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Contacts: Sara Harris, (202) 962-4087
Todd Bentsen, (202) 962-4086

STUDIES REVEAL BETTER DETECTION METHODS FOR MILD TRAUMATIC BRAIN INJURY

Early determination of severity is key to effective treatment; other findings show potential treatments for brain swelling and brain cell damage

Washington, DC — New research released today shows that scientists are developing more effective ways of detecting and determining the severity of mild traumatic brain injury (TBI). These new techniques promise to help health-care practitioners act faster to prevent and treat these currently difficult to diagnose injuries. Mild TBI affects an estimated 1.4 million Americans each year — including 10–20 percent of soldiers returning from combat in Iraq and Afghanistan **—** and often leads to a disabling loss of brain function. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Other research has uncovered a potential treatment for the swelling that occurs as a result of brain trauma and the important role that supporting brain cells called astrocytes play in the neuronal damage caused by TBI, leading to new strategies that may one day be used to prevent TBI's devastating destruction of brain cells.

Specifically, the new findings show that:

- Combining two advanced brain scanning techniques — magnetoencephalography (MEG) and diffusion tensor imaging (DTI) — makes it possible to diagnosis cases of TBI that conventional brain scans fail to detect (Mingxiong Huang, abstract 613.1, see attached summary).
- A new whole-brain method of magnetic resonance spectroscopic imaging (MRSI) can help characterize the severity of mild-to-moderate TBI soon after the injury, including neural damage not near the point of the head injury and not visible with conventional brain imaging (Andrew Maudsley, abstract 649.24, see attached summary).
- Erythropoietin (EPO), a major growth factor of blood cells, can reduce swelling of the brain known as edema, a much-feared consequence of stroke and brain trauma (Eli Gunnarson, abstract 750.4, see attached summary).
- TBI alters astrocytes in ways that eventually can lead to signaling changes in the brain and the death of neurons; furthermore, these alterations can be inhibited in mice (YungChia Chen, abstract 338.1, see attached summary).

Additional recent findings discussed at the meeting include:

- Animal research that shows progress in preventing post-traumatic epilepsy, which often develops in people who sustain a TBI weeks or even years after the injury (see attached speaker's summary).

"Although the human brain has an amazing capacity to recover from stress, damage, disease, and traumatic injuries, recovery is often incomplete," said press conference moderator David Prince, MD, of Stanford University. "This highlights the need to better understand the basic underlying neurobiological alterations and to explore methods for early detection of even mild injury so that new effective strategies to inhibit, reverse, and even prevent the effects of brain injury can be developed."

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“Sometimes a long, latent period occurs between the inciting event and the appearance of symptoms such as epilepsy, providing a window of opportunity for preventative treatment,” Prince said.

Related Presentation:

Special Lecture: **Prophylaxis of Post-Traumatic Epilepsy: Waiting for Translation**
Wednesday, November 19, 8:30–9:30 a.m., Washington Convention Center, Hall D
# # #

Abstract 613.1 Summary

Lead author: Mingxiong Huang, PhD **(858) 534-1254**
University of California, San Diego **mxhuang@ucsd.edu**
San Diego, Calif.

Combination of Two Imaging Techniques Detects Mild Traumatic Brain Injury

Previous diagnostic techniques not sensitive enough for many patients

A new study shows that by combining two advanced brain scanning techniques, magnetoencephalography (MEG) and diffusion tensor imaging (DTI), researchers were able to diagnose mild traumatic brain injury (TBI), which conventional brain scans often fail to detect. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. This discovery promises to lead to more sensitive detection of subtle neuronal injuries in mild TBI, which is important because brain trauma can trigger a series of chemical events in the brain that may lead to a secondary injury hours or even days later. This integrated neuroimaging technique can also be used to assess the effectiveness of new TBI interventions objectively.

"Conventional neuroimaging techniques have limited sensitivity to detect the subtle physiological alterations in the brain caused by TBI," said Mingxiong Huang, PhD, of the University of California, San Diego, the lead author of the study. "This creates a significant diagnostic problem. About 70 percent of civilian patients with TBI and 44 percent of military TBI patients returning from Iraq show no visible lesions with conventional imaging techniques."

For this study, 18 patients with closed head injury and mild-to-moderate symptoms of TBI, and 17 healthy study participants underwent both MEG and DTI imaging. MEG was used to measure and locate the abnormal low-frequency magnetic signals (delta waves) that are emitted by brain tissue damaged by TBI. The cause of these delta waves in TBI patients isn't fully understood, but they are believed to come from nerve cell bodies (gray matter) that are failing to communicate with each other due to disruption of the electrical signals carried by axons (white matter). The researchers then used DTI to measure damage to the axons.

"We found that MEG and DTI together were substantially more sensitive in detecting subtle neuronal injury in mild cases of TBI than conventional brain imaging techniques, such as computed tomography and magnetic resonance imaging," said Huang. In most cases, both the MEG and DTI image results were abnormal, although sometimes the MEG was abnormal while the DTI was normal. "This suggests that the current DTI technology still has room to improve," said Huang.

The research was supported by the U.S. Department of Veterans Affairs.

Scientific Presentation: Tuesday, November 18, 1–1:15 p.m., Washington Convention Center, Room 201

613.1, Detecting subtle neuronal injury in mild traumatic brain injury using integrated imaging approach with magnetoencephalography and diffusion tensor imaging
*****M. HUANG[1], S. SHARON NICHOLS[2], A. ROBB[5], A. ANGELES[5], R. J. REBECCA J. THEILMANN[3], A. DRAKE[6], M. LEVY[7], M. HOLLAND[6], T. SONG[3], S. GE[3], E. HWANG[4], R. R. LEE[3]; [1]Radiology, UCSD, San Diego, CA; [2]Neurosci., [3]Radiology, [4]Univ. of California San Diego, San Diego, CA; [5]Res., VA San Diego Healthcare Syst., San Diego, CA; [6]San Diego Naval Hosp., San Diego, CA; [7]Childrens of Hosp. San Diego, San Diego, CA

TECHNICAL ABSTRACT: Traumatic brain injury (TBI) is a leading cause of sustained cognitive deficits in the civilian population (due to motor vehicle accidents, sports, falls, and assault) and military personnel (with blast injury as an additional cause). However, conventional neuroimaging techniques have limited sensitivity to the physiological alterations caused by TBI, and poor utility for predicting long-term outcome. Mild (and some moderate) TBI can be difficult to diagnose due to lack of obvious external injuries and because the injuries are often not visible on conventional acute MRI or CT. The present study used an integrated multimodal neuroimaging approach involving Magnetoencephalography (MEG) and diffusion tensor imaging (DTI) to test their utility for diagnose and monitor mild TBI in military personnel and civilians in whom conventional CT and MRI do not show visible lesions. Injured brain tissues in TBI patients generate pathological low-

frequency neuronal magnetic signal (delta waves: 1-4 Hz) that can be measured and localized by MEG. The cause of the MEG delta-waves in TBI is not fully understood. We hypothesized that abnormal MEG delta-waves come from gray-matter neurons that experience de-afferentation due to axonal injury to the underlying white-matter fiber tracts. Here, DTI was used to detect reduced diffusion anisotropy related to axonal injuries in white matter. We also studied the neurophysiological basis of TBI-related cognitive impairments using an N-back working memory MEG task in mild TBI patients. The results show: 1) the multimodal imaging approach with MEG and DTI is substantially more sensitive than conventional CT and MRI in detecting subtle neuronal injury in mild TBI; 2) reduced DTI anisotropy in white-matter fiber tracts is highly associated with the generation of abnormal MEG delta-waves from neurons that are linked to the injured white-matter fibers; 3) DTI abnormalities and MEG delta-wave generation are closely linked to deficits in the working-memory network as measured by the MEG N-back task; 4) findings from the multimodal imaging approach is consistent with post-concussive symptoms and results of neuropsychological exams; 5) in some cases, abnormal MEG delta-waves were observed in mild TBI patients without DTI abnormality, indicating that MEG may be more sensitive than DTI in diagnosing mild TBI. In conclusion, the multimodal imaging approach with MEG and DTI can enhance our ability to detect subtle neural injuries that are invisible using conventional neuroimaging techniques, and can substantially improve our understanding of the neuronal mechanisms underlying mild TBI.

Abstract 649.24 Summary

Lead author: Andrew Maudsley, PhD **(305) 243-8080**
University of Miami **amaudsley@med.miami.edu**
Miami, Fla.

Imaging Study Shows Extensive Damage in Some Mild Traumatic Brain Injury

May lead to earlier treatment for noninvasive injuries

Using a new whole-brain method of magnetic resonance spectroscopic imaging (MRSI), scientists were able to detect for the first time the widespread brain damage that sometimes occurs in mild to moderate traumatic brain injury (TBI) — damage that fails to show up with more conventional brain scanning techniques. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"With this new technique, we were able to characterize the severity of the injury early in the process, which has been difficult to do using standard structural MRI," said Andrew Maudsley, PhD, of the University of Miami, the study's lead author.

This finding promises to expand the scientific community's knowledge of TBI and also may lead to more effective early treatments for the 1.4 million Americans who sustain one of these noninvasive head injuries each year.

For the study, MRI and MRSI data were obtained from 17 people who had been admitted to a trauma center with closed head injury and mild-to-moderate symptoms of TBI. MRSI is an advanced type of magnetic resonance imaging that creates brain images from hundreds of voxels (three-dimensional units of brain tissue) rather than a single voxel, as is the case with conventional MR spectroscopy measurements.

Using processing software developed specifically for this study, Maudsley and his colleagues analyzed the MRSI images for levels of three major brain metabolites: N-acetylaspartate, creatine, and choline. They then calculated the differences in these compounds between injured and healthy participants. They also measured the association between these neuroimaging results and the cognitive function of both groups.

"We found that the MRSI images of brain metabolite concentrations picked up diffuse metabolite changes in the TBI patients, even in people who had been classified as having a very mild injury," said Maudsley. "These differences were primarily found in white matter [nerve cell axons], but they were located throughout the brain and included regions neither near the point of the head injury nor near any injuries seen on the MRI."

The researchers also found a strong correlation between the metabolite measurements and cognitive ability. Further studies are needed, said Maudsley, to evaluate the relationship of these early imaging findings with long-term patient outcomes.

This research was supported by the U.S. National Institute of Neurological Disorders and Stroke.

Scientific Presentation: Tuesday, November 18, 4–5 p.m., Washington Convention Center, Hall A-C

649.24, Metabolic consequences of mild traumatic brain injury observed by MR spectroscopic imaging
*A. A. MAUDSLEY1, V. GOVIND1, J. JAGID2, S. GOLD3, G. SAIGAL1, L. HARRIS1; 1Radiology, 2Neurolog. Surgery, Univ. of Miami, Miami, FL; 3Neuropsychological Service, Jackson Mem. Hosp., Miami, FL
TECHNICAL ABSTRACT: The detrimental cognitive consequences of mild traumatic brain injury (TBI) are frequently uncorroborated by

structural neuroimaging findings and it is believed that an underlying widespread injury occurs that is not manifested in structural changes. In this study a whole-brain MR Spectroscopic imaging (MRSI) acquisition has been applied to detect metabolic dysfunction occurring as a consequence of mild closed head injury.
Methods: MRI and volumetric 1H MRSI data were obtained from 17 subjects admitted to the trauma center with closed head injury and GCS score between 10 and 15, within 1 to 10 weeks after injury. Metabolite images for N-Acetylaspartate (NAA), creatine, and choline were obtained, with processing including signal intensity and spatial normalization and mapping of tissue content at each MRSI voxel. Two types of analyses were carried out to examine metabolic differences between individual TBI subjects and corresponding data obtained from a normal control group. The first was based on observation of individual metabolite and metabolite ratio images, as well as the difference of these images with mean value images from the normal control group. The second analysis compared metabolite values calculated for each of the grey- and white-matter tissue, within each brain lobe, and correlation of these values with neuropsychological evaluation scores.
Results: Individual metabolite images showed evidence of widespread alterations, even in some mild injury subjects, with a patchy appearance and primarily in white matter. The Choline/NAA image showed the largest differences, which could be easily identified using a z-score image analysis method. These metabolite alterations were observed remote from any structural MRI findings. The results of the lobar-level tissue analysis confirm the widespread metabolic alterations in all brain regions, most strongly occurring in white matter. These results also showed significant correlations with neuropsychological evaluations.
Conclusions: This study demonstrates that volumetric MRSI is a sensitive method for detection of widespread metabolic alterations following mild TBI, and enables direct visualization of injury in individual subjects. These metabolite measures were significantly correlated with cognitive ability within the sub-acute time period. Further studies will evaluate the relationship of these early imaging findings with longer-term measures of outcome.

Abstract 750.4 Summary

Lead author: Eli Gunnarson, MD, PhD **(46) 8-517-77335**
Karolinska Institutet **eli.gunnarson@ki.se**
Stockholm, Sweden

Growth Factor Found to Reduce Brain Swelling Following Injury

Shows promise for addressing a leading cause of permanent damage

Erythropoietin (EPO), a major growth factor of blood cells, can reduce swelling of the brain (edema), a much-feared consequence of brain trauma and stroke, according to a new animal research study led by Eli Gunnarson, MD, PhD, of the Karolinska Institutet, in Stockholm. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. In the most common form of brain edema, water accumulates in star-shaped cells called astrocytes. Swollen astrocytes are also present in the area surrounding the central part of a stroke. "When astrocytes swell, the risk of the tissue dying increases," said Gunnarson. "By preventing or reducing the swelling, brain tissue may be rescued, making it possible to limit the extent of the final injury to the brain."

In recent years, EPO unexpectedly has emerged as a promising neuroprotective drug, minimizing brain injury in both animal and human research studies. Naturally occurring in humans, EPO has many effects in the body and for several years has been used in pharmacological doses to treat specific diseases, including anemia. EPO can be given to humans intravenously because it crosses the blood-brain barrier and acts in the brain.

In this study, Gunnarson's research team began by examining the effect of EPO in cell cultures and in excised brain tissue. In both cases, they found that, within minutes, EPO was able to prevent the excess water uptake into the astrocytes that is caused by glutamate, a major signaling substance in the brain.

They then tested whether EPO could be similarly protective — reducing the neurological symptoms caused by brain edema — in rats. The animals that received treatment with EPO or an EPO-like substance were found to have significantly fewer neurological symptoms than animals that received only a salt solution.

"The need for new treatments for brain edema is urgent," said Gunnarson. "It's a leading cause of permanent brain damage, yet current treatments are often ineffective. Our results suggest a potential new way to treat brain injuries that are accompanied by brain edema or other disturbances in brain water balance."

The research was supported by the Nordic Centers of Excellence, the Axel Tielmans Minnesfond, and the Swedish Research Council.

Scientific Presentation: Wednesday, November 19, 11 a.m.– noon, Washington Convention Center, Hall A-C

750.4, Erythropoietin modulation of astrocyte water permeability: a potential component of neuroprotection
*****E. GUNNARSON[1], M. ZELENINA[1], Y. SONG[1], J. K. KOWALEWSKI[2], H. BRISMAR[2], M. BRINES[3], A. CERAMI[3], U. ANDERSSON[1], A. APERIA[1]; [1]Dept Woman and Child Hlth., Karolinska Institutet, Stockholm, Sweden; [2]Dept Cell Physics, Royal Inst. of Technol., Stockholm, Sweden; [3]Kenneth S Warren Inst., New York, NY
TECHNICAL ABSTRACT: Disturbed brain water homeostasis is a common complication and is considered to be a leading cause of permanent brain damage following stroke, trauma, ischemia and meningitis. Swelling of astroglial cells is the main component of cytotoxic brain edema. Astroglial cells possess the water channel aquaporin-4 (AQP4), which has been shown to be rate-limiting for brain water accumulation during water intoxication. We have recently shown that glutamate, acting on group I metabotropic glutamate receptors (mGluR), increases the permeability of astrocyte AQP4. In ischemia, sustained pathological release of glutamate will therefore increase astrocyte water uptake. Here we

have examined whether the neuroprotective agent erythropoietin (EPO) antagonizes the effect of group I mGluR activation on astrocyte water permeability. First, we examined the effect of EPO in a rat astrocyte cell line transfected with AQP4 and in primary cultures of rat astrocytes that express endogenous AQP4. In both cellular models, pre-treatment with EPO abolished the increase in AQP4 water permeability caused by activation of the group I mGluR agonist DHPG. We found that activation of group I mGluR triggered fast and highly regular intracellular calcium oscillations in primary astrocytes and that EPO interfered with this signalling by altering the frequency of the oscillations. Secondly, we used acute rat hippocampal slices, where we recorded the rate of the tissue swelling following exposure to a hypotonic environment. We have previously shown that it is astrocytes that undergo the most rapid volume changes after a hypoosmotic challenge, while water uptake in neurons and microglia is much slower. Also, DHPG caused an increased rate of swelling in astrocytes only and not in neurons and microglia. In the slice study we found that exposure to DHPG significantly increased the rate of hippocampal slice swelling in response to hypotonicity and that EPO abolished the DHPG effect. Finally, we found that EPO significantly reduced neurological symptoms in a mouse model of water intoxication. Our findings indicate that EPO may reduce the risk of astrocyte swelling in stroke and other brain insults, and that the modulation of astrocyte water permeability may represent an important component of the neuroprotective effect of EPO.

Abstract 338.1 Summary

Co-author: David Meany, PhD **(215) 573-3155**
University of Pennsylvania **dmeaney@seas.upenn.edu**
Philadelphia, Penn.

Neighboring Cells May be Responsible for Damage from Traumatic Brain Injury

Suggests potential treatment for preventing cell damage

Scientists have recently come to suspect that the damage to neurons that occurs after a traumatic brain injury (TBI) comes from an unlikely source — neighboring cells called astrocytes. A new study from the University of Pennsylvania is now adding support to that theory, finding that a brain injury elicits changes in astrocytes that may eventually lead to signaling changes in the brain and the death of neurons. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Our findings suggest that targeting receptors on astrocytes may be a potential therapy for preventing the neuronal damage that occurs following traumatic brain injury — an important step in improving the outcome of patients," said David Meany, PhD, a co-author of the study.

Astrocytes typically communicate with each other via calcium waves, triggering the release of glutamate, an amino acid used by neurons as a signaling molecule. Adenosine triphosphate (ATP), an important molecule in cellular metabolism, has been shown to activate specific receptors on astrocytes — purinergic receptors — leading to an increase in calcium signaling and glutamate release.

In this mouse study, Meany and his colleagues investigated whether mechanical brain trauma could alter astrocytic calcium signaling and glutamate release, and thus alter neuronal function and, possibly, lead to neuronal death. They found that microimpacts to the cortex of the brain can elicit various levels of responses in the astrocytes. They also found that a purinergic receptor antagonist (PPADS) inhibited these responses, which suggests that the astrocytic calcium signaling following mechanical brain trauma is predominantly, though not exclusively, mediated by ATP activation of purinergic receptors.

"At first glance, it may seem contradictory that astrocytes, which are meant to provide mechanical and metabolic support to neurons, may end up damaging them," said Meany. "But that's what we and others are now finding."

Scientific Presentation: Monday, November 17, 8–9 a.m., Washington Convention Center, Hall A-C

338.1, Two-photon in vivo imaging of astrocyte calcium signaling following traumatic brain injury
Y. CHEN, A. M. CHOO, W. J. MILLER, P. G. HAYDON, *D. F. MEANEY; Univ. Pennsylvania, Philadelphia, PA

TECHNICAL ABSTRACT: Astrocytes are well-known to play an important role in supporting neuronal function but more recent studies indicate that they are also key modulators of microvascular tone and communicate with neurons at the synaptic junction. Astrocytes communicate via intercellular calcium waves, which can trigger the release of glutamate from astrocytes and contribute to excitotoxicity following neurotrauma. We used two-photon *in vivo* imaging to investigate the calcium dynamics of cortical astrocytes in response to repeated mechanical trauma. The exposed cortex of C57Bl6 mice was subjected to microcortical impacts to simulate traumatic brain injury. Calcium levels in the somata of astrocytes (detected with fluo-4) exhibited only a small increase (~25%) following two microcortical impacts that were spaced 5 minutes apart. Thirty minutes following this initial pair of impacts, a second pair of cortical impacts induced slightly greater intracellular fluo-4 fluorescence indicative of increased calcium signaling. Subsequent mechanical impacts produced heterogeneity in the astrocyte response with some somata exhibiting increased fluo-4 fluorescence while other cells bodies appeared to exhibit depletion of intracellular calcium stores or desensitization of signaling receptors. Our previous *in vitro* studies identified purinergic signaling as a common mechanism of calcium wave propagation in astrocytes following different levels of mechanical injury. Application of the purinergic receptor anatagonist PPADS onto the exposed cortex reduced fluo-4 fluorescence in most, but not all, astrocyte somata. Washout of the purinergic anatagonist resulted in a return of fluo-4 fluorescence to initial baseline levels or above. These results suggests that astrocyte calcium signaling following mechanical trauma is predominantly-though not exclusively-mediated by purinergic receptors that may be a potential therapeutic target for reducing the astrocytic release of potentially excitotoxic glutamate following neurotrauma.

Speaker's Summary

Speaker: David Prince, MD **(650) 723-5522**
Stanford University Medical Center **daprince@leland.stanford.edu**
Stanford, Calif.

Special Lecture: **Prophylaxis of Post-Traumatic Epilepsy: Waiting for Translation**
Wednesday, November 19, 8:30–9:30 a.m., Washington Convention Center, Hall D

A high proportion of people who sustain serious brain injury develop epilepsy after a delay of weeks to years. This is particularly important today because of the large numbers of service men and women who have sustained brain injury in recent conflicts and the fact that posttraumatic epilepsy can be a medical problem with significant associated human suffering and important socioeconomic impacts. Because the precise timing of the injury is known, and there is a delay between injury and onset of seizures, there is an opportunity to develop and apply preventative treatments. To develop effective strategies for prevention, it is important to know what kinds of underlying processes lead to epilepsy in the injured brain through experiments on animal models.

This lecture highlighted two key mechanisms that follow brain injury and lead to epilepsy in animal models, namely rewiring of the injured brain that leads to excessive excitatory traffic in nerve circuits, and loss of the braking influence provided by the inhibitory neurotransmitter called GABA, a chemical that quiets brain activity. We found that we could prevent epileptic activity from developing in the injured brain cortex of rats by quieting normal activity with a drug during the first 3 days after trauma. This was accompanied by a block of the excessive excitatory rewiring. This result provides proof in principle that prophylaxis of posttraumatic epilepsy is possible. Several candidate genes have been identified that might link activity, abnormal wiring and development of epilepsy.

A second potential preventative approach is suggested by the finding that the nerve cells which supply the inhibitory transmitter GABA are shrunken in the injured area. These inhibitory cells closely resemble immature ones and have fewer connections to other excitatory cells. Results lead to the hypothesis that reductions in a protein called BDNF, known to be important for development of inhibitory nerve cells, might underlie the shrunken appearance of these cells in the injured brain and their decreased quieting influence on the nerve circuits. These findings raise the interesting possibility that supplying BDNF to the injured cortex might protect or "rescue" inhibitory cells and contribute to prevention of development of epilepsy after brain injury.

The next steps in this line of research will be to identify drugs that can be applied to injured brain in man that will block the excessive new excitatory wiring, and other agents that can be delivered to the injured brain that will prevent the shrinkage and loss of function of inhibitory cells. Other important unsolved issues deal with potential adverse effects of such drugs on recovery of other functions after brain injury, and the required timing and duration of treatments.

Hopefully, translation of results such as these will allow development of successful approaches to the important problem of posttraumatic epilepsy in man.