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New Insights Into the Effects of Traumatic Brain Injury

Findings expose long-term consequences and differences between genders and age groups

CHICAGO — Research released today reveals new discoveries about the symptoms, physiology, and treatment options for traumatic brain injury (TBI). The animal studies also highlight differences in how TBI may manifest in males and females and among children and adults. The findings were presented at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Traumatic brain injury affects 1.7 million Americans each year. The majority of cases are caused by impacts during contact sports and traffic accidents or by blasts from explosive devices on the battlefield. The effects of even mild TBI can include loss of consciousness, long-term memory problems, and increased risk for diseases like Alzheimer's disease.

Today's new findings show that:

- Male and female mice display different long-term consequences of mild TBI, a finding that supports the development of gender-specific treatment options (Ramesh Raghupathi, abstract 500.03, see attached summary).
- Blast injury increases fear behaviors in mice, suggesting a link between TBI and post-traumatic stress disorder (Carmen Lin, abstract 589.09, see attached summary).
- A combination of drugs given several hours post-injury produces cognitive benefits in two rodent models of TBI, providing a more clinically useful therapeutic window (Michael Ayo Sangobowale, abstract 500.11, see attached summary).

Other recent findings discussed show that:

- TBI in juvenile mice disrupts inhibitory signaling during brain development, suggesting pediatric brain injuries may have different consequences — and require different treatments — than injuries to the adult brain (Trent Anderson, presentation 43.18, see attached speaker summary).

"Today's findings show the progress we've made in understanding TBI," said press conference moderator Akiva Cohen, PhD, of the University of Pennsylvania, an expert in brain injury. "By better understanding aspects of TBI — like gender and age differences — we can create treatments that are better targeted to the distinct problems in different populations of people."

This research was supported by national funding agencies such as the National Institutes of Health, as well as other private and philanthropic organizations. Find out more about traumatic brain injury at *BrainFacts.org*.

Related Neuroscience 2015 Presentation:
Public Advocacy Forum: Sports-Related Brain Injuries and Their Ethical, Social, and Neuroscience Considerations
Tuesday, Oct. 20, 2-4 p.m., N229

Abstract 500.03 Summary

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Male and Female Mice Display Different Symptoms Months After Mild Traumatic Brain Injury

Male mice demonstrate depressive behaviors while female mice exhibit increased sensitivity to touch

Males and females may experience different long-term consequences of mild traumatic brain injury, according to new animal research released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Mild traumatic brain injury (TBI) — a category that includes concussions — often occurs as a result of contact sports. Most of the symptoms, including nausea, dizziness, and cognitive impairments, usually disappear after a week or so, but in some people, they can last up to a year. In others, symptoms like headache, irritability, and depression don't begin until days or weeks after the initial injury. Clinical reports suggest that women and men exhibit different symptoms, with women displaying increased sensitivity to light and pain, while men are more likely to become depressed. However, sex differences have not been examined with animal models.

To address possible differences in outcomes across genders, the researchers exposed young male and female mice to mild TBI. Four and eight weeks post-injury, researchers assessed depressive behavior on a forced swim test and sensitivity to touch following gentle stimulation to the area around the eye. Up to eight weeks post-injury, injured male mice displayed more depressive-like behaviors — giving up more quickly when forced to swim — when compared with uninjured males and injured females, while injured females displayed increased sensitivity to touch — turning away or brushing the area with their forepaw — when compared with uninjured females and injured males. Additionally, the researchers found evidence suggesting decreased functionality of dopamine transporters in the injured male brain, and impaired dopamine signaling has been linked to depression. Importantly, previous work by the researchers found that the cognitive deficits from this type of injury had disappeared after a week, but the new research suggests that recovery is not complete and some symptoms are still evident months after the injury.

"This is one of the first studies showing that up to a few months after a single mild concussion, there are deficits in symptoms unrelated to cognition, like depression and pain," said senior author Ramesh Raghupathi, PhD, of Drexel University. "The sex differences suggest different treatment strategies for men and women."

Research was supported with funds from the National Institute of Child Health and Human Development and the National Institute of Neurologic Disorders and Stroke.

Scientific Presentation: Tuesday, Oct. 20, 10-11 a.m., Hall A

500.03, Sex-dependent changes in depression and facial allodynia in the chronic period following mild TBI in the mouse
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TECHNICAL ABSTRACT: Patients who have sustained a mild TBI suffer from both somatic (headache, dizziness, nausea, fatigue, sleep disturbances) and neuropsychiatric (cognitive deficits, anxiety, depression) symptoms. The effect of gender on response to and outcome after TBI has not received substantive attention both in clinical studies and in pre-clinical animal models. Importantly, a recent study suggested that men and women exhibit different behavioral deficits following sports-related concussions. In the present study, we used a well-established mouse model of mild TBI and assessed facial allodynia and depressive behavior at 4 and 8 weeks post-injury in male and female C57Bl/6 mice. Compared to their sham-injured counterparts, brain-injured male mice exhibited depressive-like behavior (using the forced swim test) at 4 and 8 weeks post-injury (Injury effect, $p<0.001$); female brain-injured mice were not different from their sham-injured counterparts. To test whether altered dopamine signaling may be the basis for this depression-like behavior, fast scan cyclic voltammetry was used to determine dopamine release kinetics in the nucleus accumbens following stimulation of the ventral tegmental area. Whereas baseline evoked dopamine release was not affected in either brain-injured male or female mice compared to sham-injured mice, cocaine-stimulated dopamine concentration in the nucleus accumbens was greater in the brain-injured male mice compared to their sham-injured counterparts ($p<0.01$), suggestive of a decreased functionality of the dopamine transporter. Female brain-injured mice exhibited increased sensitivity to periorbital stimulation (using the von Frey filament test) compared to sham-injured mice at both 4 and 8 weeks post-injury (injury effect, $p<0.001$), suggestive of post-traumatic headache; male brain-injured mice were not different from their sham-injured counterparts. Together, these data are indicative of sex-dependent differences in the response to mild TBI and underscore the importance of evaluation of behavioral and biochemical measures in the chronic post-traumatic period.

Abstract 589.09 Summary

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Mice With Mild Traumatic Brain Injury From a Blast Show Increased Fear Behaviors

Animals subjected to blast injury had increased fear and impaired memory

New animal research offers evidence that mild traumatic brain injury caused by a blast may lead to fear associations similar to those seen in post-traumatic stress disorder, according to a study released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Mild traumatic brain injury (TBI) can be caused by accidents, impacts during contact sports, or from blasts caused by improvised explosive devices. Previous research has linked mild TBI to post-traumatic stress disorder (PTSD), particularly among military personnel experiencing blast injuries.

To examine the link between TBI and PTSD, researchers compared control mice to male mice who had received a brief, high-pressure blast to the head. One day after injury, all mice were trained on fear conditioning, where they learned to associate both a particular environment and a particular sound with a foot shock. The mice were then tested for memory of these two associations, which were chosen for their representation of slightly different aspects of memory.

During learning of the fear conditioning, the blasted mice froze more often than the control mice, suggesting a greater level of fear. However, the control mice froze appropriately when placed back in the chamber during testing — indicating they had better memory of the environmental association than the blasted animals. This may indicate the blasted mice had problems in controlling their fear, as they were more fearful during the initial training, but they didn't remember the experience appropriately during testing. There were no differences between the blasted and control animals when being tested on the sound-shock association. This may be due to the two associations being processed by slightly different brain regions.

"There is some sort of emotional disturbance in fear learning for the blasted animals, which may be an indication of PTSD," said lead author Carmen Lin of Northwestern University. "To our knowledge, this has never before been shown in a mouse model of mild traumatic brain injury."

Research was supported with funds from the Department of Defense.

Scientific Presentation: Tuesday, Oct. 20, 1-2 p.m., Hall A

589.09, Mild traumatic brain injury enhances acquisition and impairs retention of fear learning
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TECHNICAL ABSTRACT: Mild traumatic brain injury (mTBI) affects 1.5 million Americans annually, with half of them experiencing an acute loss of consciousness, memory loss, and confusion following the injury. Injury can result from multiple different traumas, including a sports-related impact or from the blast of an improvised explosive device (IED), as experienced by military personnel. mTBI is a known risk factor for Alzheimer's disease (AD) and is also linked to post-traumatic stress disorder (PTSD) among veterans. Despite the growing awareness to these detrimental effects of mTBI, the neuropathology underlying these specific repercussions is still relatively unknown. In order to elucidate these mechanisms, we adopted a model for closed head mTBI using a high-pressure pneumatic cannon to simulate the high-pressure blast experienced by individuals in the military. mTBI was induced in male B6SJLF1 mice by blasting the top of the head with an overpressure of 20 psi lasting 1-2 ms. Blasted mice exhibited a longer time to wake than sham-blasted mice. Following one day of recovery after the blast, the mice were trained on trace fear conditioning. After conditioning, the mice were tested daily for memory of context and cue retention for five days. Blasted mice acquired fear-conditioning more robustly than sham-blasted mice, as determined by percent freezing. Sham-blasted mice, however, retained the contextual memory of the fear conditioning better than blasted mice. Both groups exhibited equivalent cued fear responses. The enhanced acquisition of the fear conditioning by blasted animals, which has not previously been demonstrated in a mouse model of mTBI, is indicative of more fear-like behavior and correlates with symptoms of PTSD in humans. The poorer retention of contextual memory is in accordance with previous data showing memory disturbance following mTBI. Studies are currently ongoing to investigate the effect of mTBI on a mouse model of AD (5xFAD mouse model) in fear conditioning to understand the convergence of mTBI and AD pathology on PTSD-like behavior. We hypothesize that mTBI will exacerbate the amyloid pathology and result in more severe learning and memory deficits in blasted mice. The results of this study will aid ongoing research in establishing therapeutics for those suffering from the consequences of mTBI.

Abstract 500.11 Summary

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Combination of Drugs Improves Cognition After Brain Injury in Rodents

Treatment still effective even when given hours after injury

A combination of two drugs may prevent cognitive deficits associated with traumatic brain injury, even when given hours after the injury, according to new animal research released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Previous work by the researchers showed that, when given in combination to rodents one hour after injury, two FDA-approved drugs — minocycline (MINO) and N-acetylcysteine (NAC) — improve cognition and the health of axons while reducing inflammation and cellular loss. However, a longer time window is needed to be useful in a clinical setting.

To see whether the drugs still have an effect when given later, the researchers tested the combination of MINO and NAC on two animal models of traumatic brain injury, a controlled cortical impact model in rats and a closed head injury model in mice. Rats were given either the drug combination or saline treatment 12 or 24 hours after receiving the injury; mice were dosed 6 or 12 hours post-injury. A week later, the animals were tested on a difficult active place avoidance task, where the animals had to learn to avoid an area of the chamber that gave off shocks, even as the chamber (and external clues about position) rotated. They were also tested on an easier memory task, the Barnes maze, where they had to remember the placement of an escape hole in a stationary test chamber.

The rats given the drug combination 12 hours post-injury had improved performance on the difficult active place avoidance task compared with the placebo-treated rats. These differences disappeared when the rats were dosed 24 hours post-injury. However, the rats dosed with drugs 24 hours post-injury still performed better on the easier Barnes maze than the placebo-treated rats. Similarly, the mice dosed with the drugs 6 hours post-injury performed better than the placebo-treated mice on the active place avoidance task, and the mice dosed with the drugs 12 hours post-injury performed better than the placebo-treated mice on the Barnes maze.

"There are drugs that work within the first five to 10 minutes after injury, but you're typically not able to see a patient that quickly," said lead author Michael Ayo Sangobowale of SUNY Downstate Medical Center. "What makes these results so attractive is that we're able to show that at six hours we can still dose rodents and see a therapeutic effect."

Research was supported with funds from the Department of Defense.

Scientific Presentation: Tuesday, Oct. 20, 10-11 a.m., Hall A

500.11, Minocycline plus N-acetylcysteine have a clinically useful therapeutic window in two animal models of traumatic brain injury
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TECHNICAL ABSTRACT: There are presently no drugs to treat traumatic brain injury (TBI). We have previously shown that the combination of the FDA-approved drugs minocycline (MINO) and N-acetylcysteine (NAC) synergistically improved cognition and memory, modulated inflammation, limited grey matter injury and induced remyelination when dosed 1 hour after injury in a rat controlled cortical impact model (CCI) of TBI. MINO plus NAC retained similar efficacy in a mouse closed head injury (CHI) model of TBI. The CHI model was then used to determine the lowest dose of both drugs that retained full efficacy. When dosed at one hour after CCI or CHI, the optimized MINO plus NAC dose modulated neuroinflammation and induced remyelination in the injured brain. We now report that this optimized dose of MINO plus NAC improved cognition and memory when dosed 6 hours after injury in the rat CCI and mouse CHI models. This therapeutic window was assessed using an active place avoidance task with high cognitive demand. MINO plus NAC, however, no longer improved cognition in the rat CCI model when dosed 12 hours after injury. We are testing whether the 6-hour therapeutic window of MINO plus NAC may be longer when tested using Barnes maze, a behavioral task that likely has a lower cognitive demand than active place avoidance. We are also testing whether the 6-hour dosing of MINO plus NAC modulates inflammation, limits grey matter injury and induces remyelination. These data suggest that MINO plus NAC limits brain injury with a clinically useful therapeutic window in two TBI models and in two species. These preclinical studies provide further evidence that that MINO plus NAC has sufficient potency and safety to be tested against clinical TBI.

Speaker Summary 43.18

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Pediatric Traumatic Brain Injury Induces Selective Loss of Cortical Inhibitory Function

Scientific Presentation: Saturday, Oct. 17, 2-3 p.m., Hall A

Our research indicates that when compared to adults select populations of neurons within the pediatric brain may be more vulnerable to the effects of TBI.

Traumatic brain injury (TBI) is a leading cause of death and disability in children and young adults. In the United States, each year over half a million children are seen in hospital emergency rooms for traumatic brain injuries. Studies have shown that a child's brain is more vulnerable to the effects of TBI, developing more severe cognitive and behavioral deficits and taking a longer time to recover. What is different about a child's brain that predisposes it to a worse outcome following TBI? How may we target these differences to improve clinical therapies and prevent the ensuing deficits? Answering these questions is of paramount importance to reducing the burden of brain injury on children and is a major focus of the on-going research conducted in our laboratory.

In children TBI often results in short and long term physical, emotional and cognitive deficits. Of significant clinical concern is that in the subsequent months to years following TBI, up to 20% of children will develop post-traumatic epilepsy (PTE) characterized by the presence of spontaneous recurrent seizures. These seizures are often resistant to medical intervention and have a profound negative effect on a child's development and quality of life. Little is known about the underlying cause of PTE and even less of how the physiology of a child's brain impacts the pathophysiology of the TBI. With the pediatric brain still in the process of development it is important to develop new targeted approaches to treat pediatric brain injuries without disrupting the process of neurodevelopment.

The brain is dynamically regulated to balance the need for excitatory and inhibitory activity. Epilepsy has been shown to develop from disruption in this balance that leads to a state of hyperexcitability and/or the promotion of network synchrony. In adult animals previous studies have found that TBI induces a general increase in brain excitability. However, our studies suggest the pediatric animal brain responds differently - with changes that promote synchrony in the neural network but no general increase in brain excitability. In this study, we have examined specific changes to the inhibitory neural network that may promote PTE and the sequelae of symptoms that follow in the wake of TBI. To accomplish this, mice were separated into two groups: a control group and a group that underwent a severe TBI. We found that TBI induces rapid disruption to a select population of inhibitory neurons that are thought to be important for helping to synchronize the neural network and for higher cognitive functions.

Compared to similar studies in adults, these results suggest a difference in the way the pediatric brain responds and recovers from brain injury. We are currently investigating how disruption of this select type of interneuron alters the neural and behavioral activity of pediatric mice. Long term we are developing a targeted approach to prevent the loss of interneuron function that may be of significant therapeutic value in the treatment of pediatric TBI.

The findings from this study provide further evidence that juvenile brain injuries must be examined and treated differently from adult TBI. This work adds to a growing number of studies that suggest that injuries to a developing brain are unique and can lead to long-term consequences that may persist into adulthood.

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