Botnen A, et al., unpublished observations) (27, 28).

Clearly, lack of comparability of studies because of differences in the experimental design may preclude meaningful meta-analysis. Sweeping conclusions can hardly be justified by amassing many studies with large numbers of patients when the design features of the studies are not appropriately considered. Major questions include: In goal-directed therapy, are there outcome differences in the use of normal values compared with the supranormal values of survivors? What roles are played by time factors, various associated clinical conditions such as organ failures, mortalities of the control groups, and differences in therapy between control and protocol arms? Is there a single optimal hemodynamic goal for all critically ill patients, or does this depend on age, severity of illness, physiologic reserve capacities, organ failures, and other co-morbid conditions?

The present study reviewed 21 randomized clinical trials described in 20 articles to evaluate various influences that may contribute to outcome. Inclusion criteria of this meta-analysis were randomized clinical trials of high-risk elective surgery, trauma, and acute medical sepsis. We evaluated the definition of optimal therapy, time of optimization, age, types of illness, and severity of illness. The latter, for example, was defined by the mortality rate of the control group. The differences in mortality rates in the control and protocol groups were the main criteria for evaluation of therapeutic goals in various clinical circumstances, including acute illness, high-risk surgery, or trauma vs. chronic medical illnesses, the time that the therapeutic goals were implemented during the course of acute illness, and the presence or absence of organ failures. Hemodynamic values were used to evaluate the extent or aggressiveness of therapy to achieve the targeted protocol goals compared with the same therapy given to achieve the normal control goals. The differences between control and protocol groups were principally CI and Do2, because these have been reported to differentiate early survivor from nonsurvivor patterns (6–8, 27).

**METHODS**

A search strategy was developed with the assistance of a research librarian. The database for references was MEDLINE, and the search was limited to include only references in English. The study design included randomized clinical trials of supranormal CI, pulmonary arterial occlusion pressure of <18 mm Hg, and Do2 and oxygen consumption indexed as therapeutic goals. The search terms that identified the most acceptable references were supranormal oxygen, resuscitation endpoints, cardiac output, oxygen delivery, oxygen consumption, survival and nonsurvival, and hemodynamics. The search identified 72 references; 52 of these were rejected after screening because of irrelevant interventions, patient populations, or outcome definitions.

Three inclusion criteria were used to define the patient populations, therapeutic goals, and interventions. These were: 1) critically ill patients after high-risk elective surgery, severe trauma, and septic shock; 2) therapeutic goals for resuscitation and subsequent management were either normal hemodynamic values or supranormal values observed in previous series of survivors and specified as a CI of >4.5 L min⁻¹ m⁻², pulmonary arterial occlusion pressure of <18 mm Hg, Do2 of >600 ml min⁻¹ m⁻², or oxygen consumption of >170 ml min⁻¹ m⁻² (6–8, 18, 27); and 3) initial intervention was fluid therapy, and if hemodynamic targets were not achieved, inotropes were then added. Twenty references, with 21 studies, were reviewed and accepted for meta-analysis (Table 1). Experimental designs of the studies revealed at least four different categories of patients or therapeutic regimens. These included normal vs. supranormal therapeutic goals, early vs. late administration of therapy to achieve the stated goals, and differences in severity of illness determined by the control group mortality. Late was arbitrarily defined as >12 hr after surgery, 24 hrs after injury, or after occurrence of an organ failure.

We used the following characteristics to evaluate the quality of these randomized studies. An optimum randomization process may have included a third party, a table of random numbers, or a computer-generated list to assign impaneled subjects to either the treatment or control arm. The assignment to a treatment arm was “concealed” if a third party or sealed envelopes were employed to assign subjects to the treatment or control arm. The process was “blinded” when both the investigators and the subjects were not aware of the patients’ assignment to the control or protocol arm. Finally, the withdrawal or dropout analysis was adequate if the investigators identified the number of subjects excluded, provided an explanation for exclusion, and provided the number remaining for evaluation. If the authors did not describe these processes, it was assumed that they did not employ the preferred method, and the study design was not considered optimal. The minimum criterion for inclusion was proper randomization. If the processes for concealment, blinding, or withdrawal or dropout were not described or verified by direct communication, these design components were scored as “not clear.”

All studies reviewed were randomized. There were 15 studies (4–7, 9–11, 13, 15, 17, 19, 20, 29–32) on high-risk elective surgical patients, five of these included medical patients, and two of these studies also included trauma patients. Four studied only trauma patients (14, 16, 25, 29), and two studied septic (medical) patients (12, 25). Two studies were blinded to the investigators in terms of the fluid management; the other studies were not blinded. Table 1 lists the characteristics for each study.

The general variance-based method was used to calculate the summary statistic for the meta-analysis (33). The effect size calculated was the rate difference between the protocol group and the control group. The summary statistic was the rate difference between the groups. This method is based on the fixed-effects model. A significant p value was <.05.

**RESULTS**

The results are expressed as the mortality rate difference and confidence limits, which are twice the standard error. The mortality rate differences between control and protocol groups in the series as a whole varied from –0.35 to 0.2. The average mortality rate difference for all 21 studies was –0.05 ± 0.02, indicating statistically significant improvement with the protocol groups for the series as a whole (p < .05). Table 1 lists the studies compiled from the literature in which either normal values or the optimal therapy, defined as CI > 4.5 L min⁻¹ m⁻² and Do2 > 600 ml min⁻¹ m⁻², was given to the protocol groups, and their mortalities were compared with their corresponding control groups given standard therapy. In seven studies, the values of the protocol groups reached the proscribed therapeutic goals in the allotted time frame.

Figure 1 illustrates the values of the 14 randomized studies whose control group mortalities were >20%. Seven early studies whose optimal therapy was completed before organ failure occurred had marked and significant overall reduction in the mortality rate of –0.23 ± 0.07 (p < .05). Of the seven late studies of patients who had organ failure before initiation of the studies, the overall mortality rate difference was 0.01 ± 0.06, indicating no significant improvement with therapy. In these seven studies, only the study of Yu et al. (11) of patients aged...
<table>
<thead>
<tr>
<th>Author (Reference)</th>
<th>Diagnostic Group (%)</th>
<th>Study Design* Average Age per Group, yrs</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Control groups with mortality rates &gt;20%</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Goals to supranormal values after organ failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alia et al. (24)</td>
<td>Surgical</td>
<td>Y, Y, N</td>
<td>To evaluate the effects of increased oxygen delivery on morbidity and mortality in patients with severe sepsis or septic shock</td>
</tr>
<tr>
<td>n = 63; 1999</td>
<td>Medical</td>
<td>66; 72</td>
<td></td>
</tr>
<tr>
<td>Yu et al. (10)</td>
<td>Surgical</td>
<td>Y, N, N</td>
<td>To determine whether treatment to a DO₂ of ≥800 mL/min/m² in patients unable to mount this DO₂ response affects mortality</td>
</tr>
<tr>
<td>n = 66; 1998</td>
<td></td>
<td>63; 63</td>
<td></td>
</tr>
<tr>
<td>Yu et al. (10)</td>
<td>Surgical</td>
<td>Y, N, N</td>
<td>To determine whether treatment to a DO₂ of ≥800 mL/min/m² in patients unable to mount this DO₂ response affects mortality</td>
</tr>
<tr>
<td>n = 36; 1998</td>
<td></td>
<td>81; 83</td>
<td></td>
</tr>
<tr>
<td>Gattinoni et al. (5)</td>
<td>Trauma</td>
<td>Y, Y, N</td>
<td>To determine whether targeting hemodynamic treatment to achieve either supranormal values for the cardiac index or normal values for SV̇O₂ would improve morbidity and mortality among critically ill patients</td>
</tr>
<tr>
<td>n = 762; 1995</td>
<td>Surgical</td>
<td>60; 62; 61</td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td>Medical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayes et al. (4)</td>
<td>Surgical (40)</td>
<td>Y, N, N</td>
<td>To examine the effects of treatment intended to increase the cardiac index and oxygen delivery and consumption to the previously reported median values in survivors</td>
</tr>
<tr>
<td>n = 109; 1994</td>
<td>Medical (60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>62; 64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yu et al. (9)</td>
<td>Surgical (85)</td>
<td>Y, N, N</td>
<td>To evaluate the effect of increased DO₂ to &gt;600 mL/min/m² on the morbidity and mortality of patients with sepsis, septic shock, hypovolemic shock, and ARDS</td>
</tr>
<tr>
<td>n = 72; 1993</td>
<td>Medical (15)</td>
<td>56; 57</td>
<td></td>
</tr>
<tr>
<td><strong>B. Goals to supranormal values before organ failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobo et al. (20)</td>
<td>Surgical</td>
<td>Y, N, N</td>
<td>To evaluate the effect of therapy aimed at achieving maximized oxygen transport values during the operation and in the first 24-h postoperative period on outcome in a more homogeneous set of high-risk surgical patients</td>
</tr>
<tr>
<td>n = 42; 2000</td>
<td></td>
<td>63; 63</td>
<td></td>
</tr>
<tr>
<td>Wilson et al. (19)</td>
<td>Surgical</td>
<td>Y, N, N</td>
<td>To determine whether preoperative optimization of oxygen delivery improves outcome after major elective surgery</td>
</tr>
<tr>
<td>n = 138; 1999</td>
<td></td>
<td>72; 70; 72</td>
<td></td>
</tr>
<tr>
<td>Bishop et al. (16)</td>
<td>Trauma</td>
<td>Y, N, N</td>
<td>To test prospectively supranormal values of CI, DO₂, VO₂ as resuscitation goals to improve outcome</td>
</tr>
<tr>
<td>n = 115; 1995</td>
<td></td>
<td>34; 29</td>
<td></td>
</tr>
<tr>
<td>Boyd et al. (7)</td>
<td>Surgical</td>
<td>Y, N, N</td>
<td>To assess the effect of a deliberate perioperative increase in oxygen delivery on mortality and morbidity in patients who are at high risk of both after surgery</td>
</tr>
<tr>
<td>n = 107; 1993</td>
<td></td>
<td>68; 73</td>
<td></td>
</tr>
<tr>
<td>Tuchschmidt et al. (12)</td>
<td>Medical</td>
<td>Y, N, N</td>
<td>To prospectively evaluate the therapeutic effect of augmenting cardiac output and therefore oxygen delivery on mortality in patients with septic shock</td>
</tr>
<tr>
<td>n = 70; 1992</td>
<td></td>
<td>48; 53</td>
<td></td>
</tr>
<tr>
<td>Shoemaker et al. (6)</td>
<td>Trauma (13)</td>
<td>Y, N, N</td>
<td>To test the hypothesis that the physiologic pattern empirically defined by the survivors may be the appropriate therapeutic goals for high-risk critically ill postoperative patients</td>
</tr>
<tr>
<td>Surgical (87)</td>
<td></td>
<td>56; 53</td>
<td></td>
</tr>
<tr>
<td>Schultz et al. (14)</td>
<td>Trauma</td>
<td>Y, N, Y</td>
<td>To determine whether treatment of preoperative and postoperative hemodynamic variables improves outcome after hip surgery</td>
</tr>
<tr>
<td>n = 70; 1985</td>
<td></td>
<td>76; 67</td>
<td></td>
</tr>
<tr>
<td><strong>II. Control groups with mortality rates &lt;15%</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Goals to supranormal values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velimahos et al. (20)</td>
<td>Trauma</td>
<td>Y, N, N</td>
<td>To evaluate the effect of early optimization in the survival of severely injured patients</td>
</tr>
<tr>
<td>n = 75; 2000</td>
<td></td>
<td>62; 64</td>
<td></td>
</tr>
<tr>
<td>Ueno et al. (15)</td>
<td>Hepatectomy for cirrhosis</td>
<td>Y, NC, N</td>
<td>To evaluate the response to therapy aimed at achieving supranormal cardiac and oxygen transport variables in patients with cirrhosis</td>
</tr>
<tr>
<td>n = 34; 1998</td>
<td></td>
<td>61; 58</td>
<td></td>
</tr>
<tr>
<td>Durham et al. (25)</td>
<td>Trauma (93)</td>
<td>Y, Y, N</td>
<td>To test the hypothesis that the use of supranormal values for VO₂/DO₂ as end points for resuscitation results in improved outcomes</td>
</tr>
<tr>
<td>n = 60; 1996</td>
<td>Medical (7)</td>
<td>35; 35</td>
<td></td>
</tr>
<tr>
<td>B. Goals to normal values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valentine et al. (31)</td>
<td>Aortic surgery</td>
<td>Y, Y, N</td>
<td>To evaluate the routine use of PAC in patients who undergo aortic surgery</td>
</tr>
<tr>
<td>n = 126; 1998</td>
<td></td>
<td>64; 63</td>
<td></td>
</tr>
<tr>
<td>Bender et al. (32)</td>
<td>Aortic and limb revascularization</td>
<td>Y, Y, N</td>
<td>To determine whether the preoperative placement of a pulmonary artery catheter with optimization of hemodynamics results in improved outcomes</td>
</tr>
<tr>
<td>n = 104; 1997</td>
<td></td>
<td>ND; ND</td>
<td></td>
</tr>
<tr>
<td>Ziegler et al. (30)</td>
<td>Aortic and limb salvage surgery</td>
<td>Y, N, N</td>
<td>To evaluate the effect of preoperative optimization of hemodynamic variables on outcome in patients undergoing aortic reconstruction of limb salvage procedures</td>
</tr>
<tr>
<td>n = 72; 1997</td>
<td></td>
<td>67; 64</td>
<td></td>
</tr>
<tr>
<td>Mythen and Webb (18)</td>
<td>CABG or valve surgery</td>
<td>Y, Y, N</td>
<td>To test the hypothesis that perioperative plasma volume expansion would preserve gut mucosal perfusion during elective cardiac surgery</td>
</tr>
<tr>
<td>n = 60; 1995</td>
<td></td>
<td>64; 63</td>
<td></td>
</tr>
<tr>
<td>Berlauk et al. (13)</td>
<td>Peripheral vascular surgery</td>
<td>Y, N, N</td>
<td>To answer the question in patients with peripheral vascular surgery, does the use of a PA catheter to optimize LVF improve outcome?</td>
</tr>
<tr>
<td>n = 89; 1991</td>
<td></td>
<td>66; 62; 66</td>
<td></td>
</tr>
</tbody>
</table>

*OF, organ failure; PAOP, pulmonary artery occlusion pressure; CI, cardiac index; DO₂, delivery of oxygen index; ICU, intensive care unit; SV̇O₂, mixed venous oxygen saturation; ARDS, acute respiratory distress syndrome; PAC, pulmonary artery catheters; VO₂, oxygen consumption index; CABG, coronary artery bypass graft; CVP, central venous pressure; pH, gastric intramuscular pH; PA, pulmonary artery; LVF, left ventricular function.

*Study design: randomized, concealed, and blinded were described as Y = yes, N = no, NC = not clear, and ND = no data.
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Admission to ICU with Diagnosis of Sepsis and Of</th>
<th>Targets (Protocol Group)</th>
<th>Outcomes</th>
<th>Mortality Protocol Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No, No, OF</td>
<td>PAOP 14–16</td>
<td>Yes, No, No</td>
<td>23/51 (74)</td>
</tr>
<tr>
<td></td>
<td>DoJ &gt; 600</td>
<td></td>
<td>21/52 (66)</td>
</tr>
<tr>
<td>No, No, OF</td>
<td>PAOP 15–18 (both groups)</td>
<td>ND, ND, No</td>
<td>9/43 (21)</td>
</tr>
<tr>
<td></td>
<td>DoJ &gt; 600</td>
<td></td>
<td>12/23 (52)</td>
</tr>
<tr>
<td>No, No, OF</td>
<td>PAOP 15–18 (both groups)</td>
<td>ND, ND, No</td>
<td>12/21 (57)</td>
</tr>
<tr>
<td></td>
<td>DoJ &gt; 600</td>
<td></td>
<td>11/24 (61)</td>
</tr>
<tr>
<td>No, No, OF</td>
<td>PAOP &lt; 18 (all groups)</td>
<td>Yes, Yes, Yes</td>
<td>123/253 (49)</td>
</tr>
<tr>
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<td>CI &gt; 4.5, DoJ &gt; 600</td>
<td></td>
<td>21/252 (48)</td>
</tr>
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<td>No, No, OF</td>
<td>PAOP NS (both groups)</td>
<td>ND, Yes, Yes</td>
<td>25/59 (50)</td>
</tr>
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<td></td>
<td>CI &gt; 4.5, DoJ &gt; 600</td>
<td></td>
<td>15/59 (30)</td>
</tr>
<tr>
<td>No, No, OF</td>
<td>PAOP 15–18 (both groups)</td>
<td>ND, ND, Yes</td>
<td>12/55 (34)</td>
</tr>
<tr>
<td></td>
<td>DoJ &gt; 600</td>
<td></td>
<td>12/52 (34)</td>
</tr>
<tr>
<td>Yes, Yes, Yes</td>
<td>PAOP 12–16</td>
<td>No, No, Yes</td>
<td>3/19 (16)</td>
</tr>
<tr>
<td></td>
<td>CI &gt; 4.5, DoJ &gt; 600</td>
<td></td>
<td>6/18 (33)</td>
</tr>
<tr>
<td>Yes, Yes, Yes</td>
<td>PAOP &gt; 12 (PAC group 1)</td>
<td>Yes, No, No</td>
<td>2/46 (4)</td>
</tr>
<tr>
<td></td>
<td>DoJ &gt; 60</td>
<td></td>
<td>17/46 (37)</td>
</tr>
<tr>
<td>No, No, Yes</td>
<td>PAOP &lt; 18 (group 1)</td>
<td>ND, Yes, Yes</td>
<td>9/50 (18)</td>
</tr>
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<td></td>
<td>CI &gt; 4.5, DoJ &gt; 670</td>
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<td>24/55 (47)</td>
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<td>Yes, Yes, Yes</td>
<td>PAOP ≥ 12–14 (both groups)</td>
<td>Yes, No, No</td>
<td>3/53 (6)</td>
</tr>
<tr>
<td></td>
<td>CI &gt; ↑ to plateau, DoJ &gt; 600</td>
<td></td>
<td>12/54 (22)</td>
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<tr>
<td>Admission to ICU within 4 hrs of Diagnosis of Sepsis</td>
<td>PAOP &gt; 15 (both groups)</td>
<td>Yes, Yes, Yes</td>
<td>12/25 (50)</td>
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<td>No, No, Yes</td>
<td>CI &gt; 6</td>
<td></td>
<td>18/25 (72)</td>
</tr>
<tr>
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<td>PAOP &lt; 18</td>
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<td>18/69 (29)</td>
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<td>Yes, Yes, Yes</td>
<td>PAOP ND</td>
<td>No, No, ND</td>
<td>1/05 (3)</td>
</tr>
<tr>
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<td>CI 3–3.5</td>
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<td>18/55 (29)</td>
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<td>PAOP ND</td>
<td>ND, ND, Yes</td>
<td>6/49 (15)</td>
</tr>
<tr>
<td></td>
<td>CI &gt; 4.5, DoJ &gt; 600</td>
<td></td>
<td>4/35 (11)</td>
</tr>
<tr>
<td>Yes, Yes, Yes</td>
<td>PAOP 9–18</td>
<td>Yes, Yes, Yes</td>
<td>6/16 (0)</td>
</tr>
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<td>CI &gt; 4.5, DoJ &gt; 600</td>
<td></td>
<td>2/18 (11)</td>
</tr>
<tr>
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<td>PAOP &lt; 18 (both groups)</td>
<td>Yes, Yes, Yes</td>
<td>3/27 (11)</td>
</tr>
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<td></td>
<td>DoJ &gt; 600</td>
<td></td>
<td>3/31 (19)</td>
</tr>
<tr>
<td>Yes, Yes, Yes</td>
<td>PAOP 8–15</td>
<td>ND</td>
<td>3/60 (5)</td>
</tr>
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<td>CI &gt; 2.8</td>
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<td>1/60 (2)</td>
</tr>
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<td>Yes, Yes, Yes</td>
<td>PAOP 8–14</td>
<td>ND</td>
<td>1/51 (2)</td>
</tr>
<tr>
<td></td>
<td>CI &gt; 2.8</td>
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<td>1/53 (2)</td>
</tr>
<tr>
<td>Yes, Yes, Yes</td>
<td>PAOP ≥ 12</td>
<td>ND</td>
<td>3/32 (9)</td>
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<td></td>
<td>2/40 (5)</td>
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<td>0/50 (0)</td>
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<tr>
<td>No, Yes, Yes</td>
<td>CVP: phili</td>
<td>ND</td>
<td>1/50 (3)</td>
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<td>PAOP 8–15</td>
<td>ND</td>
<td>1/68 (1.5)</td>
</tr>
<tr>
<td></td>
<td>CI &gt; 2.8</td>
<td></td>
<td>2/21 (10)</td>
</tr>
</tbody>
</table>

50–75 yrs had improved outcome with optimized therapeutic goals.

Figure 1 also illustrates mortality rate differences in three groups of studies with control group mortalities of <15% or normal values for therapeutic goals. The first group consisted of two studies with control group mortalities of 10% and 11%. One study (26) consisted of patients with organ failures before therapy, and the second study (27), which excluded patients who died within 24 hrs of admission, had a control group mortality of 11% and a protocol mortality of 15%, but there was no difference in DoJ between the control and protocol groups. The latter suggested that the treatment of control patients were similar to that of the protocol patients. If there were no differences in therapy, no outcome differences should be expected, and none were found. Neither of these studies showed significant differences in the mortality rates between the control and protocol groups; the combined rate difference of these two studies was 0.03 ± 0.11 (p > .05). The fourth group in Figure 1 studied partial heptectomy in cirrhotic patients who had an 11% control group mortality but a protocol group mortality of only 0% (16). The rate difference of −0.11 did not reach statistical significance, probably because the sample size was only 34 patients. The last group consisted of five studies that used normal values as goals and had control mortalities of <11%. Their subtotal rate difference was −0.01 ± 0.03 (p > .05). The three groups (ten studies) had control group mortalities that were <15%, with a mean of 7.1%, suggesting that these patients were not as severely ill as the first two study groups whose mean control mortality was 42.1% (Fig. 1 and Table 1). In high mortality series, fewer patients are needed to show improved outcome with different therapeutic goals.

**DISCUSSION**

Hemodynamic bedside monitoring by PACs has been considered by many as a standard for circulatory evaluation of critically ill patients, but its usefulness has recently been seriously questioned (1–5, 22–25), particularly in the late stages of illness after onset of multiple organ failures (23–25). The present review showed significantly improved outcome in randomized studies when PAC goal-directed therapy was administered early or prophylactically in patients who
were optimized preoperatively and maintained in the intraoperative and immediate postoperative period. Early studies using invasive ICU monitoring in randomized trials reported that increased CI and D\(_0\), to values characteristic of survivors of high-risk surgery in the immediate postoperative period improved outcome (6). At the initial stage in the development of this concept, it was realized that the survivors of acute critical illnesses had a wide range of higher-than-normal hemodynamic values (6, 8, 10, 18, 19, 31, 34). Because it is not possible to test a range of values, the mean or median values were arbitrarily chosen as cutoff points, not to establish a set of optimal values but to test the hypothesis that critically ill patients have high metabolic rates and therefore require greater than normal hemodynamics and oxygen transport to sustain the increased body metabolism after trauma, surgery, or sepsis. Hemodynamic goals of surviving patients were proposed as a first approximation to optimal goals for the immediate postoperative period of high-risk surgical patients. These proposed optimal therapeutic goals were not intended to be generally applied to all patients at all times because metabolic requirements are affected by age, sepsis, blood loss, preexisting cardiac and pulmonary insufficiency, and other co-morbid conditions (10, 35). Ultimately, optimal goals may be calculated for each individual patient on the basis of his or her diagnosis, co-morbid conditions, past hemodynamic deficits, and temporal stage. This is presently approached by using discriminate analysis (27) and stochastic control programs (28).

In the initial randomized trial of supranormal hemodynamic values, the mortality was decreased, but more importantly, the prevalence of organ failures was reduced from 31 cases in the control group to 1 case in the protocol group (6). Moreover, in a series of postoperative patients invasively monitored before the diagnosis of ARDS, the nonsurvivors’ CI values were in the normal range; the survivors who developed ARDS had CI values that were significantly elevated (4 L\(\text{min}^{-1}\cdot\text{m}^{-2}\)) but less than the values of survivors who did not develop ARDS (4.5 L\(\text{min}^{-1}\cdot\text{m}^{-2}\)) (34, 38, 39). Before the onset of ARDS, the mean pulmonary artery occlusion pressures were within acceptable limits for critically ill postoperative patients and none had a pulmonary arterial occlusion pressure of >18 mm Hg. Bishop et al. (16, 39) reported that supranormal goals within 24 hrs of the injury reduced the prevalence of ARDS and other organ failures after severe trauma; they reduced mortality from 39% to 18% (p < .05) and reduced prevalence of organ failure from 105 in 65 control patients (1.62 ± 0.28 organ failures per patient) to 37 in 50 protocol patients (0.74 ± 0.28 organ failures per patient) (p < .05). Less than optimal values in the early stage may lead to inadequate total blood flow and uneven microcirculatory blood flow from uneven vasoconstruction of the adrenomedullary stress response (8, 34, 38–42). Local hypoxia and acidosis of the capillary endothelium from uneven capillary blood flow is known to stimulate the systemic inflammatory response system and lead to organ failure (41, 42).

The definition of early as opposed to late studies is necessarily arbitrary. Cutoff points for the patient to reach the designated goals were: the first 12 hrs postoperatively in elective surgery, 24 hrs after injury in trauma patients, and before the onset of an organ failure. When
sepsis was the primary diagnosis, we accepted the definition of "early septic shock" proposed by Tuchschmidt et al. (12), which was within 4 hr of the time of diagnosis. However, when sepsis was a complication of elective high-risk surgery, as in the studies of Yu et al. (9–11), it was arbitrarily designated as an organ failure or dysfunction and therefore classified as late. Of these three published articles (9–11), the 1998 publication that was a continuation of their earlier studies seems to include 47 of the 50 subjects that were evaluated in the 1995 article. Therefore, to avoid redundancy, the 1995 study was not included in this meta-analysis. In the 1993 study, Yu et al. (9) demonstrated that when both the protocol and control groups were aggressively hydrated to a pulmonary artery occlusion pressure of 15–18 mm Hg, the difference in the mortality rates was insignificant. In the interim study (1995), Yu et al. (11) observed that when the subjects in both the protocol and control groups who generated a DO₂ of ≥600 mL min⁻¹ m⁻² after fluid resuscitation were excluded from the study, the mortality rate of the remaining protocol subjects was significantly less than the remaining control subjects. This difference was associated with the administration of inotropes and vasoactive drugs given to the protocol group to achieve a DO₂ of ≥600 mL min⁻¹ m⁻². In the 1998 study, Yu et al. (10) evaluated the larger series of patients randomized to protocol and control groups, and stratified the groups according to age: ≤75 yrs (50–75 yrs of age) and >75 yrs. All subjects who achieved a DO₂ of ≥600 mL min⁻¹ m⁻² after fluid resuscitation were excluded. The mortality rate of the protocol group of the subjects aged ≤75 yrs was significantly less than the control group. However, the mortality rate in the protocol and control groups of subjects aged >75 yrs was not different (p > .05). These findings suggest that the subjects aged >75 yrs did not effectively respond, in terms of outcome, to aggressive vasoactive drugs or inotropes.

In the study of Wilson et al. (19), patients undergoing major elective surgery were randomized into three groups; two groups of 42 patients received invasively monitored fluid and either adrenaline or doxapamine to increase DO₂ whereas the third group of 42 patients received routine postoperative care and served as the control. Only 3 of 92 patients (3%) in the optimized groups died, whereas 8 of 46 patients (17%) in the control group died (p < .007). The length of stay of the doxapamine group was significantly reduced compared with both the adrenaline group (p = .02) and the control group (p = .009). The authors concluded that because of the low doses of inotropes, fluid optimization was a major contributor to improved DO₂ and improved outcome in their patients (19).

Three randomized trials not included in this meta-analysis deserve mention. In a study by Takala et al. (36) of postoperative patients with 13% control mortality that used relatively normal values as goals, patients were initially brought into the normal hemodynamic range, and then two dosage levels of doxapamine were tested in randomized trials, but the outcome was not significantly improved. Sinclair et al. (37) studied length of hospital stay in patients with proximal femoral fractures after optimizing stroke volume with repeated colloid fluid challenges measured by esophageal Doppler ultrasonography. They demonstrated significantly reduced hospital stay, but there was insignificant reduction in mortality because of only two deaths in the control group and one death in the protocol group. Polenen et al. (43) used mixed venous oxygen saturation and lactate levels as criteria for adequacy of resuscitation immediately postoperatively in 403 cardiac surgical patients. The median hospital stay was shorter in the protocol group (6 vs. 7 days, p < .05), and morbidity was significantly less at the time of hospital discharge (1.1% vs. 6.1%, p < .01), but mortality was very low and not significantly affected by the study.

Low control mortalities suggest that the patients were not very ill and therefore may not respond as clearly to increased hemodynamics and, at the same time, may require much larger numbers of patients to show statistical significance. In the studies of Mythen et al. (17) and Ueno et al. (15), the protocol patients given therapy to achieve optimal goals had 0% mortality, but because of the small number of patients, statistical significance was not achieved. Moreover, in the study of Berlauk et al. (13), the mortality was reduced from 9.5% in the control group to 1.5% in the optimized group, which was not significant; however, the number of complications were significantly reduced.

Similarly, if the control and protocol patients were treated in a similar manner, no differences in outcome should be expected. In the study of Velmaños et al. (29), the difference in DO₂ between control and protocol patients was not statistically significant because the treatment of control and protocol patients were not different, and therefore, the mortality was, not unexpectedly, not different.

We conclude that increased CI and DO₂ with pulmonary arterial occlusion pressure of <18 mm Hg should be considered as goals of therapy. When implemented early and aggressively, this reduces mortality and the prevalence of organ failures in acute postoperative and posttrauma conditions. Goal-directed therapy to achieve optimal goals is ineffective in the late stages after onset of organ failure because no amount of extra oxygen will restore irreversible oxygen debts, failed organs, or dead cells. In the late stage of acute illness after organ failure has occurred, aggressive therapy directed toward achieving the survivors' supranormal values is futile. When oxygen debt is no longer reversible, increased oxygen transport is not effective. Moreover, it is difficult to demonstrate significant changes after optimization when there are no significant differences between therapy given to the control and protocol groups. That is, there must be significant differences in the type of therapy or the amount of therapy given to expect significant outcome improvement. Furthermore, outcome differences may be extremely difficult to demonstrate when the patient population is not very ill, as indicated by control mortalities of <15%. Finally, no effect should be expected in chronic medical conditions in which physiologic compensations have already had their maximum effect.

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REFERENCES
NONINVASIVE HEMODYNAMIC MONITORING OF EMERGENCY PATIENTS FOR
OUTCOME PREDICTION BY A STOCHASTIC CONTROL PROGRAM:

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ABSTRACT

Background: Time is an important factor in the resuscitation and management of critically ill emergency
patients. Early noninvasive monitoring with an outcome predictor to identify and correct cardiac,
pulmonary, and tissue perfusion deficiencies may be a useful alternative approach to invasive pulmonary
artery thermodilution catheters.

Objectives: The first aim was to present a stochastic control model, developed for noninvasive
monitoring to predict survival outcome in acute life-threatening critical illness. The second aim was to
evaluate the reliability of this stochastic model to track progress throughout the course of acute illnesses.
The third aim was to explore the feasibility of a decision support program to evaluate the relative
effectiveness of various therapies given to patients in similar clinical-hemodynamic states.

Methods: A stochastic control and decision support program was applied in 177 monitored severely
injured patients to monitor the survival probabilities at the initial resuscitation on admission to the
emergency department (ED) and at subsequent interval during the hospital stay in a county teaching
hospital. The program provides a continuous readout of the survival probabilities based on the patient's
diagnostic category, covariates, and noninvasive hemodynamic levels including cardiac output, pulse
oximetry, transcutaneous oxygen and carbon dioxide tensions, and mean arterial blood pressure. The
percentage of correct predictions was used to measure the reliability of the model. A therapeutic decision
support program was used to measure the relative effectiveness of various alternative therapies.

Results: The cardiac index, mean arterial pressure, arterial saturation, transcutaneous oxygen and carbon
dioxide tensions were higher in survivors than in nonsurvivors: a) in the initial resuscitation, b) before and
after the probability nadir, and c) during emergency surgery. The calculated Survival Probability (SP) of
those who survived averaged 75% or higher throughout first 24 hour observation period. It was below
60% for nonsurvivors during this period. Misclassifications, excluding brain dead patients and those who
died suddenly in the OR of uncontrolled hemorrhage, were 16/165 or 9.7%. However, the APACHE
scores of survivors and nonsurvivors were not significantly different during the first four ICU days in
these patients. Noninvasive systems provided continuously monitored real time displays of data for the
earliest recognition of circulatory dysfunction from the emergency department (ED) to the operating
room (OR), and to the intensive care unit (ICU). A decision support system measured the relative
effectiveness of various therapeutic modalities based on the responses of patients with very similar states.

Conclusions: The mathematical model satisfactorily measured survival probabilities and severity of
illness in acute emergencies based on noninvasively monitored values of blood flow, blood pressure,
oxygen transport in blood, and tissue oxygenation. Stochastic analysis of hemodynamic patterns provided
a feasible therapeutic decision support system.
INTRODUCTION

Invasive pulmonary artery (PA) thermodilution (Swan-Ganz) catheters provide the maximum circulatory data at the bedside, but require intensive care unit (ICU) conditions. However, when invasive monitoring is started late in the course of illness after onset of organ failures, goal-oriented studies showed no advantage of the PA catheter (1-7). Moreover, reviews of trauma patients with shock revealed that delays in correcting circulatory deficiencies have led to organ failures and death (8-15). Since time is an important factor in resuscitation and management of critically ill emergency patients, early noninvasive monitoring with an outcome predictor may be a useful approach to identify and correct hemodynamic deficiencies as early as possible (9-23). Previous studies have demonstrated early outcome prediction with discriminant function (21). Similar to early diagnosis and therapy for cancer, early diagnosis and therapy for circulatory problems is more cost-effective than therapy delayed until late stages (8-11,15,18,21).

Recently, Bayard et al (24) developed a mathematical model that used a large database of noninvasively monitored hemodynamic variables, to provide outcome prediction as well as therapeutic decision support for acute critically ill patients. The aims of the present study were to evaluate the accuracy of this mathematic model to predict survival outcome, to explore the possible use of this program to track progress as an objective measure of severity of illness, and to develop a decision support system based on responses to various therapies given to patients in similar states. The present preliminary report focuses its application on acute severely injured emergency patients under worst-case conditions. Acute injury also was selected for study because the onset of illness occurred shortly before admission and the course of circulatory events could be monitored from the time of admission (8,13,21). Moreover, it is likely that mistakes occur more frequently during periods of acute life-threatening crises.

MATERIALS AND METHODS

Clinical Series

We studied 177 severely injured patients by noninvasive hemodynamic monitoring that reflect cardiac, respiratory, and tissue perfusion functions together with a new stochastic analysis and control program based on clinical and hemodynamic values. In general, emergency patients with major blunt or penetrating truncal trauma or significant risk of mortality or morbidity were selected for monitoring shortly after emergency department (ED) admission. Monitoring was usually begun in the ED and the patients were followed to the radiology suite, when these studies were indicated, to the operating room (OR), and then to the ICU. The protocol was approved by the Institution’s Review Board.

Noninvasive Hemodynamic Monitoring

Noninvasive hemodynamic monitoring is continuous real time display of cardiac, pulmonary, and tissue perfusion functions in critically ill patients. The data was down-loaded at 30-second intervals, averaged over 5-minute periods, and then entered into the database. During the first 24-hour period, the survival probabilities were averaged over 2-hour periods.

Cardiac Output

An improved thoracic bioelectric impedance device (Yantagh Inc., Bristol, PA) was applied shortly after arrival in the ED. The noninvasive disposable prewired and hydrogen electrodes were positioned on the skin and three EKG leads were placed across the precordium and left shoulder (26,27). A 100 kHz, 4 mA alternating current was passed through the patient’s thorax by the outer pairs of electrodes and the voltage was sensed by the inner pairs of electrodes which captured the baseline impedance ($Z_0$), the first derivative of the impedance waveform ($dZ/dt$), and the EKG. The EKG and bioimpedance signals were filtered with an all-integer-coefficient technology to decrease computations and signal processing time. The signal processing algorithm used a time-frequency distribution (modified Wigner Distribution) analysis that increased signal-to-noise ratios (26,27).
Previous studies have documented satisfactory correlations between thermodilution and bioimpedance cardiac output values for trauma patients in the ED, OR, and ICU conditions (25). Pulmonary artery catheters were placed when indicated by clinical criteria.

Limitations of the impedance method include faulty electrode placement, motion artifacts, restlessness, shivering, pulmonary edema, pleural effusion, valvular heart disease, dysrhythmias, and electrical leaks from other instruments using the same circuit. These are usually apparent from inspection of the impedance waveform and by previously described criteria: baseline impedance, Zo, >15ohms and the peak impedance signal, dZ/dtmax, >0.3ohms (21).

Pulse Oximetry

Routine pulse oximetry (Nellcor, Pleasanton, Ca) was used to assess continuously arterial oxygen saturation (Sao2). Values were observed and recorded at the time of the cardiac index measurements. Appreciable or sudden changes in these values were also noted and confirmed by in vitro arterial oxygen saturation obtained by the standard blood gas analysis (25).

Transcutaneous Oxygen Tension

Conventional transcutaneous oxygen tension measurements were continuously monitored throughout the observation period. This technology uses the same Clark polarographic oxygen electrode routinely employed in standard blood gas measurements (28-31). The oxygen tensions were measured in a representative area of the skin surface heated to 44°C to increase diffusion of oxygen across the stratum corneum and to avoid vasoconstriction in the local area of the skin being measured (28,29). Previous studies demonstrated the capacity of transcutaneous oxygen tensions to reflect tissue oxygen tension (25,28-32). Transcutaneous oxygen tension (PtcO2) has been shown to reflect the delivery of oxygen to the local area of skin; it also paralleled the mixed venous oxygen tension except under terminal conditions where peripheral shunting led to high mixed venous hemoglobin saturation (SvO2) values (28). While oxygen tension of a segment of the skin does not reflect the state of oxygenation of all tissues and organs, it has the advantage of being the most sensitive early warning tissue of the adrenomedullary stress response; vasoconstriction of the skin is an early stress response of hypovolemia and other shock syndromes (28-32). Transcutaneous oxygen tension was indexed to the FiO2 to give a PtcO2/FiO2 ratio, because changes of the inspired oxygen produce marked PtcO2 changes (21,29).

Limitations of the transcutaneous methods are the thermal environment must be reasonably constant, marked changes in room temperature from drafts or open windows must be avoided; the electrode must be changed to a nearby thoracic or shoulder site and be re-calibrated at 4 to 6-hour intervals to avoid first degree skin burns.

Trauma Patient Database for Stochastic Analysis and Display Program

Database for acutely injured patients was developed to describe primary injuries, selected covariates hemodynamic patterns by invasive and noninvasive methods, and outcomes, including survival or death, organ failures, other complications, hospital days, and ICU days. Diagnostic categories included: truncal and nontruncal trauma, head injury, brain death. The database also included: age, gender, the presence of sepsis or systemic immune response system (SIRS), APACHE scores, Glasgow coma score, and injury severity score (ISS). Noninvasive monitoring was usually begun in the ED and the patient was followed to the OR, radiology department, and ICU. Invasive pulmonary artery catheterization (PAC) was instituted when clinically indicated after the patient arrived in the ICU. The time and place of monitoring, time of operations, times of ICU admission and discharge, time of hospital discharge or death, and other events were recorded in time elapsed after admission.
Calculation of Net Cumulative Deficit of Hemodynamic Variables

The area between the fluctuating monitored variables and either the normal values for blood pressure, \( \text{Sao}_2 \), and \( \text{PtcO}_2/\text{FiO}_2 \) or optimal value for cardiac index were calculated. This area was integrated over time to calculate the net cumulative amount of deficit or excess of each monitored variable for individual patient (21).

Stochastic Analysis and Control Program

The stochastic (probability) analysis and control program, system dynamics, and the calculation of the probability of survival were developed by Bayard et al (24) and are summarized in the Appendix. The program uses data from a homogeneous population, integrates a new patient's demographic characteristics, clinical diagnosis, and hemodynamic levels, and derives a survival probability for the patient in real time. The primary characteristics of this model include: a) the patient's course described by three-dimensional vectors, where baseline values are not required, b) the first derivative of the initial vector projects the patient's course if there are no inherent changes or external influences including "measurement noise", c) the second derivative tracks changes in the patient's course from either internal compensations, further deterioration, spontaneous improvement, or external influences such as changes in therapy as well as "process noise", and d) the integral sums up the total influences. The detailed formulation of the program and the derivation of the probability of survival are included in the Appendix.

Application to a Clinical Series

We tested this stochastic analysis and control program in a series of 177 severely injured patients whose hemodynamics were monitored noninvasively as early as admission to the ED. The patient population included the following diagnostic categories: truncal and nontruncal trauma, head injury, and brain death. Noninvasive monitoring was usually begun in the ED and the patient was followed to radiology department, operating room (OR), and ICU. Invasive pulmonary artery catheterization (PAC) was instituted when clinically indicated after the patient arrived in the ICU. The time and place of monitoring, time of operations, times of ICU admission and discharge or death, and other events were recorded relative to time elapsed after ED admission. In addition, the following data elements were included in the database: age, gender, presence of sepsis or activation of the systemic immune response system (SIRS), Glasgow coma score, injury severity score (ISS), primary injuries, hemodynamic patterns by invasive and noninvasive methods, organ failures, other complications, hospital days, ICU days, and hospital survival outcome. The protocol was approved by the Institution's Review Board.

Statistical Analyses

The survivors' and nonsurvivors' deficits of MAP, cardiac output, \( \text{Sao}_2 \), and transcutaneous \( \text{O}_2 \) were calculated for the periods of monitoring. For categorical variables, differences in proportions between survivors and nonsurvivors were tested using the Chi-square test or the two tailed Fischer's Exact test. For continuous variables, the equality of the means between survivors and nonsurvivors was tested the two-sample t-test or the Wilcoxon two-sample test. The effects of time (a repeated measure), outcome group, and their interaction on survival probability and on each hemodynamic parameter were analyzed by the mixed linear model using residual maximum likelihood with the unstructured covariance. The SAS statistical software (The SAS System, Release 8.2, SAS Institute Inc, Cary, NC) was used for all statistical computations.

RESULTS

Clinical Series

There were 151 males and 26 females. The mean age was \( 33.0 \pm 15.5 \) years for all patients, \( 31.5 \pm 13.7 \) years for survivors, and \( 36.7 \pm 18.9 \) for nonsurvivors. Patients who were brain dead from severe neurological injury
were evaluated separately, because disruption of the autonomic centers usually produced hyperdynamic hemodynamic responses from unopposed peripheral vasodilatory mechanisms. There were 124 survivors, 53 nonsurvivors, of whom 10 were brain dead after severe head injury. The overall mortality was 30%. There were 151 males and 26 females. Sixty-four (52%) of the 124 survivors, and 22 (42%) of the 53 nonsurvivors were operated upon as part of their initial resuscitation. Another 25% were operated subsequent to their initial resuscitation. Table 1 lists the salient clinical features.

**Temporal Patterns of Hemodynamic Values and Survival Probability from the Time of Admission**

Figure 2 displays the hemodynamic data of surviving and nonsurviving emergency trauma patients during the first 24 hours after their ED admission. Mean values ± SEM are shown for survivors and nonsurvivor for cardiac index (CI), heart rate (HR), mean arterial pressure (MAP), arterial hemoglobin saturation (Sao2) by pulse oximetry, transcutaneous oxygen tension indexed to the FiO2 (PtcO2/FiO2) and the survival probability (SP) averaged over 2-hour intervals during the first 24 hour period. The mean SP of those who survived was >75% throughout the observation period. In nonsurvivors, it was <60% during this initial period.

Table 2 lists the mean values for both groups during the first 24 hours after admission. The CI, MAP, Sao2, PtcO2/FiO2, and SP values of the survivors were significantly higher than the comparable values of those who died, while the HR was higher in the nonsurvivors. Initial studies showed that the differences between the measured SP values and the SP values predicted from the previous stage averaged 7 ± 6%, indicating satisfactory consistency and reproducibility.

**Hemodynamic and Survival Probability Patterns Before and After the Survival Probability Nadir**

Inspection of the data revealed 162 instances of abrupt hemodynamic deterioration that were identifiable by sudden reductions in the SP to 30.3% in nonsurvivors and 65.2% in survivors (Table 3). The CI, MAP, Sao2, PtcO2/FiO2, and SP values patterns before and after the SP nadir of the survivors were significantly higher than the comparable nonsurvivors' values, while HR was higher in the nonsurvivors.

**Comparison of Survival Probability with Actual Outcome**

Of the 177 trauma patients, 124 survived, and 53 died; the mortality was 30%. Of the nonsurvivors, 2 died in the OR of uncontrollable blood loss, and 10 were brain dead, leaving 41 satisfactorily monitored nonsurvivors. Of 41 patients predicted to die, 30/41 (73.2%) died. However, 11 patients predicted to survive, died of late medical complications in an average of 31 days (range 7-120 days). Complications included: ARDS (5 patients), cardiac failure (2), renal failure (1) and sepsis (3). Of those predicted to die, 5/124 (4%) survived after further resuscitation efforts. Of those who, in the initial resuscitation period, were predicted to survive 119/124 (96%) survived (Fig. 4 and Table 5). There were 16/165 (9.7%) misclassifications.

**Comparison of APACHE scores with the Stochastic Survival Probabilities**

The survivors' and nonsurvivors' APACHE scores of the present series were not significantly different during the first four days after admission, but were significantly different on the last day in the ICU (Fig. 5). However, survival probabilities of survivors and nonsurvivors were significant different during the first 24 and 48 hours after admission (Table 2 and Fig. 5).

**Hemodynamic Patterns in Severe Head Injury with Brain Death**

Table 6 lists the mean CI, MAP, HR, Sao2, PtcCO2, and PtcO2/FiO2 values of nine patients with brain death from severe head injury. There were elevated values in each of these hemodynamic variables particularly CI, MAP, HR, Sao2, and PtcO2/FiO2 suggestive of augmented peripheral tissue perfusion from absent central vasoconstriction.
Hemodynamic and Survival Probability Patterns Before, During, and After Emergency Surgery

Figure 3 and Table 4 show the temporal patterns of noninvasive hemodynamic variables and SP values of 64 survivors and 22 nonsurvivors before, during, and after emergency surgery as a part of their initial trauma resuscitation. The nonsurvivors' CI markedly decreased at the end of the operative procedure, while MAP values were approximately maintained intra-operatively. The survivors' CI, SspO₂, PtcO₂/FiO₂, and SP values were greater than the comparable values of those who died, while the HR was higher in the nonsurvivors.

Stochastic Control as a Decision Support System

The stochastic analysis of dynamic patterns also may be used for decision-making with a feedback control that, on the average, was shown to occur in similar patients, i.e., nearest neighbors, recorded in the database. Figures 6 and 7 are illustrative examples of nearest neighbors' responses to various therapeutic interventions in terms of their likely survival outcome.

DISCUSSION

A major assumption in the present approach is that circulatory deficiencies that ultimately lead to shock, organ failure, and death can be identified early by noninvasive monitoring and that the probability of survival may be predicted by stochastic analysis of dynamic patterns. The probability of survival is roughly equivalent to severity of illness. That is, the patient with a 90% likelihood of death is severely ill, while the patient with an estimated survival outcome of 90% may not be very ill. The accuracy and reliability of this approach depends on the size and comparability of the database needed to provide an adequate group of nearest neighbors.

The proposed mathematical representation of the circulation defines the patient's state by specific diagnostic categories, covariates, hemodynamic variables, their derivatives, and their integrals in a multi-dimension grid. Since the database contains over 9000 time-lines, each of which may represent a patient's state, there are many choices available for selection of the nearest neighbors. The average difference between a given patient's variables and the nearest neighbors' variable was <0.3 of the standard deviation. This suggests that we usually did not have to go far from the patient's state to find adequate numbers of nearest neighbors.

This approach was tested in the extenuating and sometimes chaotic circumstances of severely traumatized emergency patients in a large inner city public hospital. Under these circumstances, the SP was found to closely track changes at each point during the initial observation period and the SP correctly predicted outcome in over 90% of the patients during resuscitation monitoring within 24 hours of injury.

Early diagnosis and physiologic assessment is essential, because this allows therapy to be initiated sooner in the hope that earlier therapy may improve outcome in emergencies where time is crucial (8-15). Moreover, the stochastic analysis and control program provided an independent mathematical tool to evaluate therapeutic responses objectively.

REFERENCES


APPENDIX
The Stochastic Analysis Program based on a Trauma Database

Bayard et al (24) developed a stochastic analysis and control program to determine individual patients’ survival probabilities (SP), from a database of patients with similar clinical-hemodynamic “states,” which are defined in terms of the primary diagnosis, covariates, and hemodynamic variables. By “similar” is meant a group of patients, referred to as “nearest neighbors,” with the same diagnosis, who share similar covariates, and have similar hemodynamic patterns to the patient under study. Mathematically, the stochastic analysis is defined as policy iteration with respect to conventional therapeutic policy used for each patient as the database was developed. It is motivated by methods of machine learning (33,34), and methods of dynamic programming for stochastic control (35,36).

The Stochastic Analysis and Therapeutic Decision Support Program

The therapeutic decision support program utilizes the database of therapeutic responses to evaluate the relative effectiveness of various therapies by the responses of each therapy in the patient’s nearest neighbors (24). Figure 8 diagrams the stochastic analysis and control program synthesized from a database of therapeutic responses.

The state vector, \( x(t) \) at time \( t \) is described in terms of the various hemodynamic measurements, their derivatives, and their integrals. Assume that there are \( L \) different types of measurements taken on a given patient (e.g. cardiac index, blood pressure, pulse oximetry, and transcutaneous \( O_2 \) and \( CO_2 \) tensions). Specifically, for each measurement type, denoted as \( y_i \), define the state vector as a concatenation of the value \( y_i \) itself, its first and second derivatives \( y_i', y_i'' \), and its first integral \( \int_0^t y_i dt \), as follows:

\[
x(t_k) = \left[ y_i(t_k), y_i'(t_k), y_i''(t_k), \int_0^t y_i dt, \ldots, y_L(t_k), y_L'(t_k), y_L''(t_k), \int_0^t y_L dt \right]^T
\]
i.e., for L different measurement types there will be 4L states. In practice, the derivatives and integrals are approximated by finite differences and sums of the time-ordered data of the database. Specifically, we will calculate the approximations (24).

\[
y'_{i} = \frac{y_{i}(t_{k}) - y_{i}(t_{k-1})}{t_{k} - t_{k-1}}
\]

\[
y'_{i}(t_{k-1}) = \frac{y_{i}(t_{k-1}) - y_{i}(t_{k-2})}{t_{k-1} - t_{k-2}}
\]

\[
y'_{i}(t_{k}) = \frac{y_{i}(t_{k}) - y_{i}(t_{k-1})}{t_{k} - t_{k-1}}
\]

\[
\int_{0}^{t_{k}} y_{i} dt \approx y_{i}(t_{k})(t_{k} - t_{k-1}) + \int_{0}^{t_{k-1}} y_{i} dt
\]

**Control Input Definition**

The “Control Input” (mode of therapy) will be chosen from a finite set of control inputs that can be applied to the system.

**System Dynamics**

It is convenient to think of the propagation of the patient’s state \( x_{k} \) at time \( t_{k} \), to his state \( x_{k+1} \) at time \( t_{k+1} \) as obeying the following nonlinear dynamical system with process noise \( w_{k} \) and parameters \( p \), i.e.:

\[
x_{k+1} = f(x_{k}, u_{k}, p, w_{k})
\]

For simplicity, \( p \) is discrete, and is assumed to be drawn from a finite set formed by enumerating all useful combinations of clinical covariates,

\[
p \in \{p_1, \ldots, p_M\}
\]

Both the clinical covariates and process noise help to explain the variability of patient responses seen in the database. The covariates help to distinguish gross differences in responses due to patients with major differences in the nature of their disorders and complications. Process noise helps to explain small differences between patients with the same covariates but different responses to the same therapy. It is a measure of unmodeled dynamics, or intra-individual variability, due to other sources of variability in the system (24).

**Probability of Survival**

A patient’s survival probability (SP) for a given state \( x \) is denoted by \( S(x) \), which is calculated by first extracting the 40 (or 100) nearest neighbor states of patients having the same diagnosis and covariates as well as hemodynamic values that are closest to the given patients’ values. The SP is then calculated as the fraction of these nearest neighbors that survived with this treatment. The SP may also serve as an outcome predictor as well as a measure of severity of illness (24).

**LEGENDS**

**Figure 1.** Effects of resuscitation therapy on sequential hemodynamic patterns and survival probability of a 26-years old male who sustained a gunshot wound to the right chest with multiple lacerations of the liver, stomach duodenum, small bowel, and kidney. Note the initial reductions in hemodynamic values and survival
probability in the first hour after admission and recovery with control of bleeding and surgical repair of injuries. Upper row: Cardiac index (CI); second row: Mean arterial pressure; Third row: Pulse oximetry (SapO2); Fourth row: PtcO2/FiO2; Lowest row: Survival Probability. The dark areas represent the deficits of each variable. Time, in hours from ED admission, is noted below the bottom horizontal line. Therapies are outlined in boxes with vertical dotted lines marking onset an end of infusions. FFP, fresh frozen plasma; Hes, hespan (hydroxyethyl starch); rbc, packed red blood cell transfusion; alb, albumin; LR, lactated Ringer’s solution. Time in OR and ICU indicated at lowest line.

Figure 2. Survivors’ (solid line) and nonsurvivors’ (dashed line) temporal patterns are shown for the first 24 hours after their ED admission. Mean values ± SEM are shown for cardiac index (CI), heart rate (HR), mean arterial pressure (MAP), pulse oximetry (SapO2), transcutaneous oxygen tension indexed to the fractional inspired oxygen concentration (PtcO2/FiO2), and survival probability (SP). All values are keyed to the time of admission to the ED. Note the survivors’ cardiac index, MAP, SapO2, PtcO2/FiO2, and SP values were generally higher than those of the nonsurvivors. The mean survivors’ SP values were significantly higher than the mean nonsurvivors’ SP values in this initial resuscitation period.

Figure 3. Survivors’ (solid line) and nonsurvivors’ (dashed line) temporal patterns before, during, and for 24 hours after emergency surgical operations. Mean values ± SEM are shown for cardiac index (CI), heart rate (HR), mean arterial pressure (MAP), pulse oximetry (SapO2), transcutaneous oxygen tension indexed to the fractional inspired oxygen concentration (PtcO2/FiO2), and survival probability (SP). Values are keyed to the time of the surgical operation. Note the survivors’ cardiac index, MAP, SapO2, PtcO2/FiO2, and SP values were higher than those of the nonsurvivors.

Figure 4. Comparison of the actual number of survivors and nonsurvivors (dark cross-hatched columns) with the number of predicted probabilities in each of these two groups (dotted columns).

Figure 5. APACHE scores of survivors and nonsurvivors over the first 4 days after admission compared with survival probabilities of the same series of patients. Vertical bars represent SD. Statistical significance was achieved on the last ICU day, but not during the first four days.

Figure 6. Window showing an illustrative patient’s hemodynamic values at 0.43 hours after ED admission. The first column shows the number of nearest neighbors given each therapy. The second column shows the average PS of these nearest neighbors before therapy was given. Column 3 shows the number of nearest neighbors who were given each of the specified therapies in columns 5 through 12. Column 4 shows the PS of these nearest neighbors after administration of therapy specified in columns 5 through 12. WB/PRBC is whole blood or packed red cells, COLL is colloids, albumin or starch, XTALS is crystalloids, FFP fresh frozen plasma, DOB dobutamine, DOP dopamine, CRYO cryoprecipitate. Based on this information, FFP, COLL and WB/PRBC may be considered as likely to give an appropriate response. Figure 7. Left side: Values of a selected nearest neighbor (Pt. number 237), observed at 9.14 h after ED admission, with diagnostic data shown in the upper left (no blood loss, truncal injury, intra-operative monitoring, age 29, absence of head injury, male gender, and Survival outcome). Left: Hemodynamic values (CI, HR, MAP, SapO2, PtcCO2, PtcO2/FiO2, and hematocrit), integral, first derivative, and second derivative of this nearest neighbor are listed. Right: Hemodynamic values, integral, first derivative, and second derivative values of the monitored patient.

Figure 8. Flow chart showing procedures for the stochastic analysis and control program. First, the patient’s state and covariates are defined and used to extract a given number of nearest neighbors. Second, the survival probability is calculated from the percentage of the nearest neighbors who survived. Third, the data is sorted according to the therapeutic interventions (“controls”). Fourth, the state response to the selected therapy is calculated. Fifth, the survival probabilities after each of the alternative therapeutic interventions are calculated. Sixth, the stochastic control is calculated.
TABLES

Table 1. Clinical Features of the Series:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Survivors (N=124)</th>
<th>Nonsurvivors (N=53)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years; Mean ± SD</td>
<td>31.5 ±13.7</td>
<td>36.7 ±18.9</td>
<td>0.041</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, N; %</td>
<td>107/124 (86.5%)</td>
<td>44/53 (84.9%)</td>
<td>NS*</td>
</tr>
<tr>
<td>Female, N; %</td>
<td>17/124 (13.5%)</td>
<td>9/53 (15.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mechanism of Injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall, N (%)</td>
<td>5 (4%)</td>
<td>1 (2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Gunshot wound, N (%)</td>
<td>61 (49%)</td>
<td>21 (40%)</td>
<td>NS</td>
</tr>
<tr>
<td>Blunt trauma, N (%)</td>
<td>35 (28%)</td>
<td>28 (54%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Stab wound, N (%)</td>
<td>23 (19%)</td>
<td>2 (4%)</td>
<td>0.019</td>
</tr>
<tr>
<td>Bodily Injury**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head, N (%)</td>
<td>16 (9.3%)</td>
<td>19 (25.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Spinal Cord, N (%)</td>
<td>4 (2.3%)</td>
<td>1 (1.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Chest, N (%)</td>
<td>56 (42.6%)</td>
<td>15 (20%)</td>
<td>NS</td>
</tr>
<tr>
<td>Abdomen, N (%)</td>
<td>66 (38.4%)</td>
<td>26 (38%)</td>
<td>NS</td>
</tr>
<tr>
<td>Back, flank, N (%)</td>
<td>13 (7.6%)</td>
<td>4 (5.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Extremity, N (%)</td>
<td>11 (6.4%)</td>
<td>4 (5.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Fractures, N (%)</td>
<td>6 (3.5%)</td>
<td>6 (8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Injury Severity Score</td>
<td>20.2 ± 4.5</td>
<td>29.5 ± 5.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* NS, not statistically significant
** Not mutually exclusive

Table 2. Survival Probability and Hemodynamic Values for the First 24-hours After Admission

<table>
<thead>
<tr>
<th>Variable, unit</th>
<th>Optimal Value</th>
<th>Survivors (N) Mean ± SEM</th>
<th>Nonsurvivors (N) Mean ± SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP, %</td>
<td>&gt;80</td>
<td>(123) 81.5 ± 1.1</td>
<td>(52) 57.7 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>4.0</td>
<td>(123) 4.34 ± 0.07</td>
<td>(52) 3.96 ± 0.18</td>
<td>0.0005</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85</td>
<td>(122) 86 ± 1.2</td>
<td>(51) 76 ± 2.7</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>&lt;100</td>
<td>(123) 104 ± 1.6</td>
<td>(52) 117 ± 2.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SapO₂, %</td>
<td>&gt;98</td>
<td>(122) 99 ± 0.2</td>
<td>(51) 95 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PtcCO₂, torr</td>
<td>&lt;50</td>
<td>(121) 47 ± 1.2</td>
<td>(51) 65 ± 9.2</td>
<td>0.0551</td>
</tr>
<tr>
<td>PtcO₂/FiO₂</td>
<td>&gt;200</td>
<td>(120) 238 ± 12.4</td>
<td>(52) 109 ± 13.2</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

* by Wilcoxon two-sample test. SP Survival Probability, CI cardiac index, MAP mean arterial pressure, HR heart rate, SapO₂ arterial hemoglobin saturation by pulse oximetry, PtcCO₂ transcutaneous carbon dioxide tension, PtcO₂/FiO₂ transcutaneous oxygen tension indexed to FiO₂

Table 3. Survival Probability and Hemodynamic Values at the time of the Probability Nadir*

<table>
<thead>
<tr>
<th>Variable, unit</th>
<th>Optimal Value</th>
<th>Survivors (N=119) Mean ± SEM</th>
<th>Nonsurvivors (N=43) Mean ± SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP, %</td>
<td>&gt;80</td>
<td>(119) 65.2 ± 1.5</td>
<td>(43) 30.3 ± 2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>4.0</td>
<td>(119) 3.97 ± 0.11</td>
<td>(43) 3.16 ± 0.19</td>
<td>0.0002*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85</td>
<td>(118) 82 ± 1.8</td>
<td>(42) 60 ± 3.7</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>&lt;100</td>
<td>(119) 109 ± 2.8</td>
<td>(43) 122 ± 4.0</td>
<td>0.0024</td>
</tr>
<tr>
<td>SapO₂, %</td>
<td>&gt;98</td>
<td>(118) 98 ± 0.9</td>
<td>(42) 89 ± 2.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PtcCO₂, torr</td>
<td>&lt;50</td>
<td>(117) 49 ± 1.5</td>
<td>(42) 79 ± 12.1</td>
<td>0.0155</td>
</tr>
<tr>
<td>PtcO₂/FiO₂</td>
<td>200</td>
<td>(116) 188 ± 13</td>
<td>(43) 49 ± 9.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Only patients with a clearly defined nadir were included in this analysis

* by Wilcoxon two-sample test. SP Survival Probability, CI cardiac index, MAP mean arterial pressure, HR heart rate, SapO₂ arterial hemoglobin saturation by pulse oximetry, PtcCO₂ transcutaneous carbon dioxide tension, PtcO₂/FiO₂ transcutaneous oxygen tension indexed to FiO₂
Table 4. Survival Probability and Hemodynamic Values Before, During, and After Emergency Surgery

<table>
<thead>
<tr>
<th>Variable, unit</th>
<th>Survivors</th>
<th></th>
<th></th>
<th>Nonsurvivors</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (n)</td>
<td>During (n)</td>
<td>After (n)</td>
<td>Before (n)</td>
<td>During (n)</td>
<td>After (n)</td>
</tr>
<tr>
<td>Survival Percent, %</td>
<td>(34) 78.7±1.8</td>
<td>(76) 80.3±1.2</td>
<td>(53) 84.6±1.7</td>
<td>(11) 61.0±5.7</td>
<td>(27) 61.1±3.2</td>
<td>(13) 58.0±5.4</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>(34) 4.59±0.18</td>
<td>(76) 4.34±0.10</td>
<td>(53) 4.18±0.11</td>
<td>(11) 4.27±0.49</td>
<td>(27) 3.81±0.18</td>
<td>(13) 3.75±0.26</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>(34) 81±3.4</td>
<td>(76) 83±1.1</td>
<td>(52) 95±2.2</td>
<td>(11) 83±6.8</td>
<td>(26) 75±3.2</td>
<td>(13) 84±4.3</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>(34) 109±3.6</td>
<td>(76) 102±1.8</td>
<td>(53) 107±2.6</td>
<td>(11) 128±8.2</td>
<td>(27) 115±3.3</td>
<td>(13) 124±6.4</td>
</tr>
<tr>
<td>SapO₂, %</td>
<td>(34) 98±0.9</td>
<td>(76) 99±0.3</td>
<td>(52) 99±0.2</td>
<td>(11) 97±1.9</td>
<td>(27) 95±1.2</td>
<td>(13) 96±1.8</td>
</tr>
<tr>
<td>PtcCO₂, torr</td>
<td>(34) 45±2.9</td>
<td>(75) 46±1.5</td>
<td>(51) 41±1.1</td>
<td>(11) 59±6.5</td>
<td>(27) 62±8.6</td>
<td>(13) 48±6.0</td>
</tr>
<tr>
<td>PtcO₂/FiO₂</td>
<td>(34) 192±18.8</td>
<td>(74) 225±16.2</td>
<td>(51) 231±12.7</td>
<td>(11) 76±25.0</td>
<td>(27) 99±16.5</td>
<td>(13) 108±21.3</td>
</tr>
</tbody>
</table>

# P-values for tests of equality of repeated measures were derived by the mixed linear models using the residual maximum likelihood with the unstructured covariance. CI: cardiac index; MAP: mean arterial pressure; HR: heart rate; SapO₂: arterial hemoglobin saturation by pulse oximetry; PtcCO₂: transcutaneous carbon dioxide tension; PtcO₂/FiO₂: transcutaneous oxygen tension indexed to FiO₂.

Table 5. Classification Summary exclusive of brain death and death in the OR (N=165)

<table>
<thead>
<tr>
<th>Actual Outcome</th>
<th>Predicted to Die</th>
<th>Predicted to Live</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (Row%)</td>
<td>N (Row%)</td>
<td>N (Col%)</td>
</tr>
<tr>
<td>Died</td>
<td>30 73.2%</td>
<td>11 26.8%</td>
<td>41 24.8%</td>
</tr>
<tr>
<td>Lived</td>
<td>5 4%</td>
<td>119 96%</td>
<td>124 75.2%</td>
</tr>
<tr>
<td>Total (%)</td>
<td>35 21.2%</td>
<td>130 78.8%</td>
<td>165 100.0%</td>
</tr>
</tbody>
</table>

Misclassification: 16/165=9.7%

Legend: Classification summary of those predicted to live and those predicted to die by survival probabilities, exclusive of those who were brain dead and those who died in the OR of massive uncontrollable hemorrhage.

Table 6. Hemodynamic Values of Head Injured Patients with Brain Death

<table>
<thead>
<tr>
<th>Variable, unit</th>
<th>Normal Values</th>
<th>Brain Dead Patients (N=10)</th>
<th>P-values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI, L/min/m²</td>
<td>3.2±0.2</td>
<td>4.81±0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91±3</td>
<td>89±1</td>
<td>NS</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>&lt;90</td>
<td>116±2</td>
<td>0.01</td>
</tr>
<tr>
<td>SapO₂, %</td>
<td>&gt;96</td>
<td>99±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>PtcCO₂, torr</td>
<td>&lt;50</td>
<td>43±1</td>
<td>NS</td>
</tr>
<tr>
<td>PtcO₂/FiO₂</td>
<td>185±15</td>
<td>241±16</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*P-values calculated by unpaired Student's t-test

CI cardiac index, MAP mean arterial pressure, HR heart rate, SapO₂ arterial hemoglobin saturation, PtcCO₂ transcutaneous carbon dioxide tension, PtcO₂/FiO₂ transcutaneous oxygen tension indexed to FiO₂.
Figure 2

Graph showing the changes in CI, HR, MAP, Svo2, PtcO2/Fio2, and Survival Probability over time (hours) for survivors and nonsurvivors.
Figure 5

Apache

Survival Probability %

TIME

Survivors
Non-survivors
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Time (h)</th>
<th>Dose</th>
<th>Cl (mL/min)</th>
<th>HR (bpm)</th>
<th>MAP (mmHg)</th>
<th>SgO2 (%)</th>
<th>PrCO2 (mmHg)</th>
<th>PhoCO2</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.27</td>
<td></td>
<td>11300</td>
<td>144.25</td>
<td>2100</td>
<td>97.00</td>
<td>22.59</td>
<td>223.00</td>
<td>33.00</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td></td>
<td>2100</td>
<td>2100</td>
<td>2100</td>
<td>100.00</td>
<td>33.00</td>
<td>24.00</td>
<td>33.00</td>
</tr>
</tbody>
</table>
Figure 8

DATABASE OF RESPONSES

State $x_k$

Covariate $p$

- Extract $N$ Nearest Neighbors
  - $\{x_k^j\}_{j=1}^N$
  - Sort by Control Intervention
    - $\{x_{k+1}^j, u_i\}_{j=1}^{N_i}$
- Propagate State Response
  - $\{x_{k+1}^j\}_{j=1}^{N_i}$
- Calculate Survival Probability Conditioned on Intervention
  - $P(u_i, x_k, p)$
  - $i = 1, \ldots, m$
- Calculate Stochastic Control
  - $u^*(x_k, p)$

$S(x_k, p)$
Autonomic Activity in Trauma Patients Based on Variability of Heart Rate and Respiratory Rate

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Joseph Colombo, PhD

From the Department of Surgery, Division of Trauma/Critical Care, Keck School of Medicine, University of Southern California, Los Angeles

Running title: Autonomic Activity in Trauma Patients

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ABSTRACT

Objective: To evaluate the effects of sympathetic (SNS) and parasympathetic nervous system (PSNS) activity on the heart rate and other hemodynamic variables in acute emergency patients with mild to moderately severe trauma.

Setting: Fourteen trauma patients studied immediately after admission to the emergency department (ED) in a level 1 university-run trauma service.

Methods: We measured heart rate (HR) and respiratory rate (RR) variability by spectral analysis in mild to moderately injured patients and compared the patterns of the low (Lfα), and high frequency (Hfα) areas of variability. The Lfα is the area under the spectral analysis curve within the frequency range of 0.04 to 0.10 Hz. This area reflects primarily the tone of the sympathetic nervous system as mediated by the cardiac nerve. The respiratory, or high frequency area (Hfα) is a 0.12 Hz-wide frequency range centered around the fundamental respiratory frequency (FRF) defined by the peak mode of the respiratory power spectrum. It is indicative of vagal outflow reflecting parasympathetic nervous system activity. The Lfα/Hfα, or "(L/R) ratio," reflects the balance between the sympathetic and parasympathetic nervous systems. To explore the hemodynamic effects of bursts of autonomic activity in response to injury, HR and RR variability were studied along with noninvasive hemodynamic monitoring. Bioimpedance cardiac output, HR, and mean arterial pressure (MAP) were used to measure cardiac function, and transcutaneous oxygen (PtcO2) to reflect tissue perfusion.

Results: During sudden surges of autonomic activity, we observed a consistently positive relationship of HR variability to Lfα, and to a lesser degree, HR variability to Hfα. However, the L/R ratio did not show a statistically significant correlation with HR variability in this series. These HR variabilities that reflect autonomic activity were associated with increased mean arterial pressure (MAP), cardiac index (CI), as well as HR, but decreased tissue perfusion indicated by the decreased PtcO2/FiO2 ratio.

Conclusions: Surges in autonomic activity in the period immediately after ED admission of trauma patients were associated with immediate and pronounced increases in hemodynamic parameters, especially heart rates, and reduced tissue oxygenation.

Key Words: Heart rate variability; Respiratory rate variability; Estimation of autonomic nervous system activity, Parasympathetic nervous system activity; Sympathetic nervous system activity; Blunt trauma; Acute emergencies.
INTRODUCTION

The observation that heart rate and blood pressure vary from beat to beat was first made by Stephen Hales, who, in the 18th century, performed the first quantitative measurement of arterial blood pressure. He also observed correlation among respiratory cycle, beat-to-beat systolic pressure, and the intervals between beats.

Understanding of the role of autonomically mediated neural input to the heart is needed for analyses of hemodynamic responses to acute emergency conditions. A quantitative, noninvasive means of autonomic monitoring based on heart rate (HR) and respiratory rate (RR) variability techniques provides a methodology for clinically evaluating autonomic nervous activity as a whole as well as the sympathetic and parasympathetic components (1-6). Sympathetic modifying drugs and clinical tests produce repeatable and sensitive changes in the low-frequency areas (Lfa) and the (L/R ratio), suggesting that these parameters reflect sympathetic nervous system (SNS) activity. Further, parasympathetic modifying drugs and clinical tests produce repeatable and sensitive changes in the high-frequency areas (Hfa), suggesting that these Hfa values reflect parasympathetic nervous system (PSNS) activity (7-11). Cardiac autonomic monitoring leads to earlier recognition of patients’ physiologic state and may suggest timely, proactive therapy.

The present study uses noninvasive autonomic nervous system monitoring based on real-time HR variability technology in the initial period of patients recently admitted for blunt trauma. Early mild to moderate cases were selected because severe trauma cases particularly in the late stages had many associated extraneous and confounding influences that obscured underlying hemodynamic patterns. The interacting components of the ANS were evaluated by spectral analysis of HR variability and RR variability to reflect the tone of the autonomic nervous system (ANS) (1-5). The clinical usefulness of these values is based on the concept that the normal SNS and PSNS work harmoniously to maintain homeostasis which results in a stable power spectral content that maintains a balance of power contained in the Lfa and the Hfa.

In response to the long-standing recognition of beat-to-beat HR variations and their clinical relevance, Akselrod and colleagues (2) have explored the physiologic mechanisms that generate these fluctuations. Spectral analysis characterizes mathematically the physiologic mechanisms that generate variations in RR intervals. Spectral analysis calculates the frequency content of time-varying signals and offers a breakdown of the successive RR intervals into their frequency components (1-5).

The present study was designed to evaluate sympathetic and parasympathetic activity in the very early stage of acute emergencies of mild to moderate severity. Responses of patients with severe life-threatening injuries have too many confounding and conflicting influences to be easily evaluated.
METHODS

Clinical Series

We studied 14 mild to moderately severely injured patients by noninvasive monitoring of autonomic nervous system activity within the first 1 to 4 hours after admission to the emergency department (ED) before and during the initial fluid resuscitation, but before radiological diagnostic studies, and before the use of sedation, anesthesia, or vasoactive agents. There were 2 females and 12 males, mean age 33.86 years, ranging between 15 to 66 years of age. The mean injury severity score (ISS) was 10.1 ± 4.8. The patients were not under the effect of anesthesia; in three patients mild sedation or one dose of pain medication was given. All these study patients were discharged alive and well after two to 23 days of hospitalization. The demographic, salient clinical features and medications are given in table 1.

Patients were selected based on the following criteria: hypotension (systolic blood pressure <100 mmHg or mean arterial pressure < 70 mmHg), tachycardia (heart rate >100 beats/min), multiple long bone fractures, head injuries or blunt abdominal and thoracic injuries.

HR and RR Variability as Markers of Sympathetic and Parasympathetic Activities

HR variability is defined as recurrent changes in beat-to-beat, measured by RR intervals. Two skin electrodes placed on each side of the chest in the standard Lead II ECG configuration measure the instantaneous heart rate. The heart beat intervals are recorded and the HRV is plotted in the frequency domain to separate the high frequency components from the low frequency components by spectral analysis. When HR variability is plotted in the time-domain, it is difficult to distinguish the high frequency components from the low, because the curve reflects the sum total of all frequencies in that signal.

Variability in the instantaneous beat-to-beat heart rate intervals is a function of sympathetic and parasympathetic activity that regulates the cardiac functional response to the body’s level of metabolic activity. The SNS primarily generates the low frequency components of HR variability associated with more gradually increasing HR variability, because the sympathetic branch usually responds in four or five seconds, which is slower than parasympathetic responses (12,13). The PSNS primarily generates the high frequency components and has sharper increases, since it typically responds in one to two seconds (12,13).

In addition to analyzing the ECG signals, a respiratory signal is obtained by the same electrodes through impedance plethysmography estimated by chest expansion. Incorporating respiratory signal analysis enables us to independently measure each branch of the ANS. This
provides the essential dimension missing from classical heart rate variability monitoring as it pertains to independent assessment of the ANS branches.

When the low frequency and high frequency components were isolated within the HR variability spectrum, their respective areas under the curve were calculated as Lfα and Hfα. The Lfα and Hfα values were demonstrated to reflect sympathetic and parasympathetic tone, respectively, by independent digital measurements (2-5). The Lfα is computed as the area under the heart rate spectrum from 0.04 Hz to 0.10 Hz. The Hfα, sometimes referred to as the respiratory frequency area, is computed as the area within a 0.12 Hz-wide portion of the heart rate spectrum centered around the fundamental respiratory frequency (FRF), which is defined by the peak mode of the respiratory power spectrum. The Hfα is indicative of the vagal outflow and reflects the parasympathetic nervous system (PSNS) influence on heart rate control. These measurements have been demonstrated to be reliable, repeatable, and specific for sympathetic and parasympathetic function (1-14).

**Hemodynamic Monitoring**

**Cardiac Output**

An improved thoracic bioelectric impedance device (Yantagh Inc., Bristol, PA) was applied shortly after arrival in the ED. The noninvasive disposable prewired hydrogen electrodes were positioned on the skin and three ECG leads were placed across the precordium and left shoulder (15,16). A 100 kHz, 4 mA alternating current was passed through the patient’s thorax by the outer pairs of electrodes and the voltage was sensed by the inner pairs of electrodes; the voltage sensed by the inner electrodes captured the baseline impedance (Zo), the first derivative of the impedance waveform (dZ/dt), and the ECG. The signal processing algorithm used a time-frequency distribution (modified Wigner Distribution) analysis that increased signal-to-noise ratios (15,16). Previous studies have documented satisfactory correlations between thermodilution and bioimpedance cardiac output values for trauma patients in the ED, OR, and ICU conditions (17-19).

**Transcutaneous Oxygen Tension**

Standard transcutaneous oxygen tension measurements were continuously monitored throughout the observation period. This technology uses the same Clark polarographic oxygen electrode routinely employed in standard blood gas measurements (19-26). The oxygen tensions were measured in a representative area of the skin surface heated to 44°C to increase diffusion of oxygen across the stratum corneum and to avoid vasoconstriction in the local area of the skin being measured (24-25). Previous studies demonstrated the capacity of transcutaneous oxygen tensions to estimate skin oxygen tension as a reflection of tissue perfusion (19-26). Transcutaneous oxygen tension (Ptco2) has been shown to reflect the delivery of oxygen to the local area of skin; it also paralleled the mixed
venous oxygen tension (SvO₂) values (20). While oxygen tension of a segment of the skin does not reflect the state of oxygenation of all tissues and organs, it has the advantage of being the most sensitive early warning tissue for the adrenomedullary stress response; vasoconstriction of the skin is an early stress responses of hypovolemia and other shock syndromes (27-29). Transcutaneous oxygen tension was indexed to the FiO₂ to give a PtcO₂/FiO₂ ratio because of marked PtcO₂ changes produced by increased inspired oxygen. Limitations of the transcutaneous methods are the thermal environment must be reasonably constant, marked changes in room temperature from drafts or open windows must be avoided; the electrode must be changed to a nearby thoracic or shoulder site and be re-calibrated to avoid first degree skin burns.

**Experimental Design**
Continuous monitoring of HR variability with ANS-R1000 (Ansar Inc., Philadelphia, PA) was started shortly after admission and before use of anesthesia or ionotrophic agents, and when possible, before pain medication. HR variability was monitored continuously for two to four hours in each patient to identify, record, and compare patterns of Lfa, Hfa and L/R ratio with the HR changes. We made effort to exclude times when extraneous confounding events may have played a role by noting, recording, and eliminating from consideration the time periods of agitation, pain, cough, needle insertion, withdrawal of the needle from skin, local anesthesia, suturing of minor skin injuries, changes in position, dressing changes, talking, presence of friends and family members, the patient’s reaction to environmental sounds, need to urinate, and other disturbing events. Periods when the patient was quiet and stable were considered in the present analysis.

**Sequential Changes in Lfa, Hfa, L/R ratio in Relation to Heart Rate**
Spectral analysis of HRV and RRV were automatically determined and displayed as Lfa, Hfa, L/R ratio, and mean HR. The monitoring was continuous in a format of consecutive 32-second segments. Since observed changes occurred over varying lengths of time, we evaluated the patterns of changes occurring during varying time periods. Sequences of consecutive segments were used to differentiate relatively longer term patterns than the 32-second segments routinely analyzed and displayed by the monitoring device.

**Changes in Lfa, Hfa, L/R ratio and their Correlation with Hemodynamic Values**
We compared changing patterns of Lfa, Hfa and L/R with simultaneous hemodynamic changes in MAP, HR, CI, SI, PtcO₂/FiO₂ values during the initial post-admission period in the emergency department.
RESULTS

Sudden Abrupt Changes in Lfa, Hfa, L/R ratio

We studied 31.5 hours of continuous second-by-second monitoring of Lfa, Hfa and L/R ratio and compared them with simultaneous changes in CI, SI, HR, MAP, and PtcO2/FiO2. Figures 1-3 illustrate continuous simultaneous patterns of HR with Lfa, Hfa, and L/R ratios. Inspection of the continuous measurements revealed the presence of sudden bursts or surges of autonomic activity of widely varying magnitude that occurred along with increases of heart rate. The HR changes were better correlated with simultaneous Lfa values (Fig. 1) and to a lesser degree with simultaneous Hfa (Fig. 2), but not well correlated with L/R ratio (Fig. 3).

Lfa changes including relatively small changes were associated with HR changes 86% of the monitored time; range 81% to 92% (Table 4). Similarly, 65% of Hfa changes were associated with HR changes; this ranged between 55% and 86% in different patients (Table 4). L/R ratio had the least relationship (57%) with the changes in mean HR; in different patients this ranged between 45% and 75% (Table 3).

The dynamic range of HR changes did not affect the sensitivity of HR pattern to Lfa and Hfa changes. That is, there were pronounced changes in HR in the range of 70 beat/min. as well as in the tachycardia range of over 100 beat/min. The range of changes in HR was roughly proportional to the simultaneous Lfa changes (Fig. 1).

Correlation of Sudden Changes in Lfa with Hemodynamic Values

The increase of Lfa and Hfa was associated with significant increases in HR, CI, and MAP (Table 2). There was a trend toward increased SI that did not achieve statistical significance, indicating that the increased CI and HR did not occur at the expense of reduced SI. Similarly, the decreases in Lfa and Hfa was associated with significant reductions in CI, HR, and MAP, and with trends toward reduced Hfa, and SI values (Table 4).

Tissue perfusion reflected by PtcO2/FiO2 values decreased significantly with surges of increased Lfa and Hfa values, and tended to increase with the reduction in Lfa and Hfa values.

DISCUSSION

Spectral analysis of heart rate and respiratory rate variability converts the heart and respiratory time domain signals to the frequency domain signals for analysis and calculation of the LFa and the HFa. The LFa is a measurement that includes information from both sympathetic and parasympathetic components of the ANS carried by the cardiac nerve, a branch of the vagus nerve.
that receives sympathetic input (3-6). However, the LFa and the HFa, can provide insight into the cardiac sympathovagal balance (2) as well as the health and functioning of the ANS, both centrally and, to a lesser extent, peripherally (1,12,13).

The frequency of the peak mode of the respiratory spectrum in defined as the fundamental respiratory frequency, which is equivalent to the inverse of the respiratory rate at rest during normal breathing. The HR variability is affected by both SNS and PSNS activity. When the fundamental respiratory frequency is superimposed on the HR variability spectral frequency axis, the high frequency PSNS component can then be isolated. The low frequency SNS component is also isolated within the heart rate variability signal based on classical spectral analysis theory.

The clinical usefulness of spectral analysis of HR variability and RR variability is based on the hypothesis that ANS monitoring provides evidence that a particular level of treatment is adequate or insufficient. Noninvasive ANS monitoring provides this information as numerical trends in real time. The goal is to titrate intervention to the level that is most effective at that given time. Noninvasive monitoring of ANS by spectral analysis of HR and RR variability has been used in different studies in the different health conditions such as sepsis (6), trauma and shock (14), depth of anesthesia (30-32), cardiac dysfunction (32-35), cardiopulmonary diseases (35), diabetic neuropathy (36-38), pain management, neonatal development as infant monitoring (39), pharmaceutical interactions (40) and brain death (14). In these studies, patients have been monitored for short periods of time to compare the average values of Lfa, Hfa, L/R ratio and mean HR with values of normal populations.

Invasive monitoring remains the most definitive means of evaluating circulatory function in high risk patients, but it is costly, personnel intensive, has complications and is often started late in the course of illness after ICU admission and the onset of organ failure. Delays in management of trauma patients have led to circulatory deficiencies, organ failures, and death. However, noninvasive monitoring techniques that are recently coming of age may reduce the risk and cost of monitoring by invasive techniques (14,17-19). New noninvasive methodologies, such as ANS monitoring based on real-time HR variability, may also reduce delays in instituting therapy and thereby improve outcomes.

As ANS monitoring becomes more widely used and as more is learned about the underlying mechanisms driving the ANS monitored parameters, more specific and appropriate descriptive hemodynamic correlations will develop (1-14).