

AD \_\_\_\_\_

Award Number: W81XWH-11-2-0198

TITLE: Advanced Pediatric Brain Imaging Research and Training Program

PRINCIPAL INVESTIGATOR: Catherine Limperopoulos, PhD

CONTRACTING ORGANIZATION: Children's National Medical Center  
Washington, DC 20010

REPORT DATE: October 2014

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

<b>REPORT DOCUMENTATION PAGE</b>			<i>Form Approved</i> <i>OMB No. 0704-0188</i>		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
<b>1. REPORT DATE</b> October 2014		<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 15 Sept 2013 – 14 Sept 2014	
<b>4. TITLE AND SUBTITLE</b> Advanced Pediatric Brain Imaging Research á^äÁÛää↔↔^&ÃProgram			<b>5a. CONTRACT NUMBER</b>		
			<b>5b. GRANT NUMBER</b> W81XWH-11-2-0198		
			<b>5c. PROGRAM ELEMENT NUMBER</b>		
<b>6. AUTHOR(S)</b> Catherine Limperopoulos, PhD  E-Mail: <a href="mailto:climpero@childrensnational.org">climpero@childrensnational.org</a>			<b>5d. PROJECT NUMBER</b>		
			<b>5e. TASK NUMBER</b>		
			<b>5f. WORK UNIT NUMBER</b>		
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> Children's National Medical Center  Washington, DC 20010			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>		
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>		
			<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>		
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
Brain injury is a leading cause of death and disability in children. Recent advances in pediatric magnetic resonance imaging (MRI) techniques are revolutionizing our understanding of brain injury, its potential for recovery, and demonstrating enormous potential for advancing the field of neuroprotection. We have created a highly structured, collaborative, and multidisciplinary training program in <b>BRAIN (Brain Research Advanced Imaging with NMR)</b> to advance research skills of investigators from all branches of the US military focusing on pediatric brain injury. Our goal is to train, with the highest rigor, military trainees in conducting clinical research using advanced brain imaging technologies to study the causes and consequences of pediatric brain injury. Over the past year, we successfully implemented the on-site BRAIN training curriculum and we recruited and trained one civilian fellow. We developed an online learning management system, by creating and implementing methods for converting the existing in-classroom educational BRAIN seminars into self-directed online learning modules and courseware. Specifically, we developed a web-based portal site located at <a href="http://www.MilitaryMedED.com">www.MilitaryMedED.com</a> that users can search, upload, and house online training and education-related information. Our goal is to deploy this on-line BRAIN courseware to major DoD military bases to allow for a more broad-based teaching framework in which we anticipate far-reaching benefits.					
<b>15. SUBJECT TERMS</b> training program, advanced MRI, brain injury.					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b>
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>			<b>19b. TELEPHONE NUMBER</b> (include area code)
U	U	U	UU	114	USAMRMC

## Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	2
Key Research Accomplishments.....	19
Reportable Outcomes.....	20
Conclusion.....	21
References.....	22
Appendices.....	23

## **INTRODUCTION**

This report documents the activities conducted between September 2013 – September 2014 for the “Advanced Pediatric Brain Imaging Research and Training program” project. The overarching goal of this grant is to advance the training of military clinician scientists in the field of investigative brain imaging technologies to understand the causes of brain injury and the mechanisms underlying brain plasticity following injury. In this annual report we describe the development of a web-based training portal ([www.MilitaryMedED.com](http://www.MilitaryMedED.com)) to house our BRAIN (**B**rain **R**esearch **A**dvanced **I**maging with **N**uclear Magnetic Resonance) seminars courseware. Specifically, we summarize the development of our online learning management system and the methods used to convert our existing in-classroom BRAIN seminars into self-directed online learning modules. The portal site is modular with various simple and complex databases, and latest features such as SCORM-compliant training modules, learning and communication plugin widgets, and external instructional and productivity tools. With the site completed in this phase of the project, our Subject Matter Experts are beginning to create and post online BRAIN courseware, which will be made available for review and feedback to DoD and civilian trainees. Lastly, we summarize the academic progress of our on-site DoD civilian trainee.

## **BODY**

In our efforts to advance and disseminate the fundamental principles of pediatric brain injury and recovery following injury, as well as the clinical application of sophisticated MRI techniques that are improving our understanding of the causes, consequences and care of pediatric brain injury, we received Government approval (July 2013) to restructure and broaden the scope of our BRAIN training program by transforming our in-classroom BRAIN seminars into self-directed online courses. Our BRAIN program focuses on developing (i) the scientific rigor necessary to perform high-quality clinical research through instruction in epidemiology and biostatistics, (ii) an in-depth understanding of the underlying pathogenetic mechanisms of injury to the brain and its recovery, and (iii) the necessary skills to apply advanced MRI techniques to study brain injury, and to facilitate the diagnosis, management, and ultimately treatment of brain injury. While developing the web-based BRAIN curriculum, we also supported one final trainee (as per the original approved grant). Dr. An Massaro, a civilian neonatologist at Children's National Medical Center participated in our on-site BRAIN training program. Our progress over the past 12 months is summarized below.

### ***Statement of work-progress to date:***

Specific Aim 1: To advance the understanding of the fundamental principles and clinical application of sophisticated MRI techniques that is revolutionizing clinical research into the causes, consequences and care of pediatric brain injury.

Over the past year, the PI together with the scholarly oversight committee continued to implement on site clinical teaching seminars on the fundamental principles and applications of advanced MRI techniques while working on transitioning these seminars to a web-based curriculum. The program demonstrated significant training benefits to our local civilian and military residents at Children's National Medical Center. Over a dozen military residents that came through our radiology program attended these seminars. In addition, clinical and research fellows as well as junior faculty across different disciplines including fetal medicine, neonatology, neurology, critical care medicine, radiology, biomedical engineering, cardiology nursing, psychiatry and psychology participated. A detailed update on our progress on the e-learning module development is summarized below (section: Web-based BRAIN curriculum).

**Dr. Massaro**, our civilian trainee actively participated in these seminars and also benefited from a core curriculum of hands-on training sessions in quantitative MRI techniques through our MRI and Neurobehavioral training cores, which remained fully operational to

support her on-site training. Dr. Massaro has made significant progress in the data acquisition and processing phases of her research study, which focuses on the application of serial and quantitative MRI techniques to examine the microstructural and cerebral perfusion consequences following neonatal hypoxic ischemic encephalopathy. Specifically, Dr. Massaro has been supported by the diffusion and perfusion imaging cores which have provided hands-on training in advanced MRI techniques to analyze her cerebral perfusion (arterial spin labeling) MRI data and to relate measures of global and regional brain microstructural organization and perfusion to neurodevelopmental outcomes. She completed processing of all the MRI diffusion data on her subjects and already had a paper that was accepted with revisions entitled, "White matter tract integrity and developmental outcome in newborns with hypoxic ischemic encephalopathy treated with hypothermia". Her second paper examining the relationship between microstructural organization measured by quantitative diffusion tensor imaging and neonatal neurobehavioral performance in HIE is currently in preparation for peer-review submission. She is currently finalizing her cerebral perfusion measurements. This work has been conducted under the mentorship of Dr. Catherine Limperopoulos (PI), Dr. Adre du Plessis (co-investigator and Associate Director of BRAIN), Dr. Iordanis Evangelou (co-investigator and perfusion MR training core lead), and Dr. Vezina (co-investigator). Dr. Massaro presented her preliminary work at the Pediatric Academic Societies – Society of Pediatric Research (PAS-SPR May 2014) in the form of a prestigious platform presentation. She was also invited to moderate a session at PAS-SPR entitled, "Hypothermia & Hypoxic Ischemic Encephalopathy. Finally, Dr. Massaro was awarded a grant from Cerebral Palsy International Research Foundation to quantify brain injury in neonatal hypoxic ischemic encephalopathy.

*Specific Aim 2:* To enhance through didactic and clinical teaching the basic science and clinical understanding of the causes, mechanisms, and consequences of pediatric brain injury.

Clinical teaching in the form of in-classroom seminars on the principles of pediatric brain injury has taken place alongside seminars in Advanced Brain Imaging Techniques (aim 1) over the current reporting period. We demonstrated consistently high participation rates with the implementation of these seminars, often at standing room capacity. These seminars capture a wide scope of themes including normal and abnormal brain development, mechanisms of acquired brain injury, including traumatic brain injury, stroke, hypoxic-ischemic injury with a direct link made with the role of advanced brain imaging techniques in facilitating diagnosis, management and rehabilitation following brain injury. In parallel, we have been migrating these

seminars into e-learning courseware. Our progress in transitioning these seminars to web-based e-learning modules is detailed below (section: Web-based BRAIN curriculum).

*Specific Aim 3:* To provide training in clinical research methodology through courses and seminars in biostatistics and research design, and responsible conduct of clinical investigation.

Over the past year, Dr. Massaro actively participated in the Children's Research Education and Career Training (CREAT) program at Children's National. She successfully completed the on-line Collaborative Institutional Training Initiative (CITI) course on responsible conduct of research, developed her research project and obtained IRB approval (project described in aim 1) entitled, Predicting Outcomes in Patients with Hypothermia-Treated Neonatal Encephalopathy.

As part of our e-learning BRAIN modules we have incorporated our newly developed on-line FACTS (Focus on Clinical and Translational Science) curriculum with hundreds of resources (*archived lectures, tutorials, publications*) covering 14 key research thematic areas including study design, developing goals and objectives, research implementation, statistical analyses, sources of error, etc. (Appendix A).

### **Web-based BRAIN curriculum**

As described above, over the past year, we continued to host the in-classroom BRAIN seminars in the fundamental principles and clinical application of sophisticated MRI techniques *and* principles of pediatric brain injury through didactic and clinical teaching (aim 1 and 2), while developing the web-based BRAIN storyboards. Jeff Sestokas, our Senior Instructional Systems Designer, has been working closely with Ben Scalise (Multimedia Developer) and our Subject Matter Experts (SME) and overseeing the development of our on-line curriculum. Our external advisory committee came to Children's National for a site visit on April 30, 2014 at which time we provided a detailed update and demonstration of the on-line BRAIN courseware we have been developing, as well as our DoD civilian trainee's update. The committee was very impressed with our web-based BRAIN educational platform and expressed a lot of enthusiasm about our accomplishments (Appendix B). Below, we present a detailed summary of our progress with the e-learning modules to date.

During this phase (*year 3*) of the project, we achieved the following milestones:

- Developed a responsive web-based learning management system that houses the BRAIN online courseware.
  - Created an intuitive graphical user interface and mobile client for the web-based learning management portal system. The site is located at

- [www.MilitaryMedED.com](http://www.MilitaryMedED.com) (username: test, password: Demo@123 – The “D” is capitalized. The site can be access from any device web browser (personal computer, tablet or phone) and operating system (e.g. *Windows, IOS, Linux,etc*).
- Developed and uploaded six SCORM-compliant online training modules on the fundamentals of MRI and normal/abnormal brain development. Our Subject Matter Experts (SME) converted their Power Point presentations by storyboarding their content for instructional technologists and multimedia developers to begin producing interactive learning objects and assessments.
  - Incorporated our on line FACTS (Focus on Clinical and Translational Science). Developed a self-registration feature allowing users to sign-up for an account. Account requests are sent to a secure administrator’s email account for review and approval. When approved, users are sent a personalized confirmation email with instructions on how to login and use the system.
  - Implemented an automatic enrollment feature that allows course instructors to enable the system to electronically enlist users to into courses.
  - Implemented custom reporting widgets that allows administrators to view user interaction at three levels (site, course, and learning object levels)
- Performed internal field testing of the learning management system.
    - Conducted several internal field tests at Children’s National Medical Center main and satellite campuses. The field tests consisted of SMEs and content developers logging into and navigating the system and accessing their course area to begin populating it with resources and activities relevant to their BRAIN seminar topic.
  - Held extensive ongoing internal workshops to teach SMEs and Co-PIs how to design, develop, and implement online BRAIN courseware training.
    - Taught numerous ongoing instructional design workshops, in-person train the trainer workshops and one-on-one meetings discussing the roles and responsibilities for developing on-line instruction and demonstrating the portal’s capabilities (see Appendices C)
    - Created and posted best practice presentations, templates, and examples for designing effective online instruction (see Appendices C-8).

#### a. Design Phase

The primary goal in this project phase was to implement a responsive and scalable distance learning approach to the rapidly changing education needs of military medical professionals, specifically in their understanding of the causes and consequences of pediatric brain injury. Currently, advances in pediatric magnetic resonance imaging (MRI) techniques have improved our understanding of brain injury and its potential for recovery. Because of the fast emerging nature of the field, traditional in-classroom training approaches cannot support wide-area dissemination of this tacit knowledge. Electronic learning modalities such as the use of customizable, online learning management systems and web-based training modules would help bridge the gap to delivering up-to-date pediatric brain training to a worldwide clinical audience.



During the design phase, we transformed six selected Power Point presentations, developed in the first two years of the project for our seminar series (aim 1 and 2), into Shareable Content Object Reference Model (SCORM) compliant web-based training modules (Table 2). Our selection criteria for choosing the initial six modules to develop, implement, and pilot with a medical military audience are based on their universal application to learning the fundamentals of MRI and normal/abnormal brain development (see section on – Implementation Plan).

To achieve designing effective and engaging training, a five-stage design requirement approach was used to incorporate learning objectives, learner abilities, instructional methods, instructional content, and assessment methods into training delivery (Table 1). Included is an outline describing how these five design stages were implemented in the storyboarding process (Appendix D).

**Table 1.** *Incorporation of Design Requirements* into the online BRAIN curriculum

<b>Design Requirements</b>	<b>Description</b>
Scaffold Knowledge with Learning Objectives	Define and organize the knowledge and skill components for each instructional module scene in a sequence from basic to complex units of learning.
Learner’s Abilities	Account for the learner’s prior knowledge and skill development.
Instructional Methods	Establish the approach for presenting the lesson content.
Instructional Content	Focuses on the pediatric brain and MRI fundamental concepts and ideas that a medical provider would need to know.
Assessment Methods	Provide knowledge checks before, during or after user engagement with the lesson content. Assessment methods include true and false, multiple choice, multiple response, fill in the blank, drag and drop, and essay.

*Design Requirement #1: Scaffold Knowledge with Learning Objectives*

The first stage of the design process involved ensuring the instructional efficiency and proper organization of the training by breaking down the content into two categories (normal and abnormal brain development [aim 2]; modules 1-3 and MRI fundamentals [aim 1]; modules 4-6) and six instructional modules (Table 2). Module 1 examined the corpus callosum and other major cerebral commissures through the lens of normal and abnormal development. Module 2 focuses on normal and abnormal development of the cerebellum by reviewing the cerebellar anlagen, cerebral hemispheres, and vermis. Module 3 connects both categories together by

investigating brain plasticity and connectivity with structural MRI techniques following brain injury, while providing an overview of brain plasticity and describing how advanced MRI, can be used to measure changes in brain structure due to plasticity. Module 4 provides an introduction to MRI by reviewing basic magnetic physics and describes the origins of the MR signal and how precession is formed from longitudinal to transverse magnetization. Module 5 reviews the fundamentals of digital imaging by providing an overview of digital images, multidimensional data and reviewing medical imaging and their modalities. Finally, Module 6 walks learners through the important topic of pediatric MRI without sedation by summarizing key components of a successful pediatric non-sedate MRI program. Learning objectives were identified for each instructional module and Subject Matter Experts or SMEs created storyboards (Appendix D) as a visual representation of their presentation content that included navigation directions. The storyboard served as a tool to communicate the SME's narration and intended direction to the instructional systems designer and/or the multimedia specialist and what each course/lesson/learning object should actually look like online in a screen-shot format.

**Table 2.** *Web-based modules with learning objectives developed for pilot military audience*

Module Title	Learning Objectives
<b>PEDIATRIC BRAIN DEVELOPMENT</b>	
Module #1: Corpus callosum and other major commissures: anatomy, normal and abnormal development (Dr. Gilbert Vezina)	<ul style="list-style-type: none"> <li>• Discuss the corpus callosum and other major cerebral commissures looking at their anatomy through the lens of normal and abnormal development.</li> <li>• Understand why a full radiologic assessment is necessary to properly categorize a case of abnormal corpus callosum.</li> <li>• Understand the basis of the abnormal corpus callosum development and its genetic and clinical implications.</li> </ul>
Module #2: Normal and abnormal development of the cerebellum (Dr. Adre du Plessis)	Review the cerebellar anlagen <ul style="list-style-type: none"> <li>• Flexing of the rostral neural tube</li> <li>• Defining fundamental territories</li> <li>• Mesenchymal-neuroepithelial signaling</li> </ul> Describe cerebellar hemispheres and vermis <ul style="list-style-type: none"> <li>• Cellular proliferation</li> <li>• Cellular migration</li> <li>• Cellular differentiation</li> <li>• Neural organization</li> </ul>
Module #3: Investigating brain plasticity and connectivity with structural MRI techniques	<ul style="list-style-type: none"> <li>• Review the concept of brain plasticity</li> <li>• Describe how MRI can be used to</li> </ul>

(Dr. Cibu Thomas)	<p>measure changes in brain structure due to plasticity</p> <ul style="list-style-type: none"> <li>• Review the limitations of prevailing MRI studies on structural plasticity and how one can circumvent the limitations</li> </ul>
<b>MRI FUNDAMENTALS</b>	
Module #4: Introduction to MRI (Dr. Iordanis Evangelou)	<ul style="list-style-type: none"> <li>• Review basic magnetic resonance or MR physics</li> <li>• Describe the origins of the MR signal</li> <li>• Discuss the concept of protons, spin, the larmor equation</li> <li>• Review precession and how the MR signal is formed from longitudinal to transverse magnetization</li> </ul>
Module #5: Fundamentals of Digital imaging (Dr. Ahmed Serag)	<ul style="list-style-type: none"> <li>• Discuss the fundamentals of digital images and multidimensional Data</li> <li>• Review medical imaging and their modalities</li> </ul>
Module #6: Pediatric MRI without sedation: Is it the art or science? (Dr. Raymond Sze)	<ul style="list-style-type: none"> <li>• Review the role of a Certified Child Life Specialist</li> <li>• Summarize the key components of a successful pediatric non-sedate MRI program</li> <li>• Identify ideal candidates for attempting a non-sedate scan</li> <li>• Describe three major benefits of creating and implementing a pediatric non-sedate MRI program</li> </ul>

*Design Requirement #2: Learner Abilities*

The training module content is customized to be learner-centered, self-paced instruction. All pilot training modules are designed to be self-paced with a self-reflection questionnaire activity given at the conclusion of each lesson (see Appendix E) to gauge learner’s improvement with module objectives and to gather feedback on the user’s experience with the usability of the site, the content, and to gather recommendations for future module development. The premise behind the organization of the pilot module series is to provide users scaffolded instruction (Bruner, 1975) by presenting information with a guided explanation and discovery learning of the content. In certain module scenes, interactive markers are shown allowing users to access

and discover additional information about a presented topic or multimedia object. For example in module #1 at the end of scene 1.8, the scene prompts learners to click on interactive markers placed on an image of the corpus callosum to relearn the previously presented anatomical structures such as the location and function of the genu or rostrum (Appendix F).

### *Design Requirement #3: Instructional Methods*

Specific instructional methods were selected for each module based on intended learning objectives. The objectives informed the learning taxonomy for each module providing both foundational knowledge about the pediatric brain or MRI and case examples with multimedia objects incorporated throughout scenes for contextualization of concepts and assisting learners in acquiring new knowledge. For example, in module# 4 scene 2, several Flash multimedia objects containing layered graphical elements were created to illustrate the composition of magnetic resonance as 70% water with 2 hydrogen atoms. As the narrator continues within the scene, the multimedia objective transforms to illustrate the point that each atom has a single proton that rotates or pins around itself generating current and in turn induces a small magnetic field around it called a magnetic dipole moment (Figure 1). The Flash animations were created not just to better convey the instructional points, but also to promote active engagement, encourage self-reflection, and convey the personal relevance of knowledge. The text elements, interactive and composite still graphics were used to accommodate the visual learner while the audio narration supports the auditory learner.

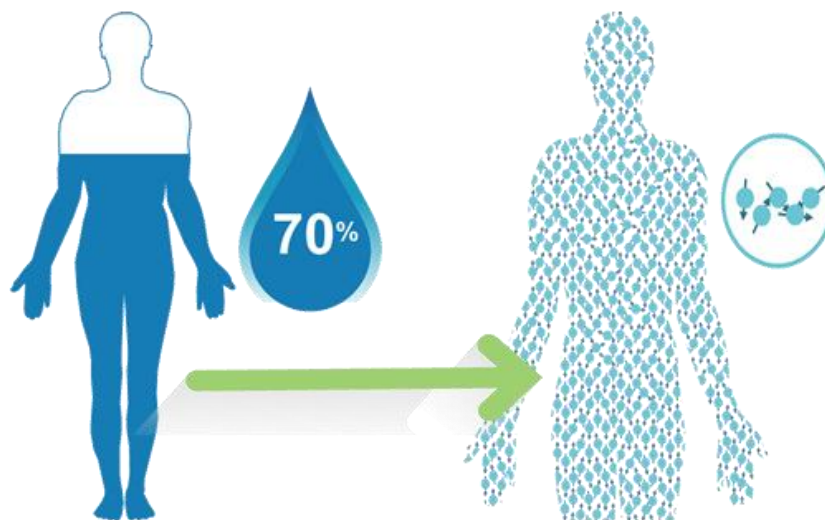


Figure1. Flash Multimedia Animation Example

Many of the graphical elements shown throughout the BRAIN modules incorporate the seven principles of design identified by Houser and DeLoach (1998):

- Contrast. Using a range of values, colors, textures, shapes, and other elements. Contrast creates visual excitement, increases interest, and places emphasis on content.
- Emphasis. The creation of a center of interest for the viewer. The center of interest attracts attention to emphasize its importance compared to the other elements in the composition.
- Balance. The appearance of visual equality in shape, form, value, and color. Balance can be symmetrical, asymmetrical, or radial.
- Unity. Enhance instruction by harmonizing sections and providing content cohesion.
- Patterns. Art elements that use planned or random repetition to enhance composition and increase users' visual experience.
- Movement. The visual flow of the content by object placement and position throughout composition.
- Rhythm. The repetition of visual movement in terms of color, shape, and lines.

#### *Design Requirement #4: Instructional Content*

Due to the amount of information presented and the visual elements needed to expand upon points or teach an objective, most module instruction is designed on a generalized content screen template. The content screen template contained some of the following elements: a slide title shown at the beginning of the module, multiple levels of bulleted text, still composite graphics, custom animations such as animated diagrams or illustrations with text or image fade-ins. The training modules are BEST viewed using the latest Adobe Flash plugin, which provides a screen visibility of the animated content. For operational purposes, the screens were designed to have a resolution of 1280 x 1024 and 1024 x 768.

#### *Design Requirement #5: Knowledge Assessments*

Assessment screens within the BRAIN training courseware are planned for development throughout years 4 & 5, but are not currently implemented into the pilot modules. Instead, we will gather participant feedback in several pilot tests at military medical facilities (see Implementation Plan) using a post-run module questionnaire accessible from inside the training portal (Appendix E). The post-run module questionnaire will gather feedback pertaining to perceived improvement of the module learning objectives, usability, organization and challenging/engaging nature of the instructional content as well as open-ended responses on what they liked and didn't like about the module, and recommendations for future module development.

## b. Development of the online portal

Development of the online portal and training modules began following the design phase. There were two primary objectives for this phase. The first was to develop a web 2.0 responsive portal that would house the instructional content. The portal can support online activity and resources such as archived lectures, SCORM-compliant training modules, quizzes, and videoconferencing and interactive capabilities. Site security policies ensure that users are safe and security is maintained. Additionally, the web portal is flexible in meeting different user needs, preferences, and situations while adhering to Government section 508 accessibility standards such as screen reader emulator compatibility (*e.g. Jaws or Nonvisual Desktop Access*) and other web browser accessibility extensions. Further, open source activity plugins such as quiz and game makers, electronic journals, discussion boards, blogs, wikis, podcasts, and live virtual classrooms are available to course creators. Site and course activity is monitored through a progress assessment engine and designated site administrators are able to run custom workflow and learning engagement analytic reports to view the completion and scoring of individual and cumulative learning objects such as training modules and assessments (Appendix G). A self-registration feature allows users to sign-up for an account (Appendix H). Account requests are sent to a secure administrator's email account for review and approval. When approved, users are sent a personalized confirmation email with instructions on how to login and use the system. Finally, an automatic enrollment feature enables course instructors to enable the system to electronically enlist users into courses (Appendix I).

Once the web portal and module content was storyboarded, the second objective was to develop the training modules with any embedded multi-media or dynamic interactions (Figure 2). The training module player includes the following features: 1.) Navigation pane, 2. Main stage, 3. Volume control, 4. Play button, 5. Control bar, 6. Rewind button, 7. Next and previous buttons.

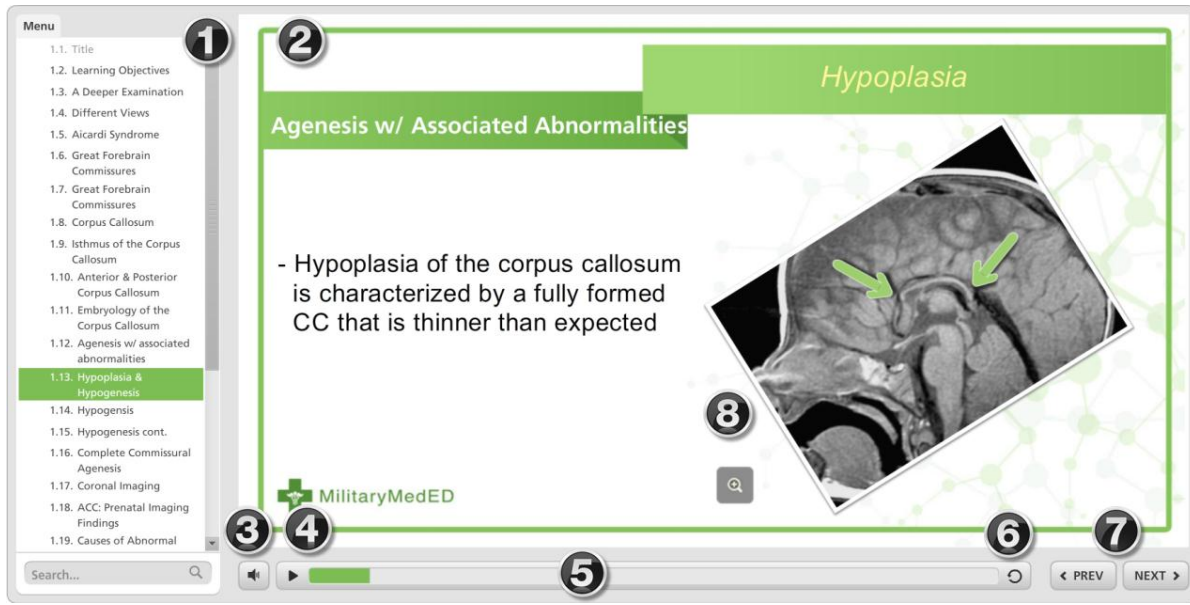


Figure 2. SCORM-compliant training module player

A combination of coding languages, including Hypertext Markup Language (HTML), Cascading Style Sheets (CSS), Extensible Markup Language (XML), Hypertext Preprocessor (PHP), Adobe Flash with Action Script (AS 1-3), Illustrator, Photoshop, Premier and various CAD Software, are used in developing the web portal (desktop, tablet, and mobile clients) and training modules. After implementation, the developed portal and modules are run through an extensive series of tests to ensure compatibility on multiple platforms and browsers.

### **c. Implementation Plan**

Another goal of this project is to provide strategic recommendations and strategies implementing the web-based portal and online content by actively promoting awareness and informing target audiences throughout the development lifecycle. The following are topics and recommendations for implementing MilitaryMedED.com and supplemental educational tools.

For the Government to incorporate MilitaryMedED.com into practice, the site must first be introduced and accepted by key stakeholders at top medical military installations as an applicable tool for providing distance learning and training on emerging medical topics, such as the developing pediatric brain and MRI fundamentals. Upper-level management support at both executive and ground levels should see the portal and associated educational tools as a modality for saving time and training costs, and provide quality training experiences and continuing education. Additionally, endorsements from intergovernmental organizations, such

as the National Institutes of Health and external medical associations such as Accreditation Council for Graduate Medical Education, would provide credibility to the site, and help accomplish the project’s objectives for creating innovative medical education on emerging medical areas such as pediatric brain development. We propose a three-step approach for obtaining organizational buy-in and implementing the training portal. Table 3 illustrates this approach by listing the steps by year, associated action(s), reasons for gaining organization acceptance, and suggested resource

**Table 3.** Three-Phased Approach for Obtaining Organizational Acceptance

STEP	ACTION(s)	RATIONALE	SUGGESTED RESOURCES
<p><b>Phase 1. Orient</b></p>	<p>➤ In <b>year 4</b>, we will set-up and attend meetings and introduce/demonstrate MilitaryMedED.com’s associated training tools at military medical bases with healthcare professionals and stakeholders. The third column represents our top five recommended bases for demonstrations.</p>	<ul style="list-style-type: none"> <li>• Familiarize target audiences at military medical installations with tool’s capabilities</li> <li>• Implement requirements or best fit possibilities for where the tool could be incorporated in the hospital at an organizational level for medical professionals</li> </ul>	<ol style="list-style-type: none"> <li>1. Walter Reed National Military Medical Center - Bethesda, Maryland</li> <li>2. San Antonio Military Medical Center - Fort Sam Houston, Texas</li> <li>3. Tripler Army Medical Center - Honolulu, Hawaii</li> <li>4. Darnall Army Medical Center - Fort Hood, Texas</li> <li>5. Landstuhl Regional Medical Center - Kaiserslautern, Germany</li> </ol>
<p><b>Phase 2. Implement</b></p>	<p>➤ In <b>year 4</b>, create and implement outreach activities such as online or in-person peer exchanges, training workshops, and stakeholder meetings.</p>	<ul style="list-style-type: none"> <li>• Maintain awareness of MilitaryMedED.com training portal with target audiences</li> <li>• Provide training support for users and facilitators</li> <li>• <i>Implement and</i></li> </ul>	<ul style="list-style-type: none"> <li>○ <i>Onsite Military Base Peer Exchanges</i></li> <li>○ <i>Regular teleconference meetings</i></li> <li>○ <i>Quarterly Refresher</i></li> </ul>



		<i>evaluate</i> best practices for using the tool	<i>Training Workshops</i>
<b>Phase 3. Inform</b>	<ul style="list-style-type: none"> <li>➤ In <b>years 4 &amp; 5</b>, document and write-up research results, best practices, and use cases for implementing the portal and supplemental tool(s) from the outreach activities.</li> <li>➤ Development Phase 2 &amp; 3 - Develop additional training content and modules based on data results.</li> </ul>	<ul style="list-style-type: none"> <li>• Continue to maintain awareness of MilitaryMedED.com and tools with target audiences</li> <li>• <b>Validate</b> the implementation approach</li> <li>• Market the tool to a wider audience and outside organizations with similar challenges</li> </ul>	<ul style="list-style-type: none"> <li>○ <i>Military Newsletter</i></li> <li>○ <i>Medical Journals</i></li> </ul>

d. Technical and Training Support

Other areas for consideration when implementing MilitaryMedED.com is to provide technical and training support to assist users in problems or challenges that may arise when operating the software. Generally, training and technical support services will attempt to help users solve specific problems with the portal or while using the training. Table 4 provides recommendations for technical and training support services that can be implemented following year 5 and beyond, if additional financial support can be obtained.

**Table 4.** Technical and training support recommendations

Technical Support	Training Support
<ul style="list-style-type: none"> <li>• Purchase a dedicated technical support phone number</li> <li>• Set-up a dedicated support email address such as <a href="mailto:help@militarymeded.com">help@militarymeded.com</a></li> <li>• Have users use the helpdesk plugin integrated in the system</li> <li>• Have users report an incident via the site’s messaging system</li> <li>• Create annual users guides that illustrate best practices and new features of the tool. These documents can also be issued to end user community as a form of technical support.</li> </ul>	<ul style="list-style-type: none"> <li>• Hold in-person train-the-trainer workshops</li> <li>• Create independent references such as coaching or facilitation guides</li> <li>• Create facilitation guides that can be used to assist medical professionals in using the site and its tools by providing instruction that concentrates on presenting teaching strategies, expert tips, and best practices for utilizing resources</li> </ul>

**e. System Sustainment**

For military or Government medical professionals to get the best use out of MilitaryMedED.com, continual updates of the hardware and software are highly advised. Continuous use of the system provides a logistical tail to assist in the implementation of new features and incorporation of user feedback, which allow the portal and modules to expand and adapt to future requirements. Implementing quarterly upgrades that address user feedback and evolving needs provide a sense of “ownership” for the user, while a system that doesn’t change over time to meet the most frequent user requests will frustrate and eventually alienate the user community. Input for these upgrades and new features come via direct user feedback to the website, after action reviews following training events, and helpdesk requests.

Table 5 depicts system sustainment needs along with strategies for fulfilling those needs.

**Table 5.** System Needs & Strategies for Need Fulfillment

System Sustainment Needs	Strategies for Need Fulfillment
<p><b>Hardware</b></p> <ul style="list-style-type: none"> <li>○ Long-term hosting</li> <li>○ Server maintenance</li> </ul>	<ul style="list-style-type: none"> <li>○ Support and sustainment from sponsoring departments and organizations</li> <li>○ Revenue stream from providing turnkey training facilitation.</li> </ul>

<b>Software</b> <ul style="list-style-type: none"> <li>○ New tools &amp; features</li> <li>○ Addressing user feedback</li> </ul>	<ul style="list-style-type: none"> <li>○ Support and sustainment from sponsoring departments and organizations</li> <li>○ Revenue stream from providing customization services</li> </ul>
--	---

Lastly, to successfully implement MilitaryMedED.com, research will be conducted to document and report the effectiveness and usage of the portal for training and teaching on the developing pediatric brain and MRI fundamentals. As part of our base demonstration meetings, we will hold up to 5 field tests with 8-10 military and civilian medical professionals. This phase of implementation will allow us to determine effectiveness within a cross-section of medical professionals, thus further validating the need and usage for creating and maintaining the portal and training tools. If discrepancies exist between the initial implemental findings identified in the early phases of field testing, we will examine these discrepancies and make design adjustments to the portal platform and/or tools. The output of the follow-up research will be requirements used to enhance the existing portal functionality, document best practices for using the tools, and present learning effectiveness results. All findings will be summarized in subsequent annual reports.

f. Potential Impediments and Impacts

Lessons learned, from implementing over two dozen online training portals here at Children’s National Medical Center and with partnering organizations, have shown an initial reluctance from users from distance learning tools. A strategy for overcoming this reluctance is to provide real-time coaching and facilitation support via field tests and onsite demonstrations (see Table 3). Our experience has shown that teaching stakeholders and learners how to properly use the training provides the necessary guidance and experience needed for long-term effective use and promotion of the portal system. For this reason, Children’s National can provide both short and long-term onsite and webinar facilitation support services. However, this will require additional financial assistance. We plan to pursue additional funding to extend this work.

Similarly, another common impediment to field testing distance learning systems is reluctance for people who are technologically-challenged and do not engage in dynamic web applications on a regular basis. Again, we have found that real-time facilitation and coaching encourages users to work in teams that can help remedy this issue and offer added benefits. For example, an inexperienced person who trains alongside an experienced person will be exposed on how to best use features and functionality in the portal interface while directly being

mentored. Mentoring involves the passing of wisdom, knowledge, and experience from the mentor to the learner. A primary goal of MilitaryMedED.com is to foster peer-to-peer and mentoring relationships over a period of time and usage of the tool to adjust learner's skill levels and needs. Mentoring teaches the learner *how* to think, rather than *what* to think, and mentors are usually people who have vast experience in a given domain. Mentoring can be an impactful teaching mechanism by providing one-on-one guidance, encouraging self-learning and reflection, and giving concise feedback after learners struggle through training and exercises.

g. Future activities

For year 4 of the project, we will complete the following tasks.

<b>Task</b>	<b>Description/Objective(s)</b>
<b>Complete e-learning storyboards</b>	Finalize the remaining storyboards that need to be converted from in-classroom lectures to e-learning modules.
<b>Hold 3-5 field tests and portal demonstration meetings at Children's National and military medical installations</b>	We will conduct three to five field tests locally at Children's National and volunteer military medical facilities (see the top five recommended locations in table 3). The recommended facilities were selected based on their expertise in pediatrics, and/or neurology, radiology, traumatic brain injury and medical education activities. The field tests will be used to generate a Kirkpatrick Level 1 evaluation, which is focused on participant reaction. We will conduct a hotwash after the field tests to elicit electronic feedback from all participants via an electronic questionnaire (Appendix C) that will focus on the following areas: <ul style="list-style-type: none"> <li>• Usability of the system</li> <li>• Accuracy of the training</li> <li>• Effectiveness of the training</li> <li>• Future training module development recommendations</li> </ul>
<b><i>Begin to iterate the existing pilot training modules and develop new modules based on user feedback from the field tests and demonstration meetings</i></b>	<ul style="list-style-type: none"> <li>• In year 4, we will document and write-up initial field test results and begin enhancing the existing training content and developing additional training modules based on data results in years 4 &amp; 5.</li> </ul>

<b>Task</b>	<b>Description/Objective(s)</b>
<b>Prepare and submit year 4 annual report</b>	We will document the work and conclusions from the year 4 tasking in the annual report. The report will contain an analysis of the field test events and Kirkpatrick training effectiveness evaluations, as well as a report on the current status of any issues and suggestions arising from the field test events. The report will also contain our draft Year 5 work plan for feedback prior to final submission.

## **KEY RESEARCH ACCOMPLISHMENTS**

- Successful recruitment of one civilian trainee in the BRAIN training program (Dr. An Massaro)
  - completed CITI and CREAT courses
  - research project was successfully developed and approved by IRB
  - completed Pediatric Neuropsychology Training Day
  - mentoring team for Dr. Massaro's training was established
  - Dr. An Massaro's research achievements over the last 12 months are summarized under the reportable outcomes section.
- Successful 2<sup>nd</sup> year implementation of our on-site BRAIN curriculum
- Successful recruitment of a multimedia developer (Ben Scalise) to work with PI and Jeff Sestokas (our Senior Instructional Systems Designer) to support the development of our web-based BRAIN curriculum.
- Initial development and implementation of the web-based BRAIN curriculum
  - developed a web-based portal site located at [www.MilitaryMedED.com](http://www.MilitaryMedED.com) that users can search, upload, and house online training and education-related information.
  - developed and uploaded six SCORM-compliant online training modules.
  - incorporated our newly developed on line FACTS (Focus on Clinical and Translational Science) curriculum onto our portal site.

## REPORTABLE OUTCOMES

Academic Accomplishments: Dr. An Massaro (BRAIN Trainee)

### Scientific/Conference Presentation:

Massaro AN, Evangelou I, Glass P, Limperopoulos C. White matter tract microstructure predicts outcome in newborns with hypoxic ischemic encephalopathy (HIE) treated with hypothermia. Presented at the 2014 Pediatric Academic Societies' Annual Meeting in Vancouver, Canada, May 3-6, (platform).

*Pediatric Academic Societies Annual Meeting-Session Moderator:*“**Hypothermia & Hypoxic Ischemic Encephalopathy**” 2014 Pediatric Academic Societies' Annual Meeting in Vancouver, Canada, May 4 2014.

### Grants:

Project Title: Quantifying Basal Ganglia and Thalamic Injury in Neonatal Hypoxic Ischemic Encephalopathy – A Method for Early Assessment of CP Risk

Funding Agency: Cerebral Palsy International Research Foundation

Date of Award: 1/1/2014-1/1/2016

### Manuscripts

**Massaro AN**, Evangelou I, Fatemi A, Vezina G, McCarter R, Glass P, Limperopoulos C. White matter tract integrity and developmental outcome in newborns with hypoxic ischemic encephalopathy (HIE) treated with hypothermia. *Dev Med Child Neurol*, accepted with revision. (Appendix J)

**Massaro AN**, Evangelou I, Fatemi A, Vezina G, McCarter R, Glass P, Limperopoulos C. White matter tract integrity is associated with neonatal neurobehavior after therapeutic hypothermia for hypoxic ischemic encephalopathy. In preparation.

## **CONCLUSION**

In summary, we have successfully developed an on online learning management system and implemented methods for converting our on-site BRAIN seminars into self-directed web-based learning modules. Our portal site, [www.MilitaryMedED.com](http://www.MilitaryMedED.com) is modular, and our Subject Matter Experts have started creating online BRAIN courseware, which will be made available for review and feedback to DoD and civilian trainees over the next year. Equally successful was a one-year on-site training of our civilian fellow, who demonstrated solid adademic accomplishments over a 12 month period. We will proceed in year 4 activities beginning with holding field tests and demonstration meetings, completing and uploading the remaining e-learning modules, and then proceed with tasking as described in the above future activities table.



## REFERENCES

Bruner, J. S. (1975). The ontogenesis of speech acts. *Journal of Child Language*, 2, 1-40.

Houser, R., & DeLoach, S. (1998). Learning from games: Seven principles of effective design. *Technical Communication*, 45, 319-329.

Mayer, R. et al. (2014). *The Cambridge Handbook of Multimedia Learning*. New York, NY. Cambridge University Press.

## **APPENDICES**

Appendix A    FACTS (Focus on Clinical and Translational Science)

Appendix B    DoD Site visit report

Appendix C    Web-based Learning Management System Portal

Appendix D    Storyboard Procedure & Template

Appendix E    Post-run Module Questionnaire

Appendix F    Guided Discovery Interactions in the TRAINING MODULE

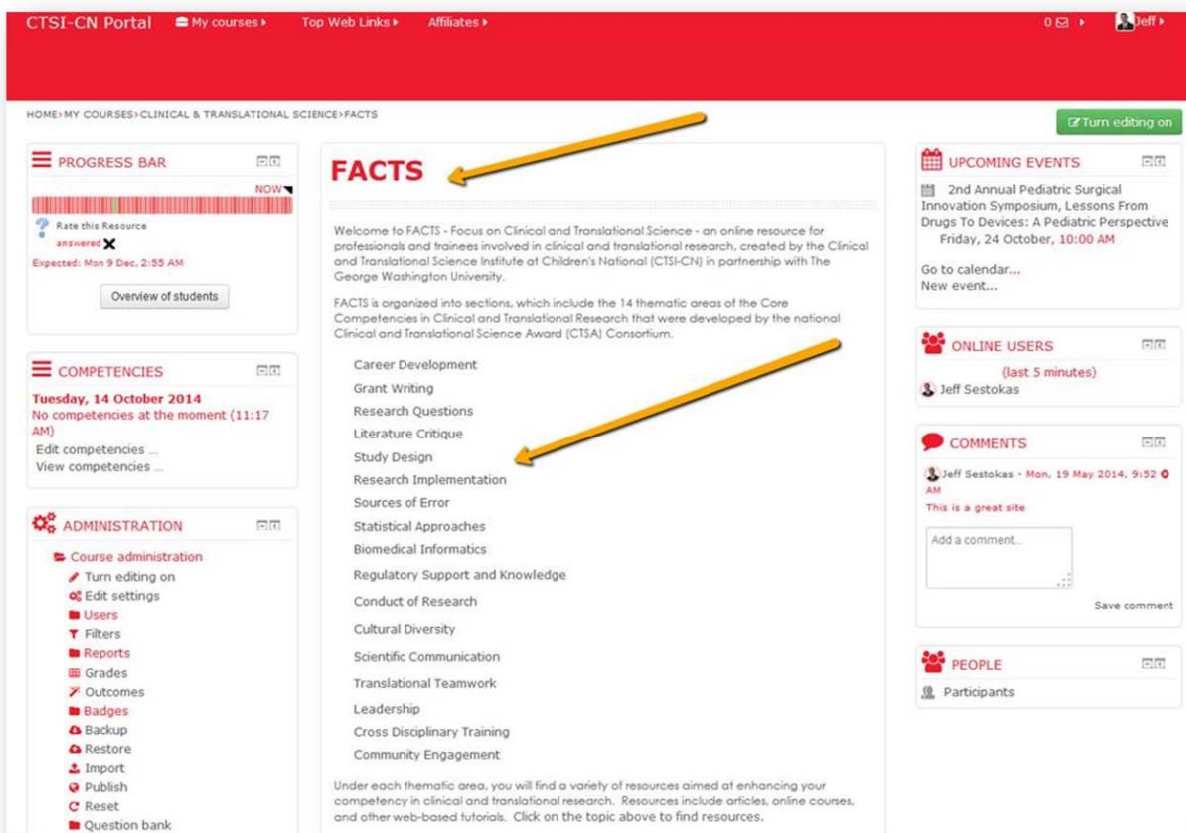
Appendix G    Three-Level reporting system

Appendix H    Self-Registration via Administrator Confirmation

Appendix I    Automatic Enrollment Feature

Appendix J    White Matter Tract Integrity and Developmental Outcome  
In Newborns with Hypoxic Ischemic Encephalopathy (HIE) treated with  
Hypothermia

**APPENDIX A:**  
**FACTS**  
**(Focus on Clinical and Translational Science)**



CTSI-CN Portal | My courses | Top Web Links | Affiliates | 0 | Jeff

HOME > MY COURSES > CLINICAL & TRANSLATIONAL SCIENCE > FACTS

**PROGRESS BAR**

Rate this Resource  
answered X  
Expected: Mon 9 Dec, 2:55 AM  
Overview of students

**COMPETENCIES**

Tuesday, 14 October 2014  
No competencies at the moment (11:17 AM)  
Edit competencies ...  
View competencies ...

**ADMINISTRATION**

- Course administration
  - Turn editing on
  - Edit settings
  - Users
  - Filters
  - Reports
  - Grades
  - Outcomes
  - Badges
  - Backup
  - Restore
  - Import
  - Publish
  - Reset
  - Question bank

**FACTS**

Welcome to FACTS - Focus on Clinical and Translational Science - an online resource for professionals and trainees involved in clinical and translational research, created by the Clinical and Translational Science Institute at Children's National (CTSI-CN) in partnership with The George Washington University.

FACTS is organized into sections, which include the 14 thematic areas of the Core Competencies in Clinical and Translational Research that were developed by the national Clinical and Translational Science Award (CTSA) Consortium.

- Career Development
- Grant Writing
- Research Questions
- Literature Critique
- Study Design
- Research Implementation
- Sources of Error
- Statistical Approaches
- Biomedical Informatics
- Regulatory Support and Knowledge
- Conduct of Research
- Cultural Diversity
- Scientific Communication
- Translational Teamwork
- Leadership
- Cross Disciplinary Training
- Community Engagement

Under each thematic area, you will find a variety of resources aimed at enhancing your competency in clinical and translational research. Resources include articles, online courses, and other web-based tutorials. Click on the topic above to find resources.

**UPCOMING EVENTS**

2nd Annual Pediatric Surgical Innovation Symposium, Lessons From Drugs To Devices: A Pediatric Perspective  
Friday, 24 October, 10:00 AM  
Go to calendar...  
New event...

**ONLINE USERS**

(last 5 minutes)  
Jeff Sestokas

**COMMENTS**

Jeff Sestokas - Mon, 19 May 2014, 9:52 AM  
This is a great site  
Add a comment...  
Save comment

**PEOPLE**

Participants

Turn editing on

## APPENDIX B



Ashok Panigrahy, MD  
Radiologist-in Chief

Children's Hospital of Pittsburgh of UPMC  
One Children's Hospital Drive  
4401 Penn Avenue  
Pittsburgh, PA 15224  
412-692-5510  
Fax: 412-864-8622

[www.chp.edu](http://www.chp.edu)

June 7, 2014

Catherine Limperopoulos, PhD  
Director, MRI Research of the Developing Brain  
Director, Advanced Pediatric Brain Imaging Research Laboratory  
Diagnostic Imaging and Radiology/Fetal and Transitional Medicine  
Children's National Medical Center  
Associate Professor of Neurology, Radiology, and Pediatrics  
George Washington University School of Medicine and Health Sciences  
111 Michigan Ave. N.W.  
Washington, D.C. 20010

Dear Catherine,

The following is the report regarding the site visit of by the External Advisory Board for your Department of Defense (DOD) training grant entitled "Advanced Pediatric Brain Imaging Research Training Program (W81XWH-11-2-0198)", which met on April 30, 2014. The board members consist of myself and Drs. Michael V. Johnston and John VanMeter, Ph.D.

Our uniform consensus is that you have formed an excellent training program for DoD trainees in the latest imaging techniques in pediatric brain injury, clinical research, and translation of these techniques to the bedside. The training program is comprehensive and incorporates experts from both Children's National Medical Center and National Institutes of Health. The topics covered in your program include diffusion tensor imaging (DTI), MR spectroscopy (MRS), morphological analyses such as voxel-based morphometry (VBM), and functional MRI (fMRI).

In terms of prior issues, the only problem identified was with respect to the numerous hurdles in working with the various branches and components of the military related to obtaining permission for the trainees to attend this training program: release from their current command to attend and the need to avoid incurring additional military service commitment. You and your team had made numerous attempts to overcome these problems by reaching out to a variety of military offices to develop a system or

mechanism to allow trainees to take advantage of this program. Over the last year following approval from the DOD to broaden the scope of your BRAIN training program, your group has been focused on developing an innovative advanced neuroimaging web-portal which can then be used by multiple DOD bases and non-DOD sites which will clearly overcome some of the prior geographic/sequestration issues encountered by DOD trainees.

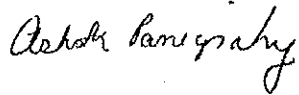
We were very impressed with the plans that your group has undertaken to develop a web-portal with the goal of transforming in-classroom BRAIN seminars into online courses. The live demonstration by Jeff Sestokas (Senior Instructional Systems Designer for the DOD BRAIN training program) of the system showed how excellent the system was. There was a review of the Dryfus 5 stages of training including going from novice to expert. The system is modular and runs on any platform and uses open source tools. The simulated Guided Learning Modules will be developed together with the expert content developers using state-of-the-art tools. This will allow for trainees to have access to an interactive platform including discussion forums, blogs, wikis, etc. Given that your DOD seminar attendance has exceeded its physical capacity, this on-line web portal training resource will allow for greater participation for civilian trainees as well. Your proposed timeline for transforming the in-classroom BRAIN educational curriculum into a web-based remote learning program, and implementing online interactive BRAIN seminars over the next 12 months seem very appropriate.

We were also very impressed with your civilian trainee, Dr. An Massaro, who presented the overall objectives of her neonatal hypoxic-ischemic encephalopathy (HIE) cooling brain project. She had lots of challenges in getting training in advanced neuroimaging techniques prior to the creating of the DOD BRAIN training program. She has enrolled 124 neonates with hypoxic-ischemic injury treated with hypothermia and has collected DTI and ASL data at 2 time points. She has collective quantitative MR imaging data (diffusion and perfusion MRI) which is being analyzed and correlated with neurodevelopment outcome. She has benefited tremendously from the DOD training program you have developed, and having access to a repository of healthy control neonatal MRI studies which is a very important component to the research. She has accomplished very much in a short time under your guidance including moderating a session and a platform presentation at the recent 2014 Pediatric Academic Society meeting. She has clearly mastered the technical details of the advanced neuroimaging techniques that she has learned via your training program. She will begin preparing an R21 grant submission on early brain imaging biomarkers for neonatal HIE, and our only recommendation is to consider having her submit and R01 given the amount of preliminary data that she has collected.

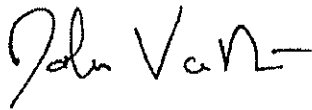
In summary, we commend you and your team for creating an excellent program providing training in the latest neuroimaging research techniques along with the essential methodologies for conducting clinical research. We laud your efforts to successfully resolve the DOD trainee recruitment/retention problems you previously encountered by creating a web-based BRAIN educational platform to advance and disseminate in a more

broad-based fashion the fundamental principles and clinical application of advanced MRI techniques to understand the causes and consequences of pediatric brain injury.

Sincerely,



Ashok Panigrahy, MD  
Radiologist-In-Chief, Department of Pediatric Radiology,  
Associate Professor of Radiology  
Children's Hospital of Pittsburgh  
University of Pittsburgh School of Medicine



John VanMeter, Ph.D.  
Associate Professor, Department of Neurology  
Director, Center for Functional and Molecular Imaging  
Georgetown University Medical Center  
Preclinical Sciences Building, Suite LM-14, GU Box 571488  
3970 Reservoir Road NW  
Washington, DC 20057-1488

*Michael V. Johnston, M.D.*

Michael V. Johnston, M.D.  
Senior Vice President and Chief Medical Officer, Kennedy Krieger Institute  
Kennedy Krieger Institute  
707 N. Broadway  
Baltimore, MD 21205

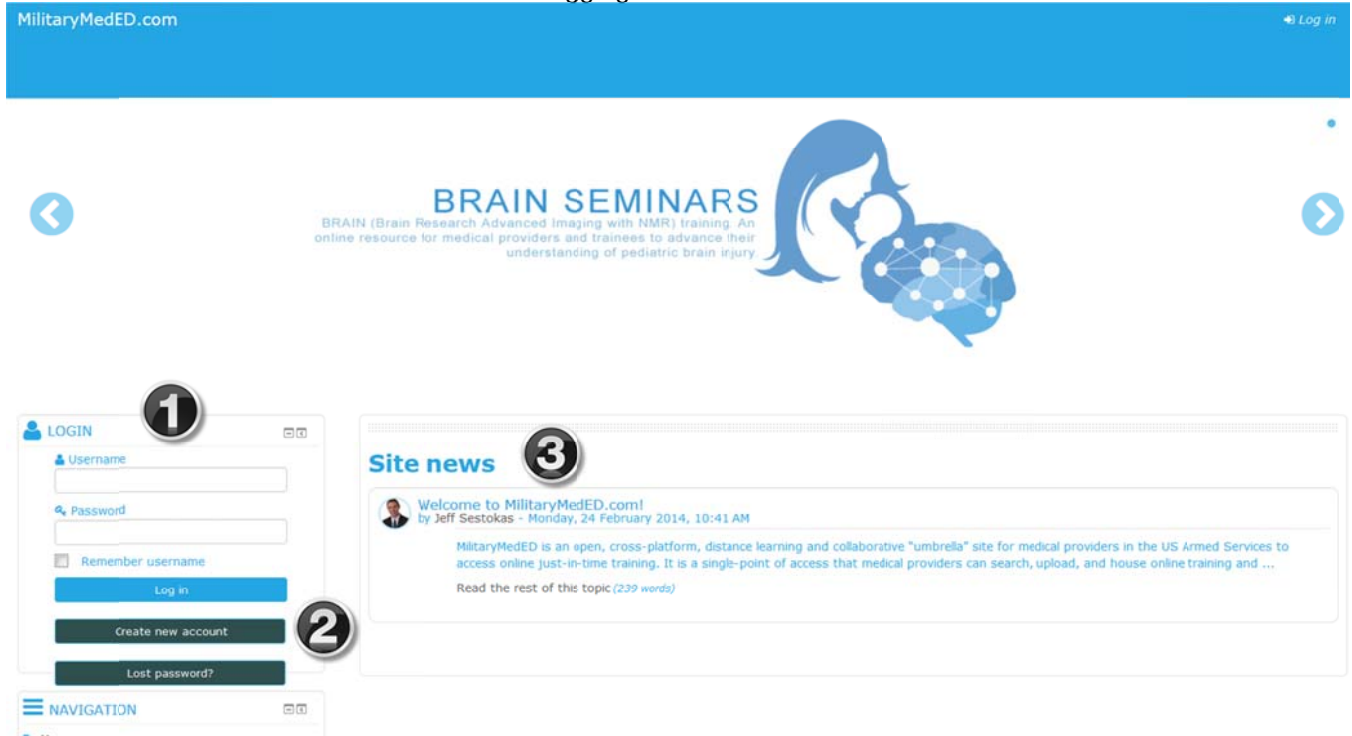


**APPENDIX C:**

**WEB-BASED LEARNING MANAGEMENT SYSTEM PORTAL**

---

C-1. Logging into the site.



The screenshot shows the MilitaryMedED.com website. At the top, there is a blue navigation bar with the site name and a 'Log in' link. Below this is a main content area with a header for 'BRAIN SEMINARS' and a graphic of a woman holding a child's head next to a brain diagram. The page is annotated with three numbered circles: 1 is over the 'LOGIN' section, 2 is over the 'Create new account' button, and 3 is over the 'Site news' section. The 'LOGIN' section includes fields for 'Username' and 'Password', a 'Remember username' checkbox, and buttons for 'Log in', 'Create new account', and 'Lost password?'. The 'Site news' section features a 'Welcome to MilitaryMedED.com!' message by Jeff Sestokas, dated Monday, 24 February 2014, 10:41 AM, followed by a brief description of the site's purpose and a link to read more.

C-2. Interface Layout





---

## APPENDIX D:

### STORYBOARD PROCEDURE & TEMPLATE

---

#### I Objectives

- List 2-3 Objectives from presentation
- Remove any content from presentation not relevant to objectives
- View an example of a slide translated into a Storyboard [see page4]

#### II Narrative

- Condense & Bullet Point Main Dialog from Objectives
- Provide Script for Voice Over [see page6]

#### III Assessment Questions

- Create 3-5 assessment questions from Objectives [see page5]

Assessment Question Options:

- a. Create Assessment Questions throughout the body (preferred with or without Post-Test)
- b. Create Post-Test only
- c. Create Post-Test with Assessment Questions throughout the body

**\*\* This information can be delivered either via Storyboard Template as subsequently provided or in the Notes Section of your PowerPoint presentation slides.**

---

This will assist in creating the 3 main sections of the Module. See [link](http://www.childrensmedicaleducation.org/cbt/complex/mod1/story.html) for example.  
[<http://www.childrensmedicaleducation.org/cbt/complex/mod1/story.html>]

#### 1. Intro

- a. Home
- b. Welcome
- c. Learning Objectives Briefing

#### 2. Body (note that the Assessment Questions can be interspersed throughout the body as shown in this example and/or included as a Post Test at the end of the 2-3 Objectives)

- a. Objective1
  - i. Assessment Question
  - ii. Assessment Question
- b. Objective2
  - i. Assessment Question
- c. Objective3
  - i. Assessment Question
  - ii. Assessment Question



- d. Post Test (Optional to include with or without interspersed Assessment Questions)
  - i. Assessment Questions

**3. Summary**


- Brief review of all content discussed

**Online Learning Module Storyboard**

<b>Course:</b>			
<b>Module:</b>			
<b>Lesson:</b>		1	
<b>Segment:</b>		1	
<b>Page Title:</b>		1	
<b>Child Page:</b>			
<b>Objective:</b>			
<b>On-Screen Text:</b>			
<b>Narration / Closed Captioning: Narrator</b>			
<b>Graphics:</b> (P – photo; G – graphic; F – flash animation; T – table/chart/graph; V – video)			
<b>Audio:</b>			
<b>Knowledge Check:</b>		<b>Remedial Screen: Page ID</b>	
Correct Feedback:			
1 <sup>st</sup> try incorrect:			
2 <sup>nd</sup> try incorrect:			
<b>Explanatory Information:</b>			
<p><i>Italics has no functional effect</i>  <b>Bold</b> is a rollover  <u>Underscore</u> is a click to pop-up with click to close</p>			

<b>Branching:</b>	<b>Back:</b>	<b>Next:</b>
-------------------	--------------	--------------

**Storyboard Example - Objective**

<b>Course:</b>	Illness Scripts		
<b>Module:</b>	<i>Using Illness Scripts to Improve Clinical Diagnostic Reasoning Skills</i>		
<b>Lesson:</b>		1	
<b>Segment:</b>		1	
<b>Page Title:</b>		3	
<b>Child Page:</b>			
<b>Objective:</b>			
<b>On-Screen Text:</b>			
Components of an Illness Script			
<ul style="list-style-type: none"> <li>1) Epidemiology</li> <li>2) Time Course</li> <li>3) Clinical Presentation</li> <li>4) Mechanisms</li> </ul>			
<b>Narration / Closed Captioning: Narrator</b>			
What makes up an illness script? The 4 elements of an illness script are epidemiology, time course, clinical presentation, and mechanism.			
<b>Graphics:</b> (P – photo; G – graphic; F – flash animation; T – table/chart/graph; V – video)			
			
<i>This graphic corresponds with the <b>Narration</b> above.</i>			
<b>Audio:</b>			
<b>Knowledge Check:</b>		<b>Remedial Screen: Page ID</b>	
Correct			
Feedback:			



1 <sup>st</sup> try incorrect:	
2 <sup>nd</sup> try incorrect:	
<b>Explanatory Information</b>	
<i>Italics has no functional effect</i>	
<b>Bold</b> is a rollover	
<u>Underscore</u> is a click to pop-up with click to close	
<b>Branching:</b>	Back: <span style="float:right">Next:</span>

**Storyboard Example – Assessment**

<b>Course:</b>	Illness Scripts				
<b>Module:</b>	<i>Using Illness Scripts to Improve Clinical Diagnostic Reasoning Skills</i>				
<b>Lesson:</b>		1			
<b>Segment:</b>		1			
<b>Page Title:</b>		10			
<b>Child Page:</b>					
<b>Objective:</b>					
<b>On-Screen Text:</b>					
Table					
<b>Narration / Closed Captioning: Narrator</b>					
Differentiating features can be found in some but not all of the diseases helping to narrow down the differential. In a 6 day old with jaundice, what are some differentiating features?					
<b>Graphics:</b> (P – photo; G – graphic; F – flash animation; T – table/chart/graph; V – video)					
Table					
Disease	Mechanism	Epidemiology	Time	Symptoms	Tests
Physiologic jaundice	Increased bilirubin from the breakdown of fetal red cells and relative deficiency of hepatocyte proteins and UDPGT combined with lack of	Can occur in all newborns	Peaks on day 3	Jaundice	Bilirubin level < 15



	intestinal flora to metabolize bile				
Breast milk jaundice	$\beta$ -glucuronidase present in breast milk deconjugates bilirubin in the intestinal tract;the unconjugated bilirubin is then reabsorbed via enterohepatic circulation.	Breast feeding infants	Begins DOL 4-7, peaks DOL 10-14	Jaundice	Bilirubin level
Hemolysis	Breakdown of RBCs	Rh incompatibility, ABO incompatibility	Evident in first few days of life	Jaundice	Coombs test Bilirubin level
Metabolic disease	Various mechanisms				Newborn screen

**Audio:**

**Knowledge Check:**

Correct Feedback:

*Timing of jaundice – physiologic jaundice usually peaks on day 3. Jaundice due to hemolysis will be evident in first few days of life. Breast milk jaundice and metabolic disease can peak at a later date. Level of hyperbilirubinemia – total serum bilirubin > 15 can rule out physiologic jaundice. A normal newborn screen can rule out metabolic disease.*

**Remedial Screen: Page ID**

1<sup>st</sup> try incorrect:

2<sup>nd</sup> try incorrect:

**Explanatory Information:**

*Italics has no functional effect*

**Bold** is a rollover

Underscore is a click to pop-up with click to close

**Branching:**

Back:

Next:

**Narration – Script Example**

**Scene 1**

- a. Hello, my name is Iordanis Evangelou. Welcome to the Introduction to MRI. For this lesson, I will (a) introduce the basics of Magnetic Resonance (MR) Physics and (b) the origin of the MR signal.

- b. By the end of this module you will understand the concept of protons, spins, the Larmor equation, precession and how the MR signal is formed from longitudinal to transverse magnetization.

### Scene 2

Starting at the molecular level, MR is based on proton (Hydrogen 1) imaging. The human body is composed of 70% water with 2 Hydrogen atoms in each water molecule (H<sub>2</sub>O) (figure 1). Each atom has a single proton that rotates around itself (spin) generating current and in turn induces a small magnetic field around it (magnetic dipole moment) (figure 2).

### Scene 3

(figure 3a) In the presence of a strong magnetic field (1.5T, 3.0T or above), the protons spin to the direction of the magnetic field ( $B_0$ ) some parallel and some antiparallel (figure 3b).

The unit of measurement of the magnetic field strength is the Tesla (T). For example, 1.5T magnetic field strength is about 30,000 times the strength ( $1.5T = 30,000$ ) of the earth's magnetic field.

### Scene 4

(figure 4) A single spin rotates around its own axis. As it aligns itself with ( $B_0$ ) the external magnetic field its axis of rotation shifts, forming a cone-like shape (figure 5a). This movement is called precession, expressed by the Larmor equation:  $\omega = \gamma \times B_0$

(figure 5b) where  $\omega$ , the precession frequency in Hz, is the number of precessions per second.  $\gamma$  is the gyromagnetic ratio specific to each nucleus and  $B_0$  is the strength of the magnetic field strength in Tesla. Protons (<sup>1</sup>H) precess at 64MHz and 128MHz at 1.5T and 3.0T respectively.

#### Aligns itself with ( $B_0$ )

Axis of Rotation shifts

Cone-Like Shape

Precession ( $\omega = \gamma \times B_0$ )

### Scene 5

Looking in a 3D (X,Y,Z) space, the external magnetic field ( $B_0$ ) is applied along the Z-axis (figure 6). Protons align parallel (positive Z axis) to the external magnetic field ( $B_0$ ) and antiparallel (negative Z-axis).

Their forces cancel each other out leaving a few protons on the positive Z-axis (figure 7). The sum of these forces forms a magnetic vector along the Z-axis called Longitudinal Magnetization ( $M_z$ ) (figure 8).



### Scene 6

(figure 9a) The Longitudinal Magnetization cannot be measured directly, as it has to be transverse (in the X-Y plane). The next step in the process is to transmit a radiofrequency (RF) pulse (figure 9b).

The precessing protons absorb some energy from the RF pulse while some protons go to a higher energy level and precess antiparallel to the magnetic field (negative Z axis). This causes the magnitude of the Longitudinal Magnetization ( $M_z$ ) to decrease(s) and to be tilted into the transverse (X-Y) plane forming Transverse Magnetization (MXY) forms (figure 9c).

*//Magnitude of longitudinal Magnetization ( $M_z$ ) Decreases//*

For this to occur, the precession frequency of the protons should be the same as the RF pulse frequency. This phenomenon is called resonance, the “R” of MRI.

*//Precession Frequency Protons = RF Pulse Frequency, Resonance//*

### Scene 7

(figure 9c) Once the RF pulse is turned off, the magnitude of the transverse magnetization decreases and longitudinal magnetization ( $M_z$ ) forms (figure 10 transition to 9a). The net magnetization vector is the addition of these two components and the current it generates is received by the RF coil as MR signal.

*// Magnetization Vector =  $M_z + M_{xy}$  //*

RF Pulse is turned Off

Transverse magnetization decreases

Longitudinal magnetization ( $M_z$ ) Forms

Magnetization Vector =  $M_z + M_{xy}$

**Scene 8, 9, 10, 11** – assessment questions

### Scene 12

I would like to thank you for joining me today. I hope that this lesson has inspired you to further explore Magnetic Resonance Imaging and I encourage you to join me as we go into a deeper exploration of this exciting field throughout discussions in the subsequent lessons.



**APPENDIX E:**

**POST-RUN MODULE QUESTIONNAIRE**

**NOTE:** These questions will appear as a web-based question form inside the learning portal.

**Three learning objectives are listed below. Please rate the improvement in your ability to accomplish the module objectives. Use the following scale:**

- 1 – None = no apparent improvement in my ability to perform this objective
- 2 – Slight = slight improvement in my ability to perform this objective
- 3 – Moderate = moderate improvement in my ability to perform this objective
- 4 – Substantial = substantial improvement in my ability to perform this objective
- 5 – Exceptional = exceptional improvement in my ability to perform this objective

<i>After completing the training module, the participant will be able to...</i>	<b>Improvement</b>				
	<b>None</b>	<b>Slight</b>	<b>Moderate</b>	<b>Substantial</b>	<b>Exceptional</b>
<ul style="list-style-type: none"> <li>• Discuss the corpus callosum and other major cerebral commissures looking at their anatomy through the lens of normal and abnormal development.</li> </ul>	1	2	3	4	5
<ul style="list-style-type: none"> <li>• Understand why a full radiologic assessment is necessary to properly categorize a case of abnormal corpus callosum.</li> </ul>	1	2	3	4	5
<ul style="list-style-type: none"> <li>• Understand the basis of the abnormal CC development and its genetic and clinical implications.</li> </ul>	1	2	3	4	5

**Please rate the following comments about the training module using the following scale:**  
**1 = Strongly Disagree 2 = Disagree 3 = Neutral 4 = Agree 5 = Strongly Agree**

<b>Training Module</b>	<b>Agreement</b>				
	<b>Strongly Disagree</b>	<b>Disagree</b>	<b>Neutral</b>	<b>Agree</b>	<b>Strongly Agree</b>
The module presented content that can be applied in real-world medical situations.	1	2	3	4	5
This lesson taught me information about the pediatric brain and MRI that I previously did not know.	1	2	3	4	5





**U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014**

Prime Contract Number: W81XWH-11-2-0198

---

I have a better understanding about the topics and concepts discussed in the module.	1	2	3	4	5
I will apply these techniques while practicing at my institution.	1	2	3	4	5
I would participate in other BRAIN training modules using this program in the future.	1	2	3	4	5



**Short Answer**

**Directions:** Please answer the following questions about using the training module.

1. Was the module presentation organized, easy-to-use, and user-friendly? Y N

Why or why not

2. To what degree did the learning environment present information in a way that was engaging?

<i>Not at all engaging</i>	1	2	3	4	5	<i>Extremely engaging</i>
----------------------------	---	---	---	---	---	---------------------------

Please provide an explanation of your rating:

3. Would you recommend that this learning environment be used for learning about the pediatric brain and MRI?

<i>Do not recommend</i>	1	2	3	4	5	<i>Highly recommend</i>
-------------------------	---	---	---	---	---	-------------------------

Why or why not

4. How challenging was the content of this module?

<i>Not at all challenging</i>	1	2	3	4	5	<i>Extremely challenging</i>
-------------------------------	---	---	---	---	---	------------------------------

Why or why not?

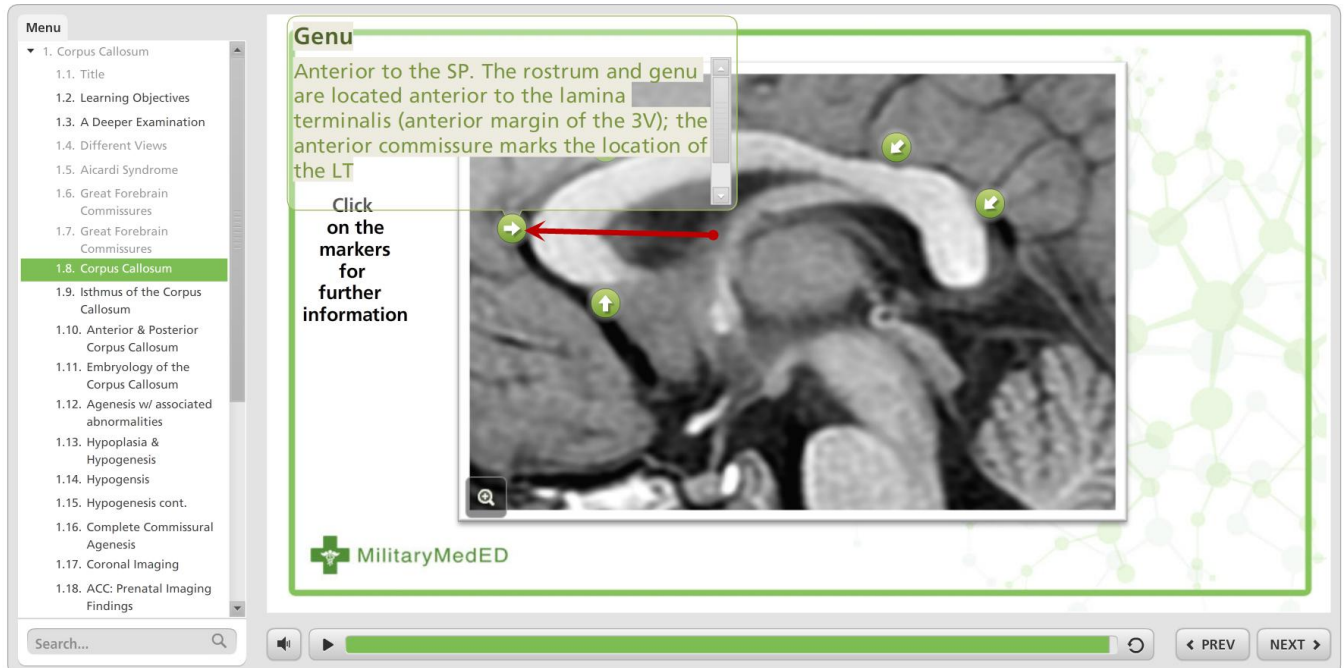
5. Please comment on skills, concepts, and techniques you learned in this module:



- 6. What did you like best about this module?**
  
- 7. What did you like least about this module?**
  
- 8. If future training modules related to the topic just learned were to be developed, which ones would you recommend? [This question will contain a list of potential topics that the user will have an opportunity to select from]**
  
- 9. Additional comments?**

## APPENDIX F:

### GUIDED DISCOVERY INTERACTIONS IN THE TRAINING MODULE



The screenshot displays a training module interface. On the left is a 'Menu' sidebar with a list of topics under '1. Corpus Callosum', including '1.8. Corpus Callosum' which is highlighted. The main content area features a text box titled 'Genu' with the text: 'Anterior to the SP. The rostrum and genu are located anterior to the lamina terminalis (anterior margin of the 3V); the anterior commissure marks the location of the LT'. Below the text is an MRI image of the brain with several green circular markers. A red arrow points to one of these markers with the instruction 'Click on the markers for further information'. The 'MilitaryMedED' logo is visible at the bottom of the image area. At the bottom of the interface are a search bar, a progress bar, and navigation buttons for 'PREV' and 'NEXT'.

[Type text]



**U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014**

Prime Contract Number: W81XWH-11-2-0198

---

**APPENDIX G:  
THREE-LEVEL REPORTING SYSTEM**

---

[Type text]



### G-1 Site Overview Reports



### G-2 Course Progress Reports

The screenshot shows the 'Overview of students' report for an online course. The table lists students and their progress. A red arrow points to the 'Last online' column, another to the 'Progress Bar' column, and a third to the 'Progress' column. The role is set to 'Student'.

First name / Surname	Last online	Progress Bar	Progress
Jeff Sestokas	Wednesday, 17 September 2014, 8:26 AM		10%
Catherine McGill	Monday, 2 June 2014, 11:30 AM		6%
Madison Berl	Never		0%
Nadja Kadom	Never		0%
William Gaillard	Never		0%
Najmeh Mahani	Never		0%
Carlo Pierpaoli	Never		0%

[Type text]



G-3 Learning Object-Level Reports (e.g. SCORM training module report)

STEP#4 - Take the Training Module

Info Reports

Basic report Graph report Interactions report Objectives report

12 attempts for 38 users, out of 38 results

First name : All A B C D E F G H I J K L M N O P Q R S T U V W X Y Z Surname : All A B C D E F G H I J K L M N O P Q R S T U V W X Y Z Page: 1 2 (Next)

Table with 7 columns: First name / Surname, Email address, Attempt, Started on, Last accessed on, Score. Rows include users like fcr12 study, fcr13 study, fcr39 study, fcr34 study, fcr21 study, fcr28 study, fcr15 study, and Mary Ottolini.

[Type text]



**U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014**

Prime Contract Number: W81XWH-11-2-0198

---





**U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014**

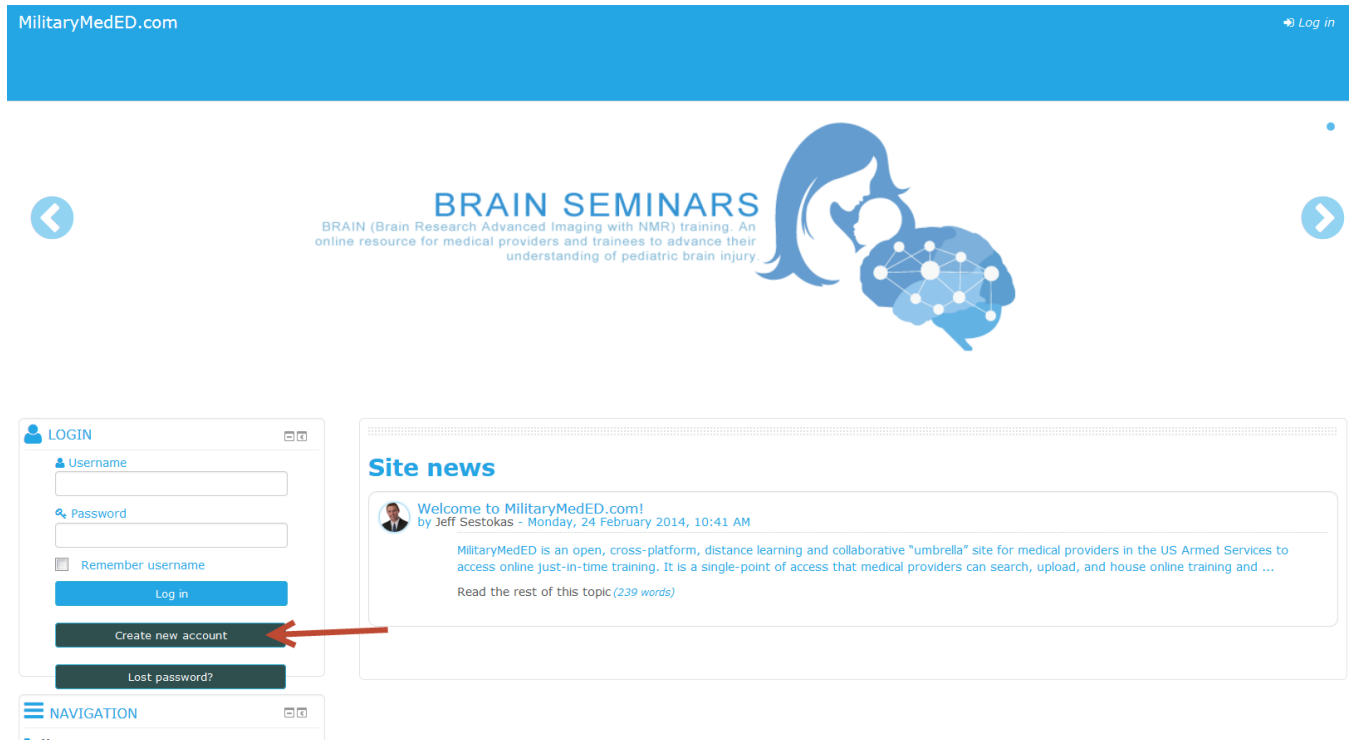
Prime Contract Number: W81XWH-11-2-0198

---

**APPENDIX H:  
SELF-REGISTRATION VIA ADMINISTRATOR CONFIRMATION**

---

### H-1 Create an account button



MilitaryMedED.com [Log in](#)

## BRAIN SEMINARS

BRAIN (Brain Research Advanced Imaging with NMR) training. An online resource for medical providers and trainees to advance their understanding of pediatric brain injury.

### LOGIN

Username

Password

Remember username

[Log in](#)


[Create new account](#)

[Lost password?](#)

### NAVIGATION

...

### Site news

 **Welcome to MilitaryMedED.com!**  
by Jeff Sestokas - Monday, 24 February 2014, 10:41 AM

MilitaryMedED is an open, cross-platform, distance learning and collaborative "umbrella" site for medical providers in the US Armed Services to access online just-in-time training. It is a single-point of access that medical providers can search, upload, and house online training and ...

[Read the rest of this topic \(239 words\)](#)

### H-2 Portal Registration Page



MilitaryMedED.com [Log in](#)

▼ Choose your username and password [Collapse all](#)

**Username\***

The password must have at least 8 characters, at least 1 digit(s), at least 1 lower case letter(s), at least 1 upper case letter(s), at least 1 non-alphanumeric character(s)

**Password\***   Unmask

▼ More details

**Email address\***

**Email (again)\***

**First name\***

**Surname\***

**City/town**

**Country**

▼ Military Information

**Branch\***

**Unit/Rank**

**Position**

**Years in this position**

**Medical specialty or subspecialty**

### H-3. Registration Confirmation Message

MilitaryMedED.com [Log in](#)

HOME > CONFIRM YOUR ACCOUNT

Your account has been registered and is pending confirmation by the administrator. You should expect to either receive a confirmation or to be contacted for further clarification.

©2014 Copyright MilitaryMedED.com

### H-4. Administrator Confirmation Email



U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014

Prime Contract Number: W81XWH-11-2-0198

**Folders**  
Last Refresh:  
Mon, 4:42 pm  
[\[Check Mail\]](#)


**INBOX (1)**  
Drafts  
Sent  
Trash

firstname: Test  
lastname: Account  
email: [test@militarymeded.com](mailto:test@militarymeded.com)  
emailstop: 0  
icq:  
skype:  
yahoo:  
aim:  
msn:  
phone1:  
phone2:  
institution:  
department:  
address:  
city: Washington DC  
country: US  
lang: en  
calendartype: gregorian  
theme:  
timezone: 99  
firstaccess: 1411418458  
lastaccess: 0  
lastlogin: 0  
currentlogin: 0  
lasttip:  
secret: 8f92uGpG19SnPcI  
picture: 0  
url:  
description:  
descriptionformat: 1  
mailformat: 1  
maildigest: 0  
maildisplay: 2  
autosubscribe: 1  
trackforums: 0  
timecreated: 1411418458  
timemodified: 0  
trustbitmask: 0  
imagealt:  
lastnamephonetic:  
firstnamephonetic:  
middlename:  
alternatename:  
Branch: Army  
Unit/Rank:  
Position:  
Years in this position:  
Medical specialty or subspecialty:

To confirm the new account, please go to this web address:  
<http://www.militarymeded.com/home/auth/emailadmin/confirm.php?data=8f92uGpG19SnPcI/test>

In most mail programs, this should appear as a blue link which you can just click on. If that doesn't work, then cut and paste the address into the address line at the top of your web browser window.

You can also confirm accounts from within the system by going to Site Administration -> Users



H-5. User Confirmation Email



U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014

Prime Contract Number: W81XWH-11-2-0198

**Current Folder:** INBOX  
[Compose](#) [Addresses](#) [Folders](#) [Options](#) [Search](#) [Help](#)

[ Previous | Next ] [ [Message List](#) ]

Reply Reply All | Forward  As Attachment | Delete  Bypass Trash

**Subject:** MilitaryMedED.com: account confirmation  
**From:** "Jeff Sestokas" <jeffsestokas@gmail.com>  
**Date:** Mon, September 22, 2014 8:51 pm  
**To:** "Test Account" <test@militarymeded.com>  
**Priority:** Normal  
**Options:** [View Full Header](#) | [Print](#) | [Download this as a file](#) | [View as HTML](#)

Greetings Test Account,

Welcome to MilitaryMedED.com! Your account has been approved. If you have not already done so, please tell us how you discovered our portal by replying to this email.

We're delighted that you have joined us and hope to see you and your learners participating in some of our projects. You are now a member of a rapidly growing community of military and non-military medical educators, professionals, and learners that are using this portal to share projects, research, and knowledge. If you ever need help, please don't hesitate to write to us at [reply@militarymeded.com](mailto:reply@militarymeded.com) <a href=mailto:reply@militarymeded.com>here</a>. We will address any questions, comments or concerns quickly and efficiently.

\* \* \*

What is MilitaryMedED?

MilitaryMedED.com is an easy-to-use, Internet-based collaborative environment that enables military and non-military medical educators and professionals to develop and manage online training or research projects, share information, and work together in a virtual environment. The portal provides a variety of web-based tools and plugins including discussion boards, video conferencing, and calendars to support collaboration among teachers and students within the system.

MilitaryMedED.com welcomes you, and looks forward to sharing a rich variety of educational projects and research activities with your participating learners and students. When you login, list of popular categories appears. Click on one of the categories to browse the projects specified by it. As you browse through these projects (click on the titles), they may help you generate an idea or two for you to use in developing a project of your own within the system or they may help you to identify projects in which you may want to enroll in.

Thank you,

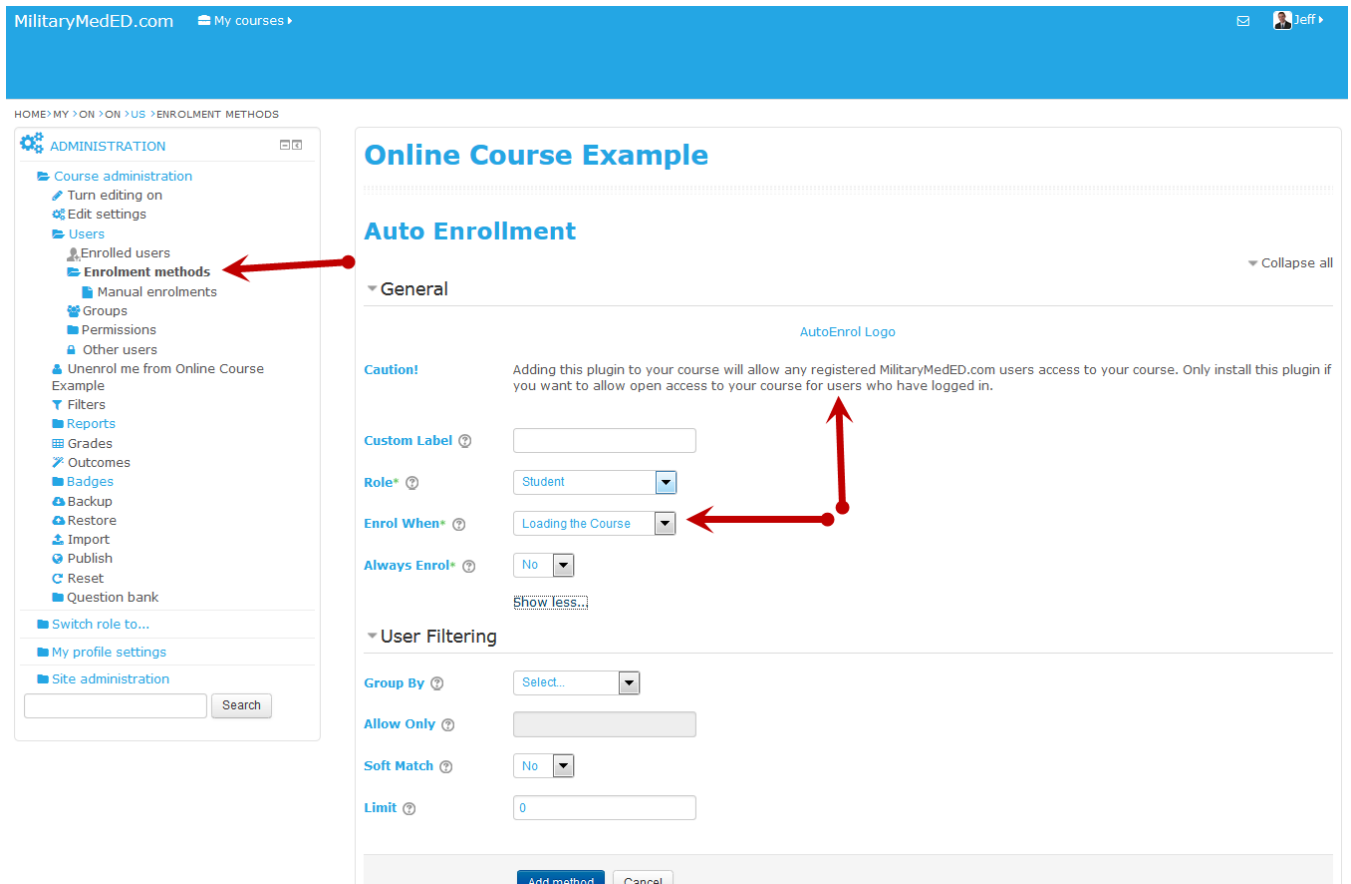
MilitaryMedED.com Administrator  
Children's National Medical Center, Medical Education  
[reply@militarymeded.com](mailto:reply@militarymeded.com)



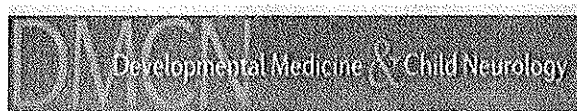
**U.S. ARMY MEDICAL RESEARCH  
AND MATERIAL COMMAND  
ANNUAL REPORT  
15 October 2014**  
Prime Contract Number: W81XWH-11-2-0198

---

## APPENDIX I: Automatic Enrollment Feature



The screenshot displays the MilitaryMedED.com administration interface. The top navigation bar includes the site name, a 'My courses' menu, and a user profile for 'Jeff'. The breadcrumb trail reads 'HOME > MY > ON > ON > US > ENROLMENT METHODS'. The left sidebar, titled 'ADMINISTRATION', lists various management options, with 'Enrolment methods' highlighted and a red arrow pointing to it. The main content area is titled 'Online Course Example' and 'Auto Enrollment'. It features a 'General' section with a 'Caution!' message, a 'Custom Label' input field, a 'Role' dropdown set to 'Student', an 'Enrol When' dropdown set to 'Loading the Course', and an 'Always Enrol' dropdown set to 'No'. A 'User Filtering' section includes 'Group By', 'Allow Only', 'Soft Match', and 'Limit' options. A red arrow points from the 'Enrol When' dropdown to the 'AutoEnrol Logo' link. At the bottom, there are 'Add method' and 'Cancel' buttons.



**WHITE MATTER TRACT INTEGRITY AND DEVELOPMENTAL  
OUTCOME IN NEWBORNS WITH HYPOXIC ISCHEMIC  
ENCEPHALOPATHY (HIE) TREATED WITH HYPOTHERMIA**

Journal:	<i>Developmental Medicine &amp; Child Neurology</i>
Manuscript ID:	DMCN-OA-14-06-0370.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	25-Sep-2014
Complete List of Authors:	Massaro, An; Children's National Medical Center, Neonatology Evangelou, Jordanis; Children's National Medical Center, Fatemi, Ali; Kennedy Krieger Institute, Johns Hopkins Medical Institutions, Vezina, Gilbert; Children's National Medical Center, Radiology and Pediatrics McCarter, Robert; Children's National Medical Center, Glass, Penny; Children's National Medical Center, Psychiatry and Behavioral Sciences Limperopoulos, Catherine; Children's National Medical Center,
Keywords:	newborn, hypothermia, magnetic resonance imaging, diffusion tensor imaging, outcome

SCHOLARONE™  
Manuscripts



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

WHITE MATTER TRACT INTEGRITY AND DEVELOPMENTAL OUTCOME IN  
NEWBORNS WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY (HIE) TREATED WITH  
HYPOTHERMIA

An N. Massaro MD,<sup>1,6</sup> Jordanis Evangelou DPhil,<sup>2,7</sup> Ali Fatemi MD,<sup>3</sup> Gilbert Vezina MD,<sup>2,6,7</sup>  
Robert McCarter ScD,<sup>4,6,7</sup> Penny Glass PhD<sup>5,6</sup>, and Catherine Limperopoulos PhD<sup>2,6</sup>  
Departments of <sup>1</sup>Neonatology, <sup>2</sup>Diagnostic Imaging and Radiology, <sup>4</sup>Biostatistics & Informatics,  
and <sup>5</sup>Psychiatry & Behavioral Sciences, Children's National Medical Center  
<sup>3</sup>Kennedy Krieger Institute, Johns Hopkins School of Medicine,  
Departments of <sup>6</sup>Pediatrics, <sup>7</sup>Radiology and <sup>8</sup>Epidemiology & Biostatistics, The George  
Washington University School of Medicine,  
Washington, DC, United States

Corresponding Author:  
An N. Massaro, MD  
Assistant Professor of Pediatrics  
The George Washington University School of Medicine  
Department of Neonatology  
Children's National Medical Center  
111 Michigan Avenue, NW  
Washington, DC 20010  
Phone (202) 476-5225  
Fax (202) 476-3459  
Email: [anguyenm@cnmc.org](mailto:anguyenm@cnmc.org)

Word Count of Abstract: 200

| Word Count of Manuscript: 33002994

**ABSTRACT**

**Aim:** To determine whether corpus callosum (CC) and corticospinal tract (CST) diffusion tensor imaging (DTI) measures relate to developmental outcome in encephalopathic newborns after therapeutic hypothermia.

**Methods:** Encephalopathic newborns enrolled in a longitudinal study underwent DTI after hypothermia. Parametric maps were generated for fractional anisotropy (FA), mean (MD), radial (RD) and axial (AD) diffusivity. CC and CST were segmented by DTI-based tractography. Multiple regression models were used to examine the association of DTI measures with Bayley-II Mental (MDI) and Psychomotor (PDI) Developmental Index at 15 and 21 months of age.

**Results:** Fifty-two infants underwent DTI at median age of 8 days. Two subjects were excluded with poor MRI quality. Outcomes were assessed in 42/50 (84%) infants at 15 months and 35/50 (70%) at 21 months. Lower CC and CST FA were associated with lower MDI and PDI respectively, even after controlling for gestational age, birth weight, gender, and socioeconomic status. There was also a direct relationship between CC AD and MDI, while CST RD was inversely related to PDI.

**Interpretation:** In encephalopathic newborns, impaired microstructural organization of the CC and CST predicts poorer cognitive and motor performance respectively. Tractography provides a reliable method for early assessment of perinatal brain injury.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**What This Paper Adds**

- DTI tractography of the corpus callosum and corticospinal tract is feasible in newborns.
- Corpus callosum integrity relates to cognitive performance in childhood.
- Corticospinal tract integrity relates to motor performance in childhood.
- DTI tractography can provide early assessment of perinatal brain injury.

For Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Hypoxic ischemic encephalopathy (HIE) is a major cause of neonatal death and long-term disability in children.<sup>1</sup> Therapeutic hypothermia is the current standard of care for newborns presenting with HIE,<sup>2</sup> however nearly half of treated infants continue to have adverse outcomes despite treatment with cooling.<sup>3</sup> Acute assessment of brain injury after neuroprotective intervention is an important aspect of neurocritical care. Such information can be used to direct adjuvant and rehabilitative therapies, provide prognostic information for families of affected children, and serve as surrogate outcomes for future neurotherapeutic trials.

Magnetic resonance imaging (MRI) is a leading source of brain injury biomarkers in perinatal HIE.<sup>4</sup> Pattern of brain injury by conventional MRI has been related to both severity and phenotype of neurodevelopmental sequelae after HIE.<sup>5,6,7,8,9</sup> However, conventional MRI requires availability of an experienced pediatric neuroradiologist, can be subjective, and provides broad severity classification on an ordinal scale. Quantitative MRI techniques such as diffusion tensor imaging (DTI) can overcome these limitations by providing continuous, reproducible measures of brain microstructural injury.

Few studies have related DTI measures and developmental outcomes in survivors of HIE.<sup>8-11,12-14</sup> Most prior studies have been limited to 2D region-of-interest approaches that are subject to inter-rater reliability. Additionally, these approaches fail to interrogate the complexity of 3D anatomical brain regions, which is important when evaluating structure-function relationships. DTI-based tractography provides a semi-automated approach for 3D delineation of major white matter pathways. It has been demonstrated to be feasible in newborns,<sup>12,13,14,15</sup> particularly when evaluating the most well developed white matter tracts.

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Formatted: Do not check spelling or grammar, Superscript

Field Code Changed

Field Code Changed

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11 This study aims to evaluate if microstructural organization measured via quantitative  
12 tractography is related to neurodevelopmental outcome in HIE. Specifically, we hypothesized  
13 that impaired brain microstructure in the corpus callosum (CC) and corticospinal tract (CST)  
14 would be associated with poorer cognitive and motor performance, respectively, in young  
15 childhood survivors of perinatal HIE.  
16  
17  
18  
19

## 20 21 22 METHODS

### 23 *Participants*

24 All patients with HIE admitted for therapeutic hypothermia to an out-born level IV regional  
25 neonatal intensive care unit (NICU) were approached for enrollment between May 2008 and  
26 October 2010 in this prospective longitudinal study. Whole-body hypothermia was provided  
27 based on established criteria<sup>3</sup> (i.e. infants were greater than 36 weeks gestational age, greater  
28 than 1800 grams at birth, demonstrated metabolic acidosis and/or low Apgar scores, and  
29 exhibited signs of moderate to severe clinical encephalopathy). Infants were excluded if they  
30 were small for gestational age or had a known or suspected chromosomal abnormality or major  
31 congenital anomaly. The study was approved by the Institutional Review Board and written  
32 informed consent was obtained from the parents of eligible participants.  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44

### 45 *Data Collection*

46 Demographic and clinical data were collected from the referral hospital and study site medical  
47 records. Clinical characteristics were recorded including umbilical cord or first-hour of life  
48 blood gas, Apgar scores, and presence of electrographic seizures. Initial clinical grade of  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Field Code Changed

encephalopathy was classified according to modified Sarnat criteria used in the NICHD trial,<sup>3</sup>  
 Socioeconomic status (SES) was classified by financial eligibility for medical assistance and  
 maternal education level.

#### *MR Image Acquisition*

MRI was performed at target 7-10 days of life on a 1.5T scanner (Signa HDx, GE Healthcare,  
 Milwaukee, WI, USA) using an 8-channel receive head coil (InVivo Corp., Gainesville, FL,  
 USA). When feasible, infants were scanned after feeding and bundling without the use of  
 sedation. Standard sequences included sagittal and axial spin echo (SE) T1-W, dual echo axial  
 SE proton density (PD) and T2-W images, and coronal fast spin echo (FSE) T2-W. In cases of  
 patient motion, periodically rotated parallel lines with enhanced reconstruction (PROPELLER)  
 T2-W and/or T1-W images were also obtained. A single shot SE echoplanar imaging (EPI) axial  
 DTI sequence was used with the following parameters: TR 10,000 ms, TE 100 ms, acquisition  
 matrix 128x128, slice thickness 4 mm, 1 volume with no diffusion weighting ( $b=0$ ) and 25  
 volumes in non-colinear gradient directions with diffusion weighting ( $b=1000 \text{ s/mm}^2$ ).

#### *Preprocessing of Diffusion Tensor Images*

DTI preprocessing was performed using an automated methodology optimized for neonatal data,  
 including calculation of parametric maps for fractional anisotropy (FA), mean (MD), axial (AD)  
 and radial (RD) diffusivity.<sup>14,15</sup> These DTI-derived metrics characterize voxel-wise the tensor  
 shape (FA) and magnitude (MD) of the underlying water diffusion within tissues. AD is a  
 measure of diffusivity parallel to the primary axis while RD measures diffusivity perpendicular  
 to the primary axis of diffusion. MD is calculated as the average diffusion along all 3 axes.<sup>15,16</sup>

Field Code Changed

Field Code Changed

Field Code Changed

### *White Matter Fiber Tracking*

Fiber tracking was performed using Fiber Assignment by Continuous Tracking (DTI Studio, Baltimore, MD)<sup>1615</sup>. Fiber trajectories were formed along the primary eigenvector between two voxels using an FA threshold of greater than 0.1. Tracts were terminated if this value dropped below 0.05 or if the angle between the primary eigenvectors of consecutive voxels exceeded 50°. CST was segmented by placing region of interest (ROI) constraints at the level of the cerebral peduncles, posterior limb of the internal capsule (PLIC) and centrum semiovale (CSO) anterior to the central sulcus on axial images (Figure 1a)<sup>1216</sup>. Measurements for the left and right tracts were averaged within each newborn for analysis. For the CC, ROI delineation was performed on a mid-sagittal image, and right and left para-sagittal images (Figure 1b). Logical exclusion masks were also drawn to prevent implausible tracts from forming across anatomical boundaries and resulting fibers were checked on the MD images for anatomical validity. Segmented fiber tracts were overlaid on the co-registered parametric maps and mean FA, MD, AD and RD were calculated for each tract using the statistical program within DTI Studio. All fiber tract measurements were performed by a single investigator (A.N.M.) masked to the clinical and outcome data of the subjects. Intra-rater reliability was assessed by repeating fiber tracking measurements in 10 random subjects. Additionally, manual ROI delineation of the PLIC (at the level of the Foramen of Monro on an axial image) and the CC (on a mid-sagittal image) was performed in duplicate in the same 10 subjects for comparison. Intraclass correlation (ICC) was calculated to evaluate the reliability of the each method.

### *Conventional MRI Scoring*

Field Code Changed

Field Code Changed

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Conventional MRI studies were reviewed by a neuroradiologist (G.V.), who was masked to the clinical characteristics, DTI measures and Bayley scores of subjects. Images were assessed for the predominant pattern of brain injury.<sup>5</sup> Additionally, the degree of deep nuclear gray and cortical/white matter injury was characterized by assigning a BG/W score ranging from 0-4 according to previously described methods.<sup>7</sup> The relationship between conventional MRI and developmental outcome scores were evaluated for comparative purposes.

*Neurodevelopmental Assessment*

Neurodevelopmental outcomes were assessed with the Bayley Scales of Infant Development-Second Edition (BSID-II) at 15 and 21 months of age.<sup>18+7</sup> The BSID-II is a standardized assessment that includes a Mental Developmental Index (MDI) that assesses the child's level of cognitive, language and personal-social skills, as well as a Psychomotor Developmental Index (PDI) that evaluates fine and gross motor development. BSID-II index scores of  $100 \pm 15$  represent the mean  $\pm$  1sd, with a basement score of 50. Children who were severely impaired and not testable at the time of evaluation were assigned an index score of 49 for analysis ( $n=8$  for MDI,  $n=9$  for PDI). Evaluations were performed by an experienced developmental psychologist who was blinded to the MRI results of the child. Although the third edition of the BSID was released in 2006,<sup>19+6</sup> the BSID-II continued to be used as the assessment tool in our developmental clinic during the study period and thus was the outcome measure used in this study.

*Statistical analysis*

Formatted: Font: Times

Formatted: Font: Times

Formatted: Do not check spelling or grammar, Superscript

Formatted: Font: Times

Formatted: Font: Times

Formatted: Font: Times

Formatted: Do not check spelling or grammar, Superscript

Formatted: Font: Times

Formatted: Font: Times, Italic

Field Code Changed

Field Code Changed



1  
2  
3  
4  
5  
6  
7  
8  
9 Descriptive statistics included standard measures of central tendency and variability for  
10 continuous data and frequencies for categorical variables. Longitudinal mixed-effects regression  
11 models were developed to evaluate the association of DTI scalars with Bayley Scores, where the  
12 dependent variable was the time-averaged Bayley score in order to account for repeated  
13 assessments over time in subjects evaluated at both 15 and 21 months, and the independent  
14 variable was the DTI scalar. Separate models were developed for each DTI scalar (FA, MD,  
15 AD, RD). Data were transformed when normality assumptions were not met. Baseline models  
16 controlled for demographic variables including birthweight, gestational age, race, gender, SES  
17 and age at MRI. Expanded models were also developed to evaluate whether DTI scalars  
18 independently related to outcome when accounting for clinical covariables including presenting  
19 pH, grade of encephalopathy. Secondary analyses were also performed to evaluate the  
20 consistency of results. In order to account for the influence of the floor effect of the Bayley index  
21 (i.e. inability to estimate developmental status at index scores <49), longitudinal logistic  
22 regression models were developed to evaluate the relationships between DTI and adverse  
23 outcome defined as Bayley score <70. Finally, the relationships between DTI and BSID-II  
24 scores from the 35 subjects evaluated at 21 months were explored via ordinal regression models.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40

#### 41 *Sample Size and Power*

42 We planned to enroll 90 subjects in this study, in order to proceed with data from 60 subjects  
43 following attrition (due to mortality, loss to follow-up and poor MRI quality). However, due to  
44 changes made to our clinical imaging protocol during the study period, we completed enrollment  
45 with 63 infants included in the study. We used PASS 11 (NCSS, Kaysville, UT, USA) to  
46 develop post hoc estimates of statistical power using One Power Correlation Analysis.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Formatted: Font: Times

Formatted: Font: Times, 12 pt

1  
2  
3  
4  
5  
6  
7  
8  
9 The magnitude of correlation between Bayley score and DTI metric was calculated based on a  
10 scenario where the 15 and 21 month data were treated as repeat observations on 50 patients, with  
11 the average patient having between 1 and 2 measurements. Statistical power was set at 80% and  
12 the 2-tailed type 1 error was set to 5%. Under these circumstances, the study was capable of  
13 detecting  
14 demonstrated that this sample size provided >80% power to detect moderate (38%) correlations  
15 between Bayley scores and DTI measures, using combined 15 and 21 month data and assuming  
16 conservatively only one observation per patient. Statistical analyses were performed with  
17  
18  
19  
20  
21  
22  
23  
24 STATA 11.0 software (StataCorp LP, College Station, TXS, USA).  
25  
26  
27

Formatted: Font: Times, 12 pt

Formatted: Font: Times

## 28 RESULTS

29  
30 Of the 63 newborns with HIE enrolled, 11 (17%) died prior to MRI. Participants underwent  
31 MRI at a median age of 8 days (range 6-16). MRI data was not analyzable in 2 infants due to  
32 motion corruption. Of the 50 infants with available DTI data, 42 (84%) were evaluated at a  
33 median age of 15.2 months (range 14.7-17.8) and 35 (70%) were also evaluated at a median age  
34 of 21.4 (20.8-30.6) months. Infants lost to follow-up were similar to those retained in the study  
35 with regards to baseline and clinical characteristics, except for indices of SES (Table 1).  
36  
37  
38  
39  
40  
41  
42

### 43 *Relationship between Corticospinal Tract Integrity and Motor Outcome*

44  
45 There was a strong positive association between CST FA and BSID-II PDI that remained  
46 significant when baseline ( $\beta=198$ ; 95% CI: 102, 293;  $p<0.001$ ) and clinical ( $\beta=128$ ; 95% CI: 34,  
47 222;  $p=0.008$ ) characteristics were controlled (Figure 2a). CST AD also trended to have a  
48 positive association with BSID-II PDI (Baseline model:  $\beta=45$ ; 95% CI: -2.5, 92;  $p=0.063$ ;  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9 Expanded model:  $\beta=36$ ; 95% CI: -6.5, 77.8;  $p=0.098$ , Figure 2b). CST RD had a negative  
10 association with BSID-II PDI ( $\beta= -87.8$ ; 95% CI: -160, -15;  $p=0.018$ , Figure 2b), which was not  
11 significant after controlling for clinical covariables ( $\beta= -45$ ; 95% CI: -116, 27;  $p=0.219$ ). CST  
12 MD was not related to motor outcome. The relationship between CST FA and PDI remained  
13 consistent in the logistic regression model ( $p=0.046$ ) and when evaluating only BSID-II PDI data  
14 from subjects evaluated at 21 months ( $p=0.001$ ), while the relationship between CST RD and  
15 PDI at 21 months was reduced to a non-significant trend (logistic regression model  $p=0.085$ , 21  
16 month PDI model  $p=0.058$ ).

#### 26 *Relationship between Corpus Callosum Integrity and Cognitive Outcome*

27  
28 FA in the CC was strongly associated with BSID-II MDI when controlling for baseline ( $\beta=149$ ;  
29 95% CI: 82.5, 216;  $p<0.001$ ), as well as clinical ( $\beta=91$ ; 95% CI: 21.6, 160.7;  $p=0.01$ )  
30 characteristics (Figure 2c). CC AD was also positively associated with BSID-II MDI ( $\beta=22.9$ ;  
31 95% CI: 2.2, 43.6;  $p=0.03$ ), but not after accounting for clinical factors ( $\beta=14$ ; 95% CI: -3.7,  
32 31.8;  $p=0.122$ , Figure 2d). RD and MD in the CC were not related to cognitive outcome. The  
33 significant association between CC FA and MDI was also demonstrated in the logistic regression  
34 model ( $p=0.022$ ) and when evaluating only 21 month MDI scores ( $p<0.001$ ), while CC AD and  
35 MDI were not significantly related in the logistic regression model ( $p=0.096$ ) and the 21 month  
36 MDI model ( $p=0.079$ ).

#### 46 *Intra-rater Reliability*

47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9 The reliability of the tractography-based approach was high and similar to the ROI-based method  
10 in the CC, but tractography of the CST was more reproducible compared to PLIC delineation by  
11 ROI (Table 2).  
12  
13

#### 14 15 16 *Conventional MRI and Developmental Outcome* 17

18 No brain injury was identified by conventional imaging in 18 (36%) subjects. Of the 32 subjects  
19 with brain injury, predominant pattern of injury was classified as watershed in 4 (13%), basal  
20 nuclei in 10 (31%), focal/multifocal in 17 (53%), and 1 patient had global brain injury. As  
21 previously described, BG/W score was significantly associated with outcome (Figure 3).  
22  
23  
24  
25

#### 26 27 28 DISCUSSION

29 This is the first study to relate microstructural injury measured by DTI tractography and  
30 developmental outcome in children with perinatal HIE treated with hypothermia. We  
31 demonstrate that lower FA and higher RD in the CST are associated with lower Bayley PDI,  
32 while lower FA and AD in the CC relate to lower Bayley MDI. Specifically, for every 0.1  
33 decrease in CST FA, we observed a 13 to 20-point decrease in Bayley PDI. Correspondingly,  
34 there was a 9 to 15 point decrease in Bayley MDI for every 0.1 decrease in CC FA. These data  
35 suggest that impaired CST and CC microstructure observed in the neonatal period predict motor  
36 and cognitive deficits, respectively, in early childhood. Assessment of brain injury severity and  
37 risk for related developmental sequelae in the neonatal period can allow clinicians and  
38 investigators to gage treatment effect immediately after intervention, and can help prioritize  
39 resources towards long-term evaluation of only the most promising interventions. This study  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

supports DTI quantitative tractography as a reliable method to measure brain injury after therapeutic hypothermia in newborns with HIE.

We demonstrated that conventional MRI scoring also relates to developmental outcome. This is consistent with While prior work that has established that conventional MRI interpretation is a good predictor of neurodevelopmental outcome in newborns with HIE after therapeutic hypothermia.<sup>6</sup> However, reliance on conventional MRI interpretation has limitations as classification of injury by this method is not only subject to inter-rater reliability, but also and only provides a discrete number of outcome category levels depending on the classification scale used. A continuous, reproducible biomarker is preferred when the goal is to evaluate small effect sizes or treatment effects in small samples, as is common in pre-clinical and early phase therapeutic trials. Other advanced MRI measures have been proposed as candidate biomarkers in answer to this need. While MR spectroscopy (MRS) measures such as Lac/NAA ratio have been related to outcome,<sup>4</sup> MRS does not provide information about the topography of injury in order to distinguish the associated developmental phenotype. It is also unclear how long the presence of lactate persists after an initial hypoxic-ischemic insult. Similarly, the apparent diffusion coefficient (ADC) from standard diffusion weighted imaging is limited by its time-dependency, and more specifically its propensity for pseudonormalization.<sup>20,21</sup>

DTI fractional anisotropy does not pseudonormalize<sup>16, 119-120</sup> and thus increasing reports have emerged relating DTI FA to developmental outcome in HIE. Brissaud et al described lower FA in the PLIC and cerebral peduncles in HIE infants with abnormal neurological exam at 1-2 weeks of life.<sup>28</sup> Malik et al reported a significant correlation between FA measured by ROI in the

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

CST and developmental quotient at 3 months of age in newborns with HIE.<sup>27</sup> Few studies have evaluated the relationship between DTI and longer term developmental outcome in HIE. Tusor et al reported the association of 1-2 year developmental outcome and DTI FA quantified by tract-based special statistics (TBSS).<sup>1149</sup> Only Ancora and colleagues reported data on all four major DTI scalars and their relation to outcome. These investigators found high predictive ability of AD in the CC and FA/ RD in the frontal and parietal white matter for distinguishing developmental outcome at 2 years in a small series of 20 patients.<sup>2120</sup> To our knowledge this is the largest study to date evaluating the relationship between DTI and developmental outcome, and the only study utilizing the tractography-based approach.

How and where DTI parametric scalars are measured is a crucial component of establishing them as viable biomarkers of brain microstructural injury. Previous DTI studies have mainly used manual 2D ROI approaches. This method only evaluates selected areas of key anatomical structures, and introduces observer error that limits reproducibility.<sup>8-107-9</sup> DTI tractography offers a semi-automated way of visualizing whole white matter fiber tracts in 3D. While processing steps still require manual placement of ROI constraints, our study and others have demonstrated that the tractography-based method has higher reliability compared to manual ROI delineation.<sup>17, 2246-24</sup> Thus, DTI tractography provides a reliable method for hypothesis-driven interrogation based on a priori knowledge of brain structure and function relationships. We selected CST and CC because of their known association with motor and cognitive function respectively.<sup>2422</sup> By quantifying whole tract microstructure, the tractography-based method allows for detection of group-wise differences that is independent of the topography of injury along the tract. This may improve sensitivity for distinguishing outcome groups as has been

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

suggested by previous reports.<sup>17, 246, 33</sup> Tusor and colleagues reported the use of tract-based special statistics (TBSS) to quantify FA.<sup>1149</sup> This method relies on normalization of subjects to a template space (which introduces interpolation), but also provides a semi-automated method for quantification of FA in WM tracts. Interrogation of microstructure via both methods may provide confirmatory or complementary information.

The other advantage of tractography-based anatomical parcellation is the ability to measure other DTI parametric scalars within the defined ROI. While FA was most significantly associated with outcome across all analyses in our study, other metrics such as RD and AD may provide more insights into pathogenesis by detailing microstructural changes after injury. RD is thought to represent the degree of myelination,<sup>2524</sup> while AD reflects fiber coherence and structure of axonal membranes.<sup>2124</sup> FA is determined by these and other factors including fiber diameter and density, as well as extracellular and interaxonal spacing.<sup>2524</sup> We found a relationship between RD in the CST and motor outcome while RD in the CC was not significantly related to cognitive outcome. This is consistent with knowledge that myelination of the CST is known to occur around and shortly after term birth whereas the CC is not myelinated until several months of age.<sup>2625</sup> That MD was not associated with outcome may represent the impact of pseudonormalization given the imaging window used in this study.<sup>2019</sup>

This study has limitations. Attrition due to loss to follow-up is a common problem in longitudinal studies. While it is reassuring that infants who were lost to follow-up were similar to those retained with regards to baseline and clinical characteristics, that infants who did not return for assessment could be differentiated by SES risk-profile remains a possible source of

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

1  
2  
3  
4  
5  
6  
7  
8  
9 bias. Attrition also impacted an already limited sample size. In order to overcome the related  
10 challenges to statistical power, we used an analytic approach that took advantage of repeated  
11 measures in participants. It is reassuring that the relationship between FA and developmental  
12 outcome was consistently confirmed when evaluating the subset of outcome data from subjects  
13 evaluated at 21 months. Given the sample size limitations, and the fact that we did not control  
14 for multiple comparisons in these analyses, the relationship between the other DTI scalars and  
15 longer-term outcome warrant further investigation. The basement effect of the Bayley index  
16 also posed analytical challenges with regard to statistical approach. The consistency of results  
17 after removal of the infants performing below the Bayley floor, and logistic regression after  
18 binarization of outcome, provide added reassurance regarding the validity of results establishing  
19 the suggestion of a relationship between DTI and developmental outcome in children with HIE.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

### 31 CONCLUSIONS

32  
33 Impaired microstructural organization of the CC and CST may predicts poorer early childhood  
34 cognitive and motor performance respectively in newborns with HIE after therapeutic  
35 hypothermia. Tractography provides a reliable method for early assessment of perinatal brain  
36 injury and can potentially ~~may~~ serve as a surrogate outcome for future neurotherapeutic trials.  
37  
38  
39  
40  
41  
42

### 43 ACKNOWLEDGEMENTS

44  
45 The authors acknowledge Yao (Iris) Cheng, M.S. for her data management and statistical  
46 support, and Judy Brown, NNP, Christen Meisel, M.S., and Maya B. Coleman Ph.D. for their  
47 efforts coordinating developmental follow-up visits for study participants. This publication was  
48 supported by the Clinical and Translational Science Institute at Children's National (NIH  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9 UL1TR000075, KL2TR000076), the Intellectual and Developmental Disabilities Research  
10 Consortium (NIH P30HD040677), and The Advanced Pediatric Brain Imaging Research and  
11 Training Program (DoD W81XWH-11-2-0198).  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Review Only

## FIGURE LEGENDS

1. Visualization of fiber tracts by diffusion tensor tractography. A) Cortical spinal tract delineation by constraints at the level of the cerebral peduncle (CP), posterior limb of the internal capsule (PLIC) and centrum semiovale (CSO). B) Corpus callosum delineation on sagittal images.

2. Relationships between Bayley scales and DTI measures. Bars represent 95% confidence intervals of regression model predictive margins. Significant associations are shown between Bayley Psychomotor Developmental Index and corticospinal tract a) fractional anisotropy ( $p < 0.001$ ) and b) radial diffusivity ( $p = 0.018$ ). Significant associations were also observed between Bayley Mental Developmental Index and corpus callosum c) fractional anisotropy ( $P < 0.001$ ) and d) axial diffusivity ( $p = 0.03$ ).

3. Relationships between Conventional MRI and Developmental Outcome. a) Bayley Psychomotor Developmental Index and b) Bayley Mental Developmental Index Scores are presented by MRI Basal Ganglia/Waterstied (BG/W) Score. Boxplots represent medians and interquartile ranges. Whiskers represent range with outliers depicted by circles. Gray and white boxes depict outcome data at 15 at 21 months respectively. Bayley scores were significantly different by MRI BG/W Score (ANOVA P-values  $< 0.001$ ).

Formatted: Font: (Default) Times New Roman

Table 1. Demographic and Clinical Characteristics of the Study Population

	Study Group (n=50)	Assessed at 15 mo (n=42)	Assessed at 21 mo (n=35)
<b>Gestational Age (Mean ± SD wks)</b>	38.8 ± 1.9	38.9 ± 1.9	39.0 ± 2.0
<b>Birth weight (Mean ± SD kg)</b>	3.4 ± 0.7	3.4 ± 0.7	3.4 ± 0.7
<b>Gender (n, % male)</b>	31 (62)	25 (60)	21 (60)
<b>Race (n, %)</b>			
Caucasian	24 (48)	23 (55)	20 (57)
Black/ African American	25 (50)	18 (43)	15 (43)
Other	1 (2)	1 (2)	
<b>Presenting pH</b>	6.91 (6.5-7.35)	6.9 (6.5-7.4)	6.9 (6.5-7.4)
<b>Presenting Base Deficit</b>	18 (8-36)	18 (9-36)	19 (9-36)
<b>Apgar Score</b>			
1 minute	1 (0-6)	1 (0-6)	1 (0-6)
5 minute	3 (0-7)	3 (0-7)	3 (0-7)
10 minute	5 (0-9) <sup>a</sup>	5 (0-9) <sup>b</sup>	5 (0-9) <sup>c</sup>
<b>Encephalopathy Grade</b>			
Moderate (n, %)	41 (82)	33 (79)	27 (77)
Severe (n, %)	9 (18)	9 (21)	8 (23)
<b>Electrographic Seizures (n, %)</b>	18 (36)	17 (40)	15 (43)
<b>Socioeconomic Status (n, %)</b>			
Need for medical assistance	8 (16)	4 (9)*	3 (8)*
<b>Maternal Education</b>			
Grade school	6 (12)	6 (14)	4 (11)
High school graduate	23 (46)	16 (38)	13 (37)
Higher level education	21 (42)	20 (48)*	18 (51)*

\* Significant difference from those lost to follow-up (p<0.05)

Table 2. Intraclass Correlation Coefficients

Parameter	CORPUS CALLOSUM		CORTICOSPINAL TRACT	
	ROI	Tractography	ROI	Tractography
FA	0.991 (0.966-0.998)	0.989 (0.953-0.997)	0.950 (0.302-0.991)	0.963 (0.857-0.991)
MD	0.962 (0.856-0.990)	0.933 (0.724-0.983)	0.798 (0.129-0.950)	0.911 (0.663-0.991)
RD	0.965 (0.866-0.991)	0.951 (0.803-0.988)	0.848 (0.408-0.962)	0.923 (0.706-0.991)
AD	0.964 (0.864-0.991)	0.944 (0.784-0.986)	0.880 (0.554-0.970)	0.913 (0.623-0.991)

Data presented as ICC estimate (95% Confidence Interval)

Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

REFERENCES

1. Dilenge ME, Majnemer A, Shevell MI. Long-term developmental outcome of asphyxiated term neonates. *J Child Neurol*. Nov 2001;16(11):781-792.

2. Higgins RD, Raju T, Edwards AD, et al. Hypothermia and other treatment options for neonatal encephalopathy: an executive summary of the Eunice Kennedy Shriver NICHD workshop. *J Pediatr*. Nov 2011;159(5):851-858 e851.

3. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. Oct 13 2005;353(15):1574-1584.

4. Azzopardi D, Edwards AD. Magnetic resonance biomarkers of neuroprotective effects in infants with hypoxic ischemic encephalopathy. *Semin Fetal Neonatal Med*. Oct 2010;15(5):261-269.

5. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. *J Pediatr*. Apr 2005;146(4):453-460.

6. Shankaran S, Barnes PD, Hintz SR, et al. Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed*. Nov 2012;97(6):F398-404.

7. Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR Am J Neuroradiol*. Jan 1998;19(1):143-149.

8. Malik GK, Trivedi R, Gupta RK, et al. Serial quantitative diffusion tensor MRI of the term neonates with hypoxic-ischemic encephalopathy (HIE). *Neuropediatrics*. Dec 2006;37(6):337-343.

Formatted: Line spacing: Double

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

9. Brissaud O, Amiraault M, Villega F, Periot O, Chateil JF, Allard M. Efficiency of fractional anisotropy and apparent diffusion coefficient on diffusion tensor imaging in prognosis of neonates with hypoxic-ischemic encephalopathy: a methodologic prospective pilot study. *AJNR Am J Neuroradiol*. Feb 2010;31(2):282-287.

10. Ward P, Counsell S, Allsop J, et al. Reduced fractional anisotropy on diffusion tensor magnetic resonance imaging after hypoxic-ischemic encephalopathy. *Pediatrics*. Apr 2006;117(4):e619-630.

11. Tusor N, Wusthoff C, Smee N, et al. Prediction of neurodevelopmental outcome after hypoxic-ischemic encephalopathy treated with hypothermia by diffusion tensor imaging analyzed using tract-based spatial statistics. *Pediatr Res*. Jul 2012;72(1):63-69.

12. Partridge SC, Mukherjee P, Berman JL, et al. Tractography-based quantitation of diffusion tensor imaging parameters in white matter tracts of preterm newborns. *J Magn Reson Imaging*. Oct 2005;22(4):467-474.

13. van Pul C, Buijs J, Vilanova A, Roos FG, Wijn PF. Infants with perinatal hypoxic ischemia: feasibility of fiber tracking at birth and 3 months. *Radiology*. Jul 2006;240(1):203-214.

14. Evangelou I, Serag A, Bouyssi-Kobar M, Du Plessis A, Limperopoulos C. Optimized methodology for neonatal diffusion tensor imaging processing and study-specific template construction. *Conf Proc IEEE Eng Med Biol Soc*. 2014;in press.

15. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B*. Jun 1996;111(3):209-219.

Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria

Formatted: Font: Cambria, Italic  
Formatted: Font: Cambria  
Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria  
Formatted: Font: Cambria, Italic  
Formatted: Font: Cambria

Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria

Formatted: Font: Cambria, Italic  
Formatted: Font: Cambria

Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria  
Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria  
Formatted: Font: Cambria, Italic  
Formatted: Font: Cambria

Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria

Formatted: Font: Cambria, Italic  
Formatted: Font: Cambria  
Formatted: Font: Cambria, Bold  
Formatted: Font: Cambria  
Formatted: Font: Cambria, Italic  
Formatted: Font: Cambria

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

16. Melhem ER, Mori S, Mukundan G, Kraut MA, Pomper MG, van Zijl PC. Diffusion tensor MR imaging of the brain and white matter tractography. *AJR Am J Roentgenol*. Jan 2002;178(1):3-16.

17. Adams E, Chau V, Poskitt KJ, Grunau RE, Synnes A, Miller SP. Tractography-based quantitation of corticospinal tract development in premature newborns. *J Pediatr*. Jun 2010;156(6):882-888, 888 e881.

18. Bayley N. *Manual for the Bayley scales of infant development*. New York: Psychological Corporation; 1969.

19. Bayley N. *Bayley Scales of Infant Development - Third Edition*. San Antonio, TX: Harcourt Assessment; 2006.

20. McKinstry RC, Miller JH, Snyder AZ, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology*. Sep 24 2002;59(6):824-833.

21. Ancora G, Testa C, Grandi S, et al. Prognostic value of brain proton MR spectroscopy and diffusion tensor imaging in newborns with hypoxic-ischemic encephalopathy treated by brain cooling. *Neuroradiology*. Aug 2013;55(8):1017-1025.

22. Gilmore JH, Liu W, Corouge J, et al. Early postnatal development of corpus callosum and corticospinal white matter assessed with quantitative tractography. *AINR Am J Neuroradiol*. Oct 2007;28(9):1789-1795.

23. Aralasmak A, Ulmer JL, Kocak M, Salvan CV, Hillis AE, Yousem DM. Association, commissural, and projection pathways and their functional deficit reported in literature. *J Comput Assist Tomogr*. Sep-Oct 2006;30(5):695-715.

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
24. Murakami A, Morimoto M, Yamada K, et al. Fiber-tracking techniques can predict the degree of neurologic impairment for periventricular leukomalacia. *Pediatrics*. Sep 2008;122(3):500-506.
  25. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage*. Nov 2002;17(3):1429-1436.
  26. Kinney HC, Brody BA, Kloman AS, Gilles FH. Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. *J Neuropathol Exp Neurol*. May 1988;47(3):217-234.
  1. Dilenge ME, Majnemer A, Shevell MI. Long-term developmental outcome of asphyxiated term neonates. *J Child Neurol*. Nov 2004;16(11):781-792.
  2. Higgins RD, Raju T, Edwards AD, et al. Hypothermia and other treatment options for neonatal encephalopathy: an executive summary of the Eunice Kennedy Shriver NICHD workshop. *J Pediatr*. Nov 2011;159(5):851-858 e851.
  3. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. Oct 13 2005;353(15):1574-1584.
  4. Azzopardi D, Edwards AD. Magnetic resonance biomarkers of neuroprotective effects in infants with hypoxic-ischemic encephalopathy. *Semin Fetal Neonatal Med*. Oct 2010;15(5):261-269.
  5. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. *J Pediatr*. Apr 2005;146(4):453-460.

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

Formatted: Font: Cambria, Bold

Formatted: Font: Cambria

Formatted: Font: Cambria, Italic

Formatted: Font: Cambria

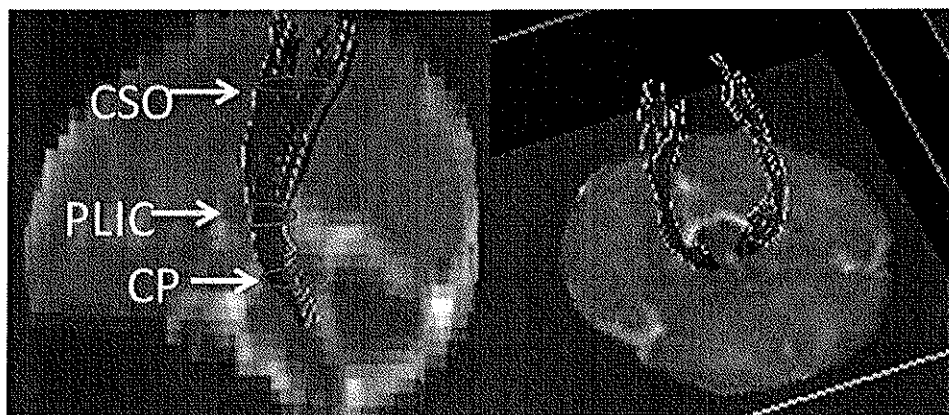


6. Shankaran S, Barnes PD, Hintz SR, et al. Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed.* Nov 2012;97(6):F398-404.
7. Malik GK, Trivedi R, Gupta RK, et al. Serial quantitative diffusion tensor MRI of the term neonates with hypoxic-ischemic encephalopathy (HIE). *Neuropediatrics.* Dec 2006;37(6):337-343.
8. Brisaud O, Amirault M, Villega F, Periot O, Chateil JF, Allard M. Efficiency of fractional anisotropy and apparent diffusion coefficient on diffusion tensor imaging in prognosis of neonates with hypoxic-ischemic encephalopathy: a methodologic prospective pilot study. *AJNR Am J Neuroradiol.* Feb 2010;31(2):282-287.
9. Ward P, Counsell S, Allsop J, et al. Reduced fractional anisotropy on diffusion tensor magnetic resonance imaging after hypoxic-ischemic encephalopathy. *Pediatrics.* Apr 2006;117(4):e619-630.
10. Tusor N, Wusthoff C, Smeets N, et al. Prediction of neurodevelopmental outcome after hypoxic-ischemic encephalopathy treated with hypothermia by diffusion tensor imaging analyzed using tract-based spatial statistics. *Pediatr Res.* Jul 2012;72(1):63-69.
11. Partridge SC, Mukherjee P, Berman JL, et al. Tractography-based quantitation of diffusion tensor imaging parameters in white matter tracts of preterm newborns. *J Magn Reson Imaging.* Oct 2005;22(4):467-474.
12. van Pul C, Buijs J, Vilanova A, Roos FG, Wijn PF. Infants with perinatal hypoxic ischemia: feasibility of fiber tracking at birth and 3 months. *Radiology.* Jul 2006;240(1):203-214.

13. Evangelou I, Serag A, Bouyssi-Kobar M, Du Plessis A, Limperopoulos C. Optimized methodology for neonatal diffusion tensor imaging processing and study-specific template construction. *Conf Proc IEEE Eng Med Biol Soc*. 2014;in press.
14. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative diffusion tensor MRI. *J Magn Reson B*. Jun 1996;111(3):209-219.
15. Melhem ER, Mori S, Mukundan G, Kraut MA, Pemper MG, van Zijl PC. Diffusion tensor MR imaging of the brain and white matter tractography. *AJR Am J Roentgenol*. Jan 2002;178(1):3-16.
16. Adams E, Chau V, Poskitt KJ, Grunau RE, Symes A, Miller SP. Tractography-based quantitation of corticospinal tract development in premature newborns. *J Pediatr*. Jun 2010;156(6):882-888, 888 e881.
17. Bayley N. *Manual for the Bayley scales of infant development*. New York: Psychological Corporation; 1969.
18. Bayley N. *Bayley Scales of Infant Development—Third Edition*. San Antonio, TX: Harcourt Assessment; 2006.
19. McKinstry RC, Miller JJ, Snyder AZ, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology*. Sep 24 2002;59(6):824-833.
20. Ancora G, Testa C, Grandi S, et al. Prognostic value of brain proton MR spectroscopy and diffusion tensor imaging in newborns with hypoxic-ischemic encephalopathy treated by brain cooling. *Neuroradiology*. Aug 2013;55(8):1017-1025.

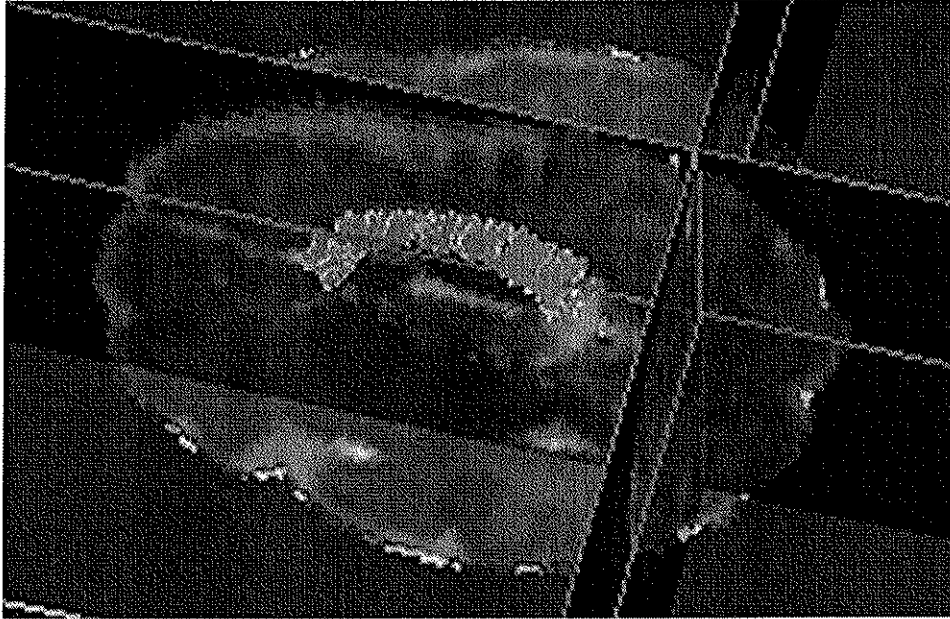
- 1  
2  
3  
4  
5  
6  
7  
8  
9 21. Gilmore JH, Lin W, Corouge L, et al. Early postnatal development of corpus callosum  
10 and corticospinal white matter assessed with quantitative tractography. *AJNR Am J*  
11 *Neuroradiol.* Oct 2007;28(9):1789-1795.  
12  
13  
14  
15 22. Aralasmak A, Ulmer JL, Kocak M, Salvan CV, Hillis AE, Yousem DM. Association,  
16 commissural, and projection pathways and their functional deficit reported in  
17 literature. *J Comput Assist Tomogr.* Sep-Oct 2006;30(5):695-715.  
18  
19  
20 23. Murakami A, Morimoto M, Yamada K, et al. Fiber tracking techniques can predict the  
21 degree of neurologic impairment for periventricular leukomalacia. *Pediatrics.* Sep  
22 2008;122(3):500-506.  
23  
24  
25  
26 24. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination  
27 revealed through MRI as increased radial (but unchanged axial) diffusion of water.  
28 *Neuroimage.* Nov 2002;17(3):1429-1436.  
29  
30  
31  
32 25. Kinney HC, Brody BA, Kloman AS, Gilles FH. Sequence of central nervous system  
33 myelination in human infancy. II. Patterns of myelination in autopsied infants. *J*  
34 *Neuropathol Exp Neurol.* May 1988;47(3):217-234.  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Formatted: Line spacing: Double



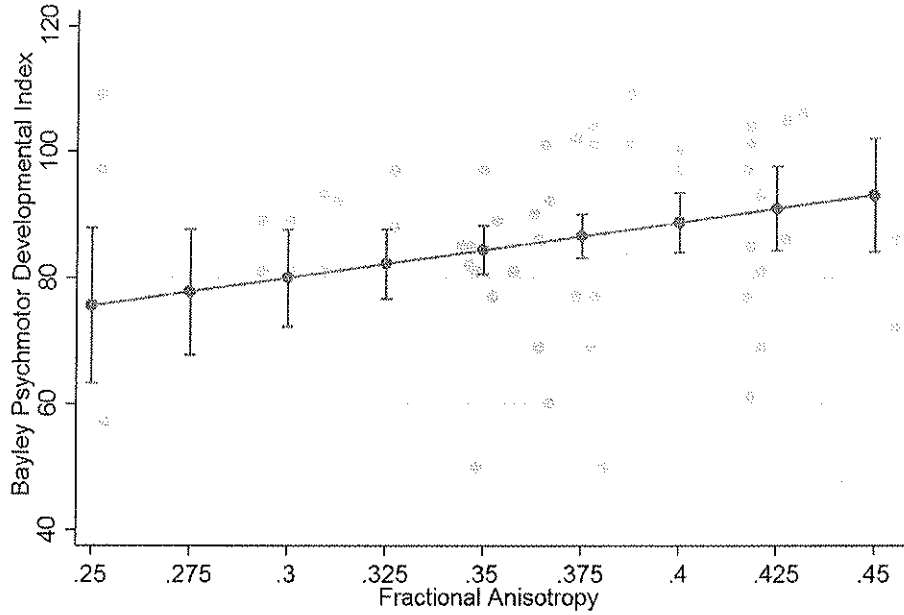
Visualization of fiber tracts by diffusion tensor tractography. A) Cortical spinal tract delineation by constraints at the level of the cerebral peduncle (CP), posterior limb of the internal capsule (PLIC) and centrum semiovale (CSO). B) Corpus callosum delineation on sagittal images.  
116x50mm (300 x 300 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



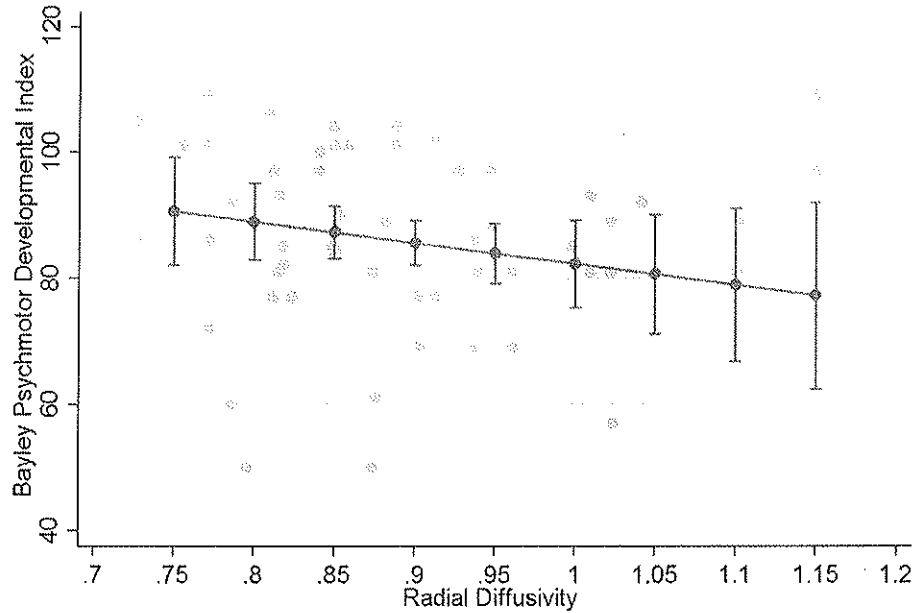
Visualization of fiber tracts by diffusion tensor tractography. A) Cortical spinal tract delineation by constraints at the level of the cerebral peduncle (CP), posterior limb of the internal capsule (PLIC) and centrum semiovale (CSO). B) Corpus callosum delineation on sagittal images.  
72x47mm (300 x 300 DPI)

PRE-PROOF ONLY



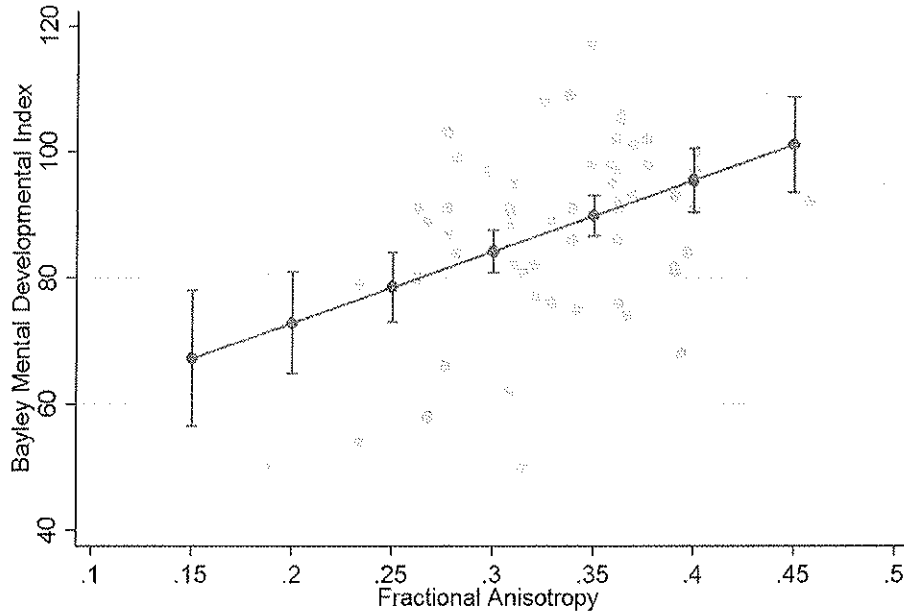
Relationships between Bayley scales and DTI measures. Bars represent 95% confidence intervals of regression model predictive margins. Significant associations are shown between Bayley Psychomotor Developmental Index and corticospinal tract a) fractional anisotropy ( $p < 0.001$ ) and b) radial diffusivity ( $p = 0.018$ ). Significant associations were also observed between Bayley Mental Developmental Index and corpus callosum c) fractional anisotropy ( $P < 0.001$ ) and d) axial diffusivity ( $p = 0.03$ ).  
203x147mm (300 x 300 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



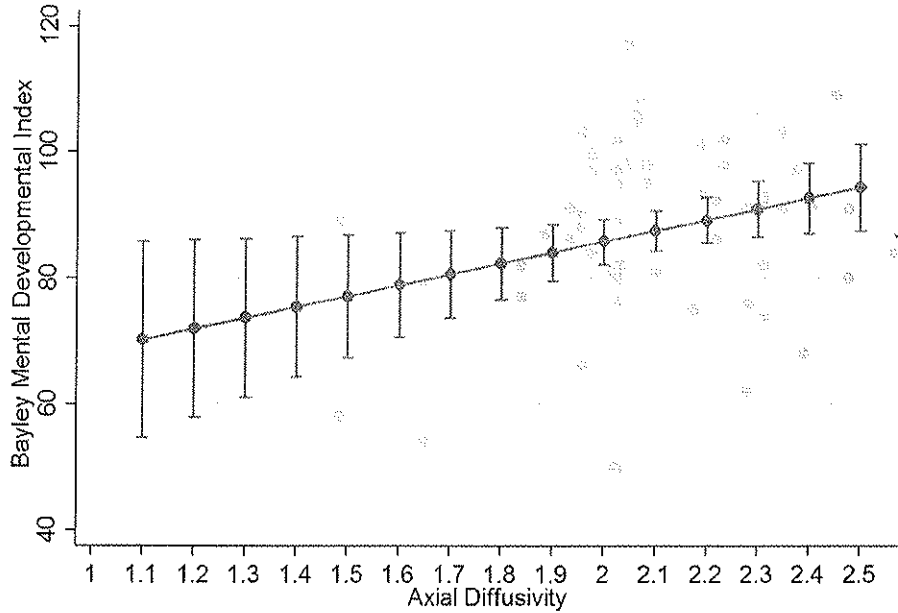
Relationships between Bayley scales and DTI measures. Bars represent 95% confidence intervals of regression model predictive margins. Significant associations are shown between Bayley Psychomotor Developmental Index and corticospinal tract a) fractional anisotropy ( $p < 0.001$ ) and b) radial diffusivity ( $p = 0.018$ ). Significant associations were also observed between Bayley Mental Developmental Index and corpus callosum c) fractional anisotropy ( $P < 0.001$ ) and d) axial diffusivity ( $p = 0.03$ ).  
203x147mm (300 x 300 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



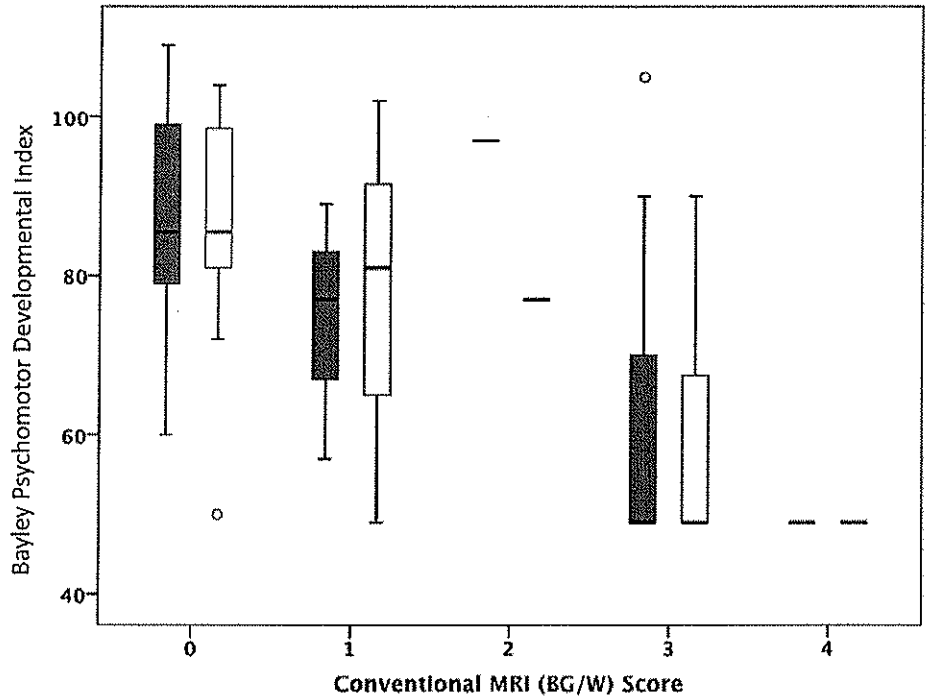
Relationships between Bayley scales and DTI measures. Bars represent 95% confidence intervals of regression model predictive margins. Significant associations are shown between Bayley Psychomotor Developmental Index and corticospinal tract a) fractional anisotropy ( $p < 0.001$ ) and b) radial diffusivity ( $p = 0.018$ ). Significant associations were also observed between Bayley Mental Developmental Index and corpus callosum c) fractional anisotropy ( $P < 0.001$ ) and d) axial diffusivity ( $p = 0.03$ ).  
203x147mm (300 x 300 DPI)





Relationships between Bayley scales and DTI measures. Bars represent 95% confidence intervals of regression model predictive margins. Significant associations are shown between Bayley Psychomotor Developmental Index and corticospinal tract a) fractional anisotropy ( $p < 0.001$ ) and b) radial diffusivity ( $p = 0.018$ ). Significant associations were also observed between Bayley Mental Developmental Index and corpus callosum c) fractional anisotropy ( $P < 0.001$ ) and d) axial diffusivity ( $p = 0.03$ ).  
203x147mm (300 x 300 DPI)

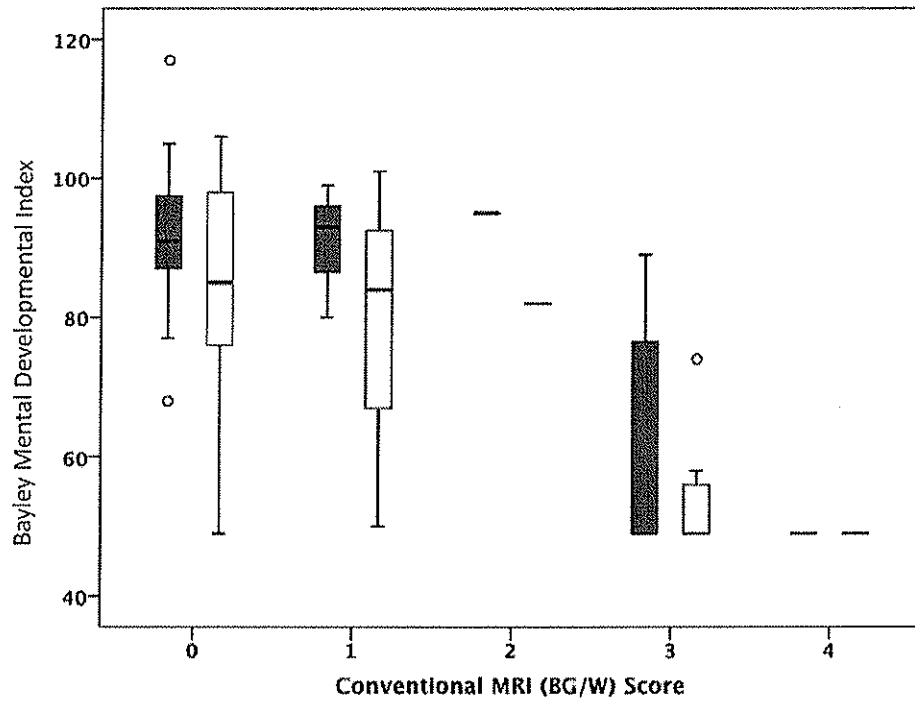
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



Relationships between Conventional MRI and Developmental Outcome. Bayley Psychomotor Developmental Index Scores are presented by MRI Basal Ganglia/Watershed (BG/W) Score. Boxplots represent medians and interquartile ranges. Whiskers represent range with outliers depicted by circles. Gray and white boxes depict outcome data at 15 at 21 months respectively. Bayley scores were significantly different by MRI BG/W Score (ANOVA P-values < 0.001).  
254x190mm (300 x 300 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



3. Relationships between Conventional MRI and Developmental Outcome. Bayley Mental Developmental Index Scores are presented by MRI Basal Ganglia/Watershed (BG/W) Score. Boxplots represent medians and interquartile ranges. Whiskers represent range with outliers depicted by circles. Gray and white boxes depict outcome data at 15 at 21 months respectively. Bayley scores were significantly different by MRI BG/W Score (ANOVA P-values < 0.001).  
254x190mm (300 x 300 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

WHITE MATTER TRACT INTEGRITY AND DEVELOPMENTAL OUTCOME IN  
NEWBORNS WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY (HIE) TREATED WITH  
HYPOTHERMIA

An N. Massaro MD,<sup>1,6</sup> Jordanis Evangelou DPhil,<sup>2,7</sup> Ali Fatemi MD,<sup>3</sup> Gilbert Vezina MD,<sup>2,6,7</sup>  
Robert McCarter ScD,<sup>4,6,7</sup> Penny Glass PhD<sup>5,6</sup>, and Catherine Limperopoulos PhD<sup>2,6</sup>  
Departments of <sup>1</sup>Neonatology, <sup>2</sup>Diagnostic Imaging and Radiology, <sup>4</sup>Biostatistics & Informatics,  
and <sup>5</sup>Psychiatry & Behavioral Sciences, Children's National Medical Center  
<sup>3</sup>Kennedy Krieger Institute, Johns Hopkins School of Medicine,  
Departments of <sup>6</sup>Pediatrics, <sup>7</sup>Radiology and <sup>8</sup>Epidemiology & Biostatistics, The George  
Washington University School of Medicine,  
Washington, DC, United States

Corresponding Author:  
An N. Massaro, MD  
Assistant Professor of Pediatrics  
The George Washington University School of Medicine  
Department of Neonatology  
Children's National Medical Center  
111 Michigan Avenue, NW  
Washington, DC 20010  
Phone (202) 476-5225  
Fax (202) 476-3459  
Email: [anguyenm@cnmc.org](mailto:anguyenm@cnmc.org)

Word Count of Abstract: 200

Word Count of Manuscript: 3300

**ABSTRACT**

**Aim:** To determine whether corpus callosum (CC) and corticospinal tract (CST) diffusion tensor imaging (DTI) measures relate to developmental outcome in encephalopathic newborns after therapeutic hypothermia.

**Methods:** Encephalopathic newborns enrolled in a longitudinal study underwent DTI after hypothermia. Parametric maps were generated for fractional anisotropy (FA), mean (MD), radial (RD) and axial (AD) diffusivity. CC and CST were segmented by DTI-based tractography.

Multiple regression models were used to examine the association of DTI measures with Bayley-II Mental (MDI) and Psychomotor (PDI) Developmental Index at 15 and 21 months of age.

**Results:** Fifty-two infants underwent DTI at median age of 8 days. Two subjects were excluded with poor MRI quality. Outcomes were assessed in 42/50 (84%) infants at 15 months and 35/50 (70%) at 21 months. Lower CC and CST FA were associated with lower MDI and PDI respectively, even after controlling for gestational age, birth weight, gender, and socioeconomic status. There was also a direct relationship between CC AD and MDI, while CST RD was inversely related to PDI.

**Interpretation:** In encephalopathic newborns, impaired microstructural organization of the CC and CST predicts poorer cognitive and motor performance respectively. Tractography provides a reliable method for early assessment of perinatal brain injury.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**What This Paper Adds**

- Corpus callosum integrity relates to cognitive performance in childhood.
- Corticospinal tract integrity relates to motor performance in childhood.
- DTI tractography can provide early assessment of perinatal brain injury.

For Review Only

1  
2  
3 Hypoxic ischemic encephalopathy (HIE) is a major cause of neonatal death and long-term  
4 disability in children.<sup>1</sup> Therapeutic hypothermia is the current standard of care for newborns  
5 presenting with HIE,<sup>2</sup> however nearly half of treated infants continue to have adverse outcomes  
6 despite treatment with cooling.<sup>3</sup> Acute assessment of brain injury after neuroprotective  
7 intervention is an important aspect of neurocritical care. Such information can be used to direct  
8 adjuvant and rehabilitative therapies, provide prognostic information for families of affected  
9 children, and serve as surrogate outcomes for future neurotherapeutic trials.

10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22 Magnetic resonance imaging (MRI) is a leading source of brain injury biomarkers in perinatal  
23 HIE.<sup>4</sup> Pattern of brain injury by conventional MRI has been related to both severity and  
24 phenotype of neurodevelopmental sequelae after HIE.<sup>5, 6, 7</sup> However, conventional MRI requires  
25 availability of an experienced pediatric neuroradiologist, can be subjective, and provides broad  
26 severity classification on an ordinal scale. Quantitative MRI techniques such as diffusion tensor  
27 imaging (DTI) can overcome these limitations by providing continuous, reproducible measures  
28 of brain microstructural injury.

29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41 Few studies have related DTI measures and developmental outcomes in survivors of HIE.<sup>8-11</sup>  
42 Most prior studies have been limited to 2D region-of-interest approaches that are subject to inter-  
43 rater reliability. Additionally, these approaches fail to interrogate the complexity of 3D  
44 anatomical brain regions, which is important when evaluating structure-function relationships.  
45 DTI-based tractography provides a semi-automated approach for 3D delineation of major white  
46 matter pathways. It has been demonstrated to be feasible in newborns,<sup>12, 13</sup> particularly when  
47 evaluating the most well developed white matter tracts.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 This study aims to evaluate if microstructural organization measured via quantitative  
7  
8 tractography is related to neurodevelopmental outcome in HIE. Specifically, we hypothesized  
9  
10 that impaired brain microstructure in the corpus callosum (CC) and corticospinal tract (CST)  
11  
12 would be associated with poorer cognitive and motor performance, respectively, in young  
13  
14 childhood survivors of perinatal HIE.  
15  
16

## 17 18 19 20 METHODS

### 21 22 *Participants*

23  
24 All patients with HIE admitted for therapeutic hypothermia to an out-born level IV regional  
25  
26 neonatal intensive care unit (NICU) were approached for enrollment between May 2008 and  
27  
28 October 2010 in this prospective longitudinal study. Whole-body hypothermia was provided  
29  
30 based on established criteria<sup>3</sup> (i.e. infants were greater than 36 weeks gestational age, greater  
31  
32 than 1800 grams at birth, demonstrated metabolic acidosis and/or low Apgar scores, and  
33  
34 exhibited signs of moderate to severe clinical encephalopathy). Infants were excluded if they  
35  
36 were small for gestational age or had a known or suspected chromosomal abnormality or major  
37  
38 congenital anomaly. The study was approved by the Institutional Review Board and written  
39  
40 informed consent was obtained from the parents of eligible participants.  
41  
42  
43  
44  
45  
46  
47

### 48 49 *Data Collection*

50  
51 Demographic and clinical data were collected from the referral hospital and study site medical  
52  
53 records. Clinical characteristics were recorded including umbilical cord or first-hour of life  
54  
55 blood gas, Apgar scores, and presence of electrographic seizures. Initial clinical grade of  
56  
57  
58  
59  
60



1  
2  
3 encephalopathy was classified according to modified Sarnat criteria used in the NICHD trial.<sup>3</sup>  
4  
5 Socioeconomic status (SES) was classified by financial eligibility for medical assistance and  
6  
7 maternal education level.  
8  
9

### 10 11 12 *MR Image Acquisition*

13  
14  
15 MRI was performed at target 7-10 days of life on a 1.5T scanner (Signa HDx, GE Healthcare,  
16  
17 Milwaukee, WI, USA) using an 8-channel receive head coil (InVivo Corp., Gainesville, FL,  
18  
19 USA). When feasible, infants were scanned after feeding and bundling without the use of  
20  
21 sedation. Standard sequences included sagittal and axial spin echo (SE) T1-W, dual echo axial  
22  
23 SE proton density (PD) and T2-W images, and coronal fast spin echo (FSE) T2-W. In cases of  
24  
25 patient motion, periodically rotated parallel lines with enhanced reconstruction (PROPELLER)  
26  
27 T2-W and/or T1-W images were also obtained. A single shot SE echoplanar imaging (EPI) axial  
28  
29 DTI sequence was used with the following parameters: TR 10,000 ms, TE 100 ms, acquisition  
30  
31 matrix 128x128, slice thickness 4 mm, 1 volume with no diffusion weighting ( $b=0$ ) and 25  
32  
33 volumes in non-colinear gradient directions with diffusion weighting ( $b=1000$  s/mm<sup>2</sup>).  
34  
35  
36  
37  
38  
39  
40

### 41 *Preprocessing of Diffusion Tensor Images*

42  
43 DTI preprocessing was performed using an automated methodology optimized for neonatal data,  
44  
45 including calculation of parametric maps for fractional anisotropy (FA), mean (MD), axial (AD)  
46  
47 and radial (RD) diffusivity.<sup>14</sup> These DTI-derived metrics characterize voxel-wise the tensor  
48  
49 shape (FA) and magnitude (MD) of the underlying water diffusion within tissues. AD is a  
50  
51 measure of diffusivity parallel to the primary axis while RD measures diffusivity perpendicular  
52  
53 to the primary axis of diffusion. MD is calculated as the average diffusion along all 3 axes.<sup>15</sup>  
54  
55  
56  
57  
58  
59  
60

### *White Matter Fiber Tracking*

Fiber tracking was performed using Fiber Assignment by Continuous Tracking (DTI Studio, Baltimore, MD).<sup>16</sup> Fiber trajectories were formed along the primary eigenvector between two voxels using an FA threshold of greater than 0.1. Tracts were terminated if this value dropped below 0.05 or if the angle between the primary eigenvectors of consecutive voxels exceed 50°. CST was segmented by placing region of interest (ROI) constraints at the level of the cerebral peduncles, posterior limb of the internal capsule (PLIC) and centrum semiovale (CSO) anterior to the central sulcus on axial images (Figure 1a).<sup>17</sup> Measurements for the left and right tracts were averaged within each newborn for analysis. For the CC, ROI delineation was performed on a mid-sagittal image, and right and left para-sagittal images (Figure 1b). Logical exclusion masks were also drawn to prevent implausible tracts from forming across anatomical boundaries and resulting fibers were checked on the MD images for anatomical validity. Segmented fiber tracts were overlaid on the co-registered parametric maps and mean FA, MD, AD and RD were calculated for each tract using the statistical program within DTI Studio. All fiber tract measurements were performed by a single investigator (A.N.M.) masked to the clinical and outcome data of the subjects. Intra-rater reliability was assessed by repeating fiber tracking measurements in 10 random subjects. Additionally, manual ROI delineation of the PLIC (at the level of the Foramen of Monro on an axial image) and the CC (on a mid-sagittal image) was performed in duplicate in the same 10 subjects for comparison. Intraclass correlation (ICC) was calculated to evaluate the reliability of the each method.

### *Conventional MRI Scoring*

1  
2  
3  
4 Conventional MRI studies were reviewed by a neuroradiologist (G.V.), who was masked to the  
5  
6 clinical characteristics, DTI measures and Bayley scores of subjects. Images were assessed for  
7  
8 the predominant pattern of brain injury.<sup>5</sup> Additionally, the degree of deep nuclear gray and  
9  
10 cortical/white matter injury was characterized by assigning a BG/W score ranging from 0-4  
11  
12 according to previously described methods.<sup>7</sup> The relationship between conventional MRI and  
13  
14 developmental outcome scores were evaluated for comparative purposes.  
15  
16

### 17 18 19 20 *Neurodevelopmental Assessment*

21  
22 Neurodevelopmental outcomes were assessed with the Bayley Scales of Infant Development-  
23  
24 Second Edition (BSID-II) at 15 and 21 months of age.<sup>18</sup> The BSID-II is a standardized  
25  
26 assessment that includes a Mental Developmental Index (MDI) that assesses the child's level of  
27  
28 cognitive, language and personal-social skills, as well as a Psychomotor Developmental Index  
29  
30 (PDI) that evaluates fine and gross motor development. BSID-II index scores of  $100 \pm 15$   
31  
32 represent the mean  $\pm$  1sd, with a basement score of 50. Children who were severely impaired  
33  
34 and not testable at the time of evaluation were assigned an index score of 49 for analysis (n=8 for  
35  
36 MDI, n=9 for PDI). Evaluations were performed by an experienced developmental psychologist  
37  
38 who was blinded to the MRI results of the child. Although the third edition of the BSID was  
39  
40 released in 2006,<sup>19</sup> the BSID-II continued to be used as the assessment tool in our developmental  
41  
42 clinic during the study period and thus was the outcome measure used in this study.  
43  
44  
45  
46  
47  
48  
49

### 50 51 *Statistical analysis*

52  
53 Descriptive statistics included standard measures of central tendency and variability for  
54  
55 continuous data and frequencies for categorical variables. Longitudinal mixed-effects regression  
56  
57  
58  
59  
60

1  
2  
3 models were developed to evaluate the association of DTI scalars with Bayley Scores, where the  
4 dependent variable was the time-averaged Bayley score in order to account for repeated  
5  
6 assessments in subjects evaluated at both 15 and 21 months, and the independent variable was  
7  
8 the DTI scalar. Separate models were developed for each DTI scalar (FA, MD, AD, RD). Data  
9  
10 were transformed when normality assumptions were not met. Baseline models controlled for  
11  
12 demographic variables including birthweight, gestational age, race, gender, SES and age at MRI.  
13  
14 Expanded models were also developed to evaluate whether DTI scalars independently related to  
15  
16 outcome when accounting for clinical covariables including presenting pH, grade of  
17  
18 encephalopathy. Secondary analyses were also performed to evaluate the consistency of results.  
19  
20 In order to account for the influence of the floor effect of the Bayley index (i.e. inability to  
21  
22 estimate developmental status at index scores <49), longitudinal logistic regression models were  
23  
24 developed to evaluate the relationships between DTI and adverse outcome defined as Bayley  
25  
26 score <70. Finally, the relationships between DTI and BSID-II scores from the 35 subjects  
27  
28 evaluated at 21 months were explored via ordinal regression models.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38

### 39 *Sample Size and Power*

40  
41 We planned to enroll 90 subjects in this study, in order to proceed with data from 60 subjects  
42  
43 following attrition (due to mortality, loss to follow-up and poor MRI quality). However, due to  
44  
45 changes made to our clinical imaging protocol during the study period, we completed enrollment  
46  
47 with 63 infants included in the study. We used PASS 11 (NCSS, Kaysville, UT, USA) to  
48  
49 develop post hoc estimates of statistical power using One Power Correlation Analysis. The  
50  
51 magnitude of correlation between Bayley score and DTI metric was calculated based on a  
52  
53 scenario where the 15 and 21 month data were treated as repeat observations on 50 patients, with  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 the average patient having between 1 and 2 measurements. Statistical power was set at 80% and  
4  
5 the 2-tailed type I error was set to 5%. Under these circumstances, the study was capable of  
6  
7 detecting moderate (38%) correlations between Bayley scores and DTI measures. Statistical  
8  
9 analyses were performed with STATA 11.0 software (StataCorp LP, College Station, TX, USA).  
10  
11  
12  
13  
14

## 15 RESULTS

16  
17 Of the 63 newborns with HIE enrolled, 11 (17%) died prior to MRI. Participants underwent  
18  
19 MRI at a median age of 8 days (range 6-16). MRI data was not analyzable in 2 infants due to  
20  
21 motion corruption. Of the 50 infants with available DTI data, 42 (84%) were evaluated at a  
22  
23 median age of 15.2 months (range 14.7-17.8) and 35 (70%) were also evaluated at a median age  
24  
25 of 21.4 (20.8-30.6) months. Infants lost to follow-up were similar to those retained in the study  
26  
27 with regards to baseline and clinical characteristics, except for indices of SES (Table 1).  
28  
29  
30  
31

### 32 *Relationship between Corticospinal Tract Integrity and Motor Outcome*

33  
34 There was a strong positive association between CST FA and BSID-II PDI that remained  
35  
36 significant when baseline ( $\beta=198$ ; 95% CI: 102, 293;  $p<0.001$ ) and clinical ( $\beta=128$ ; 95% CI: 34,  
37  
38 222;  $p=0.008$ ) characteristics were controlled (Figure 2a). CST AD also trended to have a  
39  
40 positive association with BSID-II PDI (Baseline model:  $\beta=45$ ; 95% CI: -2.5, 92;  $p=0.063$ ;  
41  
42 Expanded model:  $\beta=36$ ; 95% CI: -6.5, 77.8;  $p=0.098$ , Figure 2b). CST RD had a negative  
43  
44 association with BSID-II PDI ( $\beta= -87.8$ ; 95% CI: -160, -15;  $p=0.018$ , Figure 2b), which was not  
45  
46 significant after controlling for clinical covariables ( $\beta= -45$ ; 95% CI: -116, 27;  $p=0.219$ ). CST  
47  
48 MD was not related to motor outcome. The relationship between CST FA and PDI remained  
49  
50 consistent in the logistic regression model ( $p=0.046$ ) and when evaluating only BSID-II PDI data  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 from subjects evaluated at 21 months ( $p=0.001$ ), while the relationship between CST RD and  
4  
5 PDI at 21 months was reduced to a non-significant trend (logistic regression model  $p=0.085$ , 21  
6  
7 month PDI model  $p=0.058$ ).  
8  
9

#### 10 11 12 *Relationship between Corpus Callosum Integrity and Cognitive Outcome*

13  
14  
15 FA in the CC was strongly associated with BSID-II MDI when controlling for baseline ( $\beta=149$ ;  
16  
17 95% CI: 82.5, 216;  $p<0.001$ ), as well as clinical ( $\beta=91$ ; 95% CI: 21.6, 160.7;  $p=0.01$ )  
18  
19 characteristics (Figure 2c). CC AD was also positively associated with BSID-II MDI ( $\beta=22.9$ ;  
20  
21 95% CI: 2.2, 43.6;  $p=0.03$ ), but not after accounting for clinical factors ( $\beta=14$ ; 95% CI: -3.7,  
22  
23 31.8;  $p=0.122$ , Figure 2d). RD and MD in the CC were not related to cognitive outcome. The  
24  
25 significant association between CC FA and MDI was also demonstrated in the logistic regression  
26  
27 model ( $p=0.022$ ) and when evaluating only 21 month MDI scores ( $p<0.001$ ), while CC AD and  
28  
29 MDI were not significantly related in the logistic regression model ( $p=0.096$ ) and the 21 month  
30  
31 MDI model ( $p=0.079$ ).  
32  
33  
34  
35  
36  
37  
38  
39  
40

#### 41 *Intra-rater Reliability*

42  
43 The reliability of the tractography-based approach was high and similar to the ROI-based method  
44  
45 in the CC, but tractography of the CST was more reproducible compared to PLIC delineation by  
46  
47 ROI (Table 2).  
48  
49

#### 50 *Conventional MRI and Developmental Outcome*

51  
52 No brain injury was identified by conventional imaging in 18 (36%) subjects. Of the 32 subjects  
53  
54 with brain injury, predominant pattern of injury was classified as watershed in 4 (13%), basal  
55  
56  
57  
58  
59  
60

1  
2  
3 nuclei in 10 (31%), focal/multifocal in 17 (53%), and 1 patient had global brain injury. As  
4  
5 previously described, BG/W score was significantly associated with outcome (Figure 3).  
6  
7  
8  
9

## 10 DISCUSSION

11  
12 This is the first study to relate microstructural injury measured by DTI tractography and  
13  
14 developmental outcome in children with perinatal HIE treated with hypothermia. We  
15  
16 demonstrate that lower FA and higher RD in the CST are associated with lower Bayley PDI,  
17  
18 while lower FA and AD in the CC relate to lower Bayley MDI. Specifically, for every 0.1  
19  
20 decrease in CST FA, we observed a 13 to 20-point decrease in Bayley PDI. Correspondingly,  
21  
22 there was a 9 to 15 point decrease in Bayley MDI for every 0.1 decrease in CC FA. These data  
23  
24 suggest that impaired CST and CC microstructure observed in the neonatal period predict motor  
25  
26 and cognitive deficits, respectively, in early childhood. Assessment of brain injury severity and  
27  
28 risk for related developmental sequelae in the neonatal period can allow clinicians and  
29  
30 investigators to gage treatment effect immediately after intervention, and can help prioritize  
31  
32 resources towards long-term evaluation of only the most promising interventions. This study  
33  
34 supports DTI quantitative tractography as a reliable method to measure brain injury after  
35  
36 therapeutic hypothermia in newborns with HIE.  
37  
38  
39  
40  
41  
42  
43  
44  
45

46 We demonstrated that conventional MRI scoring also relates to developmental outcome. This is  
47  
48 consistent with prior work that has established conventional MRI as a good predictor of  
49  
50 neurodevelopmental outcome in newborns with HIE after therapeutic hypothermia.<sup>6</sup> However,  
51  
52 reliance on conventional MRI interpretation has limitations as classification of injury by this  
53  
54 method is subject to inter-rater reliability, and only provides a discrete number of outcome  
55  
56  
57  
58  
59  
60

1  
2  
3 category levels depending on the classification scale used. A continuous, reproducible  
4  
5 biomarker is preferred when the goal is to evaluate small effect sizes or treatment effects in small  
6  
7 samples, as is common in pre-clinical and early phase therapeutic trials. Other advanced MRI  
8  
9 measures have been proposed as candidate biomarkers in answer to this need. While MR  
10  
11 spectroscopy (MRS) measures such as Lac/NAA ratio have been related to outcome,<sup>4</sup> MRS does  
12  
13 not provide information about the topography of injury in order to distinguish the associated  
14  
15 developmental phenotype. It is also unclear how long the presence of lactate persists after an  
16  
17 initial hypoxic-ischemic insult. Similarly, the apparent diffusion coefficient (ADC) from  
18  
19 standard diffusion weighted imaging is limited by its time-dependency, and more specifically its  
20  
21 propensity for pseudonormalization.<sup>20</sup>

22  
23  
24  
25  
26  
27  
28  
29 DTI fractional anisotropy does not pseudonormalize,<sup>10,11</sup> and thus increasing reports have  
30  
31 emerged relating DTI FA to developmental outcome in HIE. Brissaud et al described lower FA  
32  
33 in the PLIC and cerebral peduncles in HIE infants with abnormal neurological exam at 1-2  
34  
35 weeks of life.<sup>9</sup> Malik et al reported a significant correlation between FA measured by ROI in the  
36  
37 CST and developmental quotient at 3 months of age in newborns with HIE.<sup>8</sup> Few studies have  
38  
39 evaluated the relationship between DTI and longer term developmental outcome in HIE. Tusor  
40  
41 et al reported the association of 1-2 year developmental outcome and DTI FA quantified by tract-  
42  
43 based special statistics (TBSS).<sup>11</sup> Only Ancora and colleagues reported data on all four major  
44  
45 DTI scalars and their relation to outcome. These investigators found high predictive ability of  
46  
47 AD in the CC and FA/ RD in the frontal and parietal white matter for distinguishing  
48  
49 developmental outcome at 2 years in a small series of 20 patients.<sup>21</sup> To our knowledge this is the  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 largest study to date evaluating the relationship between DTI and developmental outcome, and  
4  
5 the only study utilizing the tractography-based approach.  
6  
7  
8

9  
10 How and where DTI parametric scalars are measured is a crucial component of establishing them  
11  
12 as viable biomarkers of brain microstructural injury. Previous DTI studies have mainly used  
13  
14 manual 2D ROI approaches. This method only evaluates selected areas of key anatomical  
15  
16 structures, and introduces observer error that limits reproducibility.<sup>8-10</sup> DTI tractography offers a  
17  
18 semi-automated way of visualizing whole white matter fiber tracts in 3D. While processing  
19  
20 steps still require manual placement of ROI constraints, our study and others have demonstrated  
21  
22 that the tractography-based method has higher reliability compared to manual ROI delineation.<sup>17,</sup>  
23  
24  
25  
26  
27 <sup>22</sup> Thus, DTI tractography provides a reliable method for hypothesis-driven interrogation based  
28  
29 on a priori knowledge of brain structure and function relationships. We selected CST and CC  
30  
31 because of their known association with motor and cognitive function respectively.<sup>23</sup> By  
32  
33 quantifying whole tract microstructure, the tractography-based method allows for detection of  
34  
35 group-wise differences that is independent of the topography of injury along the tract. This may  
36  
37 improve sensitivity for distinguishing outcome groups as has been suggested by previous  
38  
39 reports.<sup>17, 24</sup> Tusor and colleagues reported the use of tract-based special statistics (TBSS) to  
40  
41 quantify FA.<sup>11</sup> This method relies on normalization of subjects to a template space (which  
42  
43 introduces interpolation), but also provides a semi-automated method for quantification of FA in  
44  
45 WM tracts. Interrogation of microstructure via both methods may provide confirmatory or  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60 complementary information.

1  
2  
3 The other advantage of tractography-based anatomical parcellation is the ability to measure other  
4 DTI parametric scalars within the defined ROI. While FA was most significantly associated  
5  
6 with outcome across all analyses in our study, other metrics such as RD and AD may provide  
7  
8 more insights into pathogenesis by detailing microstructural changes after injury. RD is thought  
9  
10 to represent the degree of myelination,<sup>25</sup> while AD reflects fiber coherence and structure of  
11  
12 axonal membranes.<sup>25</sup> FA is determined by these and other factors including fiber diameter and  
13  
14 density, as well as extracellular and interaxonal spacing.<sup>25</sup> We found a relationship between RD  
15  
16 in the CST and motor outcome while RD in the CC was not significantly related to cognitive  
17  
18 outcome. This is consistent with knowledge that myelination of the CST is known to occur  
19  
20 around and shortly after term birth whereas the CC is not myelinated until several months of  
21  
22 age.<sup>26</sup> That MD was not associated with outcome may represent the impact of  
23  
24 pseudonormalization given the imaging window used in this study.<sup>20</sup>  
25  
26  
27  
28  
29  
30  
31  
32  
33

34 This study has limitations. Attrition due to loss to follow-up is a common problem in  
35  
36 longitudinal studies. While it is reassuring that infants who were lost to follow-up were similar  
37  
38 to those retained with regards to baseline and clinical characteristics, that infants who did not  
39  
40 return for assessment could be differentiated by SES risk-profile remains a possible source of  
41  
42 bias. Attrition also impacted an already limited sample size. In order to overcome the related  
43  
44 challenges to statistical power, we used an analytic approach that took advantage of repeated  
45  
46 measures in participants. It is reassuring that the relationship between FA and developmental  
47  
48 outcome was consistent when evaluating the subset of outcome data from subjects evaluated at  
49  
50 21 months. Given the sample size limitations, and the fact that we did not control for multiple  
51  
52 comparisons in these analyses, the relationship between DTI scalars and longer-term outcome  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 warrant further investigation. The basement effect of the Bayley index also posed analytical  
4 challenges with regard to statistical approach. The consistency of results after removal of the  
5 infants performing below the Bayley floor, and logistic regression after binarization of outcome,  
6 provide added reassurance regarding the suggestion of a relationship between DTI and  
7 developmental outcome in children with HIE.  
8  
9  
10  
11  
12  
13  
14  
15  
16

## 17 CONCLUSIONS

18  
19 Impaired microstructural organization of the CC and CST may predict poorer early childhood  
20 cognitive and motor performance respectively in newborns with HIE after therapeutic  
21 hypothermia. Tractography provides a reliable method for early assessment of perinatal brain  
22 injury and can potentially serve as a surrogate outcome for future neurotherapeutic trials.  
23  
24  
25  
26  
27  
28  
29  
30

## 31 ACKNOWLEDGEMENTS

32  
33 The authors acknowledge Yao (Iris) Cheng, M.S. for her data management and statistical  
34 support, and Judy Brown, NNP, Christen Meisel, M.S., and Maya B. Coleman Ph.D. for their  
35 efforts coordinating developmental follow-up visits for study participants. This publication was  
36 supported by the Clinical and Translational Science Institute at Children's National (NIH  
37 UL1TR000075, KL2TR000076), the Intellectual and Developmental Disabilities Research  
38 Consortium (NIH P30HD040677), and The Advanced Pediatric Brain Imaging Research and  
39 Training Program (DoD W81XWH-11-2-0198).  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## FIGURE LEGENDS

1. Visualization of fiber tracts by diffusion tensor tractography. A) Cortical spinal tract delineation by constraints at the level of the cerebral peduncle (CP), posterior limb of the internal capsule (PLIC) and centrum semiovale (CSO). B) Corpus callosum delineation on sagittal images.
2. Relationships between Bayley scales and DTI measures. Bars represent 95% confidence intervals of regression model predictive margins. Significant associations are shown between Bayley Psychomotor Developmental Index and corticospinal tract a) fractional anisotropy ( $p < 0.001$ ) and b) radial diffusivity ( $p = 0.018$ ). Significant associations were also observed between Bayley Mental Developmental Index and corpus callosum c) fractional anisotropy ( $P < 0.001$ ) and d) axial diffusivity ( $p = 0.03$ ).
3. Relationships between Conventional MRI and Developmental Outcome. a) Bayley Psychomotor Developmental Index and b) Bayley Mental Developmental Index Scores are presented by MRI Basal Ganglia/Watershed (BG/W) Score. Boxplots represent medians and interquartile ranges. Whiskers represent range with outliers depicted by circles. Gray and white boxes depict outcome data at 15 at 21 months respectively. Bayley scores were significantly different by MRI BG/W Score (ANOVA P-values  $< 0.001$ ).

Table 1. Demographic and Clinical Characteristics of the Study Population

	<b>Study Group (n=50)</b>	<b>Assessed at 15 mo (n=42)</b>	<b>Assessed at 21 mo (n=35)</b>
<b>Gestational Age (Mean ± SD wks)</b>	38.8 ± 1.9	38.9 ± 1.9	39.0 ± 2.0
<b>Birth weight (Mean ± SD kg)</b>	3.4 ± 0.7	3.4 ± 0.7	3.4 ± 0.7
<b>Gender (n, % male)</b>	31 (62)	25 (60)	21 (60)
<b>Race (n, %)</b>			
Caucasian	24 (48)	23 (55)	20 (57)
Black/ African American	25 (50)	18 (43)	15 (43)
Other	1 (2)	1 (2)	
<b>Presenting pH</b>	6.91 (6.5-7.35)	6.9 (6.5-7.4)	6.9 (6.5-7.4)
<b>Presenting Base Deficit</b>	18 (8-36)	18 (9-36)	19 (9-36)
<b>Apgar Score</b>			
1 minute	1 (0-6)	1 (0-6)	1 (0-6)
5 minute	3 (0-7)	3 (0-7)	3 (0-7)
10 minute	5 (0-9) <sup>a</sup>	5 (0-9) <sup>b</sup>	5 (0-9) <sup>c</sup>
<b>Encephalopathy Grade</b>			
Moderate (n, %)	41 (82)	33 (79)	27 (77)
Severe (n, %)	9 (18)	9 (21)	8 (23)
<b>Electrographic Seizures (n, %)</b>	18 (36)	17 (40)	15 (43)
<b>Socioeconomic Status (n, %)</b>			
Need for medical assistance	8 (16)	4 (9)*	3 (8)*
<b>Maternal Education</b>			
Grade school	6 (12)	6 (14)	4 (11)
High school graduate	23 (46)	16 (38)	13 (37)
Higher level education	21 (42)	20 (48)*	18 (51)*

\* Significant difference from those lost to follow-up (p<0.05)

Table 2. Intraclass Correlation Coefficients

Parameter	CORPUS CALLOSUM		CORTICOSPINAL TRACT	
	ROI	Tractography	ROI	Tractography
FA	0.991 (0.966-0.998)	0.989 (0.953-0.997)	0.950 (0.302-0.991)	0.963 (0.857-0.991)
MD	0.962 (0.856-0.990)	0.933 (0.724-0.983)	0.798 (0.129-0.950)	0.911 (0.663-0.977)
RD	0.965 (0.866-0.991)	0.951 (0.803-0.988)	0.848 (0.408-0.962)	0.923 (0.706-0.981)
AD	0.964 (0.864-0.991)	0.944 (0.784-0.986)	0.880 (0.554-0.970)	0.913 (0.623-0.979)

Data presented as ICC estimate (95% Confidence Interval)

## REFERENCES

1. Dilenge ME, Majnemer A, Shevell MI. Long-term developmental outcome of asphyxiated term neonates. *J Child Neurol.* Nov 2001;16(11):781-792.
2. Higgins RD, Raju T, Edwards AD, et al. Hypothermia and other treatment options for neonatal encephalopathy: an executive summary of the Eunice Kennedy Shriver NICHD workshop. *J Pediatr.* Nov 2011;159(5):851-858 e851.
3. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* Oct 13 2005;353(15):1574-1584.
4. Azzopardi D, Edwards AD. Magnetic resonance biomarkers of neuroprotective effects in infants with hypoxic ischemic encephalopathy. *Semin Fetal Neonatal Med.* Oct 2010;15(5):261-269.
5. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. *J Pediatr.* Apr 2005;146(4):453-460.
6. Shankaran S, Barnes PD, Hintz SR, et al. Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed.* Nov 2012;97(6):F398-404.
7. Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR Am J Neuroradiol.* Jan 1998;19(1):143-149.
8. Malik GK, Trivedi R, Gupta RK, et al. Serial quantitative diffusion tensor MRI of the term neonates with hypoxic-ischemic encephalopathy (HIE). *Neuropediatrics.* Dec 2006;37(6):337-343.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
9. Brissaud O, Amirault M, Villega F, Periot O, Chateil JF, Allard M. Efficiency of fractional anisotropy and apparent diffusion coefficient on diffusion tensor imaging in prognosis of neonates with hypoxic-ischemic encephalopathy: a methodologic prospective pilot study. *AJNR Am J Neuroradiol*. Feb 2010;31(2):282-287.
10. Ward P, Counsell S, Allsop J, et al. Reduced fractional anisotropy on diffusion tensor magnetic resonance imaging after hypoxic-ischemic encephalopathy. *Pediatrics*. Apr 2006;117(4):e619-630.
11. Tumor N, Wusthoff C, Smee N, et al. Prediction of neurodevelopmental outcome after hypoxic-ischemic encephalopathy treated with hypothermia by diffusion tensor imaging analyzed using tract-based spatial statistics. *Pediatr Res*. Jul 2012;72(1):63-69.
12. Partridge SC, Mukherjee P, Berman JL, et al. Tractography-based quantitation of diffusion tensor imaging parameters in white matter tracts of preterm newborns. *J Magn Reson Imaging*. Oct 2005;22(4):467-474.
13. van Pul C, Buijs J, Vilanova A, Roos FG, Wijn PF. Infants with perinatal hypoxic ischemia: feasibility of fiber tracking at birth and 3 months. *Radiology*. Jul 2006;240(1):203-214.
14. Evangelou I, Serag A, Bouyssi-Kobar M, Du Plessis A, Limperopoulos C. Optimized methodology for neonatal diffusion tensor imaging processing and study-specific template construction. *Conf Proc IEEE Eng Med Biol Soc*. 2014;in press.
15. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B*. Jun 1996;111(3):209-219.



- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
16. Melhem ER, Mori S, Mukundan G, Kraut MA, Pomper MG, van Zijl PC. Diffusion tensor MR imaging of the brain and white matter tractography. *AJR Am J Roentgenol.* Jan 2002;178(1):3-16.
  17. Adams E, Chau V, Poskitt KJ, Grunau RE, Synnes A, Miller SP. Tractography-based quantitation of corticospinal tract development in premature newborns. *J Pediatr.* Jun 2010;156(6):882-888, 888 e881.
  18. Bayley N. *Manual for the Bayley scales of infant development.* New York: Psychological Corporation; 1969.
  19. Bayley N. *Bayley Scales of Infant Development - Third Edition* San Antonio, TX: Harcourt Assessment; 2006.
  20. McKinstry RC, Miller JH, Snyder AZ, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology.* Sep 24 2002;59(6):824-833.
  21. Ancora G, Testa C, Grandi S, et al. Prognostic value of brain proton MR spectroscopy and diffusion tensor imaging in newborns with hypoxic-ischemic encephalopathy treated by brain cooling. *Neuroradiology.* Aug 2013;55(8):1017-1025.
  22. Gilmore JH, Lin W, Corouge I, et al. Early postnatal development of corpus callosum and corticospinal white matter assessed with quantitative tractography. *AJNR Am J Neuroradiol.* Oct 2007;28(9):1789-1795.
  23. Aralasmak A, Ulmer JL, Kocak M, Salvan CV, Hillis AE, Yousem DM. Association, commissural, and projection pathways and their functional deficit reported in literature. *J Comput Assist Tomogr.* Sep-Oct 2006;30(5):695-715.

- 1  
2  
3  
4 24. Murakami A, Morimoto M, Yamada K, et al. Fiber-tracking techniques can predict the  
5 degree of neurologic impairment for periventricular leukomalacia. *Pediatrics*. Sep  
6 2008;122(3):500-506.  
7  
8  
9  
10 25. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination  
11 revealed through MRI as increased radial (but unchanged axial) diffusion of water.  
12 *Neuroimage*. Nov 2002;17(3):1429-1436.  
13  
14  
15 26. Kinney HC, Brody BA, Kloman AS, Gilles FH. Sequence of central nervous system  
16 myelination in human infancy. II. Patterns of myelination in autopsied infants. *J*  
17 *Neuropathol Exp Neurol*. May 1988;47(3):217-234.  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60