

Program and Abstracts

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First Meeting

Society for Research on
AFOSR-TR-88-1005

Biological Rhythms

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May 11-14, 1988
Wild Dunes Resort
and Conference Center
Charleston, South Carolina

11 JUL 1988

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Society for Research on Biological Rhythms

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University of Virginia
Charlottesville, Virginia
U.S.A. 22901

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Journal of Biological Rhythms

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REPORT DOCUMENTATION PAGE

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19. Abstract

SUMMARY OF FIRST MEETING OF SOCIETY FOR RESEARCH ON BIOLOGICAL RHYTHMS

APOSR-TR- 88-1005

From May 11-14, 1988 the Society for Research on Biological Rhythms held its inaugural meeting at the Wild Dunes Conference Center near Charleston, South Carolina. The Society was formed in 1987 to promote the advancement of basic and applied research in all aspects of biological rhythms, to disseminate important research results concerning biological rhythms to the general public, to develop and enhance the education and training of students and researchers in the field and to foster interdisciplinary communication. This first meeting was an initial success in meeting the goals of the Society, particularly in the area of interdisciplinary communication.

Researchers in the field of Biological Rhythms tend to be fragmented into many disciplines and are often divided along many different lines. One way of dividing the field is along frequency lines; while some workers study biological rhythms with a period of msec, others are interested in rhythms with periods in the range of minutes, hours (i.e. ultradian or pulsatile), a day (i.e. circadian) or a year (i.e. seasonal or circannual). The field is also divided along the lines of the major disciplines within biology since rhythm biologists can be either biochemists, molecular/cellular biologists, system physiologists, behaviorists and/or ecologists. In addition, while many workers study the basic biological mechanisms involved in generating rhythmicity, others are interested in the clinical applications of a better understanding of biological rhythmicity. Even within the clinical field, researchers fall into many traditional categories including psychiatry, endocrinology, neurology, oncology, cardiology and reproduction. This first meeting promoted the interaction of workers in the various areas in a variety of different ways. First, there was a mixture of Symposia as well as slide and poster sessions on clinical and basic research topics. The Symposia were organized to insure that the entire frequency range of biological rhythms would be presented. While some Workshops were organized to bring together researchers in a limited fast moving field, others were organized to bridge different areas and to bring together people who normally never communicate with each other. Two examples: the Workshop on "Stabilization of periodic processes through coupling of oscillators" had investigators interested in the rhythmic movement of leech swimming trading ideas with clinicians working on the multioscillatory nature of the human circadian system, while the Workshop on "Use of periodogram analysis and related procedures in biological rhythms studies" had endocrinologists interested in the significance of pulsatile hormone release communicating with sleep researchers.

In addition to providing a forum for interdisciplinary communication, this first meeting also brought together workers in various "hot areas" of biological rhythms. Progress in these areas is quite rapid due to the application of new techniques and/or because of the large number of investigators concentrating on similar problems. To name only a few of these areas: 1) neural transplants of biological clocks, 2) the study of biological clocks in vitro, 3) the molecular genetic basis for rhythmicity, 4) the effects of drugs on rhythmic functions and 5) the interaction of the sleep and circadian systems.

It should be noted that this first meeting of the Society exceeded by far the expectations of its organizers. When the Society was first formed and planning for the first meeting began, we were not sure if we would be able to attract over 100 people, and we "dreamed" of having 200 participants. The fact that about 250 scientists/clinicians attended this inaugural meeting attests to the need for this new Society and for the desire of a wide range of investigators with a common interest in biological rhythms to come together under the umbrella of this new organization.

Submitted by,
Fred W. Turek
President, SRBR

AFOSR-IR- 88-1005

FINAL TECHNICAL REPORT

**FIRST INTERNATIONAL MEETING OF
THE SOCIETY FOR RESEARCH ON BIOLOGICAL RHYTHMS**

GRANT # AFOSR-88-0133

SOCIETY FOR RESEARCH ON BIOLOGICAL RHYTHMS
First International Meeting, Wild Dunes, South Carolina
May 11 - 14, 1988

List of Attendants

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Norman Adler	Univ. of Pennsylvania
Raul Aguilar-Roblero	Inst. de Physiologica Celular UNAN, Mexico
H.E. Albers	Georgia State Univ.
Richard Allen	Hopkins Sleep Disorders Center
John J. Alleva	Div. of Research & Testing, Food & Drug Administration
Elizabeth J. Ampleford	University of Virginia
Frank Barnwell	University of Minnesota
Carey Baron	
Timothy Bartness	Worcester Foundation for Experimental Biology
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Sarah Berga	Univ. of California, San Diego, Medical Center
William Berry	Air Force Office of Science Research
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Herbert Biebach	Max-Planck-Institut fur Verhaltensphysiologie
Sue Binkley-Tatem	Temple University
Eric Bittman	Univ. of Massachusetts
Gene Block	Univ. of Virginia
Barbara Bogart	Univ. of Virginia
Alexander Borbely	Univ. of Zurich, Switzerland
Katarina Borer	Univ. of Michigan
Mary Anne Brock	Gerontology Research Center, NIA
Michael Brown	Univ. of Massachusetts
James Buggy	
Daniel Buysse	University of Pittsburgh School of Medicine
Gregory Cahill	Emory Univ. School of Medicine

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J. Patrick Card	E.I. duPont & Co.
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Anne Caufriez	Universite Libre de Bruxelles, Belgium
Christopher Chabot	Univ. of Virginia
Russell Church	Brown Univ.
R.A. Cohen	Univ. of Massachusetts Medical School
Chris Colwell	Univ. of Virginia
Linda Connell	NASA-Ames Research Center
Georges Copinschi	Universite Libre de Bruxelles, Belgium
Margo Coulter	Univ. of Kingston, Ontario, Canada
Charles Czeisler	Brigham & Women's Hospital, Boston
R.E. Dahl	Pittsburgh, PA
Janet Darrow	Wellesley College
Fred Davis	Northeastern Univ., Boston
Patricia DeCoursey	Univ. of South Carolina
William Dement	Stanford Univ. School of Medicine
John Dimitry	Univ. of Delaware
Marie Dumont	Hopital DU Sacre-Coeur, Montreal, Canada
Wallace Duncan	National Institute of Mental Health
Jay Dunlap	Dartmouth Medical School
David Earnest	Univ. of Rochester
Charmane Eastman	Rush Medical Center
Francis Ebling	Univ. of Michigan
Dale Edgar	Stanford Univ.
Leonard Eiserer	Elizabethtown College

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Guenther Fleissner	Univ. of Frankfurt, FRG
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Russell Foster	Univ. of Virginia
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Jannon Fuchs	Univ. of Texas
Robert Gallo	Univ. of Connecticut
Michael Geusz	Vanderbilt Univ.
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Rhanor Gillette	Univ. of Illinois, Urbana
Sari Gilman	WPIC, Pittsburgh, PA
Bruce Goldman	Univ. of Connecticut
Eberhard Gwinner	Max-Planck-Institut fur Verhaltensphysiologie, FRG
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Mary Harrington	Smith College, MA
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John Harsh	Univ. of Southern Mississippi

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Martha Hotz	Northwestern Univ.
Thomas Hout	Harvard Medical School
David Hudson	Univ. of Virginia
Gary Jackson	College of Vet. Medicine, Univ. of Illinois, Urbana
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Pauline Jasim	Northwestern Univ.
Amy Jetton	Northwestern Univ.
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Martin Kavaliers	Univ. of Western Ontario, Canada
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Charles McCormack	The Chicago Medical School

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Jennifer Swann	Rutgers Univ.
Joseph Takahashi	Northwestern Univ.
Barbara Tate-Ostroff	McLean Hospital, Belmont, MA
Malcolm Taylor	School of Aerospace Med.
David Terrian	VA Med. Center, Ann Arbor, MI
L. Cass Terry	Shiga Univ. of Medical Science, Japan
Jean-Christophe Thalabard	Univ. of Texas Medical School
Elie Thomas	La Trobe Univ., Australia
Tetsushi Tsujimoto	Shiga Univ. of Medical Science, Japan
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Program and Abstracts

for the
First Meeting
of the
Society for Research on

Biological **Rhythms**

Wild Dunes Resort and Conference Center
Charleston, South Carolina
May 11-14, 1988

We wish to thank the following for their contributions:

Air Canada

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Mini-Mitter Co., Inc.

National Institute of Mental Health

National Science Foundation

The Upjohn Company

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Pauline Jasim

Janet Joy

Jill Milette

Fred Turek

Franziska Wollnik

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Classified	✓
Unclassified	✓
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General Information

President's Welcome

Welcome to the first meeting of the Society for Research on Biological Rhythms. The Society was formed in 1987 to promote the advancement of basic and applied research in all aspects of biological rhythms, to disseminate important research results concerning biological rhythms to the general public, to develop and enhance the education and training of students and researchers in the field and to foster interdisciplinary communication. This inaugural meeting has been organized with these goals in mind. Every attempt has been made to provide an environment for the exchange of ideas during the scheduled scientific sessions, as well as during informal gatherings. I hope this will be the first of a long series of successful meetings of the Society for Research on Biological Rhythms.

Fred W. Turek

Fred W. Turek
President, SRBR

Registration

Registration will take place at the Gazebo outside the Wild Dunes Reception Center from 12:00 - 7:00 p.m. Wednesday, May 11.

Registration by February 1, 1988:

Regular SRBR Members	\$100.00
Non-SRBR Members	\$150.00
Student Members	\$ 50.00
Student Non-members	\$100.00
Guest Registration	\$ 60.00

Registration after February 1, 1988:

Regular SRBR Members	\$150.00
Non-SRBR Members	\$200.00
Student Members	\$ 75.00
Student Non-members	\$150.00
Guest Registration	\$ 80.00

Student rates apply to registrants who will not have received the Ph.D. or M.D. degree at the time of the meeting. Registration fees include costs for opening reception and banquet dinner, as well as use of the shuttle service on Wild Dunes property. Programs for children are available through Wild Dunes Resort and the staff will provide lists of available babysitters.

SRBR Information and Message Desk

The Society will maintain an information desk in the lobby of the Island House Conference Center from 8:00 a.m. to 1:00 p.m., May 12-14 and from 4:30 - 6:30 p.m. on May 12 and 13. Late arrivals can register during these times. A message board will be located next to the information desk. Check this board for mail, notes, and telephone messages. A telephone will be available for incoming messages. The number is: 803-886-6000 ext. 4507. All villas are provided with telephones and a list of room locations and phone numbers of all meeting registrants will be available at the information desk.

Shuttle Service

Wild Dunes maintains an excellent shuttle service for the use of their guests. Regularly scheduled shuttles will run between villas and meeting areas before and after sessions, as well as social events. During these times the shuttle busses will make several tours of the grounds. Anyone wishing transportation should wait by the roadside and the bus will stop by to pick you up. If you wish shuttle service at any other times, phone ext. 2216. Shuttle service is available daily from 7:00 a.m. to 11:00 p.m. If you phone for shuttle service, you must wait outside your villa for the bus. The bus driver will not knock on your door.

Where to Eat

Groceries, including beer and wine, are available at the Yacht Harbor and General Store, which is open 24 hours a day. Meeting registrants may charge purchases to their rooms. The shuttle bus drivers will be happy to take you to the store and wait while you do your shopping.

A continental style breakfast can be purchased on the Salon Deck of the Island House Conference Center from 7:30-8:30 a.m. on May 12-14. In addition to their regular fare, Wild Dunes has made special arrangements to provide the Society with an oceanside concession stand at the Beach Cabana which will be open for lunch from 1:00 p.m. to 3:00 p.m. on Thursday, May 12 and Friday, May 13. Several restau-

rants are available on the island. The Island House Restaurant is open for dinner and specializes in traditional Low Country cuisine and local seafood. Reservations are required for dinner (ext. 2137). The Club House Restaurant is located at the east end of Wild Dunes and serves breakfast, lunch, and dinner. Live entertainment is offered from 9:00 p.m. to 1:00 a.m. The Club House also offers a takeout menu, which must be ordered in advance. For more information call the Club House at ext. 2296. Dinner is also available at the Tradewinds Restaurant located just outside Wild Dunes. For reservations call 866-5678.

Wild Dunes Facilities

Wild Dunes facilities are described in detail in your Guest Directory. Bicycle rentals, fishing, golf, tennis, boat rentals, and tours of Charleston are all available. For further information, check with the Concierge Desk at ext. 2255.

Emergency Phone Numbers

Emergency	911
Fire Department	886-6155
Police Department	886-6522
Club Security	ext. 2128
<i>Physician</i>	
George G. Durst, MD	883-3176
John E. Emmel, MD	886-6215
<i>Dentist</i>	
Michael C. McEniry, DMD	886-6461

Social Events

The Opening Reception will feature an oceanside open bar from 7:00 - 10:00 p.m. on Wednesday, May 11 in the Beach Cabana located near the Island House Conference Center. A Seafood Buffet will be held in the Beach Cabana at 8:00 p.m. on Friday, May 13 and a cash bar will be available from 8:00 - 12:00 p.m.

Scientific Sessions

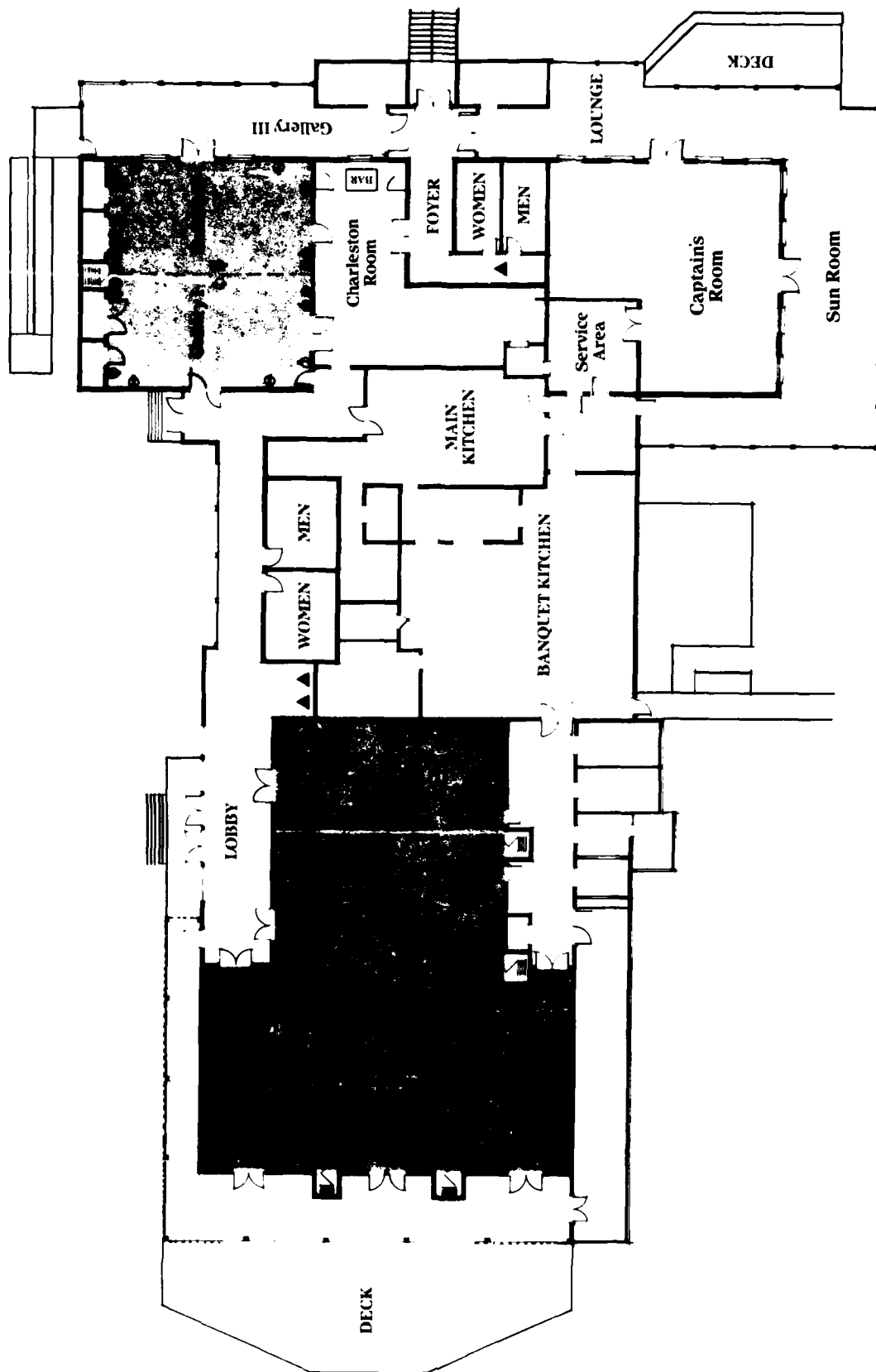
Two concurrent symposia will be held from 8:30 - 10:30 a.m. on May 12, 13, and 14. Concurrent workshops will be held from 11:00 a.m. - 1:00 p.m. on Thursday, May

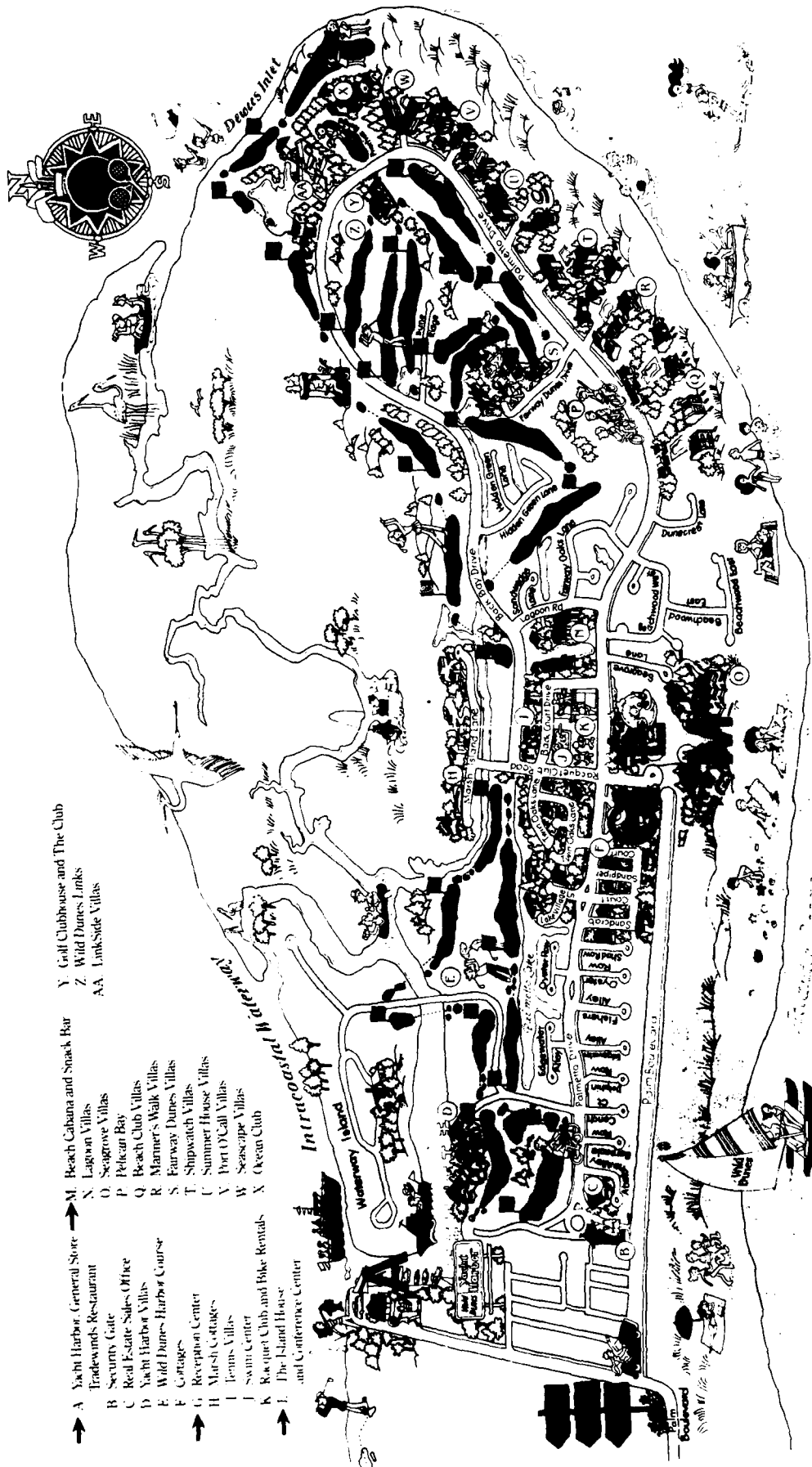
12 and from 3:30 - 5:30 p.m. on Friday, May 13. The Plenary Lecture will be held at 7:30 - 8:30 p.m. on Thursday, May 12. Contributed slide sessions will be held from 4:30 - 6:30 p.m. on Thursday, May 12 and from 11:00 a.m. - 1:00 p.m. on Saturday, May 14. Posters will be located in Salon V and in the lobby. They will be available for viewing from 3:30 p.m., Thursday May 12 until 2:00 p.m., Friday May 13. The authors will be present from 11:00 a.m. - 1:00 p.m. on Friday, May 13.

Instructions to Presenters

Posters should be assembled between 2:30 and 3:30 p.m. on Thursday, May 12. Authors are requested to attend their posters from 11:00 a.m. - 1:00 p.m. on Friday, May 13. All posters should be removed on Friday by 2:00 p.m.

Slide talk presenters should give their slides (clearly numbered and in correct order) to the projectionist 15 minutes before the start of the session. Slides should be collected immediately after the end of the session.





- A Yacht Harbor, General Store
 B Security Gate
 C Real Estate Sales Office
 D Yacht Harbor
 E Wild Dunes Harbor Course
 F Cottages
 G Reception Center
 H Marsh Cottages
 I Tennis Villas
 J Swim Center
 K Racquet Club and Bike Rentals
 L The Island House and Conference Center
 M Beach Cabana and Snack Bar
 N Lagoon Villas
 O Seagrass Villas
 P Pelican Bay
 Q Beach Club Villas
 R Mariner's Walk Villas
 S Fairway Dunes Villas
 T Shipwatch Villas
 U Summer House Villas
 V Port O'Call Villas
 W Seaside Villas
 X Ocean Club
 Y Golf Clubhouse and The Club
 Z Wild Dunes Links
 AA Linkside Villas

Program Schedule

WEDNESDAY, MAY 11

- 12:00- 7:00 **Registration:**
Reception Center Gazebo
- 7:00-10:00 **Opening Reception:** Beach Cabana

THURSDAY, MAY 12

- 8:30-10:30 **Symposia 1 & 2**
1. Organization of Animal Circadian Systems;
Salon I & 2
 2. Pulsatile Rhythms of Neuroendocrine Function;
Salon III, IV, & V
- 10:30-11:00 Coffee Break: Salon Deck
- 11:00- 1:00 **Workshops 1-6**
1. Neural Transplants and Restoration of Circadian Function;
Salon I
 2. Stabilization of Periodic Processes Through Coupling of Oscillators;
Charleston Room
 3. Photic Effects on Pacemakers;
Salon II
 4. Pineal and Retinal Oscillators In Vitro;
Salon III
 5. Computerized Data Acquisition;
Salon IV
 6. Involvement of Protein Synthesis in Circadian Rhythm Generation;
Salon V
- 3:30 **Posters available for viewing:**
Salon V and Lobby
- 4:30- 6:30 **Slide Sessions 1-4**
1. Mechanisms of Vertebrate Pacemakers;
Salon I
 2. Photoperiodism and Seasonal Rhythms;
Salon II
 3. Human Rhythms and Sleep;
Salon III
 4. Pharmacological Manipulation of Rhythms;
Salon IV
- 6:30- 7:30 Break: Salon Deck
- 7:30- 8:30 **Plenary Lecture:** "Circadian Rhythms in the 4th Decade: What Do We Want and What Can We Have?"
Michael Menaker:
Salon I-IV

FRIDAY, MAY 13

- 8:30-10:30 **Symposia 3 & 4**
3. Cellular, Molecular and Genetic Dissection of Clocks;
Salon I & II
 4. Interaction Between Sleep and the Circadian System;
Salon III & IV
- 10:30-11:00 Coffee Break: Salon Deck
- 11:00- 1:00 **Poster presentations:**
Salon V & Lobby
- 3:30- 5:30 **Workshops 7-11**
7. Entraining Effects of Melatonin;
Salon I
 8. Use of Periodogram Analysis and Related Procedures in Biological Rhythms Studies;
Salon II
 9. Cellular Analysis of Biological Oscillators;
Salon III
 10. Organization and Function of the Intergeniculate Leaflet;
Salon IV
 11. Use of In Vitro Brain Slices in Studies of Circadian Function;
Salon V
- 6:00- 7:30 **Business Meeting:** Salon I & II
- 8:00-12:00 Seafood Banquet with Cash Bar:
Beach Cabana

SATURDAY, MAY 14

- 8:30-10:30 **Symposia 5 & 6**
5. Modulation and Control of Neural Oscillators;
Salon I & II
 6. Circannual Rhythms — Properties and Mechanisms;
Salon IV & V
- 10:30-11:00 Coffee Break: Salon Deck
- 11:00- 1:00 **Slide Session 5-8**
5. Chronobiology of Depression;
Salon I
 6. Cellular and Molecular Basis of Rhythmicity;
Salon II
 7. Comparative Analysis of Rhythms;
Salon IV
 8. Entrainment;
Salon V

Scientific Program

Thursday May 12

8:30 am - 10:30 am

Salon I and II

Symposium 1:

Organization of Animal Circadian Systems

Robert Y. Moore, organizer, SUNY Stony Brook

Terry Page, Vanderbilt University

Joseph Takahashi, Northwestern University

8:30 am - 10:30 am

Salon III, IV, and V

Symposium 2:

Pulsatile Rhythms of Neuroendocrine Function

Fred Karsch, organizer, University of Michigan

Ernst Knobil, University of Texas, Houston

Robert Gallo, University of Connecticut

Michael Lehman, University of Cincinnati

11:00 am - 1:00 pm

Salon I

Workshop 1:

Neural Transplants and Restoration of Circadian Function

Patricia DeCouresy, University of South Carolina

11:00 am - 1:00 pm

Charleston Room

Workshop 2:

Stabilization of Periodic Processes Through Coupling of Oscillators

Walter Heiligenberg, University of California, San Diego

11:00 am - 1:00 pm

Salon II

Workshop 3:

Photoc Effects on Pacemakers

Benjamin Rusak, Dalhousie University

11:00 am - 1:00 pm

Salon III

Workshop 4:

Pineal and Retinal Oscillators in Vitro

Joseph Takahashi, Northwestern University

11:00 am - 1:00 pm

Salon IV

Workshop 5:

Computerized Data Acquisition

Van Gooch, University of Minnesota

11:00 am - 1:00 pm

Salon V

Workshop 6:

Involvement of Protein Synthesis in Circadian Rhythm Generation

J. Woodland Hastings, Harvard University

3:30 pm

Salon V and Lobby

Posters available for viewing

4:30 pm - 6:30 pm

Salon I

Slide Session 1:

Mechanisms of Vertebrate Pacemakers

Chairpersons: Sue Binkley

Martin Zatz

4:30

- 1 MELATONIN METABOLISM IN THE EYE OF *XENOPUS*. G.M. Cahill and J.C. Besharse. Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA.

4:45

- 2 MAPS OF SENSITIVITY TO LIGHT AND DARK PULSES FOR THE CHICK PINEAL GLAND IN VIVO. S. Binkley and K. Mosher, Biology Department, Temple U, Philadelphia, PA.

5:00

- 3 AGENTS AFFECTING VOLTAGE-SENSITIVE CALCIUM CHANNELS HAVE BIG EFFECTS ON CHICK PINEAL MELATONIN OUTPUT BUT NOT ON THE UNDERLYING PACEMAKER. M. Zatz and D.A. Mullen, Laboratory of Cell Biology, NIMH, Bethesda, MD.

5:15

- 4 SENSITIVITY AND SPECIFICITY OF THE SCN CIRCADIAN PACEMAKER TO cAMP STIMULATION. R.A. Prosser and M.U. Gillette. Neural and Behavioral Biology Program and Department of Physiology and Biophysics, University of Illinois, Urbana, IL.

5:30

- 5 CALMODULIN INHIBITORS AFFECT THE CIRCADIAN RHYTHM IN FIRING RATE OF SUPRACHIASMATIC NUCLEUS (SCN) NEURONS IN VITRO. S. Shibata and R.Y. Moore. Depts. of Neurology and Neurobiology, SUNY at Stony Brook, Stony Brook, NY.

5:45

- 6 TETRODOTOXIN ABOLISHES ELECTRICAL ACTIVITY OF SUPRACHIASMATIC NUCLEUS (SCN) NEURONS IN VITRO WITHOUT ALTERING THE GLUCOSE UTILIZATION RHYTHM OR PHASE OF THE RHYTHM IN SINGLE UNIT FIRING RATE. R.Y. Moore and S. Shibata, Depts. of Neurology and Neurobiology, SUNY at Stony Brook, Stony Brook, NY.

6:00

- 7 CIRCADIAN VARIATION OF 2-DEOXYGLUCOSE UPTAKE WITHIN THE HOUSE SPARROW SUPRACHIASMATIC NUCLEUS. V.M. Cassone. Department of Neurology, Health Sciences Center, State University of New York, Stony Brook, NY.

6:15

- 8 A COMPARATIVE STUDY OF 2-DEOXYGLUCOSE UPTAKE BY THE SUPRACHIASMATIC NUCLEUS AMONG MAMMALIAN HIBERNATORS. T.S. Kilduff, C.M. Radeke, P. Kwon and H.C. Heller. Depts. of Psychiatry and Biological Sciences, Stanford Univ., Stanford, CA.

4:30 pm - 6:30 pm

Salon II

Slide Session 2:

Photoperiodism and Seasonal Rhythms

Chairpersons: Herbert Underwood
Douglas Foster

4:30

- 9 PHOTOPERIODIC TIME MEASUREMENT IN THE MALE LIZARD *ANOLIS CAROLINENSIS*. H. Underwood. Department of Zoology, North Carolina State Univ., Raleigh, NC.

4:45

- 10 ANNUAL AND CIRCANNAL RHYTHMS IN TEMPERATE AND TROPICAL DEER SPECIES. A.S.I. Loudon, J.D. Curlewis, J.A. Milne¹ and A.S. McNeilly². Institute of Zoology, Regents Park, London, UK; ¹MLURI, Penicuik, Scotland; ²MRC Unit Reproductive Biology, Edinburgh, Scotland.

5:00

- 11 STIMULATION OF GONADOTROPIN RELEASING HORMONE (GnRH) SECRETION BY LONG DAY IN ANESTROUS PONY MARES. D.C. Sharp, W.R. Grubbaugh, J. Weithenauer, P. Sheerin and S.D. Davis. University of Florida, Gainesville, FL.

5:15

- 12 DO SOCIAL CUES PLAY A ROLE IN TIMING THE SEASONAL REPRODUCTIVE RHYTHM OF THE EWE? N.L. Wayne, B. Malpoux and F.J. Karsch. Developmental and Reproductive Biology, Dept. of Physiology, The University of Michigan, Ann Arbor, MI.

5:30

- 13 DEVELOPMENT OF REPRODUCTIVE RESPONSIVENESS TO LIGHT: INFLUENCE OF NIGHTLY AUGMENTATION OF THE AMPLITUDE OF THE MELATONIN RHYTHM IN THE NEONATAL LAMB. D.L. Foster, F.J.P. Ebling, L.E. Claypool, and R.I. Wood. Reproductive Endocrinology Program, Consortium for Research in Developmental and Reproductive Biology, and Departments of Obstetrics and Gynecology, Biology, and Physiology, The University of Michigan, Ann Arbor, MI.

5:45

- 14 THE EFFECT OF PINEALECTOMY OF THE PREGNANT EWE ON FETAL AND MATERNAL PLASMA MELATONIN CONCENTRATIONS DURING LATE GESTATION. I.C. McMillen and R. Nowak. Department of Physiology, Monash University, Clayton, Vic., Australia.

6:00

- 15 THE ROLE OF THE CIRCADIAN SYSTEM IN READING THE MELATONIN SIGNAL CONTROLLING SEASONAL REPRODUCTION IN THE SYRIAN HAMSTER. E. Maywood, C. Bonnefond, S. Davies, A. Walker, M. Hastings and J. Herbert. Department of Anatomy, University of Cambridge, Cambridge, England.

6:15

- 16 STEROID INDEPENDENT EFFECT OF PHOTO-PERIOD ON PULSATILE LUTEINIZING HORMONE (LH) RELEASE. J.M. Swann, S.L. Olson and F.W. Turek. Neurobiology and Physiology, O.T. Hogan Building, Northwestern University, Evanston, IL.

4:30 pm - 6:30 pm

Salon III

Slide Session 3:

Human Rhythms and Sleep

Chairpersons: Charles Czeisler
Georges Copinschi

4:30

- 17 AMPLITUDE MODULATION OF A BURST-LIKE MODE OF CORTISOL SECRETION GIVES RISE TO THE CIRCADIAN GLUCOCORTICOID RHYTHM IN MAN. J.D. Veldhuis, A. Iranmanesh, G. Lizarralde and M.L. Johnson. University of Virginia School of Medicine, Charlottesville, VA; Dept. of Int. Med., Veterans Administration Medical Center, Salem, VA.

4:45

- 18 EFFECT OF TRIAZOLAM ON THE ADAPTATION OF THE CIRCADIAN RHYTHMS OF CORTISOL, MELATONIN AND REM PROPENSITY TO AN 8-H DELAY OF THE SLEEP-WAKE CYCLE IN MAN. E. Van Cauter, A. Van Onderbergen, D. Bosson, M. L'Hermite-Balériaux, M. L'Hermite, M. Szyper and G. Copinschi. School of Medicine, Free University of Brussels, Belgium.

5:00

- 19 THE EFFECT OF 64 HOURS OF WAKEFULNESS ON IMMUNE FUNCTIONS IN HUMANS. F.A. Lue, H. Moldofsky, J.R. Davidson, J. Jephthah-Ochola, K. Carayanniotis and R. Gorczynski. University of Toronto, Toronto Western Hospital, Toronto, Ontario, Canada.

5:15

- 20 ENDOCRINE RHYTHMS AND SLEEP IN HUMAN AGING. A van Coevorden, E. Laurent, C. Decoster-Gervy, M. L'Hermite-Balériaux, M. Kerkhofs, P. Nève, J. Mockel and E. Van Cauter. School of Medicine, Free University of Brussels, Belgium.

5:30

- 21 ENDOGENOUS CIRCADIAN TEMPERATURE AMPLITUDE IS REDUCED AND PHASE IS DELAYED AT THE LUTEAL PHASE OF THE MENSTRUAL CYCLE IN OVULATING WOMEN. S. Rogacz, J.F. Duffy, J.M. Ronda and C.A. Czeisler. Neuroendocrinology Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

5:45

- 22 CIRCADIAN CONTROL OF SLEEP TIMING AFTER SLEEP DEPRIVATIONS. E.B. Klerman, T.A. Houpt, D.M. Edgar¹, R.E. Mistlberger and M.C. Moore-Ede. Harvard Medical School, Institute for Circadian Physiology, and ¹Stanford University, Palo Alto, CA.

6:00

- 23 THE SIGNIFICANCE OF ENDOGENOUS AMPLITUDE IN CIRCADIAN RHYTHMS. R.E. Kronauer¹ and C.A. Czeisler². ¹Harvard University, Div. of Appl. Sciences, Pierce Hall, Cambridge, MA, ²Brigham & Women's Hospital, Neuroendocrinology Lab., Boston, MA.

6:15

- 24 A MODEL OF THE HUMAN CIRCADIAN SYSTEM BASED ON INTERACTING POPULATIONS OF FEED-BACK OSCILLATORS. H. Silyn-Roberts and R.D. Lewis. Department of Mechanical Engineering and Department of Zoology, University of Auckland, New Zealand.

4:30 pm - 6:30 pm

Salon IV

Slide Session 4:

Pharmacological Manipulation of Rhythms

Chairpersons: W.J. Rietveld

Lawrence Morin

4:30

- 25 SINGLE INJECTIONS OF TRIAZOLAM, A SHORT-ACTING BENZODIAZEPINE, LENGTHEN FREE-RUNNING PERIODS IN GOLDEN HAMSTERS. J.E. Joy, S. Losee-Olson and F.W. Turek. Dept. Neurobiol. and Physiol., Northwestern University, Evanston, IL.

4:45

- 26 PHASE ADVANCING EFFECTS OF BENZODIAZEPINE ON THE HAMSTER CIRCADIAN CLOCK CAN BE BLOCKED BY PREVENTING THE ASSOCIATED HYPERACTIVITY. O. Van Reeth, J.J. Vanderhaeghen and F.W. Turek. Neuropathology and Neuropeptide Research Laboratory, Universite Libre de Bruxelles, Brussels, Belgium; Northwestern University, Evanston, IL.

5:00

- 27 LGN LESIONS BLOCK TRIAZOLAM-INDUCED PHASE SHIFTS OF HAMSTER LOCOMOTOR RHYTHMS. L. Smale, R. Johnston, R.Y. Moore and L.P. Morin. State University of New York, Stony Brook, NY.

5:15

- 28 LOCALIZATION OF THE GABA-A/BENZODIAZEPINE RECEPTOR COMPLEX IN RELATION TO THE INTERGENICULATE LEAFLET OF THE LATERAL GENICULATE NUCLEUS OF HAMSTER AND RAT. K.M. Michels, L.P. Morin and R.Y. Moore. Depts. of Neurology and Psychiatry, State University of New York at Stony Brook, Stony Brook, NY.

5:30

- 29 CIRCADIAN BENZODIAZEPINE RECEPTOR BINDING IN THE GOLDEN HAMSTER CNS. T.A. Houpt, W.F. White and M.C. Moore-Ede. Dept. Physiol., Dept. Neurosci., Harvard Med. Sch. and Institute for Circadian Physiology, Boston, MA.

5:45

- 30 ANALYSIS OF THE CIRCADIAN CONTROL OF BEHAVIOUR IN RATS. TOOLS AND TRICKS. W.J. Rietveld, J. Ruys and P. Buys. Dept. of Physiology, Leiden University, The Netherlands.

6:00

- 31 THE EFFECTS OF CONTINUOUS DIM LIGHT AND CHRONIC TREATMENT WITH THE MAOI ANTIDEPRESSANT CLORGYLINE ON WHEEL-RUNNING IN SYRIAN HAMSTERS. W.C. Duncan, P.G. Sokolove, W. Orem and T.A. Wehr. Clinical Psychobiology Branch, NIMH, Bethesda, MD and the Department of Biological Sciences, University of Maryland, Catonsville, MD.

6:15

- 32 MELATONIN ADMINISTRATION ENTRAINS FEMALE RAT ACTIVITY RHYTHMS IN CONSTANT DARKNESS, BUT NOT IN CONSTANT LIGHT. E.M.V. Thomas and S.M. Armstrong. Department of Physiology, Monash University, Clayton, Victoria, Department of Psychology, La Trobe University, Bundoora, Victoria, Australia.

7:30 pm - 8:30 pm

Salon I-IV

PLENARY LECTURE:

"Circadian Rhythms in the 4th Decade: What do we want and what can we have?"

Michael Menaker, University of Virginia

Friday May 13

8:30 am - 10:30 am

Salon I and II

Symposium 3:

Cellular, Molecular and Genetic Dissection of Clocks

Jay Dunlap, organizer, Dartmouth University

Michael Young, Rockefeller University

David Morse, Harvard University

Steve A. Kay, Rockefeller University

Kathleen Siwicki, Brandeis University

8:30 am - 10:30 am

Salon III and IV

Symposium 4:

Interaction Between Sleep and the Circadian System

Alexander Borbély, organizer, University of Zurich

Allan Rechtschaffen, University of Chicago

William Dement, Stanford University

Charles Czeisler, Harvard University

11:00 am - 1:00 pm

Salon V and Lobby

Poster Sessions:

Suprachiasmatic Nucleus

- 33 LABELING OF THE SUPRACHIASMATIC NUCLEUS AFTER INTRAOCULAR INJECTION OF HERPES SIMPLEX VIRUS. R.B. Norgren Jr., J.H. McLean, M.T. Shipley and M.N. Lehman. Department of Anatomy and Cell Biology, University of Cincinnati, Cincinnati, OH.
- 34 THE SINGLE UNIT RESPONSE OF SUPRACHIASMATIC NEURONS TO ARGININE VASOPRESSIN (AVP) IS MEDIATED BY A V1-LIKE RECEPTOR IN THE HAMSTER. S.Y. Liou and H.E. Albers. Laboratory of Neuroendocrinology and Behavior, Departments of Biology and Psychology, Georgia State University, Atlanta, GA.
- 35 PROTEIN SYNTHESIS IN THE SUPRACHIASMATIC NUCLEI (SCN) SHOWS NO CIRCADIAN RHYTHM. T.E. Scammell, C.B. Smith¹ and W.J. Schwartz. Dept. Neurol., U. Massachusetts Med. School, Worcester, MA and ¹Lab. Cerebral Metabolism, NIMH, Bethesda, MD.
- 36 THE PHASE-SHIFTING EFFECT OF MUSCIMOL ON THE CIRCADIAN RHYTHM OF ACTIVITY: ROLE OF THE SCN. R.D. Smith and F.W. Turek. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL.
- 37 DISRUPTIONS IN HUMAN CIRCADIAN AND COGNITIVE REGULATION FOLLOWING ROSTRAL HYPOTHALAMIC DAMAGE: A CASE STUDY. R.A. Cohen and H.E. Albers. Dept. of Neurol., Univ. of Mass. Med. Sch., Worcester, MA and Lab. of Neuroendocrinol. & Behav., Depts. of Biol. & Psychol., Georgia State Univ., Atlanta, GA.

38 A HORSERADISH PEROXIDASE-WHEAT GERM AGGLUTININ (HRP-WGA) STUDY OF THE EFFERENT CONNECTIONS OF THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) AND ADJACENT REGIONS OF THE MEDIAL HYPOTHALAMUS. G. Orpen, M. Steiner, and M.O. Miceli. St. Joseph's Hospital Research Institute and Departments of Psychiatry & Neurosciences, McMaster University, Hamilton, Ontario, Canada.

39 LIGHT RESPONSIVE CELLS IN THE HYPOTHALAMUS OF THE DIURNAL THIRTEEN-LINED GROUND SQUIRREL. J.H. Meijer, B. Rusak and M.E. Harrington. Dept. of Psychology, Dalhousie University, Halifax, Canada.

40 CIRCADIAN RHYTHMS IN HAMSTERS AFTER SCN ISOLATION. H. Hakim and R. Silver. Psychology Department, Barnard College of Columbia University, New York, NY.

Human Rhythms

- 41 COMPARISON OF HUMAN DAILY TEMPERATURE AND PULSE RATE MEASUREMENTS TO CLINICAL OBSERVATIONS OBTAINED ANNUALLY IN THE BALTIMORE LONGITUDINAL STUDY OF AGING. M.A. Brock¹, D.W. Denman², H.J. Hoffman², and J. van der Vate. ¹Laboratory of Clinical Physiology, NIA/NIH and ²Biometry Branch, NICHD/NIH, Washington, D.C.
- 42 SEASONAL CHANGES IN A 38-YEAR RECORD OF HUMAN DAILY TEMPERATURE AND PULSE RATE MEASUREMENTS. H.J. Hoffman¹, D.W. Denman¹, M.A. Brock², and J. van der Vate. ¹Biometry Branch, NICHD/NIH and ²Laboratory of Clinical Physiology, NIA/NIH, Washington, DC.
- 43 PEAK TIME OF ORAL TEMPERATURE RHYTHM IN AN "EVENING" PERSON: FIVE-MONTH LONGITUDINAL STUDY. L. Felver. Oregon Health Sciences University, Portland, OR.
- 44 EEG POWER AND SLOW WAVE SLEEP IN NAPS: EFFECT OF TIME AWAKE. A.W. MacLean, D. Brunet, D. Nish, M. Coulter and J.B. Knowles. Departments of Medicine and Psychology, Queen's University, Kingston, Canada.
- 45 ULTRADIAN RHYTHMS IN PERFORMANCE AND AROUSAL DURING SLEEP. P. Stone, J. Harsh, and P. Badia. USM Sleep Laboratory, University of Southern Mississippi, Hattiesburg, MS.
- 46 MOOD CHANGE FOLLOWING AN ACUTE PHASE SHIFT OF SIX HOURS: DELAY VERSUS ADVANCE. M. Surridge-David, A.W. MacLean, M.E. Coulter & J.B. Knowles. Department of Psychology, Queen's University, Kingston, Ontario, Canada.

- 47 CIRCADIAN RHYTHM MEASURED BY THE CORE TEMPERATURE IN DEPRESSION. T. Tsujimoto, N. Yamada, K. Shimoda, T. Minowada, T. Shioiri and S. Takahashi. Department of Psychiatry, Shiga University of Medical Science, Seta Tsukinowacho, Otsu, Shiga, Japan.
- 48 USEFULNESS OF THE ANALYSIS OF CIRCADIAN AND ULTRADIAN CORTISOL VARIATIONS IN THE DIFFERENTIAL DIAGNOSIS OF VARIOUS FORMS OF HYPERCORTISOLISM. S. Refetoff, R.E. Weiss, V.S. Fang, P. Linkowski and E. Van Cauter. Dept. of Medicine and Pediatrics, University of Chicago, IL and School of Medicine, Free University of Brussels, Belgium.
- 49 EFFECTS OF ABRUPT PHASE-SHIFT IN THE AMBULATORY-RECUMBENCY AND SLEEP-WAKE CYCLES ON Sm-C NYCTOHEMERAL PATTERN IN NORMAL MAN. A. Caufriez, A. Van Onderbergen, D. Bosson and G. Copinschi. Université Libre de Bruxelles, Brussels, Belgium.
- 54 SPONTANEOUS CHANGES IN THE CIRCADIAN RHYTHM OF MELATONIN SECRETION IN THE EWE: POSSIBLE GENERATION OF A CIRCAANNUAL REPRODUCTIVE RHYTHM. S.M. Moenter and F.J. Karsch. Developmental and Reproductive Biology and Dept. of Physiology, University of Michigan, Ann Arbor, MI.
- 55 RHYTHMS IN UTERINE CONTRACTILE ACTIVITY AND CORTISOL SECRETION DURING PREGNANCY IN THE SHEEP. S.M. Yellon, E.M. Apostolakis, K.E. Rice and L.D. Longo, Division of Perinatal Biology, Departments of Physiology and Pediatrics, Loma Linda University, School of Medicine, Loma Linda, CA.
- 56 DAILY MELATONIN INJECTIONS PRODUCE OBESITY AND REPRODUCTIVE REGRESSION IN IN-BRED FEMALE LSH/Ss Lak HAMSTERS. M.H. Brown and G.N. Wade. Dept. of Psychology, University of Mass., Amherst, MA.
- 57 EFFECT OF RESONANCE MELATONIN INFUSION CYCLES ON GONAD, BODY AND LIPID MASS IN PINEALECTOMIZED MALE DJUNGERAN HAMSTERS. J.A. Elliott, T.J. Bartness and B.D. Goldman, Worcester Foundation for Experimental Biology, Shrewsbury, MA.

Melatonin

- 50 SHEEP AND CIRCADIAN RHYTHM REGULATION: EFFECT OF LITHIUM ON PLASMA MELATONIN, NOREPINEPHRINE AND MHPG. J. Seggie, M. Steiner and G. Orpen. Department of Neurosciences and Psychiatry, McMaster University and St. Joseph's Hospital Research Institute, Hamilton, Ontario, Canada.
- 51 FEEDING AND LOCOMOTOR ACTIVITIES OF THE PIGEON: MELATONIN AND ITS EFFECTS ON RHYTHMICITY. C.C. Chabot and M. Menaker. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA.
- 52 EFFECTS OF PINEALECTOMY ON THE CIRCADIAN RHYTHMICITY OF THE EUROPEAN LIZARD *PODARCIS SICULA CAMPESTRIS*. G. Saviozzi and A. Foa'. Dipartimento di Scienze di Comportamento Animale, Pisa, Italy.
- 53 EFFECTS OF CONSTANT DARKNESS ON ACTIVITY AND MELATONIN RHYTHMS IN THE RAM. F.J.P. Ebling, G.A. Lincoln, F. Wollnik and N. Anderson. MRC Reproductive Biology Unit, Edinburgh, U.K., Developmental and Reproductive Biology, The University of Michigan, Ann Arbor, MI, and Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL.
- 54 EFFECTS OF SCN LESIONS ON SEASONAL RESPONSES FOLLOWING SHORT DAY EXPOSURE OR MELATONIN INFUSIONS IN SIBERIAN HAMSTERS. T.J. Bartness, B.D. Goldman and E.L. Bittman. Worcester Foundation for Experimental Biology, Shrewsbury, MA, and Dept. of Zool., Univ. Mass., Amherst, MA.
- 59 A CHRONOBIOLOGICAL STUDY ON EFFECT OF ZOTEPINE: EFFECT ON CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN RATS. T. Minowada, N. Yamada, K. Shimoda, T. Tsujimoto, T. Shioiri and S. Takahashi. Department of Psychiatry, Shiga University of Medical Science, Seta, Tsukinowacho, Otsu, Shiga, Japan.
- 60 METHAMPHETAMINE-INDUCED INTERNAL DESYNCHRONIZATION IN RATS. J.F. Ruis, T. Cambras, J.P. Buys and W.J. Rietveld. Dept. of Physiology, Div. of Medical Chronobiology, University of Leiden, The Netherlands.
- 61 CHRONIC ADMINISTRATION OF TRICYCLIC ANTIDEPRESSANTS DAMPS DOWN THE CIRCADIAN RHYTHM IN MALE RATS. K. Shimoda, N. Yamada, K. Ohi, T. Tsujimoto, K. Takahashi and S. Takahashi. Department of Psychiatry, Shiga University of Medical Science, Seta, Tsukinowacho, Otsu, Shiga, Japan.

- 62 CHRONIC LITHIUM EFFECTS ON RAT ACTIVITY RHYTHMS. D.L. McEachron, K. Stewart and N.T. Adler. Department of Psychology, University of Pennsylvania, Philadelphia, PA.
- 63 IS THE PHASE SHIFTING EFFECT OF TRIAZOLAM ON THE HAMSTER'S CIRCADIAN CLOCK DUE TO THE ACUTE INCREASE IN ACTIVITY ASSOCIATED WITH DRUG TREATMENT? C. Wickland, F. Wollnik and F. Turek. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL.
- 64 TRIAZOLAM PHASE SHIFTS SQUIRREL MONKEY CIRCADIAN ACTIVITY RHYTHMS. R.E. Mistlberger, T.A. Houpt and M.C. Moore-Ede. Dept. Physiology, Harvard Medical School and Institute for Circadian Physiology, Boston, MA.
- 71 ILLUMINANCE-THRESHOLD FOR MAINTENANCE OF TESTES IN SYRIAN HAMSTERS (*MESOCRICETUS AURATUS*) IS HIGHER IN CONTINUOUS LIGHT (LL) THAN IN LD 14:10. C.E. McCormack. Dept. of Physiol., The Chicago Med. School, N. Chicago, IL.
- 72 INTERNAL SYNCHRONIZATION OF TWO DIFFERENT RHYTHMS DURING EXPOSURE TO LD CYCLES WITH DIFFERENT PERIODS (T). A.E. Jetton, S. Losee-Olson and F.W. Turek. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL.
- 73 RELATIONSHIP BETWEEN FREE-RUNNING PERIOD AND MOTOR ACTIVITY IN BLINDED RATS. N. Yamada, K. Shimoda, T. Tsujimoto, T. Shioiri, T. Minowada and S. Takahashi. Dept. of Psychiatry, Shiga University of Medical Science, Seta-Tsukinowa, Otsu-city, Shiga, Japan.

Circadian Rhythms

- 65 PHASE RESPONSE CURVES AS PREDICTED BY A CIRCULAR LIMIT CYCLE MODEL. V.D. Gooch. Division of Science and Math, University of Minnesota, Morris, MN.
- 66 MULTISTABILITY OF CIRCADIAN ACTIVITY RHYTHMS IN BALB/c MICE: A WEAKLY COUPLED CIRCADIAN SYSTEM? A.M. Rosenwasser. Department of Psychology, University of Maine, Orono, ME.
- 67 ADVANCING SCHEDULES AND CONSTANT LIGHT PRODUCE FASTER RESYNCHRONIZATION OF CIRCADIAN RHYTHMS. S. Binkley and K. Mosher. Biology Department, Temple University, Philadelphia, PA.
- 68 POST-EMBRYONIC DEVELOPMENT OF LOCOMOTOR AND SINGING RHYTHMS IN CRICKETS. W. Loher and D. Moore. Dept. of Entomological Sciences, University of California, Berkeley, CA.
- 69 APPARENT INFRADIAN PERIODICITIES IN DIAPAUSE TERMINATION OF EMBRYONATED GYPSY MOTH EGGS ON EARTH OR AFTER LAUNCH, MICROGRAVITY AND REENTRY OF ORBITING SPACE LABORATORY. D.K. Hayes and N.O. Morgan. Livestock Insects Laboratory, Livestock and Poultry Sciences Institute, USDA, ARS, Beltsville, MD.
- 70 THE MARINE DINOFLAGELLATE, *GONYAULAX POLYEDRA*, DISPLAYS A CIRCADIAN ACTIVITY RHYTHM WITH A FREE-RUNNING τ INDEPENDENT OF LIGHT INTENSITY. J.W. Hastings, T. Roenneberg and G.N. Colfax. Harvard University Biological Laboratories, Cambridge, MA.
- 74 RUNNING WHEEL AVAILABILITY ENTRAINS SLEEP-WAKE AND DRINKING CIRCADIAN RHYTHMS IN THE MOUSE. D.M. Edgar, T.S. Kilduff and W.C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA.
- 75 NON-PHOTIC EFFECTS ON CIRCADIAN RHYTHMS: WHAT ARE THE IMPORTANT VARIABLES? N. Mrosovsky, R.A. Lavery and S.G. Reebbs. Department of Zoology, University of Toronto, Toronto, Canada.
- 76 NON-PHOTIC ENTRAINMENT OF ACTIVITY RHYTHMS WITH CORRESPONDING PHASE RESPONSE CURVES. S.G. Reebbs and N. Mrosovsky. Department of Zoology, University of Toronto, Toronto, Canada.
- 77 VISUAL EXAMINATION OF FREE RUNNING LOCOMOTOR RHYTHMS OF FEEDING ENTRAINED GOLD-FISH or I CAN SEE A FISHY AND A TIGER AND A SAILBOAT AND A . . . R.E. Spieler, Milwaukee Public Museum, Milwaukee, WI.
- 78 ENTRAINMENT OF AGED, DYSRHYTHMIC RATS TO A RESTRICTED FEEDING SCHEDULE. E.C. Walcott and B. Tate-Ostroff. Mailman Research Center, McLean Hospital, Belmont, MA.
- 79 AGE-RELATED CHANGES IN HAMSTER CIRCADIAN PERIOD, ENTRAINMENT AND RHYTHM SPLITTING. L.P. Morin. Department of Psychiatry, SUNY at Stony Brook, Stony Brook, NY.

- 80 SHORT TERM RHYTHMS AS A CONTROL FOR CIRCADIAN RHYTHMS IN THE MEADOW VOLE, *MICROTUS PENNSYLVANICUS*. L.L. Stebbins. Department of Biological Sciences, The University of Lethbridge, Lethbridge, Alberta, Canada.
- 81 TRANSGENIC MICE WITH A GENOME FOR HUMAN GROWTH HORMONE (hGH) HAVE SHORTER FREE-RUNNING PERIODS THAN NON-TRANSGENIC SIBLINGS. T.E. Wagner, A. Bartke, R.W. Steger and J.S. Ferraro. Edison Animal Biotechnology Center, Ohio University, Athens, OH and Dept. of Physiology, Southern Illinois University/School of Medicine, Carbondale, IL.
- 82 SKELETON PHOTOPERIODS SYNCHRONIZE THE ERG CIRCADIAN RHYTHM IN CRAYFISH. E. Moreno-Sáenz, F. Gutiérrez-Zepeda and B. Fuentes-Pardo. Depto. de Fisiología, Facultad de Medicina, UNAM, México, D.F. México.
- 83 EFFECT OF LIGHT ON THE ERG CIRCADIAN RHYTHM DURING THE COURSE OF THE CRAYFISH ONTOGENY. B. Fuentes-Pardo, M.L. Fanjul-Moles and E. Moreno-Sáenz. Depto. de Fisiología, Facultad de Medicina, UNAM, México, D.F. México.
- 89 CIRCADIAN RHYTHM AND AROUSAL — EFFECTS OF SEROTONINE ON OSCILLATORY AND NON-OSCILLATORY PROCESSES IN THE SCORPION. G. Fleissner, G. Fleissner and S. Michel. Zoological Institute, University of Frankfurt/Main, Frankfurt/Main, FRG.
- 90 CREATINE SHORTENS THE CIRCADIAN PERIOD IN *GONYAULAX POLYEDRA*. T. Roenneberg, H. Nakamura and J.W. Hastings. Harvard University Biological Laboratories, Cambridge, MA.
- 91 BIOLOGICAL CLOCK MUTATIONS ALTER INTER-CELLULAR JUNCTIONAL COMMUNICATION. M.W. Young, M. Baylies, T.A. Bargiello and L. Saez. Howard Hughes Medical Institute, The Rockefeller University, New York, NY.
- 92 CIRCADIAN CHANGES IN PROTEIN SYNTHESIS AND OXYDATIVE METABOLISM IN THE HAMSTER AND THE MINK SUPRACHIASMATIC NUCLEI. J. Serviere, L. Martinet and C. Bonnefond. Dept. Physiologie, INRA, Jouy-en-Josas, France.
- 93 OLFACTORY BULBECTOMY LENGTHENS TAU(dd) IN MICE. B. Possidente¹, E. deLumos¹, A.R. Lumia², L. Sterner² and M.Y. McGinnis³. ¹Dept. Biology, Skidmore College, ²Dept. Psychology, Skidmore College, Saratoga Springs, NY, ³Dept. Anatomy, Mt. Sinai School of Medicine, C.U.N.Y., NY, NY.

Cellular and Biochemical Rhythms

- 84 SEROTONIN BLOCKS LIGHT-INDUCED PHASE DELAYS BUT NOT ADVANCES. C.S. Colwell. Gilmer Hall - Biology, University of Virginia, Charlottesville, VA.
- 85 A COMPARISON OF THE CIRCADIAN OSCILLATORS IN *ACETABULARIA* AND THE EYE OF *APLYSIA*. J.C. Woolum. Department of Physics and Astronomy, California State University, Los Angeles, CA.
- 86 SPECTRAL SENSITIVITY OF THE *BULLA* EYE. M. Geusz and T. Page. Dept. of General Biology, Vanderbilt University, Nashville, TN.
- 87 INVOLVEMENT OF PROTEIN SYNTHESIS IN THE CIRCADIAN REGULATION OF MELATONIN PRODUCTION IN CHICK PINEAL CELLS. N. Murakami and J.S. Takahashi. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL.
- 88 CYCLIC AMP ELEVATES MELATONIN PRODUCTION BUT DOES NOT PHASE SHIFT THE CIRCADIAN OSCILLATOR IN CHICK PINEAL CELLS. S.S. Nikaido and J.S. Takahashi. Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL.

3:30 pm - 5:30 pm Salon I

Workshop 7:

Entraining Effects of Melatonin
Vincent Cassone, SUNY Stony Brook

3:30 pm - 5:30 pm Salon II

Workshop 8:

Use of Periodogram Analysis and Related Procedures in Biological Rhythms Studies
Eve Van Cauter, Free Univ. of Brussels/Univ. of Chicago

3:30 pm - 5:30 pm Salon III

Workshop 9:

Cellular Analysis of Biological Rhythms
Gene Block, University of Virginia

3:30 pm - 5:30 pm Salon IV

Workshop 10:

Organization and Function of the Intergeniculate Leaflet
Lawrence Morin, SUNY Stony Brook

3:30 pm - 5:30 pm

Salon V

Workshop 11:

Use of In Vitro Brain Slices in Studies of Circadian Function

Martha Gillette and Shigenobu Shibata
University of Illinois and Kyushu University

6:00 pm - 7:30 pm

Salon I and II

Business Meeting

Saturday May 14

8:30 am - 10:30 am

Salon I and II

Symposium 5:

Modulation and Control of Neural Oscillators

Otto Friesen, organizer, University of Virginia
Eve Marder, Brandeis University
Keir Pearson, University of Alberta

8:30 am - 10:30 am

Salon IV and V

Symposium 6:

Circannual Rhythms—Properties and Mechanisms

Irving Zucker, organizer, Univ. of California, Berkeley
Eberhard Gwinner, Max-Planck Institute, Andechs
Nicholas Mrosovsky, University of Toronto

11:00 am - 1:00 pm

Salon I

Slide Session 5:

Chronobiology of Depression

Chairpersons: Daniel Kripke
David Jarrett

11:00

- 94 MELATONIN ADMINISTRATION CAUSES PHASE SHIFTS IN FREE-RUNNING BLIND PEOPLE. R.L. Sack and A.J. Lewy. Oregon Health Sciences University, Portland, OR.

11:15

- 95 CHRONOBIOLOGIC MODELS FOR LIGHT TREATMENT OF DEPRESSION. D.F. Kripke. University of California, San Diego, La Jolla, CA.

11:30

- 96 SEASONAL CHANGES OF MOOD AND BEHAVIOR IN THE GENERAL POPULATION. S. Kasper, T.A. Wehr, J.J. Bartko and N.E. Rosenthal. Clinical Psychobiology Branch and Theoretical Statistics and Mathematics Branch, NIMH, Bethesda, MD.

11:45

- 97 CIRCADIAN RHYTHM IN CORTISOL SECRETION IS NOT DISTURBED IN OUTPATIENTS WITH A MAJOR DEPRESSIVE DISORDER. D. B. Jarrett and J.B. Greenhouse. Department of Psychiatry, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, PA.

12:00

- 98 PRESERVATION OF THE ULTRADIAN SLEEP CYCLE IN DEPRESSED PATIENTS. D. Buysse, D. Jarrett, and J. Miewald. Western Psychiatric Institute & Clinic, University of Pittsburgh School of Medicine, Pittsburgh, PA.

12:15

- 99 DELAYED SLEEP PHASE SYNDROME: POLYSOMNOGRAPHIC CHARACTERISTICS. R. Allen, N. Rosenthal, J.-R. Joseph-Vanderpool, J. Nadeau, K. Kelly, P. Schulz, E. Souetre. Clinical Psychobiology Branch, National Institute of Mental Health and The Johns Hopkins University Sleep Disorder Center, Baltimore, MD.

12:30

- 100 FIELD STUDIES OF HUMANS FOLLOWING 26-HR BRIGHT LIGHT AND SLEEP-WAKE SCHEDULES. C.I. Eastman. Psychology Department, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL.

11:00 am - 1:00 pm

Salon II

Slide Session 6:

Cellular and Molecular Basis of Rhythmicity

Chairpersons: Felix Strumwasser
Gene Block

11:00

- 101 EXTRACELLULAR CALCIUM MEDIATES PHASE SHIFTS OF THE *BULLA* OCULAR CIRCADIAN PACEMAKER VIA CALCIUM CHANNELS. S.B.S. Khalsa and G.D. Block. Department of Biology, University of Virginia, Charlottesville, VA.

11:15

- 102 CIRCADIAN AND LIGHT-INDUCED CHANGES IN MEMBRANE CONDUCTANCE IN *BULLA* BASAL RETINAL NEURONS. M.R. Ralph and G. D. Block. Department of Biology, University of Virginia, Charlottesville, VA.

11:30

- 103 MULTIPLE INTERACTING SECOND MESSENGER PATHWAYS IN THE STRUCTURE OF AN ENDOGENOUS OSCILLATOR. R. Gillette. Department of Physiology & Biophysics, University of Illinois, Urbana, IL.

11:45

- 104 THE TIME COURSE OF SYNTHESIS OF SPECIFIC PROTEINS CORRELATED WITH THE CIRCADIAN OSCILLATION OF NEURAL ACTIVITY IN THE EYE OF *APLYSIA*. F. Strumwasser and D.P. Vele. Marine Biological Laboratory, Woods Hole, MA.

12:00

- 105 PROTEIN PHOSPHORYLATION ASSOCIATED WITH THE PHASE SHIFT PRODUCED BY SEROTONIN IN THE *APLYSIA* EYE. R. Zwartjes and A. Eskin. Biol. Dept., Univ. of Houston, Houston, TX.

12:15

- 107 MOLECULAR CLONING OF GENES UNDER CONTROL OF THE CIRCADIAN CLOCK. J.C. Dunlap, J.J. Loros and S.A. Denome. Department of Biochemistry, Dartmouth Medical School, Hanover, NH.

12:30

- 108 MOLECULAR CLONING OF THE *FREQUENCY* AND *PERIOD-4* LOCI OF *NEUROSPORA*. C.R. McClung, O. Liu and J.C. Dunlap. Department of Biochemistry, Dartmouth Medical School, Hanover, NH.

11:00 am - 1:00 pm

Salon IV

Slide Session 7:

Comparative Analysis of Rhythms

Chairpersons: David Saunders
Eberhard Gwinner

11:00

- 109 THE HONEYBEE TIME-SENSE: EVIDENCE FOR TWO PROCESSES CONTROLLING FORAGING BEHAVIOR. D. Moore and M.A. Rankin. Department of Zoology, University of Texas, Austin, TX.

11:15

- 110 TIME-AND-PLACE LEARNING BY GARDEN WARBLERS (*SYLVIA BORIN*). H. Biebach, M. Gordijn and J.R. Krebs. Max-Planck-Institut für Verhaltensphysiologie, Vogelwarte Andechs, FRG.

11:30

- 111 CIRCADIAN ACTIVITY PATTERNS IN CAPTIVE SONGBIRDS. L.A. Eiserer¹ and R.K.R. Thompson². ¹Psychology Department, Elizabethtown College, Elizabethtown, PA and ²Psychology Department, Franklin and Marshall College, Lancaster, PA.

11:45

- 112 MELATONIN AFFECTS SUN-COMPASS ORIENTATION IN HOMING PIGEONS. A. Foa' and G. Saviozzi. Dipartimento di Scienze del Comportamento Animale, Pisa, Italy.

12:00

- 113 SIZE-SPECIFIC DIURNAL TO NOCTURNAL SHIFT OF LOCOMOTOR ACTIVITY IN RAINFOREST LEAF-LITTER FROGS OF THE GENUS *ELEUTHERODACTYLUS*. Y. Winter and F. Barnwell. Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN.

12:15

- 114 CIRCADIAN OSCILLATION IN THE POPULATION OF AIRBORNE SPORES OF FUNGAL PLANT PATHOGENS: A HALF CENTURY OF LITERATURE IN NEED OF REINTERPRETATION. B.W. Kennedy. Department of Plant Pathology, University of Minnesota, St. Paul, MN.

12:30

- 115 PERIODOGRAM ANALYSIS OF THE LOCOMOTOR RHYTHM OF *CALLIPHORA VICINA*. N.A. KENNY, R.D. LEWIS and D.S. SAUNDERS. Dept. of Zoology, University of Edinburgh, Edinburgh, U.K.

12:45

- 116 ROLE OF THE OVARY IN REPRODUCTIVE REFRACTORINESS IN THE KILLIFISH, *FUNDULUS HETEROCLITUS*. J.A. Dimitry and M.H. Taylor. School of Life and Health Sciences and College of Marine Studies, Univ. of Delaware, Newark, DE.

11:00 am - 1:00 pm

Salon V

Slide Session 8:

Entrainment

Chairpersons: Günther Fleissner
Cass Terry

11:00

- 117 THE COMMISSURAL PROJECTION BETWEEN THE INTERGENICULATE LEAFLETS OF THE RAT THALAMUS CONTAINS MET-ENKEPHALIN IMMUNOREACTIVITY. J.P. Card and R.Y. Moore. Medical Products Department, The DuPont Company, Wilmington, and Department of Neurology, SUNY Stony Brook, Stony Brook, NY.

11:15

- 118 MONOCHROMATIC PHASE RESPONSE CURVES FOR A NOCTURNAL RODENT. P.J. DeCoursey. Biology Department, University of South Carolina, Columbia, SC.

11:30

- 119 SYRIAN HAMSTERS WITH CIRCADIAN PERIODS OUTSIDE THE PREDICTED LIMITS OF ENTRAINMENT BY RESTRICTING LIGHT EXPOSURE TO THE DELAY PORTION OF THE PHASE-RESPONSE CURVE. J.S. Ferraro. Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, IL.

11:45

- 120 PHASE SHIFTING OF THE ULTRADIAN GROWTH HORMONE SECRETORY RHYTHM. L.C. Terry. Neuroendocrine Research Lab., Univ. Michigan and VA Medical Center, Ann Arbor, MI.

12:00

- 121 RESTRICTED FEEDING TIME: A ZEITGEBER IN THE RABBIT. B. Jilge and H. Stähle. University of Ulm, Ulm, FRG.

12:15

- 122 THE ROLE OF THE CIRCADIAN SYSTEM IN PHOTO-PERIODISM: A CLOCK ROLE OR A NON-CLOCK ROLE? M. Vaz Nunes and A. Veerman. Laboratory of Experimental Entomology, University of Amsterdam, The Netherlands.

12:30

- 123 COUPLING PATHWAYS IN THE SCORPION'S CIRCADIAN SYSTEM. G. Fleissner, S. Michel and G. Fleissner. Zoological Institute, University of Frankfurt/Main, Frankfurt/Main, FRG.

12:45

- 124 ERG AND LOCOMOTOR ACTIVITY RHYTHMS IN THE SCORPION: INTERACTION BETWEEN DIFFERENT CIRCADIAN PACEMAKERS. S. Michel, G. Fleissner and W. Hohmann. Zoological Institute, University of Frankfurt/Main, Frankfurt/Main, FRG.

1 **MELATONIN METABOLISM IN THE EYE OF *XENOPUS*.** Gregory M. Cahill and Joseph C. Besharse. Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

In the retina of *Xenopus* the activity of serotonin-N-acetyltransferase, the penultimate enzyme in the synthesis of melatonin, is regulated by a circadian clock that resides within the eye. Despite high synthetic activity during the subjective night, very little melatonin is released into culture medium by eyecups maintained *in vitro* (less than 10 pg/hr/eyecup). We report here that cultured eye-cups have the capacity for rapid metabolism of melatonin.

Eyecups were cultured in medium containing 8 $\mu\text{Ci/ml}$ (100 nM) [methoxy- ^3H]-melatonin for 3 hrs. Medium samples and tissue extracts were separated by reverse-phase high performance liquid chromatography (HPLC), and fractions were assayed for radioactivity. Three radiolabeled metabolite peaks were present in both tissue and medium samples. These have been tentatively identified by HPLC as 5-methoxytryptamine (5-MT), 5-methoxyindole-3-acetic acid (5-MIAA) and 5-methoxytryptophol (5-MTPL). All three metabolites were produced from labeled melatonin by isolated retinas and by retinal pigment epithelium/ choroid/sclera preparations that were cultured separately. Treatment of eyecups with the monoamine oxidase inhibitor, pargyline, blocked production of 5-MIAA and 5-MTPL, and resulted in a tenfold increase in tissue content of 5-MT. These results suggest that melatonin in the eye is first deacetylated by an aryl acylamidase to produce 5-MT, then deaminated by monoamine oxidase to produce 5-MIAA and 5-MTPL.

This pathway has previously been shown to be a minor route of melatonin metabolism in the liver, but previous attempts to demonstrate it in brain or pineal have failed. In the eye, where melatonin is a potent modulator of circadian processes, this pathway may be a mechanism for regulation of local concentrations of melatonin. Alternatively, it may be a synthetic pathway for rhythmic production of other biologically active methoxyindoles.

2 **MAPS OF SENSITIVITY TO LIGHT AND DARK PULSES FOR THE CHICK PINEAL GLAND IN VIVO** S. Binkley and K. Mosher, Biology Department, Temple U, Philadelphia, PA 19122

N-acetyltransferase activity (NAT) in chick pineal glands exhibits a circadian rhythm with peak activity occurring in the dark-time. We previously showed that in chicks kept in LD12:12, NAT was increased by dark exposure at some times of day (dark sensitive period) or decreased by light at some times of night (light sensitive period). Here, the phase, duration, and amplitude of the sensitive periods for NAT-increase in response to 2h dark pulses or the NAT-decrease in response to 10m or 30m light pulses were mapped over 24h.

Total NAT varied only 6-9% when the dark period was 8h or 16h; more activity occurred in the time period from 0-8h after lights-out (L/D) in the chicks kept in LD16:8 (79%) than in the chicks kept in LD8:16 (56-60%). The duration of dark sensitivity was up to 5h longer after LD8:16 than after LD16:8. Light sensitivity occurred during the dark sensitive time, and the duration of light sensitivity was 2.6-3.6h longer after LD8:16 than after LD16:8. The phase of sensitivity was 2h later after LD8:16 than after LD16:8. The peak amplitude of sensitivity was focussed into the first 8h after L/D following LD16:8 pretreatment.

In sum, the sensitivity of the chick pineal NAT system (which is responsible for daily melatonin rhythms) was modified by photoperiod, but the modification was more subtle than the 2-fold that might be expected in response to doubling the darklength. The results are interpreted with respect to the enzyme clock model.

Supported by NSF PCM8444352 and DCB8613594, Temple Grant-in-Aid, and Temple Research Incentive Fund.

3 AGENTS AFFECTING VOLTAGE-SENSITIVE CALCIUM CHANNELS HAVE BIG EFFECTS ON CHICK PINEAL MELATONIN OUTPUT BUT NOT ON THE UNDERLYING PACEMAKER. Martin Zatz and Deborah A. Mullen Laboratory of Cell Biology, NIMH, Bethesda, MD. 20892

Chick pineal cells in primary culture display a circadian rhythm of melatonin production and release. Melatonin output is markedly inhibited by the organic calcium channel blockers nitrendipine, nifedipine, or verapamil. We showed previously that these cells contain L-type (and other) calcium channels. Omission of calcium from the culture medium or addition of certain inorganic cations also suppressed melatonin output. The order of potency was: $\text{Cd}^{2+} > \text{Mn}^{2+} = \text{Zn}^{2+} > \text{Co}^{2+} > \text{La}^{3+}$. Conversely, the dihydropyridine calcium channel "agonist," Bay K 8644, increased melatonin output.

In contrast to pulses of light or darkness (each of which causes phase-dependent phase delays and advances of the melatonin rhythm in otherwise constant red light), 4 or 8 hour pulses of low calcium, nitrendipine, or Bay K 8644, failed to cause appreciable phase shifts. Nor did chronic Bay K 8644 block the phase-shifting effects of light pulses. These results fail to support a prominent role for (L-type) calcium channels in entrainment, though they do support a role for such calcium channels in regulation of melatonin output.

Pulses of Co^{2+} either failed to cause phase shifts or (at higher concentrations) killed the cells, as did Cd^{2+} . Pulses of Mn^{2+} did cause phase-dependent phase shifts, but these were all delays. Zn^{2+} also caused phase delays. In view of the results with other agents, we interpret these latter effects on the pacemaker as not being mediated by effects on calcium channels. One possibility is that Mn^{2+} and Zn^{2+} act via another channel present in these cells, probably a cation channel, which is not voltage-sensitive but mediates spontaneous depolarizations.

4 SENSITIVITY AND SPECIFICITY OF THE SCN CIRCADIAN PACEMAKER TO CAMP STIMULATION. R.A. Prosser and M.U. Gillette. Neural and Behavioral Biology Program and Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The mammalian circadian pacemaker in the suprachiasmatic nuclei (SCN) survives in a brain slice preparation, where it continues to produce a circadian rhythm in neuronal firing rate. This rhythm is stable *in vitro*, and so can be used to mark the phase of the underlying circadian clock. Here we characterize the specificity and sensitivity of the pacemaker to stimulation of cAMP-dependent pathways.

Hypothalamic brain slices containing the SCN were prepared from Long-Evans rats housed in a 12:12 LD cycle, and maintained in perfusion culture. The firing rates of single SCN neurons were sampled to determine the time of the peak(s). Phase-shifts were determined by comparison with the time of subsequent peaks in untreated slices.

In vitro application of cAMP analogs for 1 hr during the subjective day, but not subjective night, produces robust phase-advances. These advances are stable for 2 cycles, and so represent true shifts of the SCN pacemaker. The specificity and sensitivity of the pacemaker were tested as follows: (1) Treatment with .5mM 8-benzylamino-cAMP (BA-cAMP) or 8-bromo-cAMP at CT 7-8 advanced the rhythm $4.6 \pm .21\text{hr}$ ($N=5$) and $4.25 \pm 1.06\text{hr}$ ($N=2$), respectively, while 8-bromo-5'AMP, an inactive analog, had no effect ($.38 \pm .18\text{hr}$, $N=2$). (2) The response to BA-cAMP was dose-dependent, with $5 \times 10^{-7}\text{mM}$ needed for half-maximal response. (3) Treatments which increase endogenous cAMP levels (.05mM RO 20-1724, .1mM IBMX, or .001mM forskolin) produced similar phase-dependent effects. These data indicate that the SCN pacemaker is specifically sensitive to increases in cAMP during the day, suggesting that cAMP either entrains or is part of the SCN pacemaker.

CALMODULIN INHIBITORS AFFECT THE CIRCADIAN RHYTHM IN FIRING RATE OF SUPRACHIASMATIC NUCLEUS (SCN) NEURONS IN VITRO.

Shigenobu Shibata and Robert Y. Moore. Depts. of Neurology and Neurobiology, SUNY at Stony Brook, Stony Brook, NY 11794

Calcium is necessary for the maintenance of the circadian rhythm in firing rate and glucose metabolism of SCN neurons in hypothalamic slices in vitro (Shibata et al, 1984; 1987; Shibata and Moore, 1987). The present study analyzed the effects of calmodulin inhibitors on the circadian rhythm in firing rate. Hypothalamic slices were obtained from adult rats kept in a light dark cycle and maintained in an incubation chamber to be studied at CT3, CT9, CT15 and CT21. The calmodulin inhibitors trifluoroperazine (TFP) chlorpromazine (CPZ) and W-7 were applied at a concentration of 50 μ M for 1 hr at each CT and compared with the inactive compound, W-5 (50 μ M), water (0.1%), high K⁺ (25 μ M), pentobarbital (PB 300 μ M,) and cycloheximide (CHX 30 μ M/ml). Each of the active calmodulin inhibitors produced phase advances in firing rate at CT9 and CT15 and delays at CT21 and CT3. CHX had similar effects. In contrast, high K⁺ produced phase delays at CT9 and CT15 and a small phase advance at CT21. Water, W-5 and PB were without effect. The effects of the calmodulin inhibitors was dose dependent for both phase advances and phase delays.

These observations indicate that a calmodulin dependent process can participate in phase changes in SCN neuron firing rate and that protein synthesis inhibition and calmodulin inhibition affect the rhythm in SCN neuron firing rate in the same manner. Whether this is accomplished through the same mechanism is unknown at this time.

TETRODOTOXIN ABOLISHES ELECTRICAL ACTIVITY OF SUPRACHIASMATIC NUCLEUS (SCN) NEURONS IN VITRO WITHOUT ALTERING THE GLUCOSE UTILIZATION RHYTHM OR PHASE OF THE RHYTHM IN SINGLE UNIT FIRING RATE.

Robert Y. Moore and Shigenobu Shibata, Depts. of Neurology and Neurobiology, SUNY at Stony Brook, Stony Brook, NY 11794.

Tetrodotoxin (TTX) selectively blocks voltage-dependent Na⁺ channels in axons, inhibiting the generation of action potentials. A previous study of TTX effects on neuronal activity in hypothalamic slices in vitro demonstrated that SCN action potentials are reversibly abolished when TTX is added to the medium (Sugimori et al, 1984). In the present study hypothalamic slices including the SCN were obtained and maintained in an incubation chamber to be studied at CT3, CT9, CT15 and CT21 from adult rats maintained in a light-dark cycle. The slices were maintained for single unit recording as described previously (Shibata et al, 1982) with one set of slices kept in control medium and another set exposed to 1 μ M TTX. Single unit recordings were continued for 6 to 12 hours to determine the timing of the next circadian change in firing rate. Two additional sets of slices, obtained at CT6 and CT18 were maintained in either control medium or TTX and glucose metabolism was determined using a modification of the 2-deoxyglucose technique (Newman and Hospod, 1986; Shibata et al, 1987).

TTX abolished single unit activity of SCN neurons throughout the period of exposure and for several minutes thereafter when firing rate spontaneously recovered to the pre-exposure level. A phase response curve indicates that TTX had no effect on the phase of the next circadian change in firing rate. TTX and control slices did not differ. Similarly, TTX had no effect on glucose utilization; the values for control and TTX treated SCNs at CT6 were nearly identical as were those at CT18. Thus, circadian function of SCN neurons is maintained independent of Na⁺ dependent action potentials.

- 7 CIRCADIAN VARIATION OF 2-DEOXYGLUCOSE UPTAKE WITHIN THE HOUSE SPARROW SUPRACHIASMATIC NUCLEUS. Vincent M. Cassone. Department of Neurology, Health Sciences Center, State University of New York, Stony Brook, NY 11794-8121.

Very little is known about the mechanisms by which the avian suprachiasmatic nucleus (SCN) influences circadian rhythmicity. In fact the location and intrinsic anatomy of the avian SCN have only recently been described (Cassone and Moore, 1987, J. Comp. Neurol. 266:171-182). To determine whether the SCN of the house sparrow exhibit circadian variations in activity, metabolism within these and several other diencephalic structures was studied using the 2-deoxyglucose (2DG) method at several times of day. Uptake of 2DG was high at midday and mid-subjective day, but low at midnight within the SCN. Uptake was high at midday and low at midnight and mid-subjective day within the ventral lateral geniculate nucleus and lateral anterior nucleus, while no daily or circadian variation was observed within the periventricular preoptic nucleus, previously thought to be the SCN. These data indicate that the avian SCN, as in mammals, express circadian rhythmicity. It is not known, however, whether these changes in SCN 2DG uptake represent independent oscillatory capacities for the sparrow SCN or merely circadian variations in activity that are driven by circadian oscillators located elsewhere. The birds in this study, for example, had intact pineal glands, which are known to be independent oscillators in birds and reptiles, that could drive SCN activity via melatonin or possibly other hormonal factors. Current work is directed toward elucidating these and other considerations.

Supported by NSF Grant BNS 85-19660.

- 8 A COMPARATIVE STUDY OF 2-DEOXYGLUCOSE UPTAKE BY THE SUPRACHIASMATIC NUCLEUS AMONG MAMMALIAN HIBERNATORS. T.S. Kilduff, C.M. Radeke, P. Kwon, and H.C. Heller. Depts. of Psychiatry and Biological Sciences, Stanford Univ., Stanford, CA 94305.

The existence of a rhythm of 2-deoxyglucose (2DG) uptake by the suprachiasmatic nucleus (SCN) of the rat, cat and squirrel monkey strongly supports the concept that the SCN is a circadian pacemaker. We have previously reported that the SCN of the squirrel Citellus lateralis has high levels of 2DG uptake during hibernation relative to other brain regions. However, we were unable to find evidence of a rhythm of 2DG uptake of the euthermic (non-hibernating) state. To examine whether high levels of 2DG uptake in the SCN during hibernation and the absence of a circadian rhythm of 2DG uptake in euthermia is a general property of hibernators or specific to C. lateralis, we conducted 2DG experiments during both hibernation and euthermia in two other hibernating species: Belding's ground squirrel (C. beldingi) and the Turkish hamster (Mesocricetus brandti). All animals were entrained to LD 12:12 and implanted with venous jugular catheters and a subcutaneous thermocouple re-entrant tube. For euthermic experiments, a 15W bulb covered by a Kodak 1A filter was left constantly on for at least 7 d prior to 2DG injection. On the day of the experiment, the fluorescent room lights were not turned on. Animals were injected during the middle of either the subjective day or subjective night. The 2DG incubation period was 45 min for euthermic experiments. Hibernation 2DG injections were conducted in a cold chamber at 5°C. Body temperature was less than 10°C for all hibernation incubations; hence, the 2DG incubation period was much longer (24 hr) than in euthermia. In all three species, the SCN was highly labelled relative to other brain structures during hibernation. In the euthermic experiments, the SCN was highly labelled in M. brandti and weakly labelled but above background in C. beldingi during the subjective day. The SCN was not labelled above background during the subjective night in either species. The presence of a rhythm of 2DG uptake during euthermia in M. brandti contrasts with the apparent absence of such a rhythm in the conspecific golden hamster (M. auratus) except in the anesthetized state. Similarly, the weak rhythm in C. beldingi contrasts with the apparent absence of such a rhythm in C. lateralis. It is of particular interest that, in all three species examined to date, the SCN is clearly labelled and one of the most active brain regions during hibernation.

9 PHOTOPERIODIC TIME MEASUREMENT IN THE MALE LIZARD ANOLIS CAROLINENSIS. Herbert Underwood. Department of Zoology, North Carolina State Univ., Raleigh 27695

Previous studies in *Anolis* failed to show a circadian involvement in photoperiodic time measurement. That is, none of the resonance (LD 8:16, LD 8:28, LD 8:40, LD 8:52) or T-cycle (LD 6:12, LD 6:14, LD 6:16, LD 6:18, LD 6:20, LD 6:22) protocols employed were inductive (i.e., stimulated testicular growth). Subsequently, it was observed that the activity patterns of anoles entrained to short-duration (6-8 hr) photoperiods are "disorganized" whereas entrainment patterns on longer-duration (11-12 hr) photoperiods appear to be normal. Accordingly, it was hypothesized that the failure of prior resonance or T-cycles to show circadian involvement may have resulted since the short-duration photoperiods used were incapable of keeping the circadian system sufficiently organized to measure photoperiodic time. In fact, night-break, resonance, and T-cycle protocols using longer duration (10-11 hr) photoperiods strongly support a role for the circadian system in photoperiodism in *Anolis*. First, one hour night breaks placed 8-11 hrs after dark onset (of LD 10:14) are inductive whereas pulses placed at other times of night are not. Second, resonance cycles (11 hr photoperiods) of T=24 hr or T=28 hr are not inductive whereas T=36 is strongly inductive. Third, T-cycles (11 hr photoperiods) either 20 or 30 hrs in length are strongly inductive whereas T-cycles 24 hr, 26 hr, or 28 hr in duration are not.

10 ANNUAL AND CIRCANNUAL RHYTHMS IN TEMPERATE AND TROPICAL DEER SPECIES.

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Seasonally breeding deer are characterised by pronounced rhythms of reproduction, appetite and metabolic rate and growth of the pelage. We have studied 2 seasonal species (the female Pere David (P.D.) and red deer) which have different breeding seasons (July and October respectively) and the tropical male Axis deer. The breeding season (based on progesterone cycles) of P.D. and red deer lasted 159 and 161 days respectively but was advanced by 88 days in the P.D. Seasonal appetite rhythms, changes in prolactin and T3 concentration and moult of pelage all showed a similar advance. Thus, both the seasonal reproductive and metabolic axis is linked to a common rhythm which is advanced in P.D. deer. Advance of breeding in red deer using melatonin was also associated with a shift in appetite cycle. Studies of the young P.D. and red deer calf over the first year of life indicated that the species differences only developed with the approach of puberty.

Work on Axis stags indicated that individual animals exhibited circannual cycles of antler and testicular growth. Within the herd, these cycles were not synchronised. Treatment with melatonin failed to advance antler cleaning or cause increase rate of testicular recrudescence. Thus, tropical deer may exhibit aphotoperiodic circannual rhythmicity which is entrained by melatonin in seasonal species.

STIMULATION OF GONADOTROPIN RELEASING HORMONE (GnRH) SECRETION BY LONG DAY IN ANESTROUS PONY MARES. Sharp DC, Grubaugh, WR, Weithenauer, J, Sheerin, P, and SD Davis. University of Florida, Gainesville, FL 32611.

The transition from anestrus to the breeding season in mares is characterized by increased FSH secretion and marked follicular development, but failure of LH secretion and hence, anovulation. After multiple waves of follicle growth, LH is eventually secreted, resulting in ovulation of one of the developing follicles, and culmination of the transition period. This experiment sought information as to the temporal sequence and the role of GnRH secretion in the early stages of sexual recrudescence in mares. Eight anestrus pony mares (180 kg) were fitted with push-pull perfusion devices, positioned within the pituitary, and were housed in light rooms (n=4/room) beginning in December. Mares were randomly assigned to one of two treatments: Group 1 (n=4) was exposed to "short day" (L:D 8:16) and Group 2 was exposed to L:D 8:16 until December 22, when the photoperiod was altered to L:D 16:8. Blood samples were collected once daily to assess circulating LH, FSH, estradiol and progesterone. Ovaries were palpated to assess follicular development and incidence of ovulation. GnRH was collected via push-pull perfusion just prior to the photoperiod change and again 3 weeks later. LH, FSH and GnRH were assayed by RIA using equine gonadotropins donated by H. Papkoff, and antisera donated by G.D. Niswender, D.L. Thompson and W. Ellinwood, respectively. LH was not different between Group 1 and Group 2, ($4.3 \pm .4$ vs $4.1 \pm .5$, $x \pm SEM$ ng/ml for Group 1 and 2, respectively) but FSH was significantly increased in Group 2 ($4.2 \pm .6$ vs $6.9 \pm .7$, $x \pm SEM$ ng/ml for Group 1 and 2 respectively). GnRH was significantly ($P < .05$) increased in perfusate of Group 2 mares after 3 weeks of exposure to long photoperiod but was not significantly changed in Group 1 mares, (3.3 ± 7.4 vs 7.8 ± 8.6 , Group 1, 1st vs 2nd perfusion; and $.4 \pm 8.6$ vs 26.1 ± 8.6 , Group 2, 1st vs 2nd perfusion; $x \pm SEM$, pg/ml perfusate/10 min). These data indicate that GnRH secretion occurred shortly after exposure of anestrus mares to long photoperiod, and suggest that increased FSH secretion reflects the increased GnRH secretion. Supported by NIH 10862.

DO SOCIAL CUES PLAY A ROLE IN TIMING THE SEASONAL REPRODUCTIVE RHYTHM OF THE EWE ? Nancy L. Wayne, Benoit Malpoux and Fred J. Karsch. Developmental and Reproductive Biology, Dept. of Physiology, The University of Michigan, Ann Arbor, MI 48109.

The annual cycle of photoperiod is the primary environmental factor that entrains the seasonal reproductive rhythm in sheep. This photoperiodic effect is mediated via the pineal gland. The purpose of the present study was to test the hypothesis that, in the absence of photoperiodic signals, *social cues* from gonadal-intact sheep can synchronize the reproductive season to the appropriate time in the ewe. To test this hypothesis, ewes were pinealectomized around the spring equinox 1987 (early anestrus season) and then either kept with, or isolated from, the normal flock that contained gonadal-intact ewes and rams. The effect on the onset of the reproductive season in the autumn was then established. A control group of pineal-intact ewes was kept with the normal flock. Ewes were ovariectomized and implanted with estradiol capsules; reproductive neuroendocrine activity was assessed by the ability of estradiol to suppress luteinizing hormone (LH) secretion. Results indicate that the control group (n=8) showed an onset of the reproductive season on September 8 ± 2 days (mean $\pm SEM$), with rises in circulating LH increasing 30-fold. The group that was pinealectomized and kept with normal sheep (n=5) showed an onset of the LH rise on September 7 ± 11 days; the timing of this onset of the seasonal reproductive activity was not different from that of the control group. In marked contrast, the group that was pinealectomized and isolated from normal sheep (n=6) showed an onset of the LH rise on November 5 ± 14 days; the timing of this onset of reproductive induction was significantly delayed compared with that of the control group and the pinealectomized-isolated group ($p < 0.025$; Mann-Whitney U test). These results suggest that social cues from gonadal-intact sheep play a role in modulating the timing of the onset of the seasonal reproductive rhythm in ewes not able to respond to photoperiodic signals. This study, however, leaves several unanswered questions: What is the social cue responsible for timing the seasonal reproductive rhythm? Does this cue emanate from both the ewe and the ram? Is the cue acting as an entraining agent or as a driving force? Finally, to what extent does the social cue contribute to timing the annual breeding season in ewes that are responsive to photoperiodic signals? (Supported by NIH-HD-18337 and HD-18258).

- 13 DEVELOPMENT OF REPRODUCTIVE RESPONSIVENESS TO LIGHT: INFLUENCE OF NIGHTLY AUGMENTATION OF THE AMPLITUDE OF THE MELATONIN RHYTHM IN THE NEONATAL LAMB. D.L. Foster, F.J.P. Ebling, L.E. Claypool, and R.I. Wood. Reproductive Endocrinology Program, Consortium for Research in Developmental and Reproductive Biology, and Departments of Obstetrics and Gynecology, Biology, and Physiology, The University of Michigan, Ann Arbor, Michigan 48109-0404.

Photoperiod is an important external cue governing the tempo of sexual maturation in the female sheep. Exposure to artificial long days (16L:8D) induces the first reproductive cycles during subsequent short day lengths (8L:16D); thus, the long summer day lengths provide a seasonal reference to time puberty to autumn in this short-day breeder. However, if long days are presented early (0-10 weeks of age, short days thereafter), puberty is delayed (*Endocrinology* 116: 2090, 1985). In older lambs, the pineal gland conveys photoperiodic information to the reproductive system via the pattern of rhythmic melatonin secretion (*Endocrinology* 119:44, 1986); this is not known for very young lambs in which the amplitude of the melatonin rhythm is lower than in the adult (unpublished). Thus, the ineffectiveness of long-day exposure early in postnatal life to induce puberty during subsequent short-day exposure might result from 1) an inability of the pineal gland at this age to transduce photoperiod and/or 2) an inability of the reproductive neuroendocrine system at this age to respond to photoperiod information. This study addressed the first possibility that the very young lamb fails to distinguish day length because of subthreshold melatonin rises. The approach was to increase the low nighttime rises of melatonin during the neonatal period to adult levels. Control lambs (n=8) were exposed to 5 weeks of long days between 17 and 22 weeks of age (short days before and after); this well-characterized light treatment induced puberty at the normal age (33±1 weeks; ≥ 3 repetitive reproductive cycles based upon circulating progesterone). Experimental females (n=16) were exposed to 5 weeks of long days between 2 and 7 weeks of age and short days thereafter. Half (n=8) received no further treatment. The other half (n=8) were infused nightly during the 8-hour dark phase of the 5-week, long-day photoperiod with melatonin (1.0 ug/h/kg) by means of a self-contained, computerized syringe-pump in a backpack. This increased the nighttime melatonin rise 4-5 fold, into the adult range of 200-300 pg/ml. In uninfused lambs exposed to long days as neonates, puberty was delayed as expected relative to controls, and only 1 of 8 lambs exhibited repetitive reproductive cycles by 44 weeks of age (end of study). Supplementation of the nocturnal melatonin rises neonatally was without effect; only 1 of 8 melatonin-infused lambs attained puberty by 44 weeks, a delay of at least 11 weeks, much like that for uninfused females. The inference from these results is that the failure of early long-day exposure to induce puberty in the lamb cannot simply be attributed to low amplitude melatonin secretion. Rather, it must be a post-pineal gland deficiency related to immaturity of the reproductive axis itself because of inadequate growth. (Supported by NIH HD-18258 and HD-18394 and Fogarty TW0 3766.)

- 14 THE EFFECT OF PINEALECTOMY OF THE PREGNANT EWE ON FETAL AND MATERNAL PLASMA MELATONIN CONCENTRATIONS DURING LATE GESTATION. I.C. McMillen and R. Nowak, Department of Physiology, Monash University, Clayton, Vic., 3168. Australia.

We have investigated the effect of pinealectomy of the late pregnant ewe on the presence of the diurnal rhythm in fetal and maternal plasma melatonin (MT) concentrations.

Six pregnant ewes were pinealectomised (PINX) between 110 and 118 days (d) gestation. Fetal and maternal arterial vascular catheters were inserted in the PINX group and also in a control group of 4 intact pregnant ewes (CONTROL). All ewes were kept in a 12 hour light:12 hour dark regime (lights on 07.00h). Fetal (2ml) and maternal (5ml) arterial blood samples were collected every 2 hours (h) for 24h (09.00-09.00) at two gestational ages between 125 and 140d. Fetal and maternal arterial samples were also collected frequently (6/24h) for 3-7d before delivery in 5 PINX ewes and 2 CONTROL ewes.

There was a significant diurnal rhythm in maternal and fetal plasma MT concentrations in the CONTROL ewes. In the CONTROL group, plasma MT concentrations were higher in the fetus than in the ewe during the day (eg. at 11.00h fetal MT = 44.4 ± 8.8 pmol/l; ewe MT = 25.2 ± 3.8 pmol/l), whereas at night the plasma MT concentrations were higher in the ewe than in the fetus (eg. at 23.00h fetal MT = 118.9 ± 13.9 pmol/l; ewe MT = 215.7 ± 27.3 pmol/l). The maternal and fetal MT concentrations were significantly higher ($p < 0.001$) in the control group when compared with the PINX group. There was no diurnal rhythm in either maternal or fetal MT concentrations in the PINX ewes. In this group there was also no difference between the MT concentrations in the ewe and fetus during the day. In the 3-7d preceding delivery there was no diurnal rhythm in fetal plasma MT concentrations in the PINX ewes. The mean length of gestation in the PINX group was 146.5d which is in the normal range for intact pregnant sheep (147 ± 3 days). Therefore we have demonstrated that in late pregnancy, the source of the diurnal rhythm in fetal and maternal plasma MT concentrations is the maternal pineal.

15 THE ROLE OF THE CIRCADIAN SYSTEM IN READING THE MELATONIN SIGNAL CONTROLLING SEASONAL REPRODUCTION IN THE SYRIAN HAMSTER.

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Neuroendocrine responses to seasonal changes in daylength are mediated by the pineal hormone melatonin (MEL). The duration of the nocturnal secretion of MEL is directly proportional to the length of the night and this duration is read by some form of timing mechanism within the brain. The circadian system may play a role in reading the MEL signal, in part by defining phases of the 24h cycle during which the brain is sensitive or insensitive to MEL. The role of the circadian system in reading an artificial MEL signal applied to hamsters was investigated, firstly by testing for a differential sensitivity in animals receiving MEL at different times of day, and secondly by investigating the effect of lesions of the suprachiasmatic nuclei (SCN), on the response to MEL. Hamsters held on a photoperiod of 8L:16D for 7 weeks to induce testicular regression were castrated, pinealectomized (PX) and fitted with chronic sub-cutaneous cannulae through which MEL or saline could be delivered. Animals received programmed infusions of MEL (1 μ g/500 μ l) or saline delivered over 10h commencing during either the light phase (1h before lights on) or dark phase (3h after lights off). After 15 days, serum LH levels were significantly higher in animals receiving saline (1.7 \pm 0.4 ng/ml) than in animals receiving MEL during either the light (0.6 \pm 0.3) or dark phase (0.4 \pm 0.1). However, there was no significant difference in LH levels from the 2 MEL groups indicating that there was no differential sensitivity to MEL applied at different phases of the LD cycle. In exp. 2, animals were PX, castrated and received infusions of MEL (100 ng/500 μ l over 10h) or saline during the dark phase in combination with electrolytic or sham lesions of the SCN. The response to infusion was determined by the increase in serum FSH levels observed over the 15 days of the experiment. The rise in FSH in intact animals infused with saline (15.0 \pm 2.5 ng/ml) was significantly higher than in the MEL group (5.4 \pm 3.0 ng/ml). The same effect of MEL was observed in SCNX animals (saline 19.5 \pm 1.1, vs MEL 4.5 \pm 0.9 ng/ml) indicating that the SCN are not essential for MEL to exert photoperiodic control of FSH secretion.

These experiments suggest that the mechanisms which time a MEL signal are not dependent upon the circadian clock and that daily changes in sensitivity of the brain to MEL are not involved in the detection of a MEL signal.

16 STEROID INDEPENDENT EFFECT OF PHOTOPERIOD ON PULSATILE LUTEINIZING HORMONE (LH) RELEASE Swann, J. M., Olson, S. L. and Turek, F. W. Neurobiology and Physiology, O.T. Hogan Building, Northwestern University, Evanston Ill. 60201

The reproductive state of the golden hamster, like those of most seasonal breeders, is regulated by the length of day. Prolonged exposure to a short day (less than 12 hours of light per 24 hours) results in gonadal involution and eliminates pulsatile LH release. Hamsters castrated before exposure to short days show LH pulses equivalent to long day castrates, suggesting that the inhibitory effects of short day exposure are mediated by gonadal steroids. The present study indicates that LH pulse height is inhibited in hamsters castrated during prolonged exposure to short days. Adult, male hamsters (*Mesocricetus auratus*) were divided into four groups. Groups 2, 3 and 4 were transferred to short days; group 1 remained on long days. Ten (groups 1 and 2) or 11 (groups 3 and 4) weeks after transfer all hamsters were castrated. Groups 2 and 4 were returned to short days; groups 1 and 3 were exposed to long days. Two weeks after castration hamsters were bled every 10 minutes for 4 hours via intra-atrial cannulas. Serum LH levels were determined via RIA with RP2 as the standard. Castrates from all four groups had hourly pulses of LH release. Castrates maintained on long days (group 1) had a mean pulse height of 14.1 \pm 1.38 ng/ml and mean LH levels of 54.67 \pm 4.66 ng/ml/hr. Exposure to short days before castration (groups 2 and 4) decreased LH pulse height (5.2 \pm 1.13 ng/ml, $p < .01$) and mean LH (17.96 \pm 5.01 ng/ml/hr, $p < .01$). Transfer from short to long days after castration (group 3) restored LH pulse height (15.07 \pm 1.59 ng/ml) and mean LH levels (52.68 \pm 2.86 ng/ml/hr) to that of long day castrates. There was no difference in pulse frequency between the groups. These results support the concept of a steroid independent effect of short days on pulsatile LH release in the male golden hamster.

- 17 AMPLITUDE MODULATION OF A BURST-LIKE MODE OF CORTISOL SECRETION GIVES RISE TO THE CIRCADIAN GLUCOCORTICOID RHYTHM IN MAN. JD Veldhuis, A Iranmanesh, G Lizaralde, ML Johnson. University of Virginia School of Medicine, Charlottesville, VA 22908; Dept of Int Med, Veterans Administration Medical Center, Salem, VA 24153.

We examined mechanisms subserving the *in vivo* circadian rhythm of cortisol in man. Blood samples were withdrawn at 10 min intervals for 24 hr in each of 6 men to yield well defined profiles of episodic cortisol release. A novel multiple-parameter deconvolution model was applied to discriminate the number, amplitudes, and durations of all significant underlying cortisol secretory bursts, and simultaneously estimate the endogenous half-life of cortisol disappearance in each subject. These experiments disclosed that the 24-hr profile of pulsatile cortisol release could be attributed to distinct and delimited cortisol secretory bursts with a mean frequency of 19 ± 0.82 secretory events per day and a corresponding mean interpulse interval of 77 ± 4.0 min. The estimated mean amplitude of endogenous cortisol secretory bursts was 0.45 ± 0.044 (mcg/dl/min). Resolved secretory bursts showed a mean half-duration (duration at half-maximal amplitude) of only 16 ± 0.61 min. Estimating secretion and clearance values from all plasma hormone concentrations and their variances considered simultaneously, we calculated a mean half-time of endogenous cortisol disappearance of 73 ± 5.3 min (range 62-97 min) and an *in vivo* production rate of 142 ± 14 (mcg/dl/day) or 16 ± 1.4 mg cortisol secreted/day assuming a nominal distribution volume of 11.3 L. Cortisol secretory burst frequency varied approximately 2.2 fold over 24 hr with an acrophase at 0415 clock time. Nyctohemeral variations in cortisol secretory burst amplitude averaged about 6.6 fold, with an acrophase at 0815 clock time. The latter value is congruent with timing of maximal serum cortisol concentrations in the same men, *viz.* 0849 clock time. We conclude that the robust nyctohemeral pattern of cortisol variation characteristic of healthy men can be accounted for by a parsimonious model of amplitude-modulated burst-like cortisol secretion. This concept eliminates the need to postulate a tonic mode of cortisol secretion.

- 18 EFFECT OF TRIAZOLAM ON THE ADAPTATION OF THE CIRCADIAN RHYTHMS OF CORTISOL, MELATONIN AND REM PROPENSITY TO AN 8-H DELAY OF THE SLEEP-WAKE CYCLE IN MAN. E. Van Cauter, A. Van Onderbergen, D. Bosson, M. L'Hermite-Baleriaux, M. L'Hermite, M. Szyper and G. Copinschi. School of Medicine, Free University of Brussels, Belgium.

The aim of the present study was to determine whether triazolam would accelerate the adaptation of circadian rhythms and sleep to abrupt shifts of the sleep-wake cycle such as those involved in rapid transmeridian transportation. Six normal male volunteers, aged 21-30 yrs, were studied once with triazolam and once with placebo, in random order. The two studies were spaced 2 months apart. In each study, the 24-h profiles of plasma cortisol and melatonin were obtained at 20-min intervals under basal conditions, and 1, 3 and 5 days after a 8-h delay of the sleep-wake cycle obtained by sleep deprivation from 23:00 to 07:00 on the first day. These conditions mimicked in the laboratory the shift in sleep-wake cycle experienced in the course of a westward flight crossing 8 time zones. A bedtime schedule of 07:00 to 15:00 in total darkness was enforced for 5 consecutive 24-h periods. Sleep was polygraphically recorded. During waking hours, the subjects were exposed to light intensities between 1,000 and 2,000 lux. Triazolam (0.5 mg) or placebo was given at 04:00 on the first shifted night and at 07:00 on the following nights. Reference time points on 24-h profiles were the end of the quiescent period for cortisol and the onset of the "nocturnal" rise for melatonin. On the first day after the shift, after triazolam administration, the quiescent period of cortisol secretion ended on average 2h03 later than under placebo ($p < 0.02$), the REM latency was lengthened by an average of 32 min ($p < 0.02$) and the melatonin onset was delayed by 1h00 to 2h40 in 4 of the 6 subjects. On days 3 and 5 after the shift, differences between triazolam and placebo were no longer significant. These findings suggest that triazolam may facilitate the early stage of adaptation to abrupt time shifts. On day 5, the adaptation of both hormonal rhythms to the time shift was almost complete, with reference time points within one hour of expected timing. These results indicate that adaptation of human circadian rhythmicity to shifts of the sleep-wake/light-dark cycles can be studied in ordinary laboratory conditions.

THE EFFECT OF 64 HOURS OF WAKEFULNESS ON IMMUNE FUNCTIONS IN HUMANS

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Sleep has been proposed to be a time of immunologic significance. We have reported that circadian lymphokines and peripheral blood monocyte immune functions are changed with sleep, & these can be altered during 40 hours of continuous wakefulness. We hypothesized that extended wakefulness would be associated with changes in "chrono-immunology".

Six normal, male subjects (mean age 23.2 yrs.) were investigated over 96 hrs. with continuous rectal temp. and polygraphic recordings. After a 24-hrs. baseline that included their usual nocturnal sleep, subjects remained awake for 64 hrs., then resumed nocturnal sleep. Serial venous blood samples were taken, every 2 hrs. from 0800 to 2400 and half hourly from 2400 to 0730, during the first 24 hrs. & final 48 hrs. They were assayed for pokeweed mitogen (PWM) and phytohaemagglutinin (PHA) mitogen responses and natural killer (NK, K562) cell activity. Mean Δ CPMs were Z-transformed & averaged for 12 time segments (MHour) and subjected to analysis of variance.

Consistent with our earlier studies, PWM response increases during sleep. However with 64 hours of wakefulness the usual nocturnal increase in PWM response is shifted to an earlier time (1900-2300) during both sleep deprivation and recovery days. PHA mitogen response during baseline was similar to our previous report, where response rises in early morning (0600). After 64 hrs. of wakefulness only slight changes in PHA response were observed with diminished response in early morning during both sleep deprivation and recovery sleep (day x MHour: $F=1.7$, $df=24$, $p<.05$). NK cell activity during baseline shows the peak with sleep onset (2400) and trough (0100-0300) while asleep. During 64 hrs. of wakefulness, peak of NK activity occurs earlier (1500-2300), but the nocturnal trough is unchanged. With return to sleep there is prolonged NK depression (1900-0600) (day x MHour: $F=2.3$, $df=23$, $p<.005$).

As with 40 hrs. of wakefulness PHA mitogen response shows only subtle changes with 64 hrs. of wakefulness. However following 64 hrs. of wakefulness, PWM mitogen & NK cell activity show more pronounced sleep related changes.

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ENDOCRINE RHYTHMS AND SLEEP IN HUMAN AGING.

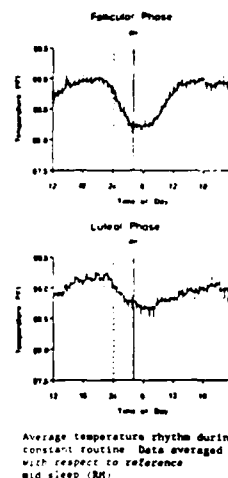
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The present investigation was undertaken to determine whether aging is associated with disorders of amplitude and/or synchronization of circadian rhythms of hormonal secretion. Eight healthy elderly male subjects (ages 67-84 yrs) and eight young normal men (ages 20-27 yrs) were submitted to blood sampling at 15-min intervals over a 24-h span. Each study was preceded by 3-5 nights of habituation to laboratory conditions. Sleep was polygraphically recorded for 3 consecutive nights, including the night of blood sampling. Plasma levels of cortisol, thyrotropin (TSH), prolactin (PRL), growth hormone (GH) and melatonin were determined for each sample. For cortisol, TSH, PRL and melatonin, the circadian waveshape was quantified in terms of amplitude, acrophase and nadir using a procedure based on periodogram calculations. Hormonal pulses were identified using the ULTRA algorithm. Sleep was significantly perturbed in the older subjects, with an increase in number and duration of awakenings and a decrease in stages III and IV. In older subjects, the mean TSH level was diminished by half ($0.78 \pm 0.37 \mu\text{U/ml}$ vs $1.43 \pm 0.41 \mu\text{U/ml}$, mean \pm SD, $p<.01$) and the total amount of GH secreted was decreased by two-thirds ($99 \pm 53 \mu\text{g/24 h}$ vs $301 \pm 178 \mu\text{g/24 h}$, $p<.01$). Mean levels of cortisol and daytime levels of PRL and melatonin were similar in both groups of subjects. For all hormones studied, even when overall secretion was markedly reduced, the normal circadian waveshape was still present in old age. A modest reduction in relative circadian amplitude was observed for all hormones, but was statistically significant for cortisol and PRL only. For both TSH and cortisol, the circadian secretory rise started earlier in older than in younger subjects. When corrected for differences in times of sleep onset, these phase advances averaged 60 min for cortisol and 51 min for TSH. In contrast, the timing of the onset and peak of nocturnal melatonin secretion was unchanged. These results demonstrate the occurrence of alterations of both amplitude and phase of hormonal rhythms in aging.

ENDOGENOUS CIRCADIAN TEMPERATURE AMPLITUDE IS REDUCED AND PHASE IS DELAYED AT THE LUTEAL PHASE OF THE MENSTRUAL CYCLE IN OVULATING WOMEN

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Animal studies have shown that changes in the levels of estrogen and progesterone can induce changes in the characteristics of the circadian timing system. This study was designed to determine whether the follicular and luteal phases of the menstrual cycle, each characterized by different levels of endogenous estrogen and progesterone, are associated with changes in phase or amplitude of the body temperature cycle. 10 healthy ovulating women 18-35 years of age were studied. Endogenous circadian phase and amplitude assessments (ECAP) were performed on all subjects using a 40-hour constant routine. The average endogenous circadian temperature amplitude at the luteal phase ($0.22 \text{ degrees F} \pm 0.02 \text{ SEM}$) ($N=6$) was significantly lower than at the follicular phase ($0.39 \text{ degrees F} \pm 0.02 \text{ SEM}$, $p<0.01$) ($N=9$) (see Figure). Women at both phases of the menstrual cycle had a significantly lower temperature amplitude than 39 young male control subjects (0.49 ± 0.03 , $p<0.01$) studied previously. Mid-trough of the circadian temperature cycle occurred at a significantly later hour at the luteal phase (3.78 ± 0.46 hours after mid-sleep) than at the follicular phase (1.97 ± 0.22 hours after mid-sleep, $p<0.01$). We hypothesize that the observed changes in the phase and amplitude of the body temperature cycle are due to the difference in the levels of gonadal steroids associated with menstrual cyclicity. These studies suggest that the menstrual cycle has a significant impact on the function of the circadian timing system in ovulating women.



CIRCADIAN CONTROL OF SLEEP TIMING AFTER SLEEP DEPRIVATIONS

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While the sleep-wake cycle is the most obvious mammalian circadian rhythm, the type of oscillator controlling these states is disputed. Does behavioral state change at specific phases of an endogenous circadian clock, or after a set level is reached in a relaxation oscillator? Alternatively, some combination may be involved. These hypotheses were tested using sleep deprivations (SDs) of different lengths and ending at different circadian phases in squirrel monkeys, diurnal primates with consolidated sleep periods. Data collection and analysis are described in Klerman and Moore-Ede, Sleep Research 16:618, 1987. Six SD lengths representing three pairs of termination phase were studied: 0, 90, 180, 360, 450 and 540 circadian degrees. All SDs began at predicted time of consolidated sleep (CS) onset. Sleep latency after SD was dependent on circadian phase and not on length of time awake. The duration of the first recovery CS episode was also highly circadian phase dependent; CS was of approximate normal length after 0, 360, 180 and 540 degree SDs, but was shorter after 90 and 450 degree SDs. No changes in NREM or REM percent during recovery CS were seen, even in recovery CS occurring at phases when REM probability would be expected to be high (after 90° and 450° SD). After all but 0° SDs, there was decreased average REM latency. We conclude that an endogenous circadian rather than a relaxation-type oscillator governs the timing of sleep and wake in squirrel monkeys.

THE SIGNIFICANCE OF ENDOGENOUS AMPLITUDE IN CIRCADIAN RHYTHMS.

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The amplitude of the endogenous circadian activity rhythm in individual mosquitoes is reduced close to zero by a light pulse of about 1.5h duration. This reduction has been unambiguously implicated in "Type-O" phase-resetting in Culex (1). Bright light stimuli of 8h and 16h duration have produced type-O resetting in Rattus exulans (2), where "disrupted" activity (reduced circadian component) was seen near the critical phase of stimulus application. We have demonstrated type-O phase-resetting in man, using as the stimulus a 72h protocol employing 3 circadian L/D cycles. Furthermore, at the reference stimulus phase for which the 72h protocol produces phase shifts close to 12h (near maximal) we have shown that a 48h protocol (only 2 circadian L/D cycles) produces a large reduction in the amplitude of the endogenous circadian temperature rhythm (A_T). In agreement with basic mathematical concepts, phase resetting capacity is greatly enhanced when endogenous circadian amplitude is strongly reduced.

Spontaneous reduction in A_T has been observed in women in the luteal phase of their menstrual cycle (3). [Comparable reductions in the amplitudes of circadian rhythms of alertness and slow-wave-sleep are seen in rats in the 2nd day after estrous (4).] We have other data which show that, in men, aging significantly reduces A_T . While phase within the circadian cycle is undeniably important we infer that endogenous rhythm amplitude (seldom measured or reported) may have equally important implications for circadian function.

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A MODEL OF THE HUMAN CIRCADIAN SYSTEM BASED ON INTERACTING POPULATIONS OF FEEDBACK OSCILLATORS

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The human circadian sleep-wake system has been modelled as an interaction between two populations of loosely coupled feedback oscillators. One population (X) of six oscillators has a mean free-running period of 24.5 h and simulates the behaviour of the circadian temperature regulation system. This is linked with unequal bilateral coupling to the population of eleven oscillators which regulates the sleep-wake system (Y), which has a mean period of 30 h. The periods of the individual oscillators of both X and Y populations are normally distributed. The system is light entrainable through the perturbation of the Y population by light pulses; there is no direct effect of light on the X's.

The model simulates several critical features of human free-running rhythms, and in particular the desynchronization of the X and Y oscillators after ten days or so in constant conditions. The length of sleep in desynchronization is correlated with the phase of the temperature oscillator at the onset of the sleep phase, and periodic long sleep phases of low amplitude are also exhibited in the simulated data. The simulated data also show napping as a result of partial desynchronization of the Y group.

SINGLE INJECTIONS OF TRIAZOLAM LENGTHEN FREE-RUNNING PERIODS IN GOLDEN HAMSTERS

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Light pulses induce both phase shifts and period changes in golden hamster circadian rhythms. The changes in period depend on the magnitude and direction of the phase shifts. Single injections of triazolam, a short-acting benzodiazepine, also produce reliable phase shifts in golden hamsters. However, the shape of the phase response curve (PRC) is quite different from the PRC to light. This allowed us to address the question: Are the changes in period seen after phase shifts intrinsic properties of phase shifts—regardless of how they are induced—or, are they specific to light-induced phase shifts?

Triazolam-induced phase shifts were followed by an increase in the free-running period (τ). This was seen after both phase advances (mean change at CT3 = $0.16 \text{ h} \pm 0.04$) and phase delays (mean change at CT21 = $0.15 \text{ h} \pm 0.04$). τ was not increased after vehicle injections or after triazolam injections that did not cause phase shifts. This is in contrast to light-induced phase shifts in which phase advances are associated with decreases in τ and phase delays are associated with increases in τ . Furthermore, changes in τ after light pulses are only about 0.05 h, much less than the triazolam-induced changes.

It appears that the circadian system responds differently to triazolam-induced phase shifts than to light-induced phase shifts. Like light, benzodiazepines may prove to be a powerful tool to elucidate formal properties of the circadian system.

PHASE ADVANCING EFFECTS OF BENZODIAZEPINE ON THE HAMSTER CIRCADIAN CLOCK CAN BE BLOCKED BY PREVENTING THE ASSOCIATED HYPERACTIVITY.

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A single injection of the short acting benzodiazepine, triazolam (Tz), can induce pronounced phase shifts in the circadian rhythm of wheel running activity in hamsters free running in either constant darkness or constant light. The phase advancing property of Tz was successfully used to reduce by about 50% the time it takes to resynchronize the biological clock of hamsters after an 8-hr advance shift in the LD cycle. The observation that Tz often induces hamsters to run in their wheel, as well as recent reports indicating that activity during the normal rest period also accelerates the rate of reentrainment after a shift in the LD cycle, raises the possibility that benzodiazepines may act on the biological clock through the hyperactivity they induce. To test this hypothesis, the phase shifting effect of a single injection of vehicle (V) or 2.5 mg Tz (given 6 hours before the onset of activity) was assessed in hamsters restrained for 6 hrs after the injection, as compared to unrestrained animals. While Tz induced large phase advances in the onset of activity in unrestrained animals, no significant phase shift was observed in either restrained animals treated with Tz or in hamsters of both groups treated with V. In a second series of experiments, hamsters subjected to an 8 hr advance in the LD cycle received a single injection of V or 2.5mg Tz (6-7 hrs before the expected onset of activity the day of the shift) before being either restrained for 6 hrs or left undisturbed. While injection of Tz in unrestrained animals reduced by about 50% the time for reentrainment, Tz treatment did not alter the rate of reentrainment in restrained animals. These findings suggest that benzodiazepines can induce behavioral events which in turn can induce phase shifts in the circadian system. Supported by FRSM (34-523), FMRE (86-88) and the Upjohn Company. OVR is a Research Fellow of the FNRS.

LGN lesions block triazolam-induced phase shifts of hamster locomotor rhythms. Smale L, Johnston R, Moore RY, Morin LP, State University of New York, Stony Brook

Intraperitoneal injections of triazolam (TZ) produce a phase response curve similar to that following intraventricular injections of NPY (1), injections of the excitatory neurotoxin NMDA into the lateral geniculate nucleus (LGN; 2), and electrical stimulation of the LGN (3). Because of these similarities, we hypothesized that the LGN mediate the effect of TZ on circadian rhythms of hamsters. To test this, we examined the effects of TZ on free running rhythms of enucleated LGN lesioned and control hamsters. Administration of TZ 6 h before activity onset (CT 6) induced large phase advances (>30 min) in 6 out of 7 control animals (1.9 ± 0.2 h). Only 3 out of 17 LGN lesioned animals responded to TZ at this time with a measurable phase change (1.9 ± 0.29 h advance for these 3). TZ injections at CT 21 induced significantly larger delays in control than in LGN lesioned hamsters (-0.69 ± 0.19 h and -0.14 ± 0.13 respectively). The 3 experimental hamsters that advanced after TZ injections at CT 6 all had incomplete lesions. We conclude that the LGN are involved in mediating the effect of TZ on hamster circadian rhythms. The nature of this involvement remains to be elucidated and several alternative possibilities will be discussed.

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LOCALIZATION OF THE GABA-A/BENZODIAZEPINE RECEPTOR COMPLEX IN RELATION TO THE INTERGENICULATE LEAFLET OF THE LATERAL GENICULATE NUCLEUS OF HAMSTER AND RAT. K.M. Michels, L.P. Morin and R.Y. Moore. Depts. of Neurology and Psychiatry, State University of New York at Stony Brook, Stony Brook, NY 11794-8121.

Involvement of the GABA-A/benzodiazepine receptor complex in neural pathways which regulate the mammalian circadian system is suggested by studies involving GABA-A receptor antagonists and benzodiazepine receptor agonists. In golden Hamsters the phase-response-curve (PRC) of locomotion to a peripheral injection of the benzodiazepine receptor agonist triazolam is similar to PRC's produced by electrical stimulation of the lateral geniculate nucleus (LGN), injections of N-methyl aspartate into the LGN and neuropeptide-Y (NPY) infused near the suprachiasmatic nucleus (SCN). The intergeniculate leaflet (IGL) of the LGN of hamster and rat contains NPY cells which project to the SCN and lesions including the IGL block the phase-shifting effects of triazolam in the hamster. These converging lines of evidence suggest a role for the IGL in mediating the effects of GABA-A and benzodiazepine drugs on the circadian system.

To determine whether these drugs may directly target IGL neurons we looked at the relationship of the GABA-A/benzodiazepine receptor complex to the IGL using a monoclonal antibody specific to the receptor complex (Vitorica et al., 1987), NPY immunocytochemistry and [3 H]diazepam binding and autoradiography.

In both hamster and rat immunoreactivity for the receptor complex is absent from the IGL and does not overlap with NPY immunoreactive cells. The medial division of the ventral LGN is also devoid of immunoreactivity. The dorsal lateral geniculate and lateral division of the ventral lateral geniculate, however, contain a dense finely granular immunoreactivity with the profiles of some cells clearly outlined. Semiquantitative analysis of the autoradiograms for the binding of [3 H]diazepam show close to background levels in the IGL while the binding density in the dorsal LGN and the lateral region of the ventral LGN is approximately 2.5 times greater. This pattern of [3 H]diazepam binding is consistent with the pattern of immunoreactivity obtained with the receptor monoclonal antibody.

These results suggest that the IGL may not be the initial site in the pathway mediating GABAergic interaction with the circadian system but do not rule out an integral role for the IGL in this pathway.

29 CIRCADIAN BENZODIAZEPINE RECEPTOR BINDING IN THE GOLDEN HAMSTER CNS T.A. Houpt, W.F. White, M.C. Moore-Ede, Dept. Physiol., Dept. Neurosci., Harvard Med. Sch. and Institute for Circadian Physiology, Boston, MA.

In the hamster, benzodiazepines (BZD) can induce phase shifts in activity rhythms and may modulate light input to the circadian system. Circadian rhythms in BZD receptor binding have been seen in the rat CNS. We studied BZD receptor binding in the hamster retina, cortex, caudate, and cerebellum at various circadian times. Rat cerebellum was also examined. Male hamsters (100-200g) and rats (200-400g) were group housed under a 12 hr light, 12 hr dark cycle for 3 to 4 weeks. Following 24 hrs light, hamsters (6/time pt) were decapitated under halothane at 4 hr intervals; similarly, rats (5/time pt) were sacrificed at 6 hr intervals. After 24 hrs darkness, hamsters (6/time pt) were enucleated and the retinae removed at 4 hr intervals under halothane in dim red light. The samples were placed in 4 °C 50 mM KPO₄ buffer at pH 7.4, and stored at -80 °C. To determine BZD receptor binding, tissue samples were homogenized, sonicated, and centrifuged at 45,000 x g for 15 minutes. Pellets were resuspended in fresh buffer and repelleted 3 times, then resuspended and incubated with 1.2 nM ³H flunitrazepam for 1 hr at 4 °C. Clonazepam, 10 µM, was used to determine nonspecific binding. Samples were filtered, washed 3 times with ice cold buffer, and counted in a scintillation counter. Assays were run in quadruplicate with all circadian times in parallel. Protein concentration was determined with Bradford's method. Specific BZD binding for hamsters was similar to previously reported rat receptor densities: 150 fmoles/mg protein for both light- and dark- adapted retina, 250 fmoles/mg protein for cerebellum, 600 for cortex, and 350 for caudate. No significant circadian rhythms were seen in any hamster regions or rat cerebellum. Although other studies have reported circadian rhythms in BZD binding to regions of the rat CNS, the amplitudes are small and variable in phase. It seems unlikely that gross changes in BZD receptor binding modulate the circadian effects of BZDs. Autoradiographic localization of BZD receptors may define such changes in more discrete, functionally significant regions.

30 ANALYSIS OF THE CIRCADIAN CONTROL OF BEHAVIOUR IN RATS. TOOLS AND TRICKS. W.J. Rietveld, J. Ruys, P. Buys. Dept. of Physiology, Leiden University.

There is ample evidence that the circadian control of overt behaviour is exerted by the hypothalamic suprachiasmatic nuclei (SCN), at least in rodents.

However, many experiments mostly done in rats and hamsters, point towards the presence of other, subordinate oscillator systems. In order to unravel the network involved in the circadian control of behaviour, a set of experiments have been designed combining lesion experiments with drug studies.

While recording food intake, water intake, locomotor activity as well as deep body temperature in blinded rats, it has been confirmed that in this species the SCN acts as the main driving oscillator for the circadian rhythmicity in all forms of activity (including deep body temperature).

In addition it has been found that the dorsomedial hypothalamic nucleus acts as a specific relay station for the circadian control of food intake. This nucleus also generates the so-called anticipatory locomotor activity during food restriction experiments, even in SCN-lesioned animals.

The effect of drugs (antidepressants like Clorgyline, GABA-ergic drugs) depend on the species (rat or hamster) as well as on the mode of application. Experiments using methamphetamine (MA) demonstrate a differential effect on various components of the circadian activity pattern resulting in an internal desynchronisation in normal rats and rats that received a partial lesion of the SCN.

The results from experiments in rats with a complete lesion of the SCN suggest that the effects of MA in the control of behaviour are neither due to an effect on the SCN itself nor on a different subordinate oscillator.

- 31 THE EFFECTS OF CONTINUOUS DIM LIGHT AND CHRONIC TREATMENT WITH THE MAOI ANTIDEPRESSANT CLORGYLIN ON WHEEL-RUNNING IN SYRIAN HAMSTERS. Wallace C. Duncan, P.G. Sokolove, W. Orem, T.A. Wehr. Clinical Psychobiology Branch, NIMH, Bethesda MD 20892 and the Department of Biological Sciences, University of Maryland, Catonsville, MD.

Chemical antidepressants alter the expression of the mammalian biological clock. The clinical antidepressant clorgyline (CLG), an irreversible type A MAOI, increases the intrinsic period of wheel-running and the duration of the active phase when measured in continuous darkness. In addition, CLG alters the phase-response curve (PRC) to brief light pulses. The effect of this compound on the light PRC suggests that CLG treatment might also affect the expression of the circadian pacemaker in continuous light.

CLG (2 mg/kg/day; n=20) or saline (n=24) was administered s.c. via osmotic mini-pumps, to male hamsters housed in LD 14.5:9.5 for two-three weeks prior to running-wheel access. Animals were then transferred to the circadian facility where drug treatment continued, and hamsters had ad-lib access to running-wheels, food and water through the rest of the experiment. Following 1-2 weeks in LD 14.5:9.5 (300:0 lux), the lighting schedule was changed to LL (1-5 lux). The period of wheel-running was estimated for days 15-24 of LL treatment by chi-square periodogram analysis. The difference between the estimated period for the saline and CLG groups was not significant ($\tau_{\text{CLG}} = 24.20 \pm .28$ hours, $\tau_{\text{S}} = 24.15 \pm .22$ hours). Between the fourth and fifth week of LL, the width of the right testis was measured through the skin covering the scrotal area. At this time point, the testis width of CLG treated hamsters ($12.18 \pm .81$ mm) was significantly smaller ($p < .001$) than the width of saline treated hamsters (13.26 ± 1.02 mm).

Tau of CLG treated hamsters housed in dim LL did not differ significantly from tau measured in CLG treated hamsters housed in DD. In contrast, saline treated hamsters exhibited an increase of tau in dim LL compared to tau measured in DD. CLG treated hamsters may be less sensitive to light at this intensity than saline treated hamsters. This interpretation is supported by the preliminary data which suggests the testes of CLG treated hamsters are in the early stages of regression.

- 32 MELATONIN ADMINISTRATION ENTRAINS FEMALE RAT ACTIVITY RHYTHMS IN CONSTANT DARKNESS, BUT NOT IN CONSTANT LIGHT

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Entrainment of the mammalian circadian system by exogenous melatonin (MT) has only been demonstrated in male rats and only in continuous darkness (DD). The present experiments investigate the effect of daily MT injections on the running and drinking activity rhythms of female rats in continuous dim white light (LL) (LL <20 lux) and in DD. In the female rat the luteinizing hormone (LH) surge is under circadian control. The LH surge is followed by a display of intense oestrogen induced running behaviour (pro-oestrous running), thus the rat oestrous cycle can be monitored from wheel running activity records. In LL, neither MT injections (50 $\mu\text{g/kg}$ or 1 mg/kg) nor vehicle saline injections had any discernable effect on free-running activity rhythms or incidence of pro-oestrous running. In DD, four out of six MT treated rats (100 $\mu\text{g/kg}$) entrained to injection and a fifth rat showed phase advance in its activity rhythm when onset of activity passed through injection time. Running, drinking and pro-oestrous activity rhythms were similarly affected. The sixth MT treated rat was not injected at activity onset time. None of the saline injected rats showed injection effects. Therefore, daily injections of MT are capable of entraining the activity rhythms and, by inference, the oestrous cycle of female rats in DD but not in LL.

LABELING OF THE SUPRACHIASMATIC NUCLEUS AFTER INTRAOCULAR INJECTION OF HERPES SIMPLEX VIRUS. Norgren, Robert B., Jr., John H. McLean, Michael T. Shipley and Michael N. Lehman, Department of Anatomy and Cell Biology, University of Cincinnati, Cincinnati, Ohio.

Herpes simplex virus has attracted great interest recently as a possible transneuronal tracer. We have evaluated its potential use in studying retinohypothalamic projections. Adult male golden hamsters received unilateral intraocular injections of 5 μ l of Herpes simplex virus (type 1, McIntyre strain). After survival times of 2 to 5 days, animals were sacrificed and brains were processed for immunocytochemical detection of HSV 1 using an avidin-biotin-HRP procedure (Vectastain). After 2 day survival no labeled neurons were observed in the hypothalamus. After 3 days a few labeled neurons were observed dorsal-lateral to the suprachiasmatic nucleus (SCN) and in the paraventricular nucleus. After 4 days, additional labeling was present in neurons in the SCN. After 5 days the SCN was heavily labeled, and appeared to be necrotic. Patchy labeling was consistently observed in the optic tract and, in two instances, in the superior colliculus. Retinae from animals at 2 days and longer were necrotic. Labeling in nuclei related to cranial nerves other than the optic, most notably the trigeminal nucleus, was observed after intraocular injection of Herpes virus but has not been reported after intraocular injection of other tracers. Electron microscopic examination demonstrated viral particles in both optic nerve axons and within the nuclei and cytoplasm of SCN neurons. In several animals in which the optic nerve was cut and HSV1 applied to the cut nerve, we observed no labeling in the distal optic nerve or SCN. These observations are suggestive of anterograde transport of HSV1 from the retina; unexplained by this interpretation is the absence of labeling in the lateral geniculate. (Supported by NIH Grants NS24292 [M.N.L.] and NIH NS23348, 20643 and U.S. Army DAMD 17-86C-6005 [M.T.S.]).

THE SINGLE UNIT RESPONSE OF SUPRACHIASMATIC NEURONS TO ARGININE VASOPRESSIN (AVP) IS MEDIATED BY A V1-LIKE RECEPTOR IN THE HAMSTER. Liou, S.Y., and H.E. Albers. Laboratory of Neuroendocrinology and Behavior, Departments of Biology and Psychology, Georgia State University, Atlanta, GA 30303.

A large population of local circuit neurons within the suprachiasmatic nucleus (SCN) exhibit immunopositive staining for AVP. AVP appears to be released from SCN neurons in a circadian pattern that peaks during the subjective day. The purpose of the present study was to examine how SCN neurons respond to AVP over the circadian cycle, and to determine whether the response to AVP is mediated by V1- or V2-like AVP receptors. Extracellular single unit recordings were made from spontaneously firing cells in hamster hypothalamic slice preparations. Coronal brain slices containing the SCN (400-450 μ m in thickness) were cut during the light or dark phase of the LD 14:10 cycle (CT 12.00 refers to lights off). Immediately after sectioning, the slices were placed in incubation medium oxygenated with 95% O₂ and 5% CO₂ at 35°C for at least 1 h and then transferred to a recording chamber. Exposure of the slice to AVP (10⁻⁸ M as verified by RIA of media samples) produced excitatory responses in 62% of the 58 SCN neurons examined. The threshold concentration of AVP ranged from 10⁻⁹ to 10⁻¹⁰ M. A statistically significant day-night difference in the percentage of SCN units responding to AVP was observed ($\chi^2=11.34$; $p<0.01$). During the subjective night (CT 12.00-22.00 h) 83% were excited by AVP, whereas during the subjective day (CT 22.00-12.00 h) only 33% had excitatory responses. In a second experiment the effects of selective V1 and V2 agonists and antagonists were determined. A V1, but not a V2 receptor antagonist was found to block the effects of AVP on single unit activity (N=5). Similarly, a V1 but not a V2 receptor agonist mimicked the effects of AVP (N=3). The present results indicate that 1) a larger percentage of SCN neurons have excitatory responses to AVP during the subjective night than during the subjective day, and 2) the receptor mediating the effects of AVP within the SCN is more similar to the V1 than the V2 AVP receptor. (Supported by ONR-N00014-87-K-0172)

PROTEIN SYNTHESIS IN THE SUPRACHIASMATIC NUCLEI (SCN) SHOWS NO CIRCADIAN RHYTHM. T.E. Scammell, C.B. Smith*, & W.J. Schwartz. Dept. Neurol., U. Massachusetts Med. School, Worcester MA 01655 & *Lab. Cerebral Metabolism, NIMH, Bethesda MD 20892.

Recent interest has focused on the anatomy and molecular biology of neuroactive peptides in the SCN. To provide a foundation for these and future studies of peptide expression, we measured overall protein synthesis in the SCN using a fully quantitative autoradiographic method (Smith et al, J Neurosci 4:2489-2496, 1984).

Ten male Sprague-Dawley rats (150 g at delivery) were entrained to a 12 h:12 h light-dark (LD) cycle (n=5) or a reversed (DL) cycle (n=5) for 2 weeks before they were blinded under ether during the light portion of their cycles. On each of 5 separate days (days 11 to 15 of free run), an LD/DL rat pair underwent femoral arteriovenous cannulation under halothane. After 2 to 3 h recovery, rats were injected i.v. at a time corresponding to mid-subjective light/dark with 100 μ Ci/kg L-[1- 14 C]leucine. Timed arterial blood samples were collected over 60 min, brains removed & frozen, and 20 μ m coronal sections cut & autoradiographed. From the time courses of [14 C]leucine and leucine in deproteinized plasma and the local tissue concentrations of 14 C, rates of protein synthesis were calculated as previously described.

There was no difference in overall protein synthesis in the SCN at these two time points (4.9 ± 0.4 nmol/gm/min, mean \pm S.D., for subjective light versus 4.3 ± 0.7 nmol/gm/min for subjective dark). Similarly, there was no difference in the paraventricular nuclei (10.0 ± 1.5 versus 9.6 ± 1.3) or in the brain as a whole, weighted for the masses of its component parts (3.2 ± 0.4 versus 2.9 ± 0.4).

Unlike the rhythms of glucose utilization and electrical activity at these times, overall protein synthesis in the SCN remains constant. We suspect that the bulk of proteins synthesized by the nuclei are directed to "housekeeping" functions performed at all hours.

THE PHASE-SHIFTING EFFECT OF MUSCIMOL ON THE CIRCADIAN RHYTHM OF ACTIVITY: ROLE OF THE SCN. Richard D. Smith and Fred W. Turek. Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208.

The suprachiasmatic nuclei (SCN) of the hypothalamus contain a neural oscillatory system which regulates many circadian rhythms in mammals. Immunohistochemical evidence indicates that a relatively high density of GABAergic neurons exist in the SCN region. We have previously demonstrated that when aimed at the region of the SCN, microinjections of muscimol, a specific agonist for gamma-aminobutyric acid (GABA), can induce phase shifts in the circadian rhythm of locomotor activity in the hamster (Neurosci. Abstr. p.209, 1986). The present investigation was conducted to determine a dose response curve for muscimol and to investigate the relationship between phase shifts induced by muscimol and the site of microinjection.

Stereotactically implanted guide cannulae aimed at the region of the SCN were used to deliver repeated microinjections of either muscimol or vehicle (1 μ l saline) at Circadian Time 6 (CT 12 = activity onset) in 17 blinded hamsters. A total of 75 injections of muscimol ranging in dose from 0.088 to 8.80 nmoles muscimol was sufficient to induce maximal phase advances (mean \pm SEM: 38 ± 12 min., n = 12 injections). Doses of 0.88 nmoles or less, did not produce statistically reliable phase shifts. Thus, the active range for muscimol delivered to the SCN region was within a single order of magnitude.

Phase shifts induced by 8.8 nmoles muscimol or vehicle administered at CT 6, were plotted as a function of the distance of the end of a cannula track from the peripheral border of the SCN. Phase advances induced by muscimol were positively correlated with distance from the SCN ($r = 0.51$, $N = 21$, $p < 0.05$). Generally, the magnitude of a phase advance was found to diminish as the distance from the SCN increased.

These data indicate that a GABAergic system may exist within the suprachiasmatic region as part of a central biological clock responsible for the regulation of the circadian rhythm of locomotor activity in the golden hamster.

- 37 DISRUPTIONS IN HUMAN CIRCADIAN AND COGNITIVE REGULATION FOLLOWING ROSTRAL HYPOTHALAMIC DAMAGE: A CASE STUDY. R.A. Cohen and H.E. Albers, Dept. of Neurol., Univ. of Mass. Med. Sch., Worcester, MA 01605 and Lab. of Neuroendocrinol. & Behav., Depts. of Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Little is known about circadian and behavioral dysfunction in humans following hypothalamic lesions. In the present study the sleep-wake cycle, cognitive/behavioral functioning and neuroanatomic findings were documented in a 34 yr. old female patient (AH) with damage resulting from surgical removal of a craniopharyngioma. Initial neurologic examination revealed a right visual field cut, diabetes insipidus, reduced thirst sensation, amenorrhea, major disruptions in sleep and wakefulness, and changes in cognitive function. The sleep-wake disorder was evaluated by electrophysiological recordings and a two week sleep-wake diary. A reduction in the percentage of stage I and II sleep and increased percentages in REM and stage IV sleep were noted as well as, an early REM onset. Sleep diaries revealed a severe disruption in the 24 hr. sleep-wake pattern. Cognitive and behavioral functioning was evaluated with a battery of neuropsychological measures repeated on three separate occasions over a 9 month period. AH demonstrated global cognitive dysfunction. The unusual feature of the disturbance was the extreme fluctuations in performance on a range of measures including memory, motor performance and logical processing, as determined by analysis of response consistency across assessments. Behaviorally, AH exhibited rapid alterations in mood, activity level and subjective arousal. Magnetic resonance and CAT imaging indicating damage to the rostral hypothalamus including the suprachiasmatic region and pituitary. In summary, disruptions in the normal temporal organization of the sleep-wake cycle, cognition and behavior were associated with damage to the rostral hypothalamus. These data are consistent with the hypothesis that the suprachiasmatic nucleus is essential for circadian control of human behavior, and suggest that circadian organization is necessary for regulation of normal cognitive functioning.

- 38 A HORSE RADISH PEROXIDASE-WHEAT GERM AGGLUTININ (HRP-WGA) STUDY OF THE EFFERENT CONNECTIONS OF THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) AND ADJACENT REGIONS OF THE MEDIAL HYPOTHALAMUS. Gail Orpen, Meir Steiner, and Mario O. Miceli. St. Joseph's Hospital Research Institute and Departments of Psychiatry & Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 4A6.

The efferent connections of the SCN have not been previously studied in the golden hamster, a common model for biological rhythm research. We therefore undertook a study of the efferent connections of the hamster SCN and of adjacent regions of the medial hypothalamus following the anterograde transport of HRP-WGA. Eight hamsters were given intra-SCN injections of 10% HRP-WGA (5-20 nL) that were largely confined to the nucleus. Ten hamsters received injections centered in the region of the medial anterior hypothalamus (MAH) ventral to the paraventricular nucleus and dorsal to the SCN (the subparaventricular zone [SPZ]) and another 2 hamsters received injections centered in the retrochiasmatic area (RCA). Control injection sites were centered in regions of the MAH lateral or dorsolateral to the SCN and in a region of the MAH rostral to the SPZ. Hamsters were perfused after a 36-48 hr survival period and cryostat-cut coronal brain sections were collected for the TMB histochemical reaction.

Following HRP-WGA injection into the SCN, anterograde labelling (presumptive terminals) was noted in the lateral septum; bed nucleus of the stria terminalis; medial preoptic area; SPZ and other regions of the MAH; paraventricular, dorsomedial, ventromedial, and arcuate hypothalamic nuclei; premammillary nuclei; and the thalamic paratenial and paraventricular nuclei. The same regions were also labelled after tracer injection into the SPZ and RCA. Efferents of the RCA, however, were more widely distributed, and included projections to the anterior and medial nuclei of the amygdala, periaqueductal grey, dorsal raphe, and dorsal parabrachial area. In contrast to the terminal distributions in the SCN, SPZ and RCA cases, anterograde labelling after control injections into neighbouring regions of the SCN and SPZ was restricted to hypothalamic areas adjacent to the injection sites. The hamster SCN neurons have limited extrahypothalamic projections. Furthermore, these projections overlap with those from adjacent hypothalamic regions, which in turn receive SCN input. These findings suggest potential new sites for modulation and diversification of the SCN circadian signal.

Supported by a St. Joseph's Hospital Foundation grant to M.O.M. and an Ontario Mental Health Foundation Fellowship to G.O.

LIGHT RESPONSIVE CELLS IN THE HYPOTHALAMUS OF THE DIURNAL THIRTEEN-LINED GROUND SQUIRREL. J.H. Meijer, B. Rusak and M.E. Harrington, Dept. of Psychology, Dalhousie University, Halifax, Canada

The hypothalamic suprachiasmatic nucleus (SCN) drives circadian rhythms in mammals. The pacemaker of the SCN is entrained to the daily light-dark cycle in the environment via the retino-hypothalamic projection and via the geniculo-suprachiasmatic tract. Behavioural studies have indicated that the circadian system of diurnal mammals responds differently to light as compared with the circadian system of nocturnal animals. We have investigated whether visual cells in a diurnal squirrel exhibit the same properties as in the nocturnal rodents.

20 Wildly trapped thirteen-lined ground squirrels were anaesthetized with thiopental sodium to perform extracellular single unit activity recordings. A total number of 325 hypothalamic cells were recorded with 83 cells inside the SCN. These hypothalamic cells exhibited spontaneous discharge rates between 0.1 and 2 Hz. 15 Cells were responsive to bilateral illumination of the eyes and responded to increasing levels of illumination with either an increase (n=7) or a decrease (n=8) of their discharge rate. All the light activated and most light suppressed cells displayed a tonic response to light pulses up to 4 minutes. Histological verification of the recording sites indicated that 7 visual cells were located inside the SCN. No relation was observed between the location of the visual cells and their response to light. These results show that visual cells of a diurnal squirrel are also tonically activated and suppressed by enduring light stimuli. The results moreover suggest that a larger proportion of visual cells in this species is suppressed by light.

40 CIRCADIAN RHYTHMS IN HAMSTERS AFTER SCN ISOLATION

Harvey Hakim and Rae Silver, Psychology Department, Barnard College of Columbia University, New York, NY 10027.

Transection of efferents from the suprachiasmatic nucleus (SCN) results in the loss of circadian activity patterns (Nishio, et al., 1979; Stephan & Nunez, 1977). Furthermore, following creation of a hypothalamic "island" that contains the SCN, circadian rhythmicity of multiple unit neural activity persists in the SCN but not outside the island (Inouye & Kawamura, 1979). These results suggest that efferents from the SCN are necessary for the expression of circadian rhythmicity.

We examined locomotor activity in male golden hamsters after isolating the SCN using a Halasz knife. Hamsters were housed for at least six weeks in constant dim illumination and one week in LD 12:12. Brains were then processed immunocytochemically for the presence of two SCN peptides: vasoactive intestinal polypeptide (VIP) and vasopressin (VP). Antisera to glial fibrillary acidic protein (GFAP) was used to stain the border of the knife cut, and rhodamine isothiocyanate (RITC) was used to detect retinal input to the island.

Preliminary results indicate that in some animals, tissue within the island stains positively for VIP and VP. Some of these animals continue to express free-running rhythms of locomotor activity. (Supported by NIH grant NS24292).

COMPARISON OF HUMAN DAILY TEMPERATURE AND PULSE RATE MEASUREMENTS TO CLINICAL OBSERVATIONS OBTAINED ANNUALLY IN THE BALTIMORE LONGITUDINAL STUDY OF AGING

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Except for determinations of many age-related physiological changes, few studies have explored relationships between physiological measurements spanning 20-30 years. Oral temperature and pulse rate have been assiduously recorded by one of us (JvdV) since August 1950. The data set analyzed for this report began in June 1960 when he enrolled in the Baltimore Longitudinal Study on Aging (BLSA) and has continued to this date. He is now almost 90 years old and continues to live independently in a state of good health. Comparisons have been made between the various physiological parameters obtained by the BLSA and the daily, self-recorded oral temperature and pulse rate. Mean basal morning and evening temperatures and pulse rates, corresponding to one week before and after each BLSA measurement, were abstracted from the continuous record. The BLSA measurements included pulse, height, weight, blood pressure, hematological values, respiratory rate, but not temperature.

After adjusting for age-related changes, correlations were estimated between all parameters. Although morning and evening pulse rates were highly correlated with each other (.72), no BLSA parameters were found to be closely associated with either. Pulse measurements were less highly correlated with temperature (~.6). Diastolic blood pressure was not significantly correlated to either pulse or temperature. Morning and evening temperatures were highly correlated (.90) and were also strongly correlated with hematocrit (.77, .74), hemoglobin (.78, .81), systolic blood pressure (-.84, -.79) and weight (-.77, -.75). These relationships between temperature and BLSA-determined parameters may be due to common underlying metabolic processes.

SEASONAL CHANGES IN A 38-YEAR RECORD OF HUMAN DAILY TEMPERATURE AND PULSE RATE MEASUREMENTS

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This study is based upon a unique human record of oral temperature and pulse rate recorded at least twice daily at approximately 0530 and 2230 while in a resting state. The data record began in 1943, however, the almost continuous record analyzed in this report began in August, 1950 and is current through December, 1987. A remarkably consistent protocol has been followed throughout this time period for the collection of data by one of the co-authors (JvdV).

Basal body temperature from the morning readings was on average 0.3°F higher than evening temperature recorded just prior to falling asleep at night with no significant trend over the time period. However, the standard deviation for evening temperatures was 48% higher than for the morning and, also, there was a significant increase in this measure of dispersion as the subject aged (between 20-30% increase). In contrast to temperature, morning pulse rates were 0.7 beats per minute lower on average than evening values but, again, a larger standard deviation was noted for evening pulse rates. Marked seasonal patterns appeared in both measures. In the first 15 years of the record, evening temperatures peaked during the summer, whereas morning temperatures peaked during the winter. A similar pattern was noted for pulse rate. The amplitude of the seasonal component for evening temperature (and pulse rate) was twice that for the morning values. During the last 20 years, morning and evening temperatures both peaked during the summer months, whereas the seasonal pattern of morning and evening pulse rate was unchanged. Whether these results are due to aging or to other intervening factors is unknown, however, dissociation between physiological parameters has been found previously in studies of both daily and annual rhythms in aging humans.

43 PEAK TIME OF ORAL TEMPERATURE RHYTHM IN AN "EVENING" PERSON: FIVE-MONTH LONGITUDINAL STUDY. Felver, Linda, Oregon Health Sciences University, Portland, OR

Day-active persons have been categorized into "morning" and "evening" people depending on their times of peak performance. In "morning" persons, the peak of the oral temperature rhythm frequently occurs between 4:00 and 6:00 p.m. This expected time of peak temperature is often used clinically as a standard for all day-active persons. The purpose of this study was to provide initial data on the appropriateness of using 4:00 - 6:00 p.m. as the standard peak time for the body temperature of a day-active "evening" person.

The oral temperature of a 42-year-old healthy female "evening" person was measured hourly during waking hours for five months. Oral temperature was measured with an IVAC TempPlus electronic thermometer. The thermometer was calibrated before use in a well-stirred water bath against a National Bureau of Standards certified thermometer; calibration was checked daily with an IVAC calibration plug. Measurements were not taken within 20 minutes of eating or drinking. Sleep-wake pattern, meals, exercise, and other variables that could affect body temperature were noted. "Eveningness" was established with the Horne-Ostberg Morningness-Eveningness Questionnaire.

The peak times of the daily oral temperature rhythm were determined using graphical techniques. The peak of the daily oral temperature rhythm occurred consistently later than 6:00 p.m. A one-sample t test showed a significant difference ($p < 0.001$) from a population mean of 6:00 p.m. These data indicate that use of a 4:00 - 6:00 p.m. standard for peak oral temperature may be inappropriate for an "evening" person. The body temperature rhythm in day-active "evening" persons may peak later than in "morning" persons. Assessment of the morningness-eveningness of day-active persons may be a useful adjunct to interpretation of oral temperature measurements obtained in clinical practice.

44 EEG POWER AND SLOW WAVE SLEEP IN NAPS: EFFECT OF TIME AWAKE. A.W. MacLean, D. Brunet, D. Nish, M. Coulter and J.B. Knowles, Departments of Medicine and Psychology, Queen's University, Kingston, Canada.

Daan et al. (1) have proposed that sleep is regulated, in part, by a Process S that increases exponentially as a function of time awake. To determine the time course of Process S, sleep was recorded during naps taken at 0900, 1400, 1900, and 2400. The resulting records, identified by number only, were scored visually, and the EEG submitted to spectral analysis using fast Fourier transform. To allow comparison with Daan et al., the dependent measures (mins. slow wave sleep and integrated power 0-25 Hz in the first hour of sleep) were transformed to a zero to one scale. Exponential equations were then fitted to the mean data using the method of least squares. When defined by integrated power density, the equation is

$$s = 1 - [e^{-(0.182 - 0.039 * \text{prior wake time})}]$$

When defined by slow wave sleep, the equation is

$$s = 1 - [e^{-(0.074 - 0.038 * \text{prior wake time})}]$$

The value for r' (the growth rate of the function) given by Daan et al. (0.055) falls within the 95% confidence limits of those reported here. Therefore, these data confirm their hypothesis that slow wave activity reflects a sleep intensity process that increases exponentially as a function of time awake. In addition, it appears that visually scored SWS and computed EEG power are equally sensitive to changes in prior wake time.

(1) Daan, S., Beersma, D.G.M. and Borbély, A.A. (1984). Timing of human sleep: recovery process gated by a circadian pacemaker. Amer. J. Physiol. 246: R161-178.

The purpose of the present study was to investigate the relationship between ultradian rhythmicity in behavioral response latencies and in measures of cortical arousal during sleep. Eight subjects were instructed while awake to take a deep breath to terminate a tone which was subsequently presented after every four min of continuous sleep. The subjects responded to nearly all (>99%) tones. Awakenings and shifts to stage 1 sleep (cortical arousal) occurred following approximately 25% of the trials. For analysis, the recording period was divided into 10-min intervals. Mean response latencies and number of minutes spent awake or in stage 1 sleep were calculated for each interval. The data for each subject were then submitted to time series analysis.

An ultradian rhythm was found in behavioral responsiveness with a 100-min period. A similar rhythm was found in the arousal measure. Analysis of cross-covariation indicated that, for two subjects, the rhythms were out of phase, i.e., short response latencies were associated with arousal while long latencies were associated with undisrupted sleep. For the remaining subjects, the rhythms were more nearly in phase, i.e., short response latencies were associated with undisrupted sleep and long response latencies were associated with arousal. In sum, there are ultradian rhythms in the behavioral and physiological responses to stimuli presented during sleep. The relationship between these rhythms varies across subjects.

A phase advance of some circadian rhythms (e.g. body temperature and REM (Rapid Eye Movement) sleep propensity) relative to the sleep-wake cycle is thought to be implicated in the pathophysiology and pathogenesis of affective illness.

Using a six hour phase delay of bedtime, Surridge-David et al. (1987)¹ tested this hypothesis in ten healthy males, and found a general lowering of mood which was modest but reliable. Two subjects, however, became noticeably depressed when interviewed following the first or second day of the shift.

In the study reported here, twelve healthy males (mean age 21) were studied for five consecutive nights under each of two conditions. On the first, second and fifth days, subjects slept between 2400 and 0800. On the third and fourth days, sleep was either advanced to 1800 or delayed until 0600. Mood was assessed by the Beck Depression Inventory, and by self-ratings on visual analogue scales. Interviews conducted at these times were videotaped and scored blindly by two independent raters, using a depression-hypomania visual analogue scale.

When testing time was held constant, reliable changes in mood (and sleepiness) were associated with a six hour phase delay, but not with a comparable phase advance. This lowering of mood was not independent of concurrent changes in subjective sleepiness, as assessed by the Stanford Sleepiness Scale. In this respect, and with one exception ("overall feeling"), these findings replicate those of the first study. In the first study, "overall feeling" was significantly lower following a phase delay, and remained so when sleepiness was entered as a covariate; in the present study it did not remain so. It remains unclear whether the observed mood change is a direct consequence of the phase delay, or an indirect consequence of the sleepiness caused by an acute delay of bedtime.

- 47 CIRCADIAN RHYTHM MEASURED BY THE CORE TEMPERATURE IN DEPRESSION
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In recent years, there has been growing interest in the disturbance of circadian rhythms for physiological variables in affective illnesses. In order to investigate the disturbance, we measured the body temperature by a technique of the core temperature. The core temperature revealed to fluctuate less, and had better correlation with the rectal temperature than either oral or axillary temperature, also to have less physiological and psychological burdens than the rectal temperature, thus, to be a more ideal measure applicable to clinical research.

On the basis of this result, we investigated longitudinally the circadian rhythm in the core temperature of 25 inpatients including 14 depressed patients. The data was analyzed by methods including maximum entropy spectral analysis (MEM) and sinusoid curve fitting technique (COSINOR). A predominant 24 hour component was found in most of all normal subjects, schizophrenic and neurotic patients, while it was not clearly observed in severely depressed patients. This component emerged the more obviously when the depressive symptoms became the milder, suggesting unstability of the circadian rhythm to be present in depressive state. The acrophase of the core temperature rhythm was found to advance in only 29% of the depressed patients studied, while it delayed in 29% and stayed within a normal range in 42% of them.

- 48 USEFULNESS OF THE ANALYSIS OF CIRCADIAN AND PULSATILE CORTISOL VARIATIONS IN THE DIFFERENTIAL DIAGNOSIS OF VARIOUS FORMS OF HYPERCORTISOLISM. S. Refetoff, R.E. Weiss, V.S. Fang, P. Linkowski and E. Van Cauter. Dept of Medicine, University of Chicago, Illinois - and - School of Medicine, Free University of Brussels, Belgium.

Depressed subjects and patients with Cushing's disease frequently present similar ranges of basal ACTH and cortisol levels and similar resistance to low dose dexamethasone suppression. Accordingly, elaborate and as yet unstandardized diagnostic tests have been proposed to differentiate these two conditions of hypercortisolism. We show here that indices derived from the analysis of a basal 24-h profile of plasma cortisol (a simple procedure using a well-standardized assay available in all laboratories) can discriminate between these two conditions. The 24-h profile of plasma cortisol was analyzed in 26 depressed patients and 50 patients with Cushing's disease. Blood sampling intervals were 15-30 min. For each of the 76 subjects, mean cortisol levels were calculated over the 24-h span and over the 18:00-00:00 interval ("nocturnal mean"), and the circadian variation was quantified by a best-fit curve based on periodogram calculations. Circadian rhythmicity was considered to be present if the 24-h component was significant ($p < 0.05$) and the best-fit curve had an acrophase between 06:00 and 12:00 and a nadir between 19:00 and 03:00. The absolute amplitude was defined as 50% of the difference between the level at the acrophase and the level at the nadir and relative amplitude as the absolute amplitude divided by the 24-h mean. In each individual, significant cortisol pulses were identified using the ULTRA algorithm. The overall pulsatility in each profile was characterized by the median of absolute pulse increments, the median of relative pulse increments and the sum of absolute pulse increments. Circadian rhythmicity was present in all depressed patients but only in one third of patients with Cushing's disease. Pulse magnitude was increased in depressed patients but dampened in the majority of patients with Cushing's disease. The combination of the nocturnal mean, the relative circadian amplitude and the median of relative pulse increments discriminated between the two groups in more than 95% of the cases.

- 49 EFFECTS OF ABRUPT PHASE-SHIFT IN THE AMBULATORY-RECUMBENCY AND SLEEP-WAKE CYCLES ON Sm-C NYCTOHEMERAL PATTERN IN NORMAL MAN.
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In a previous study (Ann. Endocr. 46, 1985, 173) we had found positive correlations between circadian variations of somatomedin-C (Sm-C) and total plasma proteins (TPP) levels but not between Sm-C and growth hormone, suggesting that Sm-C variations are related to postural TPP fluctuations. In order to further investigate this hypothesis, we studied six normal men, 21 - 30 y.o. The 24-h profiles of plasma Sm-C and TPP were obtained at 40-min intervals under basal conditions (Do) (sleep from 23:00 to 07:00) and 1, 3 and 5 days after an 8-h delay of the usual sleep-wake and ambulatory-recumbency cycles. This delay was obtained by sleep and recumbency deprivation from 23:00 to 07:00 on the first day. A sleep schedule of 07:00 to 15:00 was then enforced for 5 consecutive 24-h periods. During ambulatory periods, recumbency and naps were prohibited. At Do, 23:00 - 07:00 and 07:00 - 23:00 Sm-C levels averaged 0.85 ± 0.12 U/ml (mean \pm SEM) and 0.95 ± 0.13 U/ml respectively ($p < 0.05$). Corresponding TPP values averaged 6.10 ± 0.25 g/dl and 6.69 ± 0.17 g/dl respectively ($p < 0.005$). On day 1 after the phase shift, 07:00 - 15:00 SmC and TPP levels were lower than 15:00 - 07:00 levels ($p < 0.01$ and < 0.05 respectively) while no difference was observed between 23:00 - 07:00 and 07:00 - 23:00 values. Similar results were found at days 3 and 5, indicating a complete adaptation to the time shift at day 1 already. These results strengthen our hypothesis of the preponderant role of postural conditions on Sm-C circadian fluctuations, in a manner comparable to that seen for TPP.

- 50 SHEEP AND CIRCADIAN RHYTHM REGULATION: Effect of lithium on plasma melatonin, norepinephrine and MHPG. Jo Seggie, Meir Steiner and Gail Orpen. Department of Neurosciences and Psychiatry, McMaster University and St. Joseph's Hospital Research Institute, Hamilton, Ontario, Canada L8N 4A6

Considerable rodent data suggest that melatonin and the retinal-hypothalamic pineal axis may be important mediators of the chronobiological properties described for prophylactic drugs such as lithium. Limitations of rodent models however preclude certain research protocols of interest in this area. We have investigated the effects of lithium carbonate on the circadian pattern of plasma melatonin, norepinephrine and MHPG in sheep around the time period of switch from light to dark under a controlled lighting cycle of 10L:14D.

Administration of lithium carbonate in food at the rate of 600, 900, 1200, 1500 and 1800 mg/day/in weekly intervals resulted in a linear dose response curve for lithium levels in plasma and red blood cells. Polydipsia was not evident and weight loss was seen only at the maximal dose. In contrast to the rodent model but in agreement with the human condition plasma lithium levels exceeded those of red blood cells. Daily dosage of lithium in the 1200-1500mg range produced plasma lithium levels of 0.7 - 0.9 mEq/l which is equivalent to the human therapeutic range. Once lithium levels were stabilized at these levels blood samples were taken by venipuncture at approximately 30 min. intervals during the period 90 minutes before lights out to the middle of the dark period. Preliminary results indicate that lithium treated sheep in comparison to controls demonstrated increased melatonin levels, with a suggestion of a phase advance while norepinephrine and MHPG levels were unaffected.

Our data suggest the sheep to be a useful model for the study of chronobiological properties of pharmacological agents and their probable mechanisms of action.

This work was supported in part by the St. Joseph's Hospital Foundation and Seggie's Shire Farm. J.S. and G.O. hold Ontario Mental Health Foundation Research Career Awards.

FEEDING AND LOCOMOTOR ACTIVITIES OF THE PIGEON: MELATONIN AND ITS EFFECTS ON RHYTHMICITY. C.C.Chabot and M.Menaker. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA. 22901.

Although a circadian rhythm of locomotor activity has been measured in the homing pigeon, this measurement of general activity often does not allow for an accurate interpretation of period or phase. Since feeding activity, a more specific type of behavior, has been measured and found to be clearly rhythmic in at least two other species of birds, we measured both feeding and locomotor activity in the pigeon. Feeding activity of the pigeon is rhythmic in both a 12 hour light/ 12 hour dark cycle (LD) and in constant darkness (DD). Pigeons exposed to a variety of constant light intensities exhibit clear changes in both feeding and locomotor rhythms. In general, these rhythms persist at low intensities of LL, while at higher intensities the birds become arrhythmic. Pinealectomy appears to have little effect on either feeding or locomotor rhythms. The implantation of melatonin filled capsules into intact pigeons abolishes rhythmicity in both behaviors, while their removal immediately restores it. In all of these experiments in which both feeding and locomotor rhythms could be measured, a constant phase relationship between the two rhythms was maintained. These results are similar to results found in the house sparrow.

MELATONIN AFFECTS SUN-COMPASS ORIENTATION IN HOMING PIGEONS

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Endocrinological and behavioral data suggest that maintenance of circadian locomotor rhythms in birds depends on rhythms of circulating melatonin (Menaker, 1985, for review).

The aim of the present study was to test whether melatonin is also involved in sun compass orientation, which is known to rely on a circadian rhythm (Aschoff, 1954).

The navigational performances of 50 homing pigeons were tested under sun from unfamiliar sites. Birds were kept in continuous dim LL < 1 lux or in the natural LD cycle for 7 days before the test releases. They were implanted subcutaneously with 60 mm long sylastic capsules filled with crystalline melatonin (ME-birds) or with empty capsules (C-birds). Birds in LL were also carried to the release site inside light-tight containers and drawn out singly immediately before being released. C-birds in LD and LL, and ME-birds in LD were capable of homeward orientation. ME-birds in LL, on the contrary, were completely disoriented, or deviated almost 180° from the home direction.

Inability of homeward orientation of pigeons released at unfamiliar sites can be attributed to a defect either in map or in compass working, or both. As the sole difference between ME-birds in LD and ME-birds in LL consisted in depriving ME-birds in LL of Zeitgebers, we conclude that the circadian rhythm controlling the sun compass mechanism is involved in the poor navigational performances of ME-birds in LL: such a mechanism is heavily damaged by continuous administration of melatonin in constant conditions.

The present results demonstrate for the first time the involvement of melatonin in bird navigation.

EFFECTS OF CONSTANT DARKNESS ON ACTIVITY AND MELATONIN RHYTHMS IN THE RAM. F. J. P. Ebling, G. A. Lincoln, F. Wollnik and N. Anderson. MRC Reproductive Biology Unit, Edinburgh, U.K., Developmental and Reproductive Biology, The University of Michigan, Ann Arbor, MI., and Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL.

The relationship between rhythms in plasma concentrations of melatonin and rhythms of activity was studied in adult male Soay sheep. Rams were exposed to 16-week periods of short days (8h light: 16h darkness, 8L:16D) or long days (16L:8D), followed by a 16-week period of constant darkness (dim red light, DD). The rams were penned individually, and an event recorder documented each time the ram broke an infra-red beam which was reflected across the pen. As expected, on both 8L:16D and 16L:8D there was a clearly defined 24-hour rhythm in plasma concentrations of melatonin, with elevated levels occurring throughout the dark period. Periodogram analysis of activity records revealed significant 24-h rhythms in activity in 8L:16D and 16L:8D. A crepuscular pattern was evident; activity bouts occurred following lights on and also preceding lights off. The periodogram analysis revealed that a significant activity rhythm persisted in all rams over the first 10 days on DD following transfer from an entraining photoperiod. In 7 of 8 rams, the period of this rhythm (23.50 ± 0.08 h, mean \pm SEM) was shorter on DD than on 8L:16D or 16L:8D (23.98 ± 0.02 h). However, both activity and melatonin rhythms became disorganized after prolonged exposure to DD. After 8 weeks on DD, most rams had significant melatonin peaks, but they were of variable duration and period between onsets of successive peaks, and were not synchronized between rams. The introduction of a one-hour light pulse every 24 h for 2 weeks (1L:23D) after 8 weeks on DD entrained the melatonin rhythms, and induced a significant 24-h activity rhythm. The main bout of activity appeared to precede the light pulse, whereas the rise in melatonin followed the light pulse. This suggests that the 1-h light pulse provided a "dusk" zeitgeber for each rhythm, rather than a "dawn" signal. Under appropriate housing conditions, activity rhythms can provide a useful marker of circadian organization in the ram.

54 SPONTANEOUS CHANGES IN THE CIRCADIAN RHYTHM OF MELATONIN SECRETION IN THE EWE: POSSIBLE GENERATION OF A CIRCANNUAL REPRODUCTIVE RHYTHM. Suzanne M. Moenter and Fred J. Karsch. Developmental and Reproductive Biology and Dept. of Physiology, University of Michigan, Ann Arbor, 48109.

The annual cycle of photoperiod synchronizes the seasonal reproductive cycle of the ewe via pineal melatonin secretion. Although circannual rhythms of reproductive activity persist in ewes housed in a constant photoperiod, the mechanism by which these cycles are generated is unknown. We hypothesized that spontaneous changes in the circadian rhythm of melatonin exist under long-term exposure to short days, and these changes may generate the circannual rhythm of reproductive activity. As an initial test of this hypothesis, the melatonin pattern was determined by taking hourly blood samples for 24-48 hours approximately once a month from five ewes which were exhibiting circannual cycles of reproductive activity as measured by serum luteinizing hormone concentration. These ewes had been exposed to short days (8L:16D) for four years as of April 1987, the time when melatonin sampling began. Results to date indicate there has been a major alteration in the melatonin rhythm in three of the five animals studied. This alteration was manifested either as a complete lack of the nightly melatonin rise or as a delay of 5-14 hours in the time from lights off to the melatonin rise. The change in melatonin pattern was uniform from one night to the next within a sampling period, and was observed to persist over 3 consecutive sampling periods (41 days) in one animal. Subsequently, these three ewes have exhibited melatonin patterns in which the elevation filled the entire dark period. No elevated secretion of melatonin has ever been observed during the light phase of the photocycle in these ewes. The changes in the melatonin rhythm in each of these animals occurred during a period of reproductive inactivity, and they were followed by reproductive induction within 34-85 days. Our results are consistent with the hypothesis that spontaneous changes in pattern of melatonin secretion exist in ewes after several years of exposure to a constant photoperiod, providing our first indication that melatonin secretion may not always be an accurate indicator of photoperiod. This preliminary observation suggests several questions which merit further investigation. Why have we observed no changes in melatonin in ewes during development of photorefractoriness? Do changes in the melatonin pattern reflect an underlying circannual rhythm in melatonin secretion? If so, do changes in melatonin secretion have a causal relationship to changes in reproductive activity? (NSF-PCM-8316364, NIH-HD-18337, HD-18258)

RHYTHMS IN UTERINE CONTRACTILE ACTIVITY AND CORTISOL SECRETION DURING PREGNANCY IN THE SHEEP. S.M. Yellon, E.M. Apostolakis, K.E. Rice and L.D. Longo, Division of Perinatal Biology, Departments of Physiology and Pediatrics, Loma Linda University, School of Medicine, Loma Linda, CA, 92350.

Diurnal rhythms in uterine myometrial activity and circulating adrenal corticosteroids occur during pregnancy in the primate. In the sheep, increased cortisol late in gestation is hypothesized to initiate parturition, but little is known about the temporal relation between circadian cortisol secretion and uterine contractile activity. The present study determined whether 24 h rhythms in cortisol and uterine activity were present in sheep during pregnancy. After one week acclimation to artificial short days (10L:14D, lights off 1700 h PST), catheters were surgically implanted in the pregnant ewe and fetus at about 113 d gestation. Additionally, a catheter placed in the amniotic cavity was used to assess intrauterine pressure; average hourly rate and amplitude of contraction (> 5 mmHg) were scored from Gould polygraph records. Plasma was collected over 48 h (every 1-4 h; 25 blood samples) at weekly intervals preceding birth (120, 127, 135 days gestation; 6, 4 and 3 ewes and fetuses, respectively), and cortisol quantified by RIA. No significant pattern related to time of day or light/dark cycle was detected in maternal or fetal cortisol concentrations ($p > 0.05$, ANOVA). A rise in mean fetal cortisol levels was apparent within several days preceding birth. At 120 d gestation, a low-amplitude nocturnal rhythm in contraction rate was significant. However, in the two weeks prior to birth, no rhythm in uterine contraction rate or amplitude was present. Mean contraction rate remained constant ($\sim 12/h$) after 127 d while daily mean amplitude rose linearly with gestational age. Therefore, increases in mean fetal cortisol concentrations are correlated with a rise in uterine contraction amplitude. In the apparent absence of circadian patterns for cortisol and uterine contractile activity, the data suggest that amplitude of uterine contractions associated with high fetal cortisol levels near term are important for initiation of birth. These findings contrast with data in the Rhesus monkey which suggest that rhythmicity in uterine activity and in circulating adrenal steroids may be important for the initiation of parturition. (Supported by Dept. Pediatrics and NIH HD 03807.)

DAILY MELATONIN INJECTIONS PRODUCE OBESITY AND REPRODUCTIVE REGRESSION IN INBRED FEMALE LSH/Ss Lak HAMSTERS. M. H. Brown and G. N. Wade Dept. of Psychology, University of Mass., Amherst, MA 01003.

Exposure of female Syrian hamsters to short days results in cessation of estrous cyclicity, reduction of uterine weight, and increased body weight and carcass fat content. While outbred hamsters typically require 3-10 weeks to show these responses, Hauser and Benson (e.g., *Endocrinology* 120 239-246, 1987) have recently reported that inbred females of the LSH/Ss Lak strain show reproductive responses within 14-34 days of exposure to short photoperiod.

In the present experiment, we examined the time course of reproductive and metabolic responses of LSH hamsters to daily injections of melatonin. Two groups ($n=7$ each) of adult female LSH hamsters matched for baseline body weight were housed individually in a photoperiod of 16 hrs light: 8 hrs dark. One group received daily injections of 10 ug of melatonin (MEL) in 0.1 ml ethanolic saline 2.5 hrs before lights off while control hamsters received injections of the saline vehicle. Animals were inspected daily for the postovulatory discharge and body weight and food intake were recorded weekly. After 45 days of injections, uteri, parametrial white fat (PWAT), and interscapular brown fat (IBAT) pads were removed and weighed and carcass composition determined.

While all of the control hamsters showed estrous cycles throughout the experiment, all of the hamsters in the MEL group stopped cycling after 28-37 days of the injections. Mean body weight gain for the MEL-injected hamsters was significantly greater than for controls at weeks 3-6 of the injections. The animals that received MEL injections also showed significantly lower uterine weight and significantly higher carcass fat content than control hamsters. Wet weight of PWAT and IBAT did not differ significantly between groups.

These results indicate that LSH hamsters show rapid body weight and reproductive responses to exogenous melatonin. (Supported by NS 10873, DK 32976, MH 00321, and MH 09564.)

57 EFFECT OF RESONANCE MELATONIN INFUSION CYCLES ON GONAD, BODY AND LIPID MASS IN PINEALECTOMIZED MALE DJUNGARIAN HAMSTERS, Jeffrey A. Elliott, Timothy J. Bartness and Bruce D. Goldman, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

The goal of the experiment was to discriminate between two hypotheses regarding how the circadian rhythm of pineal melatonin production transmits photoperiodic information. The hypotheses considered were: 1) a circadian rhythm of sensitivity to melatonin regulates the hormone's effect, 2) the duration of the melatonin signal, rather than its circadian timing, is the critical parameter of the melatonin rhythm. The experiment examined the response of pinealectomized Djungarian hamsters to long (10h) vs. short (6h) duration melatonin (MEL) infusions (10ng/infusion) in cycles with periods of T=18, 24, 36 and 48h. After cannula implantation, animals were moved from 16L:8D to 10L:14D (lights 0500-1500 EST) and were attached via a swivel to a clock-driven syringe pump. Additional T=24h cycles included as controls were: 18h MEL, 18h saline (SAL), and 10h SAL infusions. In each treatment, the first infusion began at 1400 on the day of implantation. This timing was chosen to provide maximum temporal coincidence of the infusions with the circadian phase of enhanced melatonin sensitivity which Stetson and co-workers postulate is induced under short photoperiods. Body weight and food intake were measured every 2 weeks. After 6 weeks animals were killed, blood samples taken for RIA of serum FSH and PRL, and terminal body, paired testes, and epididymal white adipose tissue (EWAT) weights were recorded. 6h MEL infusions failed to induce short day effects regardless of the period (T) of the infusion cycle. In contrast, compared to SAL infusion, 10h MEL resulted in decreases in body, testes and EWAT weights in T24, but not in T36 or T48. In T18, testes, body and EWAT mass were decreased but not to the same extent as in T24. Similarly, 18h MEL (T=24h) was less effective as a short day stimulus than was 10h MEL. Thus, 6h MEL infusions were never effective as short-day stimuli even though T24 and T48 infusions always occurred during the proposed melatonin responsive phase, while in T18 they occurred at all circadian phases. 10h MEL infusions were effective in T18 and T24 but not in the longer T36 and T48 cycles. The effectiveness of 10h, but not 6h, MEL in T18 and T24 is consistent with the duration hypothesis and argues against the circadian hypothesis. Because the results suggest that a long duration melatonin signal must occur with a frequency close to once/day (i.e. T<36h) in order to elicit a short-day response, the experiment did not prove as strong a test of the circadian hypothesis as anticipated.

58 EFFECTS OF SCN LESIONS ON SEASONAL RESPONSES FOLLOWING SHORT DAY EXPOSURE OR MELATONIN INFUSIONS IN SIBERIAN HAMSTERS Timothy J. Bartness,* Bruce D. Goldman* and Eric L. Bittman** *Worcester Fndn. for Exptl. Biol., Shrewsbury, MA 01545 and **Dept. of Zool., Univ. Mass., Amherst, MA 01003

Photoperiod regulates the peak nocturnal duration of pineal melatonin (MEL) secretion in Siberian hamsters and controls a variety of seasonal responses. To determine if the SCN mediates photoperiodic responses in this, as in other species, DC SCN lesions (SCNX) were made in adult male Siberian hamsters. After 10 wks of short day exposure (LD 10:14), the % change in body weight (%BW), testes weight (in mg; TW) and epididymal white adipose tissue weight (in mg; EPIWAT W) of SCNX hamsters were similar to those of long day (LD 16:8) intact controls (6.4±1.1 vs 8.5±3.4, 1056.2±113.2 vs 797.1±19.9, 476.9±10 vs 546.2±63.7, respectively). Non-lesioned, short day-housed controls and hamsters with lesions sparing the SCN had decreased %BW (-9.54±4.5), TW (344.2±141.6), and EPIWAT W (403.8±71.4). SCNX hamsters had arrhythmic or very low levels of locomotor activity. To determine if the SCN is required as a target site for MEL-induced seasonal responses, long day-housed hamsters were pinealectomized, given SCN lesions, and implanted with s.c. catheters. MEL (10 ng/daily infusion) was infused for 5 wks at durations of 10, 8.5, or 5 h to mimic pineal secretory patterns of short, intermediate, or long days, respectively. Two 5 h MEL infusions separated by 2 h (5 x 2) and saline infusions (10 h) were also used. %BW, TW, and EPIWAT W were similar among MEL- 5, 8.5, 5 x 2 h, or saline-infused SCNX hamsters (11.2±1.7, 820.7±48.4, and 439±35.9, respectively). However, 10 h MEL-infused, SCN-intact and SCNX hamsters had decreased %BW (-6.2±2.8 vs -0.4±3.5), TW (185.4±30.6 vs 485.4±88.3), and EPIWAT W (421.9±86.9 vs 672±88.8), with the SCNX values intermediate between their SCN-intact counterparts and all the other groups. Some 10 h MEL-infused SCNX hamsters with complete lesions had regressed gonads, and others with largely spared SCN failed to regress. These data suggest the SCN are required for photoperiodic responses, but are an unlikely required MEL target site in this species.

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A CHRONOBIOLOGICAL STUDY ON EFFECT OF ZOTEPINE : EFFECT ON CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN RATS.
Tamotsu Minowada, Naoto Yamada, Kazutaka Shimoda, Tetsushi Tsujimoto, Toshiki Shioiri and Saburo Takahashi.
 Department of Psychiatry, Shiga University of Medical Science, Seta, Tsukinowacho, Otsu, Shiga, 520-21, Japan.

In order to investigate the effect of zotepine, which is known to be antiserotonergic and clinically antimanic agent, on endogenous circadian oscillator, blinded rats (n=11) were subjected to oral administration of the drug (17.6 mg/kg), and the free-running pattern of circadian rhythm of locomotor activity was examined. Although the amount of locomotor activity per day was reduced to 74.1 % level of baseline, the amount of water consumption was not. Nine out of 11 rats represented a shorter period of free-running rhythm during drug administration than the baseline level. When the period of free-running rhythm after drug termination was compared to that during its administration, six of 11 rats represented no change in the period, while two animals represented a shorter period and one represented a longer period after termination. These effects on the free-running period during and after drug administration may be related to its antiserotonergic effect.

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METHAMPHETAMINE-INDUCED INTERNAL DESYNCHRONIZATION IN RATS.
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In earlier studies we found that chronic Methamphetamine (MA), administered to the drinking water of rats, induced infradian rhythmicity (periods ranging from about 28-56 hours) while ultradian rhythmicity was reduced. The infradian rhythms were observed in body temperature, eating, drinking and wheel-running activity. These functions were all in phase with each other.

In this study we tested the dependence of the MA-induced infradian rhythms on the period of the LD cycle. Rats were initially placed in LD 11.5:11.5 (T=23 hrs). Body temperature, eating, drinking and wheel-running were recorded continuously and automatically. After all rats were entrained to the LD cycle, a MA solution (0.01% to females, 0.015% to males) was given daily as drinking water. After a few months, the light regime was changed to LD 12.5:12.5 (T=25 hr). A few months later MA treatment was stopped but the registrations continued. Periodogram analysis revealed circadian (the period of the LD cycle) as well as infradian rhythmicity during MA treatment. The infradian periods were not constant but showed phase-jumps at multiples of the circadian periods. Circadian rhythms of 46 hrs (in LD 23 hrs) and 50 hrs (in LD 25 hrs) were also observed. In some rats the temperature and the activity actograms showed both the infradian and the circadian patterns, a phenomenon resembling internal desynchronization as has been observed in humans.

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CHRONIC ADMINISTRATION OF TRICYCLIC ANTIDEPRESSANTS DAMPS DOWN THE CIRCADIAN RHYTHM IN MALE RATS.

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Department of Psychiatry, Shiga University of Medical Science, Seta, Tsukinowacho, Otsu, Shiga, 520-21, Japan.

Effect of chronic administration of clinically effective tricyclic antidepressants, Imipramine, Clomipramine, Desipramine on circadian rhythm in male rats was investigated. Chronic administration of Imipramine, Clomipramine or Desipramine for 12 days at dose of 20 mg/kg B.W./day (1400h i.p.) abolished circadian fluctuation of blood corticosterone release when the rhythm was entrained to 12-12 hr light-dark alteration (LD), while not at a dose of 2 mg. Normal circadian variation of blood corticosterone release was observed 7 days after termination of administration of Imipramine. Power spectrum analysis revealed that circadian fluctuation of locomotor activity was damped down during a period of Imipramine administration (20 mg/kg B.W./day at 1400h i.p. for 12 days) while the rhythm being either entrained to LD cycle or free-ran, and that ultradian components were increased under both of these two conditions. Change in free-running period was recognized in some rats during and after administration of Imipramine. These findings suggest that chronic administration or withdrawal of tricyclic antidepressants may have some effects on the endogenous circadian clock.

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CHRONIC LITHIUM EFFECTS ON RAT ACTIVITY RHYTHMS. D.L. McEachron, Karen Stewart, and Norman T. Adler. Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104

In a pilot study, male rats were placed in individual running wheel cages and exposed to a 12/12 light/dark cycle for approximately 21 days followed by exposure to constant dim red light for 10 days. Lithium carbonate was then introduced into the diet in steps to avoid flavor aversion. The concentration was 0.075% (by weight) for 5 days, followed by 0.15% for 5 days, followed by a final concentration of 0.3%. This concentration was achieved by July 16 and the determination of the periods was calculated using the single cosinor for the time between July 26 and Sept. 1. The mean values were 24.63 hours \pm 0.144 hrs. (sd) for the lithium-fed animals and 24.35 hours \pm 0.176 hrs (sd) for the control animals, a significant difference ($t = 4.14$, $df = 21$, $p < 0.001$). On Sept. 4, 1/2 of the lithium-treated animals were switched to the control diet and 1/2 of the control animals were begun on the lithium diet. By Sept. 14, all the lithium-fed animals were receiving 0.3% lithium carbonate by weight. Determination of the periods covered the time between Sept. 25 and Nov. 7. The subsequent analysis recognized the existence of four groups, lithium only, lithium switched to control diet, control switched to lithium diet, and control only. The results were (in hrs.): lithium only 24.40 \pm 0.29, a **decrease** from the previous value for these animals of 24.58 \pm 0.17, lithium switched to control 24.52 \pm 0.14, a **decrease** from the previous value of 24.68 \pm 0.12, control switched to lithium 24.42 \pm 0.29, an **increase** from the previous value of 24.39 \pm 0.14 and control only 24.45 \pm 0.13, an **increase** from the previous value of 24.31 \pm 0.21.

In summary, those animals who were exposed to lithium early in the experiment shortened their tau values during the later part of the experiment irrespective of whether lithium treatment was continued or not while those who were fed a control diet early lengthened tau regardless of whether a lithium diet was instituted or normal food was provided. These changes eliminated the significant difference between the lithium-fed and control groups. Plausible hypotheses include the possibility that there is an age-lithium interaction effect in which lithium loses its effectiveness with age or that the lithium effect is more complex than a simple lengthening of the free-running period.

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Is the phase shifting effect of triazolam on the hamster's circadian clock due to the acute increase in activity associated with drug treatment? C. Wickland, E. Wollnik, E. Turek, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208

Treatment with the short-acting benzodiazepine, triazolam, induces phase shifts in the circadian rhythm of locomotor activity of golden hamsters. Phase advances occur when triazolam (Tz) is injected at CT 6 (CT 12 = activity onset), and these advances are associated with an acute increase in locomotor activity that persists for several hours after the injection time. By using the "Automex II" system for measuring total locomotor activity, we have examined (1) the relationship between the amount of increase in activity and the magnitude of the phase shift, and (2) whether an increase in activity itself can phase shift the circadian clock.

Following an injection of 2.5 mg of Tz at CT 6, activity measurements were taken from that time until 6 hours later and compared to a corresponding period on the previous day. The level of activity on the control day was defined as 100%. A statistically significant increase in overall activity (median increase 1700%; $p < .01$) was observed, and there was also a significant increase in wheel running activity (median increase 950%; $p < .05$) for that same period. Injections of vehicle alone at CT 6 did not produce significant changes in activity level, and in most cases slightly decreased activity (median 28% of control). Phase advances ranged from 0 to 6 hours, and the magnitude of the phase shift was correlated with the amount of increase in activity ($r = .82$; $p < .05$).

Hamsters that had never had wheels in their cages received a 3 hour wheel "pulse" centered near CT 6, and activity levels were increased in all 5 animals tested (median increase 1570%; $p < .01$). However, while phase advances of 4 to 6 hours were observed in 3 of the animals, 2 did not phase shift at all, and there was no apparent correlation between increase in activity and magnitude of the phase shift.

These results indicate that an increase in locomotor activity may be associated with Tz induced phase shifts, but that increased activity alone will not necessarily result in a phase shift in the circadian clock regulating locomotor activity.

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TRIAZOLAM PHASE SHIFTS SQUIRREL MONKEY CIRCADIAN ACTIVITY RHYTHMS
R.E.Mistlberger, T.A.Haupt and M.C.Moore-Ede, Dept. Physiology, Harvard Medical School and Institute for Circadian Physiology, Boston, MA.

Acute administration of the benzodiazepines (BZDs) triazolam or diazepam can induce phase shifts in the activity rhythms of hamsters under free-running (non-entrained) conditions. The direction and magnitude of the phase shifts are phase dependent and the phase response curve (PRC) is similar to that for 2 or 6 h dark pulses presented to hamsters in constant dark. BZDs are used widely for their anxiolytic and hypnogenic properties but their effects on human activity rhythms are unknown. As a preliminary to human trials, we have explored the effects of triazolam on the activity rhythms of another diurnal primate, the squirrel monkey, with a monophasic sleep-wake cycle similar to humans.

Male squirrel monkeys (1 kg) were housed in isolation chambers in constant light. Each chamber was equipped with one or two perches. Movements on the perch were detected by microswitches and monitored by computer. Triazolam (.1-.2mg in DMSO vehicle) was injected intraperitoneally every 3-4 weeks. Sedation occurred rapidly and usually lasted 1-3 h. Phase shifts of 1-3 h magnitude occurred after 23 of 36 injections. The typical low precision of monkey activity rhythms largely precluded detection of possible smaller shifts. The PRC is similar to that observed for hamsters; phase advances occurred after injections in the latter half of the subjective day, phase delays after injections in the subjective night and early subjective day. Tau changes occurred on occasion. Nocturnal, polyphasic hamsters and a diurnal, monophasic primate appear to be similar in the response of their circadian systems to BZDs. Dose response trials are in progress.

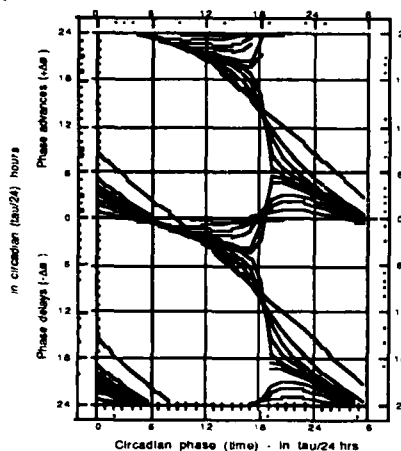
PHASE RESPONSE CURVES AS PREDICTED BY A CIRCULAR LIMIT CYCLE MODEL;
Van D. Gooch; Division of Science and Math, University of Minnesota, Morris, MN 56267

Several classical circadian rhythm experimental approaches are being simulated using a simple circular limit cycle model. In this particular work, the experiments resulting in phase shifts due to light, temperature, or chemical pulses have been investigated. There is no intention that the equations of this model represent kinetic equations of a biochemical circadian process. Rather, the intent is to see what features of experimental data can be represented by this simplest of models.

The equations of the model are in polar coordinates:

$d\Theta/dt = 360 \text{ degrees per } \tau \text{ hours}$ where Θ is the angle about the singularity at any given time and τ is the natural period length of the rhythm.

$dR/dt = \epsilon R (A - R)$ where R is the distance from the singularity at any given time, A is the natural radius of the limit cycle, and ϵ is a constant representing how fast the system returns to the limit cycle. In the dark the singularity is set at $x=0, y=0$, and $A=D$. In the light $x=-L, y=-L$, and $A=0.5*L+1$ where L is an arbitrary light intensity. The effects of light intensity (L) (see figure), light duration, and transient effects (ϵ) have been determined. The pros and cons of different ways of plotting pulse data is also considered.

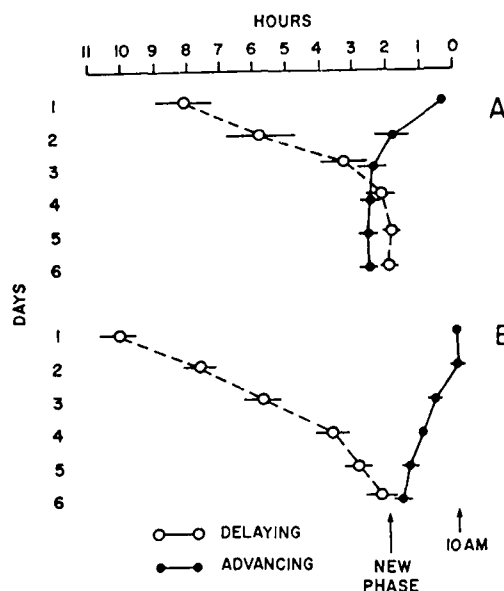


MULTISTABILITY OF CIRCADIAN ACTIVITY RHYTHMS IN BALB/c MICE: A WEAKLY COUPLED CIRCADIAN SYSTEM? Alan M. Rosenwasser, Department of Psychology, University of Maine, Orono, ME 04469

Inbred strains of laboratory mice differ in the expression of free-running circadian rhythmicity. Strain differences have been identified in free-running period as well as in the dependence of period on light intensity. In addition, strains may also differ in the stability or coherence of free-running activity rhythms. For example, it has been reported that BALB/c mice fail to sustain coherent rhythmicity under constant light, and that mice of the DDK strain display unusual intra-individual lability in free-running period and rhythm coherence under constant darkness. In the present study, male and female BALB/c mice of two different substrains, and female C57Bl/6 mice, were individually maintained in running wheel cages under constant dim light for extended observations. Animals of the BALB/c strain displayed dramatic, and apparently spontaneous, alterations in the expression of rhythmicity which included changes in free-running period, rhythm splitting, circadian arrhythmicity, and the emergence of dominant ultradian periodicities. Transitions between these various states of circadian organization and disorganization often occurred abruptly, and there was no trend towards increasing desynchrony with time in constant light. Rather, transitions to more coherent states occurred as often as transitions to less coherent states. As expected from the literature, C57Bl/6 mice displayed stable and persistent free-running rhythmicity under these conditions. These results indicate that certain strains of laboratory mice are characterized by naturally weak coupling relationships between component circadian oscillators. Such strains should prove valuable in subsequent genetic, physiological, and anatomical investigations of the multi-oscillatory nature of the vertebrate circadian system. (Supported by a Faculty Research Fund Award from the University of Maine.)

67 ADVANCING SCHEDULES AND CONSTANT LIGHT PRODUCE FASTER RESYNCHRONIZATION OF CIRCADIAN RHYTHMS, S.Binkley and K.Mosher, Biology Department, Temple University, Philadelphia, Pa. 19122

Perch-hopping rhythms of house sparrows, Passer domesticus, were recorded continuously while the sparrows were subjected to six-to-nine, once-a-week 8 hour advancing (closed circles) or delaying (open circles) phase shifts of 5 days of LD8:16; constant light (A) or dark (B) intervened between the shifted schedules; onsets of activity were determined during reentrainment. Most rapid resynchronization occurred when the LD8:16 was advanced and constant light intervened (closed circles, A). Sparrows were able to begin activity at lights-on of the first day of a shifted cycle when it was advanced; the anticipatory activity which normally occurs in LD8:16 was reestablished as early as the third day when constant light intervened between two schedules. The ordinate is days on new cycle; the abscissa is hours before lights-on of the new cycle; points represent the average activity onset for 5 animals plus/minus one SEM. Supported by NSF PCM8444352 and DCB8613594, Temple Grant-in-Aid, and Research Incentive Fund.



68 POST-EMBRYONIC DEVELOPMENT OF LOCOMOTOR AND SINGING RHYTHMS IN CRICKETS. W. Loher and D. Moore. Dept. of Entomological Sciences, University of California, Berkeley, CA 94720

Circadian rhythms in the calling song and locomotor (walking) activity of adult male crickets Teleogryllus commodus are well-known. Under LD conditions, singing is predominantly nocturnal and walking is mostly diurnal. One aspect that has received relatively little attention in this species is the change that occurs in circadian behavior following the imaginal molt (early post-embryonic life).

On the day of the imaginal molt (day 0), male crickets were housed in cages equipped with both a running wheel and a microphone, permitting the simultaneous monitoring of locomotor and stridulatory activity. Under LD 12:12, both behaviors began at extremely low levels but demonstrated a sharp, steady increase in activity over days 1-10, followed by a slower increase until about day 20, and thereafter a plateau at about 9 hr/day. Some crickets showed dynamic changes in activity phase positions. Several exhibited an abrupt switch from nocturnal to diurnal locomotor behavior, or vice versa. These locomotor rhythm reversals were accompanied by stridulatory reversals or phase changes in some cases but not in others.

Stridulation activity levels were measured in crickets after surgical removal of both optic lobes on day 0 (eliminating the bilaterally paired circadian pacemakers). Although rendered arrhythmic, all operated animals showed the steady development of activity levels seen in intact animals. Under LD 12:12 or LL, operated crickets exhibited a plateau level of about 8 hr/day; those kept in DD showed a plateau of about 14 hr/day. The activity levels were influenced through light detection from the ocelli, as controls (ocellar nerves cut in addition to bilobectomy) showed no activity level differences between LL and DD groups. These results suggest that the circadian clock has no effect on the development of the amount of behavior, but serves only to partition behavior into time-frames.

- 69 APPARENT INFRADIAN PERIODICITIES IN DIAPAUSE TERMINATION OF EMBRYONATED GYPSY MOTH EGGS ON EARTH OR AFTER LAUNCH, MICROGRAVITY AND REENTRY OF ORBITING SPACE LABORATORY. Hayes, D. K. and N. O Morgan, Livestock Insects Laboratory, Livestock and Poultry Sciences Institute, USDA, ARS, Beltsville, MD 20705.

The gypsy moth, *Lymantria dispar* (L.)(GM), of northeastern U.S., exhibits an obligatory diapause as an embryonated egg. This diapause is terminated in a major portion of the overwintering population only by 90 days of chilling of the fully embryonated eggs. In 1975 diapause was terminated prematurely in a small but significant fraction of GM eggs by exposing them to launch, microgravity and re-entry in Skylab IV (Bull. Entomol. Soc. Amer. 22:15, 1975). In a second test, G-470, in A Get-Away-Special (GAS) container on the space shuttle, Columbia, Flight 61-C, diapause was terminated in a significantly larger fraction of eggs exposed to launch, microgravity and re-entry than in ground controls. From 30 egg masses obtained from lab-reared insects, a total of 4153 larvae eclosed; from 10 egg masses collected in the wild in Maryland 454 larvae were obtained. In the lab-reared strain, maximum emergence of 8.1% of total emergence occurred at 17 days after opening the GAS can, while in the wild strain 2 major peaks, each 10.6% of total emergence, were observed 24 and 31 days after opening the GAS cannister. The average time between all peaks of eclosion was 3.9 days for the laboratory-reared strain and 3.6 days for the field-collected insects. The eclosion rate in ground controls was low, being 1 larvae or less over several days. The data suggest that the emergence pattern is probably not quite so regular in the ground controls as is that found in insects exposed to flight conditions in the GAS can. The unusual stresses which occur during space shuttle flights may influence responses of endocrine mechanisms as well as optimize rhythmicity and eclosion rates for gypsy moth eggs in diapause.

- 70 THE MARINE DINOFLLAGELLATE, *GONYAULAX POLYEDRA*, DISPLAYS A CIRCADIAN ACTIVITY RHYTHM WITH A FREE-RUNNING τ INDEPENDENT OF LIGHT INTENSITY. J. Woodland Hastings, Till Roenneberg and Grant N. Colfax Harvard University Biological Laboratories 16 Divinity Ave. Cambridge MA 02138

Like other phytoplankton, *Gonyaulax polyedra* exhibits diurnal vertical migration. We have investigated such movements in petri dishes illuminated from the side, using time lapse video recordings. At night, a 'lawn' of quiescent cells forms at the bottom of the dish. Before dawn, cells become active, rise and aggregate in motile, but structured formations below the surface. The exact pattern of these formations depends on shape and volume of the container; in petri dishes, cells form round or Maltese-cross-shaped clusters. Within these clusters, cells move downward in the highly dense center and rise up at the periphery. In addition to the vertical migration of the populations, which occurs independent of the light direction, cells show a light dependent orientation: the nightly 'lawn' forms as a crescent towards the light, the daily swarms situate themselves at a distance from the light, depending on light intensity, with clusters forming every day at the same location within the dish.

This phenomenon has proved to be most suitable for an analysis of the circadian properties such as limits of entrainment, phase-angles between rhythm and *Zeitgeber*, as well as investigating the dependence of the free-running period (τ) on different physical and chemical conditions. In constant light, free-running activity rhythms exhibit period lengths comparable to those of the circadian rhythm of bioluminescence. But whereas the τ of the bioluminescence rhythm depends on intensity, the free-running τ of the activity rhythm does not change significantly with light intensity. A possible explanation for these apparently contradictory results could relate to the possibility for the cells to selfselect intensity: Measurements of bioluminescence are made in small scintillation vials, illuminated from below, so cells are unable to selfselect light intensity by horizontal localization. In the set up used for measurements of the activity rhythm, light is provided from the side and *Gonyaulax* can selfselect light intensity during the day by horizontal displacement.

ILLUMINANCE-THRESHOLD FOR MAINTENANCE OF TESTES IN SYRIAN HAMSTERS (*MESOCRICETUS AURATUS*) IS HIGHER IN CONTINUOUS LIGHT (LL) THAN IN LD 14:10. C.E. McCormack, Dept. of Physiol., The Chicago Med. Sch., N. Chicago, IL 60064.

Elliott (p217, "Biol. Clocks in Seasonal Reprod. Cycles" Ed. B.K.&D.E. Follett, 1981) found that the illuminance-threshold for maintenance of testes in Syrian hamsters was higher in LL than in LD 14:10. To investigate this further, mature males (70 days old, LVG, Charles River) were exposed for 9 wk prior to autopsy to various illuminances of cool white fluorescent light. Each hamster was kept in an isolated activity apparatus, and illuminance was adjusted by inserting partially opaque screens below the light. In the table, LD refers to LD 14:10 (on 0500, off 1900); SK to skeleton LD 14:10 with lights on for 15 min at 0500 & 1900. The results show that the illuminance-threshold for testicular maintenance in LL is at least an order of magnitude higher than in LD or SK. Also, in the LD and SK groups, most hamsters which failed to maintain an entrained (Ent.) 24h locomotor rhythm (LR) underwent gonadal regression. These results show that for light signals to be maximally photoinductive, they must entrain circadian rhythms to 24h.

Illuminance (lux)*	n	LR tau** h±SE	Percent with			Wt of Testes (g/100g±SE)	(Supported by 1-R01-HD13131 & S07 RR05366-24)
			Ent. LR	Testes >2g	Split LR		
LL 300	13	24.67±.05	0	100	62	2.85±.10	
LL 30	7	24.67±.11	0	86	29	2.51±.30	*for this light
LL 3	15	24.44±.06	0	20	0	1.00±.22	1 lux = approx.
LL 0.3	16	24.25±.07	0	6	0	.65±.14	.29 μ W/cm ²
LD 3	7	-	100	86	0	2.65±.18	**measured prior
LD 0.3	6	-	50	50	0	1.34±.48	to splitting of
SK 3	8	-	88	88	0	2.61±.16	LR
SK 0.3	8	-	62	50	0	1.45±.43	
DD	19	24.13±.03	0	0	0	.35±.10	

INTERNAL SYNCHRONIZATION OF TWO DIFFERENT RHYTHMS DURING EXPOSURE TO LD CYCLES WITH DIFFERENT PERIODS (T) AE Jetton, S Losee-Olson and FW Turek, Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

At the present time, the hierarchical organization of the circadian system and the interrelationships between different circadian functions are poorly understood. Although many theoretical models of the circadian system are based on mechanisms involving multiple circadian oscillators/pacemakers, little is known about the coupling between these putative oscillators. In order to gain information about the coupling properties of two different circadian rhythms, we examined the circadian timing of the luteinizing hormone (LH) surge and of wheel running activity in the golden hamster during entrainment to different T cycles.

Females, individually housed in running wheel cages on LD 14:10, were ovariectomized and allowed to recover at least one week before being placed on one of the following T cycles in groups of 6: LD 2:21.3 (T=23.3); LD 2:22 (T=24.0); LD 2:22.75 (T=24.75). After three weeks on a given cycle the animals were surgically implanted with 4-5 mm estradiol silastic capsules and intraatrial cannulas during their light period. Beginning at 8 am CST two days after surgery, 0.5 ml blood samples were taken every 45 min. for the next 24 (T=23.3 and T=24.0 groups) or 24.75 (T=24.75 group) hours. Plasma was assayed for LH by RIA, and the LH surge onset was designated as the time at which LH values rose above 15 ng/ml (RP2 standard). An eyefit line through activity onset for the 10 days prior to cannulation was used to project activity onset on the day before, the day of and the day after blood sampling. Phase angle relationships were calculated for activity onset to light, LH surge onset to light, activity onset prior to the surge to LH surge onset and LH surge onset to activity onset following the surge. The Watson-Williams test was used to compare phase angle relationships between groups on different light cycles.

There were definite differences in the phase angles of each of the rhythms to light under the different T cycles, and the phase angle between the LH surge and activity onsets was significantly different on T=23.3 compared to T=24.0 ($p<0.05$). The difference in phase angle between the LH surge and the activity rhythms for T=23.3 and T=24.0 indicates that the phase relationship between different circadian rhythms is not fixed and that under different T cycles different phase relationships between internal rhythms can occur.

RELATIONSHIP BETWEEN FREE-RUNNING PERIOD AND MOTOR ACTIVITY IN BLINDED RATS

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Free-running rhythm of motor activity in blinded rats were measured by two different devices, an Automex or a running wheel. The period of the free-running rhythm measured by a running wheel was likely to be shorter than that measured by an Automex, indicating subtle environmental difference, whether a cage is equipped with a wheel or not, could affect the free-running period. In addition, we found negative correlation not only between the free-running period measured by a running wheel and that measured by an Automex, but between the free-running period and the number of revolutions per day.

This is the first evidence for that motor activity, other than the external factors such as light intensity and temperature, may be related to change in the free-running period.

RUNNING WHEEL AVAILABILITY ENTRAINS SLEEP-WAKE AND DRINKING CIRCADIAN RHYTHMS IN THE MOUSE. Dale M. Edgar, Thomas S. Kilduff and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94305.

The circadian control system modulates the regulated level of most physiological and behavioral variables and synchronizes their timing with the external environment. Several environmental factors are known to entrain circadian rhythms (light, food, social, etc.). However, it is unclear whether the circadian timekeeping system, like most physiological control systems, utilizes physiological or behavioral feedback in the process of entrainment.

In this study, we addressed this question by examining the influence of limited running wheel availability (wheels locked 12 hrs and free 12 hrs; LF 12:12) on the circadian timing of sleep-wakefulness and drinking rhythms in male mice (C57BL/6NNia; age 6-7 months). Each animal was housed in a separate cage equipped with a commutator and running wheel. Food and water were available ad libitum. Ambient temperature was 24 ± 1 °C. Animals were surgically prepared with a miniature chronic skull implant which permitted continuous EEG and EMG monitoring. Sleep stages were determined automatically with a validated sleep-wake bioassay system. Drinking and wheel running activity were also monitored with this system. Mice were entrained to a 24 hr light-dark cycle (LD 12:12) for several weeks prior to the initiation of constant darkness (DD). Automatic wheel locking control was enabled one day prior to DD, and was continued for at least 17 days. Wheels were locked at times corresponding to when lights were on in LD (8:00-20:00).

On release into DD, sleep-wake and drinking rhythms continued to exhibit 24 hr periodicity. Sleep-wake and drinking onsets advanced 30-60 minutes relative to the onset of running activity, and the daily interval of active behavior (alpha) increased by 1-2 hours in DD. Harmonic analysis showed no significant change in the acrophase of each variable 17 days after release into DD, and total sleep time was unchanged (approx 650 min/day).

These results suggest that the opportunity to run on a running wheel, or perhaps the kinesthetics afforded by this opportunity, provides temporal feedback to the circadian timekeeping system of the mouse.

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NON-PHOTIC EFFECTS ON CIRCADIAN RHYTHMS: WHAT ARE THE IMPORTANT VARIABLES?

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Non-phototic events are capable of affecting the circadian rhythms in a variety of ways: phase-shifting free-running rhythms, acting as entraining agents, and enhancing the rate of re-entrainment to phase-shifted light-dark cycles. With hamsters (*Mesocricetus auratus*), cage changing and social interaction with conspecifics are effective manipulations. However, these manipulations are complex in that they involve sleep deprivation, arousal, and motor activity. It is not known which of these aspects is the most important. The term non-phototic describes only by exclusion: it does not tell one which aspects of these manipulations are most important. New experiments bearing on this issue will be reported. Hamsters were placed in an unfamiliar running wheel for 3 hours, but the wheel was prevented from rotating. The results showed that wheel-running itself is not essential for enhancement of re-entrainment rate to shifted light-dark cycles. In another experiment, hamsters were kept in continuous darkness. There were individual differences in the phase response curves to bouts of induced wheel-running activity. The results showed that more than sleep deprivation is sometimes required to produce phase-shifts. Individual variability complicates the analysis of non-phototic effects but also offers a window through which to look at the phenomenon. (Support came from the Natural Sciences and Engineering Research Council of Canada).

NON-PHOTIC ENTRAINMENT OF ACTIVITY RHYTHMS WITH CORRESPONDING PHASE RESPONSE CURVES. Stéphan G. Reebbs, and N. Mrosovsky. Department of Zoology, University of Toronto, Toronto, M5S 1A1, Canada.

This poster will present four examples of entrainment of activity rhythms by non-phototic zeitgebers, with corresponding phase response curves (PRCs). The zeitgebers include daily cage changing, daily bouts of social interaction, daily bouts of sleep deprivation (all in Syrian hamsters), and daily acoustical disturbance (in house sparrows). The former two come from LL experiments, whereas the latter two come from DD experiments. During entrainment the stimuli coincided with part of the subjects' normal sleep time. No anticipatory activity preceded the daily stimuli. Not all animals entrained however. The four PRCs are similar in shape and amplitude (most shifts < 1 hr). In almost all cases, advances took place when the stimuli were given during the last 6-8 hr of the sleep period, and delays occurred when the stimuli were given during the first 4-6 hr of the sleep period. This is consistent with the phase angle difference observed between rhythm and zeitgeber during entrainment. A common effect of all of these zeitgebers, and probably of other social zeitgebers, may be part of the mechanism involved in non-phototic entrainment. (Financial support came from the Natural Sciences and Engineering Research Council Of Canada).

or

I CAN SEE A FISHY AND A TIGER AND A SAILBOAT AND A . . .

Richard E. Spieler Milwaukee Public Museum Milwaukee WI USA 53233

Meal-feeding can synchronize a host of daily rhythms in vertebrates. In many cases feeding is a more potent entrainer of the expressed rhythms than the light-dark cycle. Whether meal-feeding is entraining an endogenous oscillator responsible for the expression of the rhythms is, however, not clear and may be species dependent. Previous research has shown the locomotor activity of goldfish can be entrained by meal-feeding and will maintain a consistent phase angle relationship with the light-dark cycle during periods of starvation. Present research examined free running locomotor rhythms of feeding entrained goldfish on constant light or dark.

Juvenile goldfish (body weight approx. 6 gm) of mixed sexes were held in 18 aquaria (12 fish/aquarium) on a 12L: 12D photoperiod and fed once daily (2.5% body weight Biodiet). Fish were fed at 1600 CST, however, the onset of light was staggered amongst the aquaria so that the fish received the food at one of four subjective times-of-day: light onset, 6 h after onset, 12 h after onset or 18 h after onset. After 46 days on the feeding regime the fish were allowed to free run under constant light or darkness for 5 days. During this period the animals were not fed or disturbed and daily activity patterns were remote monitored (ultrasound).

The activity rhythm held similar phase relationships to the time-of-feeding for at least two days, regardless of the differing times of light onset. Thus meal feeding does, as a minimum, partially entrain an endogenous component of the circadian mechanism(s) involved in expression of the activity rhythm. The remaining 3 days of activity, however, differed considerably amongst, and within, the groups of fish. Depending on the group selected the data supports a variety of differing hypotheses, i.e., an endogenous oscillator system strongly entrained by feeding, an oscillator (or suboscillator) system weakly entrained by feeding, splitting and reemerging of differentially entrained aspects of circadian activity, etc.

ENTRAINMENT OF AGED, DYSRHYTHMIC RATS TO A RESTRICTED FEEDING SCHEDULE. E.C. Walcott and B. Tate-Ostroff. Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Aged female rats show abnormal activity rhythms. Entrained animals experience a loss in amplitude of the circadian rhythm and an overall increase in activity. An oscillator responsible for regulating these rhythms is believed to be light entrainable and located in the suprachiasmatic nuclei. The objective of this study was to examine whether a food-entrained oscillator, thought to be separate but linked to a light entrained oscillator, was still functional in these aged, dysrhythmic rats.

A group of 4-month old female rats showing normal circadian rhythms and a group of 16-18 month old dysrhythmic females were placed on a food restriction schedule (FR) for 14 days. Food was available for 2 hours during the light phase, 5 hours after lights on. Water was available ad lib. Following FR the animals were given food ad libitum for 3 days, and then deprived of food for 2 days. This ad libitum/deprivation schedule was then repeated. Activity was measured by telemetry.

Animals entrained to FR as measured by anticipatory activity on deprivation days whose onset occurred several hours before the time of food availability on FR. In several cases aged animals showed restoration of nocturnal activity patterns that were not present prior to entrainment to FR. Young animals entrained to FR as well, exhibiting an anticipatory bout of activity in addition to some loss of nocturnal activity late in the dark phase.

Our results support the conclusion that aged dysrhythmic rats possess an intact food-entrainable oscillator. This oscillator may have restorative properties enabling aged animals to entrain to food as well as to regain a nocturnal activity pattern. Further studies will investigate possible mechanisms for reinstating rhythmicity in the aged animal. Supported by NSF grant #DCB-8508187 and an AFAR grant to BTO.

- 79 AGE-RELATED CHANGES IN HAMSTER CIRCADIAN PERIOD, ENTRAINMENT AND RHYTHM SPLITTING. L.P. Morin. Department of Psychiatry, SUNY at Stony Brook, Stony Brook, New York 11794

Age has not been sufficiently explored as a variable which can influence circadian periodicity. The present experiments 1) reevaluated changes in hamster circadian period with age while controlling for access to wheels; 2) studied whether the limits of entrainment differ between young and old animals; and 3) examined whether age influences the probability of rhythm splitting under prolonged constant light conditions. The circadian wheel-running period shortened with age, generally regardless of prior wheel access. Access to wheels was associated with increased weight gain. Animals with prolonged access to wheels died younger than animals without wheel access. While equal proportions of young and old hamsters entrained equally to all T cycles used, loss of entrainment to long T cycles was more rapid for old than for young animals. About 60% of young animals showed rhythm splitting compared to 7% of old animals. The data are consistent with the observation that the circadian rhythm period of the male hamster shortens with age.

Supported by grant AG05773

- 80 SHORT TERM RHYTHMS AS A CONTROL FOR CIRCADIAN RHYTHMS IN THE MEADOW VOLE, MICROTUS PENNSYLVANICUS. Dr. Lucius L. Stebbins, Department of Biological Sciences, The University of Lethbridge, Lethbridge, Alberta, Canada.

Activity of Microtus pennsylvanicus, the meadow vole, were studied under natural meteorological conditions between August 1 and December 10, 1977 at Lethbridge, Alberta, Canada 49° 42' N, 112° 50' W. Basic characteristics of circadian rhythms, including definition and timing of daily peaks of activity, daily levels of activity, synchrony of activity among test animals, and seasonal changes in characteristics were all controlled by the basic nature of the short-term rhythms. Detailed analysis of these showed they provided the framework for the occurrence of all activity and that changes in their internal structure controlled occurrence and daily and seasonal adjustments of circadian rhythms. Duration and timing of short active periods are suggested as the most important controlling elements of circadian rhythms.

TRANSGENIC MICE WITH A GENOME FOR HUMAN GROWTH HORMONE (hGH) HAVE SHORTER FREE-RUNNING PERIODS THAN NON-TRANSGENIC SIBLINGS. Thomas E. Wagner, Andrzej Bartke, Richard W. Steger and James S. Ferraro, Edison Animal Biotechnology Center, Ohio University, Athens, OH 45701 and Dept. of Physiology, Southern Illinois University/ School of Medicine, Carbondale, IL 62901.

A hybrid strain of mice (C3H/C57BL6) had its genome altered by the insertion of a 1.9Kb DNA fragment, which codes for hGH and is regulated by a metallothionein promotor. Mice which have this segment incorporated into their genome (transgenic) have substantial levels of plasma hGH (2.9 ± 0.2 ng/ml), which results in mice that are much larger than normal (from hypersomatotropism; 37.36 ± 1.19 and 25.50 ± 0.89 grams respectively; $P < 0.00001$). Transgenic mice have a reduced reproductive fitness, probably the result of inadequate luteal function due to suppressed endogenous prolactin (i.e., there is an increase in median eminence dopamine turnover). Normal and transgenic female mice were exposed to constant dark (DD) or constant light (LL) for durations exceeding 2 weeks. The locomotor activity was continuously monitored on Apple computers. The acrophases of sine waves fit to the data were used to draw a linear regression line used in the determination of the period (τ). The transgenic and non-transgenic mice free-ran in LL and DD with a τ_{DD} of 23.5 ± 0.2 and 23.7 ± 0.1 and a τ_{LL} of 25.4 ± 0.2 and 25.9 ± 0.2 respectively. The free-running period was shorter for the transgenic mice in both LL and DD; however, the difference was significant only in LL ($P < 0.01$). At present it is not known if the decreased period in transgenic mice is due to direct somatotrophic (or lactogenic) effects or due to secondary effects, such as alterations in steroid content. Supported by NIH Grants HD20001, HD09042 and NS23128.

SKELETON PHOTOPERIODS SYNCHRONIZE THE ERG CIRCADIAN RHYTHM IN CRAYFISH. Enrique Moreno-Sáenz, Francisco Gutiérrez-Zepeda and Beatriz Fuentes-Pardo. Depto. de Fisiología, Facultad de Medicina, UNAM. 04510 México, D.F. México.

The fact that alternate light-darkness (L:D) cycles (complete photoperiods, PPc) of different photofractions, with the same total period ($T=24$ hs), can induce synchronization in the ERG circadian rhythm in crayfish (data obtained in our laboratory) raises the possibility that two short light signals instead of the total photofraction (skeleton photoperiod, PPs), be able to entrain the oscillators responsible of the ERG circadian rhythm. To test this hypothesis, the ERG from adult crayfish Procambarus digueti, of either sex, housed individually in a constant temperature chamber, was recorded by means of conventional procedures for periods up to 10 days. The PPc used were: 4:20, 8:16, 11:13, 12:12, 13:11, 16:8 and 20:4. The PPs consisted in two light signals, one hour duration each, applied at the beginning and at the ending of the equivalent PPc. In a first experimental group, the animals were initially submitted to free run, then stimulated with PPs and finally returned to free run. In a second group, the crayfish were stimulated with different PPc (the first three days), then stimulated with the corresponding PPs (from 4th to 7th day) and later left under constant environmental conditions (from the 8th day to the end of the record). The results indicated that the ERG circadian rhythm oscillators were synchronized to all the PPc and to all the PPs applied, except those PPs corresponding to PPc 16:8 and 20:4, i.e. when the time elapsed between the two light signals, determining the night length, was too short ($D \leq 8$ hs), the crayfish showed a change in the phase of the ERG rhythm (jump), which signified the inversion of both subjective day and subjective night. These results imply that, under some of the PPs applied, the crayfish, as many other species, tries to increase its subjective night to a minimum length.

This work was supported by a grant from Ricardo J. Zevada Foundation.

- 83 EFFECT OF LIGHT ON THE ERG CIRCADIAN RHYTHM DURING THE COURSE OF THE CRAYFISH ONTOGENY. Beatriz Fuentes-Pardo, Ma. Luisa Fanjul-Moles and Enrique Moreno-Sáenz. Depto. de Fisiología, Facultad de Medicina, UNAM. 04510 México, D.F. México.

Light is known to affect circadian rhythms in a variety of ways, ranging from phase shifts, when applied as a single pulse, to changes in the circadian period, when is turned on periodically. In this work, we analyze the phasic response to single light pulses and synchronization to light: dark cycles (L:D 12:12) in the amplitude rhythm of the electrical response to light (ERG) in adult and juvenile crayfish forms aged from 1 day to 8 weeks. The experiments were conducted crayfish *Procambarus clarkii*, of either sex. The ERG was recorded by conventional procedures for up to 8 days from crayfish placed in a constant temperature chamber. Single light pulses or alternate L:D cycles were externally controlled and their effects on amplitude, phase and period of the ERG rhythm was measured. The results showed that the ERGs from the youngest crayfish (1 day to 2 weeks) have only ultradian rhythms (τ = 0.5 to 4 hs) and, therefore failed to follow the light signals for the post-stimulation period, regardless of whether light was applied as a single pulse or as L:D cycles. Crayfish aged between 2 to 4 weeks show ultradian rhythms superimposed on an irregular, inverted circadian rhythm which showed only phase advances when the light pulse was applied in the subjective night. At this age, the crayfish followed the L:D cycles; however, when these last were suspended, and the animal was returned to constant conditions, it failed to "remember" the previous signals. Later on, the circadian rhythm expected from a nocturnal species appears. Finally (up to 8 weeks), ERG displayed the main properties of a circadian system; i.e., the amplitude was rhythmic under constant environmental conditions (free run), showed advances or delays, depending on the circadian time of the light application, and it was synchronized to the L:D cycles (entrainment). These results support that the ERG circadian rhythm emerges from ultradian oscillators along different developmental stages until the crayfish reaches the adult age and that, during the same time in ontogeny, their main properties appear. Granted by Ricardo J. Zevada Foundation.

- 84 SEROTONIN BLOCKS LIGHT-INDUCED PHASE DELAYS BUT NOT ADVANCES C.S. Colwell
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Experiments were undertaken to examine the hypothesis that serotonin (5-HT) can act as a neuromodulator of the entrainment pathway in *Aplysia*. The experiments reported here suggest that light-induced phase delays but not phase advances can be blocked by 5-HT. Further light-induced phase advances can be enhanced by giving a 5-HT pulse immediately after the light pulse. The effects of light and serotonin do not appear to be additive.

The phase shifts induced by 6 hour treatments of light (200lx), 5-HT (10⁻⁵M), and light in combination with 5-HT were determined. These treatments were given at two time points: one during Ct 6-12 when light causes a phase delay of 1.37 hr (\pm 0.1, n=6) and one during Ct 18-24 when light causes a phase advance of 2.64 hr (\pm 0.2, n=7). Serotonin produces an advance of 1.66 hr (\pm 0.2, n=6) at Ct 6-12 and a 1.5 hr (\pm 0.2, n=6) delay at Ct 18-24. Serotonin plus light at Ct 6-12 results in no phase shift (0.08 hr, \pm 0.2, n=6) while at Ct 18-24 light plus serotonin results in a 3.56 hr (\pm 0.3, n=6) phase advance. This phase advance is not distinguishable from a phase shift induced by light alone. However, a light pulse followed by a 5-HT pulse produces a significantly larger phase advance of 6.06 hr (\pm 0.5, n=4). These results suggest that the phase shifts produced by light and 5-HT are non-additive.

Previous work has focused on 5-HT as an entraining agent and used it as a probe of the circadian system in the *Aplysia* eye. Although the function of 5-HT in this system is unknown, current results suggest that 5-HT may be involved in modulation of circadian responses to light in *Aplysia*.

A COMPARISON OF THE CIRCADIAN OSCILLATORS IN ACETABULARIA AND THE EYE OF APLYSIA.

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The effects of a number perturbations on the circadian rhythm (CR) of the rate of compound action potentials (CAPs) in the eye of the mollusc, Aplysia and the circadian migration of chloroplasts in the green alga, Acetabularia are compared. It has been shown that a rather small dose of x-rays (about 6 kRad) immediately stops the circadian modulation of CAP rate without having much effect on other eye functions (Proc. Natl. Acad. Sci. USA 77, 5542) implying some rather large structure(s) may be involved in generating the CR. In contrast, a quite large dose of x-rays (about 100 kRad) are required to stop the CR in Acetabularia. Even at this dose, the CR persists for as much as 6 or 7 days. In addition, the effects of a number of mutagens/carcinogens on the two circadian oscillators have been measured. Ethidium bromide which binds to nuclear DNA and uncoils mitochondrial DNA lengthens the circadian period in the Aplysia eye but has no effect on the period (or amplitude) of the CR in Acetabularia. Acriflavin and N-methyl-N'-nitro N-nitroso guanidine inhibit the CR in the Aplysia eye without affecting the size or average number of CAPs. These agents when administered in a similar way to Acetabularia have no effect on its CR. These results may imply the involvement of nucleic acids in the generation of the CR in Aplysia but seem to suggest that the mechanism for generating the CR in Acetabularia may be quite different.

SPECTRAL SENSITIVITY OF THE BULLA EYE. Michael Geusz and Terry Page. Dept. of General Biology, Vanderbilt University, Nashville, TN 37235.

The basal retinal neurons (BRNs) of the eye of the mollusk Bulla gouldiana are believed to (1) generate the circadian rhythm in compound action potential (CAP) frequency in the optic nerve (2) contain biochemical processes comprising the circadian pacemaker and (3) act as photoreceptors functioning in entrainment. To further examine BRN photoresponses and to identify the pigments involved in transduction, spectral sensitivity measurements were made of isolated eyes in culture. The latency of the first CAP following the onset of a 0.6 second light pulse was used as a reliable measure of response to monochromatic light at 6-8 wavelengths between 452 and 620 nm and over a 3 log unit intensity range. Eyes were dark adapted for 10 minutes between intensities and for 30 minutes between wavelengths. When eyes were tested during the subjective day (n=4) or subjective night (n=4) the resulting action spectra indicated a single rhodopsin absorbing maximally near 490 nm was sufficient to mediate the BRN photoresponse. To examine the response with the BRNs isolated from chemical synaptic input, the spectral sensitivities of eyes in a high Mg^{++} /low Ca^{++} artificial seawater were found during the subjective night (n=4). Also to isolate the BRNs, spectral sensitivities of eyes surgically reduced (lens and photoreceptor layer removed) were found during the subjective night (n=4). In both treatments the action spectra were indistinguishable from those of intact eyes in normal ASW. Supported by NIH NS 15264.

87 INVOLVEMENT OF PROTEIN SYNTHESIS IN THE CIRCADIAN REGULATION OF MELATONIN PRODUCTION IN CHICK PINEAL CELLS. N. Murakami and J. S. Takahashi. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208

Dissociated chick pineal cells express a circadian oscillation of melatonin release with peak values occurring in the night. To determine whether protein or RNA synthesis is involved in the regulation of the nocturnal increase in melatonin, several inhibitors of 80S ribosomal protein synthesis or RNA synthesis were added to cultured cells maintained under LD 12:12 or constant darkness. Treatments for 12 hrs either during day or the night were examined first. Anisomycin and cycloheximide inhibited the nocturnal melatonin increase in a dose-dependent manner, with more potent inhibition occurring with night time treatment. This dose-dependent inhibition by anisomycin was correlated with the inhibition of overall protein synthesis as assessed by incorporation of [³⁵S]methionine into TCA precipitable protein. Actinomycin D and α, β -amanitin also inhibited melatonin production, however, with RNA synthesis inhibitors, more potent inhibition was observed with day time treatment.

In the detailed experiments using either 3 or 6 hr pulse treatments with inhibitors of protein or RNA synthesis, a 24-hr rhythm of sensitivity to these inhibitors was found. Maximal sensitivity of melatonin synthesis to inhibitors of protein synthesis occurred during the first half of the dark period; whereas, maximal sensitivity to RNA synthesis inhibitors occurred earlier near the light-dark transition period. The phase-dependent effects of these inhibitors were observed in pineal cells maintained in either LD 12:12 or constant darkness.

These results suggest that transcriptional and translational processes may be involved in the circadian regulation of melatonin production in chick pineal cells. (Supported by NIMH grant MH39592, Searle Scholars award 85-H-107 and NSF PYI award DCB-8451642)

88 CYCLIC AMP ELEVATES MELATONIN PRODUCTION BUT DOES NOT PHASE SHIFT THE CIRCADIAN OSCILLATOR IN CHICK PINEAL CELLS. S. S. Nikaido and J. S. Takahashi. Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

Dissociated chick pineal cells express a circadian oscillation of melatonin release in vitro. In chick pineal cell cultures, agents that elevate cAMP cause increases in melatonin production. Analogues of cAMP (8-Br-cAMP and 8-(p-chlorophenylthio)cAMP) are potent stimulators of melatonin synthesis; whereas, analogues of cGMP and other related nucleotides are ineffective. Forskolin, an activator of adenylate cyclase, and 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase, also stimulate melatonin synthesis. The pharmacology of this response strongly suggests that melatonin production in the chick pineal is a cAMP-dependent process. In addition, the level of cAMP oscillates with a 24-hour rhythm which is correlated with the rhythm of melatonin release. All of these results suggest that cAMP may be involved in the regulation of either: 1) the output from the circadian oscillator, or 2) the circadian oscillator itself.

To test whether cAMP may be a component of the circadian oscillator, we determined whether pulses of 8-Br-cAMP would cause phase shifts of the circadian rhythm of melatonin release. Six-hour pulses of 1 mM 8-Br-cAMP administered at 8 different phases did not cause detectable phase shifts. However, treatment with 8-Br-cAMP during the subjective daytime did elevate melatonin levels. Thus, treatments with 8-Br-cAMP are effective in stimulating melatonin production, but do not phase shift the circadian rhythm. These results suggest that cAMP may be involved in relaying the output of the circadian oscillator, and that cAMP does not appear to be a component of the circadian pacemaking system.

(Supported by NIMH grant R37 MH39592, Searle Scholars award 85-H-107, NSF PYI award DCB-8451642 to JST and NIMH grant F31 MH09572 to SSN.)

CIRCADIAN RHYTHM AND AROUSAL - EFFECTS OF SEROTONINE ON OSCILLATORY AND NON-OSCILLATORY PROCESSES IN THE SCORPION

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In earlier papers we presented an octopaminergic efferent fiber system which is closely associated with the visual pathways and conducts circadian information to the eyes. In addition a well-developed system of 5HT fibers in the brain of the scorpion intensively innervates the optic ganglia and the central body. There is evidence that the 5HT fibers are good candidates for a component presynaptic to the 'circadian' octopaminergic efferent fibers.

Pharmacological and electrophysiological data suggest the participation of serotonergic fibers in circadian information processing in the scorpion. 5HT applied to the hemolymph is able to simulate night state sensitivity in the eyes. Arousal can enhance the visual sensitivity up to the same level. Experiments in which those pharmacological and behavioral stimulations were combined with central lesioning of the efferent aminergic fibers demonstrate that 5HT and arousal only share the final section of their pathway to the eyes. Lesions proximal to the optic neuropils interrupt the arousal reaction in the eyes but not the 5HT effect. Arousal most likely is processed in the central body and seems to be switched over to the octopaminergic efferent fibers either directly within the central body or by serotonergic components at the level of the optic neuropils whereas 5HT applied via the hemolymph has access to those octopaminergic fibers at the level of optic neuropils through intracerebral fluid.

90 CREATINE SHORTENS THE CIRCADIAN PERIOD IN *GONYAULAX POLYEDRA* Till Roenneberg, Hideshi Nakamura and J. Woodland Hastings Harvard University Biological Laboratories 16 Divinity Ave. Cambridge MA 02138

The period (τ) of free-running circadian rhythms in the unicellular marine dinoflagellate *Gonyaulax polyedra* can be shortened by extracts of eucaryotic tissues and cells (including *G. polyedra*). This effect depends both on the concentration in the medium and on the cell density of the culture. Under conditions of constant blue or white light the rhythm is accelerated up to 4.5 hours per day, whereas the extracts have no effect under red light. The τ -shortening extracts affect equally different expressions of the circadian rhythm: the two rhythms in bioluminescence (glow and flashes) as well as in the behavioral rhythm of activity (see separate abstract: Hastings, Roenneberg and Colfax).

The effect of the extracts is associated with a single substance which we have isolated from bovine muscle and identified as creatine (by help of FAB mass-spectroscopy as well as ^1H - and ^{13}C -NMR). The effects of authentic creatine on the circadian period at micromolar concentrations are identical with those of the extracts. In addition to shortening τ , creatine increases the daily levels of activity.

(Phospho)creatine is known as a phosphogen in vertebrates and some invertebrates. Recent studies show that this molecule is important not only for intracellular storage of chemical energy but functions as an energy-shuttle molecule between mitochondria and sites of energy consumption. In vertebrates creatine is taken up by cells against a concentration gradient, presumably via specific ports and uptake experiments suggest that the same is true in *Gonyaulax*. The finding that a molecule known for its role in the maintenance and intracellular distribution of energy charge has a substantial effect on the free-running period of circadian rhythms suggests that the molecular mechanism responsible for these daily oscillations may be related to the cell's energy metabolism.

TITLE: Biological Clock Mutations Alter Intercellular Junctional Communication
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Summary: Mutations of the per locus of Drosophila modify expression of ultradian and circadian behavioral rhythms. Biochemical analyses have shown that the product of the gene is a proteoglycan associated with cell borders. The level of production of per protein can be modulated by genetic transformation. The level of per made in transformants is correlated with the pace of the biological clock. Higher levels of the gene product generate shorter period locomotor activity rhythms. In adults per transcripts and proteins are generated in discrete regions of the brain. However, in embryos per is most abundantly expressed in developing salivary glands. Formation of the protein persists in salivary glands through the larval instars. We have examined several aspects of the physiology of isolated salivary glands in per mutants with the results that striking effects on gap junctional communication have been discovered. The behaviorally arrhythmic mutant per⁰ shows lowest levels, and per^S highest levels of junctional communication as assessed by dye permeability and measurement of electrical conductances between cells. Glands derived from genetically transformed larvae show levels of conductance and dye permeability that are correlated with both periodicity of their behavioral rhythms and abundance of per protein produced. It is proposed that per mutations affect biological rhythms by altering the conductance of electrical synapses in pacemaker tissues of the Drosophila brain.

CIRCADIAN CHANGES IN PROTEIN SYNTHESIS AND OXYDATIVE METABOLISM IN THE HAMSTER AND THE MINK SUPRACHIASMATIC NUCLEI. SERVIERE, J., MARTINET, L. & BONNEFOND, C.
 Dept. Physiologie - INRA - 78350 Jouy-en-Josas - France.

Metabolic correlates of SCN circadian mechanisms can be described by the fluctuation of either glucose consumption or amino acid incorporation in newly synthesized proteins. It has also been demonstrated that amino acids have a neuromodulatory effect on the regulation of circadian phase. Nevertheless according to the type of amino acid used (lysine or leucine) an hypothalamic rhythmic component of protein synthesis was not always reported. This contradictory data prompted us to use methionine. The present study addresses four questions; (1) Does methionine uptake in the SCN exhibit a circadian fluctuation. (2) What is the phase relationship between the rate of glucose utilization and the rate of protein synthesis. (3) Are these patterns of SCN metabolic fluctuations identical in the Mink (a short day breeder) and the Hamster (a long day breeder). (4) Does melatonin affect protein synthesis activity within SCN. To address questions n°1, 3 & 4 we used a simplified version of the quantitative protein synthesis method of Lestage et al. (1987). Animals were entrained under LD16:8, put under DD during the last 24h cycle. Then they received either 2h before or 2h after subjective dusk and intra-cardiac pulse of L-(35S) methionine (600uCi/Kg). For experiments related to (4) animals received a subcutaneous injection of melatonin (1mg/Kg) 15 min before methionine injection. After 90min the animals were decapitated, brains removed, frozen at -60°C, cut at 20um in a cryostat, the tissue was then exposed on SBfilms Kodak. (1) SCN radioactive labelling tends to be higher at night than during the day; if confirmed, this pattern would be reverse to the one reported using different amino acids. (2) Glucose consumption is higher during subjective day. (3) Initial data obtained on the Hamster after combined injections of melatonin and methionine indicate that animals injected during subjective day have a methionine uptake similar to the one observed at night. Such a result would indicate that melatonin increases protein synthesis rate within SCN. Further experiments and optical density measurements are in progress on Hamster and Mink (4).

OLFACTORY BULBECTOMY LENGTHENS TAU(DD) IN MICE

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Bilateral olfactory bulbectomy in SWR mice lengthened tau(DD) for wheel running activity and reduced the proportion of total activity occurring during the dark phase of a 12:12 LD cycle. The free running period in constant darkness during 25 consecutive days following release from a 12:12 LD cycle was $23.89 \pm .05$ (SEM) hr in 12 bulbectomized mice versus $23.17 \pm .10$ hr in 12 sham operated controls. The proportion of total activity during the dark phase of the 12:12 LD cycle was $0.74 \pm .04$ (SEM) for bulbectomized mice compared to $0.83 \pm .03$ in controls. No significant difference in phase of activity onset in the 12:12 LD cycle was observed. These results indicate a role for the olfactory bulbs, or neural pathways through them in regulating circadian rhythms in mice.

MELATONIN ADMINISTRATION CAUSES PHASE SHIFTS IN FREE-RUNNING BLIND PEOPLE

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Melatonin has been shown to entrain the activity rhythms of free-running rodents and lizards. In this study we tested the effects of daily oral melatonin administration (5 mg) on the free-running endogenous melatonin rhythms of totally blind people.

Methods: Five men (average age 35.4 years) who were in good health but without any light perception, had blood samples drawn hourly every one to two weeks. Plasma melatonin was measured by a highly specific and sensitive gas chromatography-mass spectrometry method. The onset of melatonin production was used to determine phase of the melatonin rhythm on each sampling day. The period of the melatonin rhythm was determined by fitting a regression to the successive melatonin onsets.

The melatonin trial was single-blind and placebo-controlled; the study drug was always taken at bedtime (2200). Throughout the study, the subjects had full access to social cues; most were regularly employed and kept conventional hours for sleeping and waking.

Results: All five subjects had free-running melatonin rhythms during the pre-treatment period. Cortisol was also measured in four of the subjects and found to be free-running and phase-locked to the melatonin rhythm. The free-running melatonin rhythm was found to be very stable and precise.

After three weeks of treatment, phase advances were observed in four of the five subjects ranging from 3 to 12 hours. Following treatment, rhythms reverted to the previous free-running period. Cortisol rhythms advanced in parallel with melatonin rhythms. There was no apparent phase delay effect of melatonin. Melatonin was well-tolerated by all the subjects.

Recent studies have shown that bright light treatments are effective in reducing the symptoms of patients with nonseasonal major depressive disorders, as well as the selected group of patients with winter depressions. Preliminary results suggest that 1 week of treatment with evening (2000-2300) bright (>2000 lux) white light may be as effective among inpatients as 4 weeks treatment with standard antidepressants. It is desirable to determine the chronobiologic mechanism of action of light in order to optimize the treatment pattern. Possible mechanisms to be considered include both external coincidence and internal coincidence photoperiodic mechanisms (which might involve phase advance or phase delays), abnormalities of the amplitude, duration, or phase timing of the nocturnal melatonin elevation, a photoperiodic window model (as in the Turkish hamster), an insufficient light model, and a model suggesting an inadequate amplitude for circadian rhythms. The consideration of these models presents an exciting opportunity for interchange between clinicians and basic scientists.

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SEASONAL CHANGES OF MOOD AND BEHAVIOR IN THE GENERAL POPULATION

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In a recent telephone survey (random digit dialing, completion rate 92%, $n = 416$) of the residents of Montgomery County (Maryland) we found that 92% of the population notice seasonal changes of mood and behavior to varying degrees. These changes included energy (66% of the population), mood (63%), social activity (60%), weight (47%), appetite (47%) and sleep (42%). Sleep length was significantly ($F = 35.7$, $p < 0.0001$) different in the seasons with the longest time in winter (7.41 ± 1.2) and the shortest in summer (7.05 ± 1.2). 68% of the population reported seasonal changes in food preference, with 81% reporting a preference for starchy foods and 66% a preference for sweet foods in the winter. 94% of the population expressed a preference for high-protein foods in the summer months. Inquiries into sensitivity to weather revealed that 77% of the population reported feeling worse in humid weather, 58% on grey, cloudy days, 48% in hot weather, 47% on short days and 43% in cold weather. For 27% of the population these changes were a problem and approximately one third (8% of the total population) of this group rated it as marked, severe or disabling, a degree of impairment equivalent to that of patients with *seasonal affective disorders* (SAD). The seasonal pattern of "feeling worst" revealed a bimodal distribution with a greater winter and a substantially lower summer peak (relation 4:1).

It is apparent from our study that SAD represents the extreme end of the spectrum of seasonality that affects a large percentage of the general population. Several studies have shown the antidepressant effects of bright light for individuals with SAD and SAD-like symptoms (subsyndromal SAD). The influence of environmental factors on mood disorders and mood changes in the general population might provide valuable insight into pathogenesis, treatment and prevention of affective illness. The results of our study will be discussed in relation to mechanisms by which mood changes are triggered and sustained and at times cured by changes in the physical environment.

97 CIRCADIAN RHYTHM IN CORTISOL SECRETION IS NOT DISTURBED IN OUTPATIENTS WITH A MAJOR DEPRESSIVE DISORDER.

David B. Jarrett and Joel B. Greenhouse

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Disturbances in pituitary hormone secretion have frequently been reported in depressed patients. Increased secretion in the hypothalamic-pituitary-adrenal axis results in a reduced amplitude in the circadian rhythm of serum cortisol concentration. It has been suggested that this abnormality is associated with an alteration in the phase and period of the rhythm consistent with a disturbance in circadian regulation. This hypothesis was examined in 12 depressed outpatients and 13 healthy subjects who were studied in a protocol in which blood samples, collected every 20 minutes for 48 hours, were assayed for cortisol. There was no statistically significant difference in the mean cumulative amount of cortisol secreted between these groups in each circadian period (patients = 65 ± 17 , 63 ± 17 ; controls = 58 ± 22 , 52 ± 24 ng/ml). The mean minimum serum cortisol concentration was not statistically different between groups (patients = 16 ± 11 , 14 ± 14 ; controls = 18 ± 11 , 16 ± 9 ng/ml). A repeated measures ANOVA showed no statistically significant difference in the amount or pattern of cortisol secreted in each 24 hour period (patients $F=0.05$, $p=0.83$; controls $F=2.61$, $p=0.15$). The serum cortisol data for each subject was also analyzed using a statistical model which included higher order harmonics as well as ARMA models for the errors. This model eliminates noise by producing, for each subject, a smooth curve that represents the systematic component of the circadian variation in the serum cortisol concentration. This time series model was applied to each series of data and the estimates of the parameters were determined based upon individual and intersubject variability (using a random effects model) in each group. The smooth curve for each subject varies about a population average which was used to determine the amplitude, phase and period for the data from each group. Using this model, no statistically significant difference in the circadian pattern of cortisol secretion was found between the depressed and control subjects. These data are inconsistent with a "phase advance" hypothesis for depressive illness and indicate that the circadian regulation of cortisol secretion is preserved in these depressed outpatients.

98 PRESERVATION OF THE ULTRADIAN SLEEP CYCLE IN DEPRESSED PATIENTS

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Human sleep is characterized by an ultradian rhythm of alternating Non-Rapid Eye Movement (NREM) and Rapid Eye Movement (REM) sleep episodes. Depressed patients characteristically have a short first NREM sleep episode and, less consistently, a long first REM episode. However, it is not known whether these changes in episode lengths affect NREM/REM cycle lengths. We examined the hypothesis that the ultradian NREM/REM rhythm is altered in depressive illness, against the competing hypothesis that depressive sleep changes do not affect the ultradian NREM/REM rhythm *per se*.

Polysomnography was performed on 41 inpatients with major depression and 48 healthy control subjects. Groups did not differ in mean age (33.7 years) or sex ratio. NREM Cycle (NC) length was defined as the interval between the onset of successive NREM sleep episodes; sleep onset marked the start of the first NC. REM Cycle (RC) length was defined as the interval between the onset of successive REM sleep episodes. Cycle lengths were compared for patients and controls having 3 or 4 complete NCs, and for those having 2 or 3 complete RCs during one post-adaptation night of sleep (Table).

Total recording period and median number of NCs and RCs did not differ between groups. The first NREM episode was shorter in depressives than in controls (mean: 40.8 vs. 70.1 min; $t=-5.78$, $p<.001$), but duration of the first REM episode was not different between groups. The first NC was significantly shorter in depressives than controls among those with 4 NCs ($t=-2.67$, $p<.02$), but not among those with 3 NCs ($t=-1.57$, N.S.). There were no significant group differences in subsequent NC lengths. Similarly, no group differences were seen in any RC lengths across the night.

These results do not support the hypothesis of a disturbance in the ultradian NREM/REM rhythm of depressed patients. The short first NC found in some depressives is the result of a short first NREM episode. The preservation of RC and subsequent NC lengths indicates that the NREM/REM oscillator functions normally in depressed patients.

GROUP*	n	NC1*	NC2	NC3	NC4	GROUP	n	RC1	RC2	RC3
NC=3 D	19	81.9 (18.1)	106.5 (15.6)	109.8 (18.3)		RC=2 D	13	107.4 (20.1)	109.7 (24.0)	
C	29	92.7 (29.5)	112.9 (25.3)	108.6 (22.7)		C	11	117.6 (40.4)	121.7 (22.4)	
NC=4 D	12	49.6 (27.4)	105.2 (33.1)	101.5 (16.9)	93.0 (22.1)	RC=3 D	16	104.9 (38.3)	91.3 (26.5)	103.8 (20.2)
C	13	74.1 (16.7)	98.8 (13.8)	98.7 (20.5)	91.8 (23.7)	C	31	96.4 (18.3)	101.7 (17.9)	99.2 (21.5)

D=Depressed; C=control. All cycle lengths are given as mean (S.D.) in minutes.

DELAYED SLEEP PHASE SYNDROME: POLYSOMNOGRAPHIC CHARACTERISTICS

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Weitzman et al (1981) defined the Delayed Sleep Phase Syndrome (DSPS) to include: 1) a chronic inability to fall asleep at the desired clock time, 2) normal sleep pattern, normal sleep length, and spontaneous awakenings feeling refreshed when sleep occurs at times delayed compared to the normal schedule, and 3) persistent history of the problem for at least the past 6 months. The patients in this study were selected to meet these criteria and also report: disturbance of normal functioning owing to sleep problems (eg: disturbance of work or interpersonal relations), repeated unsuccessful attempts to go to sleep and wake up at earlier times, and poor morning alertness particularly during 7:00 to 9:00 am. Patients with significant sleep apnea or sleep related myoclonus were excluded. Eighteen patients who responded to a public add and met the above criteria were studied with all night clinical polysomnography (PSG) from 11:30 pm until spontaneous awakening the next day. The mean age was 38.4 yrs, 50% were males and mean age of onset of the problem was 13.5 yrs.

The PSG results were both compared to data from age matched norms and correlated with severity of the clinical symptoms of DSPS. Compared to norms DSPS patients showed: increased latencies to stages 2, 3 and REM; increased awakening in the first cycle of sleep, decreased Slow Wave Sleep (SWS) and decreased sleep efficiency. Correlated with greater severity of symptoms were longer stage 2 latency, more SWS, lower sleep efficiency and younger age. Sleep onset to 1.5 mins of stage 1 or deeper sleep did not differ significantly from norms. The data suggest that waking in the first cycle of sleep and latency to stage 2 sleep are better PSG indicators of this disorder than the usual measures of sleep onset.

FIELD STUDIES OF HUMANS FOLLOWING 26-HR BRIGHT LIGHT AND SLEEP-WAKE SCHEDULES

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The entrainment of human circadian rhythms to non-24-hr zeitgebers is usually studied in temporal isolation units. In the present studies 26-hr sleep and bright light schedules were tested in the field since these schedules may be useful building blocks in practical work-sleep schedules for shift-workers.

Twenty subjects followed a 26-hr schedule for 12 consecutive days; 18 of them did so on two separate occasions so that different patterns of bright light exposure could be compared. In one protocol light exposure from a large florescent light bank for two hrs before bed ("evening light"; EL) was compared with natural light (NL) exposure. In another protocol EL was compared with light exposure for 2 hrs after waking ("morning light"; ML). Subjects wore dark goggles to block out sunlight in the "morning" during the EL condition and in the "evening" during the ML condition. Body temperature was continuously measured with a rectal probe, and periodograms indicated the degree of entrainment to the 26-hr schedule.

Entrainment of the temperature rhythm to the 26-hr schedule was significantly better during EL than during NL, and significantly better during EL than during ML. Combining all subjects successfully tested in EL, 14/19 or 74% had their temperature rhythm entrained to the 26-hr schedule. These results show that many people can be entrained to a 26-hr schedule while living at home exposed to the conflicting 24-hr zeitgebers, and support the existence of a human phase response curve to bright light in which evening light delays the circadian rhythm of temperature.

101 EXTRACELLULAR CALCIUM MEDIATES PHASE SHIFTS OF THE BULLA OCULAR CIRCADIAN PACEMAKER VIA CALCIUM CHANNELS Sat Bir S. Khalsa and Gene D. Block
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The in vitro ocular circadian pacemaker of Bulla is phase-shifted by light and other treatments which depolarize the membrane of the putative pacemaker cells (the basal retinal neurons).

McMahon & Block (J. Comp. Physiol., 161: 335-346, 1987) observed that phase delays to light pulses in the early subjective night (CT 14-16) were blocked in the presence of low calcium EGTA ($[Ca]=0.13 \mu M$) artificial seawater (ASW) suggesting that extracellular calcium is essential in the phase-shifting mechanism. We have added support to this hypothesis with data to show that light phase advances and both delays and advances to high K^+ ASW (a depolarizing treatment) are also blocked in the presence of low calcium EGTA ASW. Low calcium ASW without EGTA ($[Ca]<50 \mu M$) is also effective in blocking light phase shifts.

Furthermore, we now have data which support the extended hypothesis that voltage sensitive calcium channels are involved. The inorganic calcium channel blocker Ni^{2+} (5mM $NiCl$) is effective in blocking light-induced phase delays and advances. Organic calcium channel blockers (nitrendipine, verapamil, diltiazem) have generated their own shifts or have been toxic, as have other inorganic blockers (La^{3+} , Co^{2+}). Our current hypothesis suggests that calcium acts as an intracellular second messenger in the phase shifting mechanism of the Bulla pacemaker.

DATA

<u>Pulses delivered at CT 13-16</u>				<u>Pulses delivered at CT 21-24</u>			
Treatment	Average	95% C.I.	N	Treatment	Average	95% C.I.	N
Light	-1.7 hr.	0.2 hr.	9	Light	+1.1 hr.	0.3 hr.	9
Light + Low Ca	0.0 hr.	0.5 hr.	3	Light + EGTA	-0.4 hr.	0.2 hr.	5
Light + $NiCl$	-0.3 hr.	0.2 hr.	8	Light + Low Ca	-0.1 hr.	0.5 hr.	4
Hi K	-1.3 hr.	0.5 hr.	8	Light + $NiCl$	+0.2 hr.	0.5 hr.	7
Hi K + EGTA	-0.1 hr.	0.2 hr.	6	Hi K	+1.0 hr.	0.3 hr.	7
				Hi K + EGTA	-0.3 hr.	0.4 hr.	8

102 CIRCADIAN AND LIGHT-INDUCED CHANGES IN MEMBRANE CONDUCTANCE IN BULLA BASAL RETINAL NEURONS Martin R. Ralph and Gene D. Block, Department of Biology, University of Virginia, Charlottesville, VA 22901.

Circadian rhythms in the frequency of compound action potentials (CAP's) can be recorded from the isolated eye of Bulla gouldiana. The CAP's arise from a population of electrically coupled basal retinal neurons (BRN's) whose change in frequency is driven by a circadian rhythm in membrane potential. BRN's are most hyperpolarized during the subjective night and depolarize near dawn. Depolarization of the membrane is required also for light-induced phase shifts of the rhythm at night. Thus, changes in membrane potential are critical for both regulation and expression of rhythmicity.

We have hypothesized that changes in specific ion conductances underly both the light-induced depolarization at night and the rhythmic changes in membrane potential. We have measured first the total membrane conductance of BRN's in situ during the subjective night and day and in both the dark and light-adapted state. Conductance was found to be highest during the subjective night when the cells were most hyperpolarized, and lowest during the day. A decrease in conductance was measured at dawn which could account for the total difference between night and day. Light given during the subjective night which causes a sustained depolarization, resulted in a sustained decrease in conductance compared to the dark adapted state. Light had little effect on total conductance during the subjective day. Thus, membrane depolarizations which occur at dawn and in response to [nighttime] light are accompanied by decreases in membrane conductance. This is most likely due to a K^+ current which is decreased at dawn and in response to light pulses at night.

We have also found that drugs which reduce Ca^{2+} entry (5mM Ni^{2+} , $N=1$; $10 \mu M$ EGTA, $N=2$) block the light-induced conductance decrease. This raises the possibility that Ca^{2+} entry in response to light leads to the sustained depolarization by reducing a specific K^+ conductance (see Kramer and Levitan, (1986) Soc. Neurosci. Abstr. 13:1439). Such a mechanism could be responsible for a continued Ca^{2+} flux which is essential for light-induced phase shifts.

MULTIPLE INTERACTING SECOND MESSENGER PATHWAYS IN THE STRUCTURE OF AN ENDOGENOUS OSCILLATOR. Rhanor Gillette. Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.

Oscillatory mechanisms in single neurons arise from activities in positive and negative feedback loops. Such loops consist of multiple second messenger pathways and ion channels. The feedback loops are completed by interactions occurring between the different second messenger pathways. These interactions determine the character and expression of cyclic activity, as well as shape the neuromodulatory response.

In an endogenously oscillatory neuron of the predatory mollusk Pleurobranchaea the oscillator is formed by multiple interactions between cyclic AMP, Ca^{2+} and H^+ . Recurrent burst activity is induced by neuromodulatory inputs. Bursts are sustained by a cyclic AMP-dependent Na^+ current whose voltage dependence is conferred by at least two actions of Ca^{2+} current. Ca^{2+} also functions to terminate bursting activity via phosphodiesterase activation and yet another effect on the ion channel. High levels of cyclic AMP also interact with Ca^{2+} in an additional stimulatory fashion. Intracellular pH regulates cyclic AMP levels through pH-sensitive and Ca^{2+} /calmodulin-dependent phosphodiesterase activity. A model of oscillator structure based on these interactions is presented.

THE TIME COURSE OF SYNTHESIS OF SPECIFIC PROTEINS CORRELATED WITH THE CIRCADIAN OSCILLATION OF NEURAL ACTIVITY IN THE EYE OF Aplysia. Felix Strumwasser and D.P. Vile, Marine Biological Laboratory, Woods Hole, MA 02543.

The isolated eye of Aplysia, under conditions of total darkness and constant temperature, produces a circadian rhythm (CR) in the frequency of compound action potentials (CAPs) conducted along its optic nerve. Past evidence indicated the likelihood that the CR is generated by and dependent on the daily synthesis of proteins possibly due to short-lived mRNAs. More direct evidence for the hypothesis that the CR is correlated with the synthesis of a specific set of proteins has been obtained by quantitative examination of fluorograms after two-dimensional PAGE of 35S-methionine labelled eyes. Since ionizing radiation from radiolabelled precursors can compromise the CR, we used a dose of 35S-methionine (150 uCi/ml) for 2 hours that allowed the expression of the next cycle of the CR. Five time points were explored (CT 2, 8, 11, 14 and 20). Each time point had four or six independent samples, each consisting of 4 eyes. Each dried gel containing scintillator was exposed for three different time periods to increase the dynamic range of the film. The digitized film images were merged and analyzed by quantitative densitometry using PDQUEST, software developed by PDI. Fifty-three protein spots were altered as a function of CT using statistical tests with $P < 0.01$. The largest number of changes observed, when the quantity of label incorporated into specific proteins was compared between the different CTs, occurred at CT2 versus CT14 (17 spots) and CT14 versus CT20 (19 spots). The former pair correspond to the peak and trough of the electrical rhythm, respectively. The most "abundant" protein (0.2%) that showed altered synthesis as a function of CT had a m.w. of 17 kdaltons (pI 4.0) and by these characteristics could be a portion of a peptide precursor. The largest fold change in the CT2/CT14 group was a protein with m.w. 25.3 kdaltons (pI 6.8). The largest fold change in any pairwise comparison was a protein with m.w. 74.9 (pI > 7) which incorporated maximal counts at CT2 and minimal counts at CT20 and CT8.

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PROTEIN PHOSPHORYLATION ASSOCIATED WITH THE PHASE SHIFT PRODUCED BY SEROTONIN IN THE APLYSIA EYE. R. Zwartjes and A. Eskin. Biol. Dept., Univ. of Houston, Houston, TX 77004.

Serotonin (5-HT) shifts the phase of the circadian rhythm of the eye of Aplysia californica. This effect was shown to be mediated by an increase in intracellular cAMP. Since cAMP usually works by activating a kinase, we have investigated changes in protein phosphorylation which occur in the eye.

Control and experimental groups of isolated eyes were incubated in inorganic P-32, then treated. After treatment the eyes were ground and the homogenate run on two dimensional polyacrylamide gels. The autoradiograms produced from these gels were then analyzed with a densitometer programmed for 2-D gels.

A spot corresponding to a 57,000 Dalton protein, consistently increased in incorporation when eyes were treated with 5-HT at a phase when it produces its largest advance shift. Agents which increase intracellular cAMP, forskolin and 8-bt-cAMP, also increased incorporation into this spot. TPA, an activator of protein kinase C, does not change the 57kD spot. Light also increased the 57kD spot when given at this phase. Light does not produce a phase shift of the rhythm at this phase.

We also found that incorporation into the 57kD spot increases at a phase when 5-HT produces a delay shift, and preliminary data appears to show an increase at a phase when 5-HT has no effect on the rhythm. This increase, then, seems to be independent of phase.

A spot on silver stained gels corresponding to the 57kD spot has been identified. The silver stained gels indicate that low picogram amounts of the 57kD protein are present in each eye.

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106 WITHDRAWN

MOLECULAR CLONING OF GENES UNDER CONTROL OF THE CIRCADIAN CLOCK

J.C. Dunlap, J.J. Loros and S.A. Denome, Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756

It is clear from a cursory examination of the biology of circadian rhythms that a wide variety of processes are being controlled, directly or indirectly, by these clocks. Doubtlessly one important aspect of this temporal control within a rhythmic cell's metabolic network is the daily control of gene expression. Experiments are underway in the *Neurospora* clock system to study the flow of information from the clock to timed target genes- "clock-controlled genes"- whose levels of expression are controlled by the clock. As a first step to this end, we have undertaken the systematic isolation of morning and evening specific genes. Polyadenylated RNA from both subjective morning [circadian time (CT) 1] and evening (CT13) cultures was isolated and used for subtractive hybridizations and for differential hybridizations to generate morning and evening specific cDNA's. The circadian time-specific cDNA's from the subtractive procedure were used as probes in the screening of both a genomic and a cDNA library. For the differential hybridizations time-specific cDNA libraries made from CT1 and CT13 mRNA were generated and then screened with CT-specific cDNA's. Unexpectedly, we have reproducibly identified only three genes that are strongly regulated by the clock at the level of mRNA abundance.

By Northern analysis over two circadian cycles, the abundance of the mRNA's have been shown to oscillate with a periodicity of 22 hours in a clock wild-type strain and with a periodicity of 29 hours in the long-period clock mutant *frq-7*. Two of these genes, 7C1 and 6C1, are specific to the subjective morning while the other, 13-8-16, is evening specific. Interestingly, Northern blots indicate that the evening specific gene may also be regulated by either light or changes in culture conditions, and in both 13-8-16 and one of the morning genes, 7C1, the absolute mRNA abundance is considerably reduced in *frq-7* as compared to the wild-type strain. Sequencing of both the genomic regions defined by these three genes and the corresponding cDNA's is in progress. We are also developing a method for transcriptional analysis by nuclear run-on assay in *Neurospora* that might be applicable to several microbial systems. Preliminary results for the two morning specific genes indicate that regulation of mRNA abundance is at least partially at the transcriptional level.

MOLECULAR CLONING OF THE *FREQUENCY* AND *PERIOD-4* LOCI OF *NEUROSPORA*

C.R. McClung, Q. Liu & J.C. Dunlap. Department of Biochemistry, Dartmouth Medical School, Hanover NH 03756.

The *frq* (*frequency*) locus of *Neurospora crassa* is thought to be of central importance to the biological circadian clock and therefore represented the initial target in a molecular dissection of the *Neurospora* clock. We established the physical map of a 200 kbp region of DNA extending from *oli* (*oligomycin resistant*) to *for* (*formate*), which had been shown genetically to flank *frq* on linkage group VIIR. *frq* was identified by transformation and phenotypic rescue of strains carrying a recessive *frq*⁰ allele, and shown by transformation with subclones to be contained within an 8 kbp region. We have confirmed that the DNA sequence complementing *frq*⁰ originated on linkage group VIIR by Restriction Fragment Length Polymorphism (RFLP) mapping. Transformants in which the *frq*⁰ phenotype was complemented to *frq*⁺ showed circadian banding with a wild type period length, and this period length was temperature compensated over the range of 20 to 30°C. The DNA sequence of the *frq* genomic region has been determined. Analysis of *frq* expression is in progress.

We have used a similar approach towards the isolation of the *prd-4* (*period-4*) locus, a second gene identified through mutational analysis of period length. Genetically, *prd-4* was mapped between *arg-13* (*arginine-13*) and *os-1* (*osmotic-1*) on linkage group IR. We identified a cosmid able to phenotypically rescue a strain carrying the recessive *arg-13* allele by sib selection (Vollmer and Yanofsky, 1986 PNAS USA 83:4869) and used this clone to initiate a chromosomal walk. A physical map of 100 kbp was determined and was shown to extend 80 kbp in the direction of *os-1* (centromere proximal) by RFLP mapping. Cloned DNAs able to phenotypically rescue a strain carrying the recessive *os-1* allele were identified by DNA mediated transformation. Thus the physical map extends from *arg-13* to *os-1* and spans the region shown genetically to include *prd-4*. Identification of the *prd-4* DNA region within the 40 kbp separating *arg-13* and *os-1* is in progress, as is characterization of the *arg-13* and *os-1* loci.

- 109 THE HONEYBEE TIME-SENSE: EVIDENCE FOR TWO PROCESSES CONTROLLING FORAGING BEHAVIOR. Darrell Moore and Mary Ann Rankin. Department of Zoology, University of Texas, Austin, TX 78712

The classical experiments of Beling (1929) demonstrated that bees can be trained to collect food at virtually any time of day and that they will visit the food source at this time on consecutive days. Subsequent work established that the foraging rhythm is controlled by an endogenous circadian clock.

Recently, we showed that groups of bees trained early in the day are apparently more accurate than bees trained at midday or late afternoon. Bees always anticipated the onset of an unrewarded training period, but the duration of the anticipatory component was always greater for late-day training times than for earlier training periods.

In the present study, experiments show that the change in foraging accuracy across the day persists in an artificial flight room, in the absence of several potential environmental time-cues. However, individual bees, irrespective of training time, visit the unrewarded food source with increasing frequency until the arrival of the training time. Afterwards, the visitation frequency decreases. Similarly, members of foraging groups monitored in an observation colony cluster in the vicinity of the "dance floor" in anticipation of the training time, but disperse from this cluster soon after the arrival of the training time. These results suggest that early-day and late-day bees have equal or nearly equal time-keeping capabilities, but late-day bees are programmed to forage with greater anticipation. Thus, foraging behavior may be controlled by two separate processes - one premised on accurate time-keeping at all times of day and the other acting to change the anticipation as the day progresses.

- 110 TIME-AND-PLACE LEARNING BY GARDEN WARBLERS (SYLVIA BORIN)
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We have trained captive garden warblers (*Sylvia borin*) to go from a central living room to four different feeding places, each one at a different time of day (room A from 6:00 to 9:00, B from 9:00 to 12:00, C from 12:00 to 15:00, D from 15:00 to 18:00 hr). The birds are capable of learning this temporal and spatial pattern of food availability (better than 70% correct choices within 9 days).

During test-days with food available in all places and at all times of day the daily temporal and spatial pattern still persists, showing that some kind of timing mechanism is involved.

Phase-shift-experiments indicate that the training is based on a circadian oscillator rather than on an hourglass.

CIRCADIAN ACTIVITY PATTERNS IN CAPTIVE SCNGBIRDS, by Leonard A. Eiserer (Psychology Department, Elizabethtown College, Elizabethtown, Pa. 17022) and Roger K.R. Thompson (Psychology Department, Franklin and Marshall College, Lancaster, Pa. 17604).

American Robins (*Turdus migratorius*), European Starlings (*Sturnus vulgaris*), and Redwinged Blackbirds (*Agelaius phoeniceus*) were maintained in captivity under conditions of natural illumination. The Robins showed progressively less motor activity across the daylight hours, but then generated a marked activity peak at dusk. This peak is thought to reflect an "urge" to travel to the communal roostsite in this species. Such "roosttime restlessness" may be comparable in some respects to the migratory restlessness that many songbirds display seasonally when they are maintained in captivity.

Although Starlings and Redwings, like Robins, roost communally in the wild, only the captive Redwings showed roosttime restlessness at dusk. This species difference may be related to the fact that both Robins and Redwings are territorial, while Starlings are not.

112 EFFECTS OF PINEALECTOMY ON THE CIRCADIAN RHYTHMICITY OF THE EUROPEAN LIZARD *PODARCIS SICULA CAMPESTRIS*

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Inter-species variability in the circadian organization of Iguanidae mainly concerns differences between species in the roles played by pineal, retina, and melatonin (Underwood, 1983; Janik, 1987). Comparative investigations by Underwood and Menaker (1976) revealed also striking differences in the mechanisms of entrainment between Iguanidae and Lacertidae, a phylogenetically more recent family of lizards.

To shed light on the evolutionary meaning of such a variability in circadian organization we decided to test the effect of pinealectomy on activity rhythms in a species of Lacertidae.

Free-running locomotor activity of the species Podarcis sicula campestris was recorded in tilt-cages placed inside environmental chambers maintained in constant darkness and constant temperature (28° C). Pinealectomy has one of three effects on locomotor activity: (i) arrhythmicity; (ii) splitting of the activity rhythm into two components; (iii) change in the period of the free-running rhythm.

These preliminary data indicate that the behavior of pinealectomized Podarcis sicula is similar to the behavior of pinealectomized Sceloporus.

As regards relative dominance of pineal and non-pineal components in the circadian organization of lizards, Sceloporus (and perhaps Podarcis) occupy an intermediate position between Anolis at one extreme, where the pineal is dominant, and Dipsosaurus at the other extreme, where non-pineal components are dominant.

- 113 SIZE-SPECIFIC DIURNAL TO NOCTURNAL SHIFT OF LOCOMOTOR ACTIVITY IN RAINFOREST LEAF-LITTER FROGS OF THE GENUS ELEUTHERODACTYLUS. York Winter and Frank Barnwell. Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN 55455.

Rhythms of locomotor activity were recorded in 74 individuals belonging to six species of Eleutherodactylus (bransfordii, biporcatus, crassidigitus, fitzingeri, ridens, and talamancae). Animals were freshly collected from the lowland rainforest at La Selva Biological Station in northeastern Costa Rica. Experiments were conducted during the 1985 rainy season and 1986 dry season. Activity was monitored in actographs under natural illumination for durations of three to nine days.

Individual frogs exhibited bimodal diel rhythms that were consistent in waveform but ranged across a spectrum from diurnal to nocturnal patterns between animals. This difference was significantly correlated with body size. Small frogs of all species were predominantly day-active while larger animals were night-active. This ontogenetic shift in activity occurred at a fixed, species-independent switch-size of 18 to 22 mm snout-vent length. Diel patterns monitored in specimens of E. bransfordii in leaf-litter plots exhibited a similar relationship between body size and day-night occurrence of activity.

It is paradoxical that juveniles of Eleutherodactylus are day-active when it is their size class that should be most prone to desiccation because of a high surface area-to-volume ratio. The explanation may lie in the opportunity for juveniles to rehydrate in small moist crevices and holes that are inaccessible to adults because of their larger size.

- 114 CIRCADIAN OSCILLATION IN THE POPULATION OF AIRBORNE SPORES OF FUNGAL PLANT PATHOGENS: A HALF CENTURY OF LITERATURE IN NEED OF REINTERPRETATION. B.W. Kennedy Department Plant Pathology, University of Minnesota, St Paul, Mn. 55108.

The basic importance of inoculum load to development of epidemics of fungus diseases in plants has led to measurements of spore numbers in the atmosphere around economic crop plants and these investigations reveal striking cyclic (circadian) oscillations in spore load. Technology for measuring microparticulates in the air has greatly improved over the span of time when most of these studies were made and more recent studies support earlier ones. Clock time peaks in the population vary enormously with the particular fungus involved and the specific disease under consideration but reports invariably attribute rise and fall of spore numbers to prevailing changes in environmental variables such as temperature, humidity, rain, dew, wind and light. Chronobiology is not the pedigree of research in plant pathology and the concept that acrophase peaks could, in part at least, be due to entrained or endogenous rhythmic behavior of these fungi (or in conjunction with any of the varied resistance and (or) susceptible tendencies of the host plant) and thus continue to oscillate for a time regardless of the environmental synchronizers, is a new and unrecognized approach that needs to be researched. A literature search reveals a single study of free running (constant) environmental conditions and this example, even though bioperiodicity was clearly demonstrated, consisted of a study of only one fungus and one treatment replication.

Investigations on the role of rhythmic release of spores, and the probable rhythmic formation of spores as well, are potentially productive pursuits needed in order to elicit a more complete interpretation of former efforts and to evaluate more completely the role of environmental parameters contributing substantially to epidemiology of plant disease.

PERIDODGRAM ANALYSIS OF THE LOCOMOTOR RHYTHM OF CALLIPHORA VICINA

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The locomotion of Calliphora vicina is almost entirely restricted to the illuminated portion of light/dark (LD) cycles, with constant activity occurring in constant light (LL) and a free-running rhythm in constant dark (DD). The focus of the work was on the period of this free-running rhythm and how it might be effected by various physical and chemical conditions.

A standard form of free-run emerged, namely an initial period of less than 24hrs followed by a gradual lengthening to about 24hrs or longer. The exact form of this may be effected by the prior LD regimes experienced by the adult flies but it is independent of those which the larvae may receive. Keeping the flies together during these LD cycles, or, feeding them meat produced qualitative rather than quantitative effects on the free-running rhythm.

Chemical disturbance of the rhythm was attempted with a variety of compounds and resulted in an array of effects. Deuterium Oxide, for example, produced a notable degree of lengthening of the rhythm, and the results suggest that this change may be dose dependent. Other effects produced by chemical disturbance included clear rhythm-splitting and ultradian rhythms.

ROLE OF THE OVARY IN REPRODUCTIVE REFRACTORINESS IN THE KILLIFISH, FUNDULUS

HETEROCLITUS. John A. Dimitry and Malcolm H. Taylor, School of Life and Health Sciences and College of Marine Studies, Univ. of Delaware, Newark, DE 19716.

Several species of fish with extended reproductive seasons have been shown to enter a "refractory period" at the termination of spawning. During this time, normal stimulatory cues, such as long photoperiods, fail to induce gonadal recrudescence. The aim of the present study is to confirm the existence of a refractory period in the mid-atlantic population of the salt marsh killifish, Fundulus heteroclitus, and determine the role of the ovary in refractoriness.

In order to document the occurrence of a refractory period, F. heteroclitus females were brought into the laboratory in July and exposed to various temperature-photoperiod regimens. None of the treatments maintained sexually active fish past the end of the natural spawning season (mid-August) even though those animals tested at a cooler temperature (15 vs. 20°C) regressed more slowly.

The responsiveness of the ovary was tested by bringing fish into the laboratory at various times during the year and attempting to induce recrudescence with daily intraperitoneal injections of (50 IU/fish) Human Chorionic Gonadotropin. Fish treated immediately prior to the spawning season recrudesced in 1 week while those tested during the refractory period required 4 weeks to produce mature oocytes. This difference may simply reflect the regressed condition of the ovary at the termination of the breeding season. At that time none of the pre-injection control fish contained "vitellogenic" oocytes (>0.50 mm), while fish collected 4-6 weeks prior to the natural spawning season contained many oocytes in the 0.5 - 0.7 mm. size range. Although reproductive refractoriness in fish may include reduced sensitivity of the hypothalamic-pituitary control system to normally effective environmental stimuli, our results indicate that the condition of the ovary can be an important factor.

- 117 THE COMMISSURAL PROJECTION BETWEEN THE INTERGENICULATE LEAFLETS OF THE RAT THALAMUS CONTAINS MET-ENKEPHALIN IMMUNOREACTIVITY. J.P. Card and R.Y. Moore. Medical Products Department, The Du Pont Company, Wilmington, Delaware 19898 and Department of Neurology, SUNY @ Stony Brook, Stony Brook, New York, 11790.

Two major efferent projection pathways from the IGL have been demonstrated. The most extensively studied projection arising from each IGL terminates bilaterally within the suprachiasmatic nuclei (SCN). A substantial number of these neurons contain neuropeptide Y and are known to participate in the entrainment of SCN neuronal activity. The other projection system consists of neurons which form commissural connections between the two IGLs. Recent data has demonstrated that these neurons have no ascending collaterals terminating in the SCN and do not contain NPY. In the present investigation we have combined retrograde tract-tracing of fluorescent dyes with immunocytochemical localization of met-enkephalin (mENK) to gain further insight into the organization of these two projections systems. Immunohistochemical localization of mENK, both alone and in combination with retrograde tracers, confirmed the previous demonstration (Mantyh and Kemp, 1983) of mENK-containing neurons in the IGL. No double labeling of IGL neurons was observed following bilateral tracer injection into the SCN and immunohistochemical localization of mENK. In contrast, numerous double labeled neurons were observed in the IGL following tracer injection into the contralateral IGL and immunohistochemical localization of mENK. These findings demonstrate that the commissural connection between the IGLs is distinct from the geniculohypothalamic projection, both in the source and chemical content of the projection system.

- 118 MONOCHROMATIC PHASE RESPONSE CURVES FOR A NOCTURNAL RODENT
Patricia J. DeCoursey, Biology Department, University of South Carolina,
Columbia, SC 29208

Phase response curves (PRCs) were constructed for the flying squirrel, *Glaucomys volans*, using single 15-min dim light pulses for animals free-running in constant darkness. Initially white-light curves were made with sequential pulses (intensity = 3.8×10^{16} photons \times cm⁻²) for 8 individuals every 7-10 days, testing throughout the animals circadian day at 2-hour phase points. Considerable interindividual variation in amplitude and shape of curves was found, but all demonstrated a non-response zone from approximately CT02 to CT11.5, a prominent delay zone from 11.5 to CT18 with a maximum 1-hour delay at CT13, and a shallow advance zone from CT18-CT02. The high precision and long-lasting nature of the squirrels' circadian rhythms permitted construction of complete PRC's at 2-hour phase intervals for 4 individuals using 15 min pulses of monochromatic red (620nm) or green (500nm) light. The white and monochromatic PRC's were very similar, differing primarily in amplitude of phase shifting response; maximum delay for red was 45 min at CT12, and maximum delay for green was 70 min at CT13. The data do not support the hypothesis that separate red and green photoreceptor systems exist for advance and delay portions of the phase response curve.

SYRIAN HAMSTERS WITH CIRCADIAN PERIODS OUTSIDE THE PREDICTED LIMITS OF ENTRAINMENT BY RESTRICTING LIGHT EXPOSURE TO THE DELAY PORTION OF THE PHASE-RESPONSE CURVE. James S. Ferraro, Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, IL 62901.

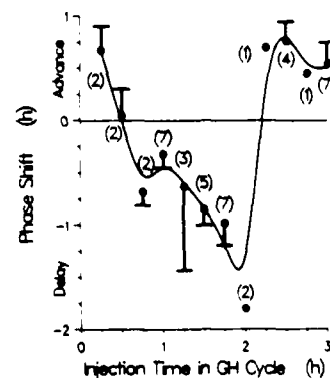
Adult male Syrian hamsters (*Mesocricetus auratus*) free-run in constant dark (DD) with a period (τ) very close to 24 h (24.02 to 24.22 h). The maximum phase delay of the hamster, as defined by the phase-response curve (PRC), is approximately 1.1 h for 15 min light pulses (Pittendrigh and Daan, 1976; Takahashi *et al.*, 1984) and 1.45 h for 60 min light pulses (Elliott 1974). The maximum period, of a light-dark cycle (T-cycle) that would be predicted capable of entraining the circadian oscillator would be, τ_{DD} plus the maximum phase delay. Therefore, the predicted limit of entrainment (50%) would be approximately 25.1 h for 15 min pulses and 25.7 h for 1 h pulses. Experimental results for 15 min pulses have showed stable entrainment breaks down at 25.0 h in the majority of the hamsters. It has been suggested that the limit of entrainment is dependent upon of the photoperiod length of the T-cycle and the length of transition time (Carmichael *et al.*, 1981). It was hypothesized that 1) a light pulse that exposed the entire delay portion would produce the longest τ possible and 2) increasing the photoperiod of a T-cycle longer than 1 hour would do little to increase the exposure of the delay portion of the PRC. In order to test this hypothesis, hamsters were exposed to light pulses, 1 to 8 h in duration, with "lights-on" 1 h after the onset of activity. The onset of the light pulse was phase locked with the onset of activity. This method of illumination resulted in periods as long as 26.5 h with 1 h light pulses and 28.5 h with 8 h pulses. These periods are longer than predicted and suggest that the phase shifts described by the PRC can be integrated to change the oscillators velocity during the light exposure. Supported by NIH Grant NS23128.

PHASE SHIFTING OF THE ULTRADIAN GROWTH HORMONE SECRETORY RHYTHM. L. Cass Terry. Neuroendocrine Research Lab., Univ. Michigan & VA Medical Center, Ann Arbor, MI 48105.

Pituitary growth hormone (GH) secretion is regulated by GH-releasing hormone (GRH) and GH-inhibiting hormone (somatostatin, GIH) release into the hypothalamic-pituitary portal system. In the male rat GH is released episodically with secretory bursts every three hours. An endogenous ultradian neuronal oscillator is believed to stimulate GRH which, in turn, causes GH release. The role of GIH in control of this rhythm is not entirely clear, but its rhythm is most likely driven by high levels of GH and/or GRH. Although the GH rhythm is purported to be light-entrained, phase shifting has not been shown by photic or any other stimuli. **The purpose of this investigation was to demonstrate that the GH rhythm is generated by an endogenous ultradian oscillator.** Intravenous (iv) pulses of human GH (hGH, 35 ug) were administered to chronically cannulated adult male albino rats in an attempt to phase shift the rat GH (rGH) rhythm. hGH was given during various phases of the rGH rhythm, and blood samples were removed every 15 min from 800-2000h. hGH was selected because it has biological activity in the rat and does not interfere with the rGH radioimmunoassay.

The mean interpeak period between rGH secretory events was $3.08 \pm .08$ h. Injection of hGH caused significant phase shifts in the GH rhythm. The threshold for detection of a spontaneous rGH burst was 100 ng/ml. Phase delay was detected in the first and phase advance in the second spontaneous rGH peak after hGH injections. Phase delays were caused by hGH injections within a 2 h window beginning at the onset of a rGH burst and advances by injections between 2 and 3 h. The normal rhythm of GH release resumed after being advanced or delayed, confirming a phase shift of the neuronal oscillator. A phase response curve (PRC, figure) was then constructed from the data of 43 animals. **This PRC of the rGH rhythm is the first direct evidence that an internal ultradian oscillator generates rhythmic GRF release and suggests that its sensitivity is phase dependent.** Studies are in progress to ascertain if hGH pulses phase shift the rGH rhythm independently of GIH.

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RESTRICTED FEEDING TIME: A ZEITGEBER IN THE RABBIT

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Restricted feeding time (RFT) with a period length of 24 h, 23 h 51 min and 24 h 09 min did not only mask but also entrain the free-running rhythms of locomotor activity, visits at the food box, hard faeces excretion, contacts with the water tube and urine excretion. Masking occurred immediately after insertion of RFT. Five characteristics indicate, however, that RFT did not only mask but did indeed entrain the circadian rhythms: 1. in spite of the camouflage the circadian rhythm was shifted until it had reached the RFT; 2. when the circadian rhythm had reached the corresponding phase of RFT (day 10-50 of RFT), an anticipatory peak of locomotor activity was built up; 3. during the last 20 days of each RFT regimen (lasting for a total of 70 - 100 days) all five functions had definite phase relationships to RFT; 4. following return to ad libitum food access the phase of the circadian rhythm was set by the precedent RFT; 5. period length of the free running rhythm was obviously affected by the period length of the preceding RFT regimen (aftereffect).

The results demonstrate that RFT in the rabbit exhibits the properties of a true zeitgeber. While the five circadian rhythms remained internally synchronized during the whole experiment (>700 days), we do not know, however, whether this is the consequence of a common oscillator system or whether it is due, at least in part, to mechanical or metabolic processes.

(Supported by grants of the Deutsche Forschungsgemeinschaft)

THE ROLE OF THE CIRCADIAN SYSTEM IN PHOTOPERIODISM: A CLOCK ROLE OR A NON-CLOCK ROLE?

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Nanda-Hamner experiments have shown that the circadian system is involved in photoperiodic processes in a variety of plant and animal species. Response curves of these experiments revealed peaks and troughs of incidences of the measured photoperiodic reaction at intervals of approximately 24 h. The number of peaks may vary, dependent on the organism, but also on temperature. In those instances where temperature effects on Nanda-Hamner experiments have been studied, lowering the temperature resulted generally in a "filling-up" of the troughs and a rise of the peaks, until an "hourglass-like" response curve appeared.

If it is assumed that the circadian system acts as the clock itself, the above results may be described by a damping circadian oscillator with a temperature-dependent damping rate. At low temperatures the oscillator then damps out so quickly in continuous darkness, that effectively it becomes undistinguishable from an "hourglass".

Nanda-Hamner experiments undertaken with the spider mite, Tetranychus urticae, revealed 4 peaks of diapause incidences, about 20 h apart. Therefore, if the clock is a damping oscillator, it must run at least 4 cycles before damping out. However, it has recently been shown experimentally that the spider-mite clock runs only one cycle in continuous dark. That means that in this species the observed peaks and troughs can only be described by a non-clock role of the circadian system.

COUPLING PATHWAYS IN THE SCORPION'S CIRCADIAN SYSTEM

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Based on neuroanatomical and electrophysiological data from a system of efferent fibers in the scorpion brain a model will be discussed dealing with essential parts of information processing within a circadian system.

These fibers are likely to be the coupling pathways between left and right sided pacemaker centers, and between them and the eyes as well as the centers of locomotor control. At the level of the first optic neuropil those fibers seem to be involved in LD-Zeitgeber processing via the eyes.

Affecting this fiber system by controlled microsurgery or pharmacology alters the overt circadian rhythmicity in both the eye sensitivity and the locomotor activity in a way as it can be predicted by the model: All components can be selectively disconnected from the rest of the complex multioscillatory system resulting in desynchrony or arrhythmicity of the respective component.

Obviously octopaminergic and serotonergic fibers closely interact, the role of a so far only histologically identified peptidergic system is still unclear.

ERG AND LOCOMOTOR ACTIVITY RHYTHMS IN THE SCORPION: INTERACTION BETWEEN DIFFERENT CIRCADIAN PACEMAKERS

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Electroretinogram amplitude (ERG) and locomotor activity of scorpions were recorded simultaneously using a computer based data acquisition system.

Previous anatomical and physiological experiments combined with the results presented here suggest that there are at least two distinct types of bilaterally circadian oscillators: One controlling the visual sensitivity and another one for locomotor rhythmicity. In addition there is evidence for morning and evening oscillators within both of them. The phase angle difference and the amplitude of the overt rhythm of both parameters depend on the coupling strength in this multioscillatory circadian system. The phase relation of locomotor activity to the ERG rhythm is variable, often positive, depending on the locomotor pattern and light conditions. Switching between different stable phase angle differences is achieved by transient changes of period length or by phase jumps.

Locomotor and ERG rhythm of one scorpion can continuously show interactions. Increasing positive phase angle of running is compensated by the eye rhythm with the lengthening of subjective night followed by phase jumps.

The phase response of locomotor activity and ERG rhythm to light pulses is complex and can only be explained as a reaction of a mutually coupled pacemaker system.

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