The Effects of Mild Traumatic Brain Injury on the Activation of a Diffuse Noxious Inhibitory Control System in Rats

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INTRODUCTION

The sensation of pain involves the transmission of nerve impulses from the source of tissue damage or injury, up through the spinal cord, and through multiple pain-processing pathways in the brain. These pathways have a protective function as they help detect sources of injury or potentially tissue damaging stimuli before it is too late. Take for example people with Riley-Day syndrome. Fifty percent of individuals with this condition only live to the age of 20 because they are not equipped with a functioning pain-processing system vital for the recognition of harmful or injurious stimuli. At the other end of the pain continuum are individuals suffering from chronic pain conditions such as Post Concussive Syndrome (PCS). These patients present symptoms and impairments such as poor concentration, dizziness, along with head and neck pain. One theory as to how this chronic pain condition develops involves a cascade of secondary events associated with mild traumatic brain injury (MTBI). However, the effects of MTBI on pain are not understood because most of the research related to MTBI focuses on cognitive function and recovery, thereby making studies related to altered sensation, including pain, a novel area of research in the field of brain injury.

MTBIs are often due to vehicular accidents, falls, sports injuries, and acts of violence, and most commonly affect young men between the ages of 15 and 24. MTBI, like other types of brain injury, involve both primary and secondary events. The primary event is the direct, mechanical disruption of brain tissue that occurs at the time of injury. The secondary phase encompasses a wide range of chemical and anatomical events in the central nervous system (CNS) that occur after brain injury. Secondary injury in the field of central nervous system trauma is of great interest because it has the potential of being, at least in part, prevented. However, despite significant scientific strides in this direction, there exists no effective treatment strategy. Treating brain injury is extremely challenging, in part, due to the wide range of physiological changes associated with brain trauma. A well-characterized effect of TBI is diffuse axonal injury. Trauma to the head causes a shearing and stretching of axons in the brain. This physical stress on nerve processes causes a variety of secondary events including axonal swelling, structural damage, compromises in axonal transport, changes in phosphorylation states, mitochondrial damage, and, in some cases, axonal disconnection from post-synaptic targets.
information, including that related to the sensation of pain. The reason for this is that processing of nociceptive signals occurs in a decentralized manner and involves many areas of the brain and brainstem. The intensity of the pain experience, for example, is represented in the somatosensory and anterior cingulate cortex, and in the periventricular area. The S1 somatosensory cortex is believed to be responsible for discriminating between nociceptive and non-nociceptive stimuli, the coding of stimulus intensity, and the localization of sensory events on the body. At cortical levels, the temporally integrated response to repetitive or prolonged nociceptive stimuli is encoded in Brodman’s area 3a. A bi-directional inhibitory interaction has also been proposed between area 3a and adjacent areas of the somatosensory cortex, based on cortical activity mapping studies. It is therefore possible that MTBI could lead to abnormal sensory states by disturbing the functional balance existing between different parts of the somatosensory cortex as well as connections with subcortical structures. Therefore, the goal of the present study was to investigate how recent MTBI interferes with these circuits to cause changes in the processing of sensory and affective aspects of pain perception.

METHODS

Traumatic Brain Injury

Twelve female Sprague-Dawley rats, weighing between 280 and 300 grams, were housed in standard cages with ad libitum food and water. All animals were treated in accordance with the guidelines set forth by the University of Florida IACUC. Six rats were initially anesthetized in a large glass container using a gauze pad saturated with 3-5 ml of liquid halothane (2-Bromo-2-chloro-1,1,1-triluoroethane) that was taped to the top of the container. Once the animal was sedated, a subcutaneous injection of a Ketamine, Xylazine, and Acepromazine mixture (Rat Cocktail) was administered. The dosages of each drug per animal were 27.8 mg/kg of Ketamine, 5.57 mg/kg of Xylazine and 0.91 mg/kg of Acepromazine (10 mg/ml). Once the animal was deeply anesthetized, the scalp was shaved to allow for a midline incision in the scalp. The skin was reflected and the periosteum covering the bone was removed. The skull was scraped to ensure adequate fixation of a 10 mm stainless-steel disc to the skull on the coronal suture between lambda and bregma with dental acrylic. The animal was placed in a prone position on a foam bed of known spring constant within a Plexiglas frame. A 450 gram brass weight was dropped onto the disk from a height of 1 meter through a Plexiglas tube held in place with a ring stand onto the disc (Figure 1). The weight impacted the skull with a velocity of 4-6 m/s, causing a maximum skull compression of 0.2 mm as calculated by Marmarou et al. After injury, the head incision was closed with staples and the animal was placed in an enclosed circular area for 6 hours to monitor recovery from the above procedures. In addition to the six animals undergoing TBI, a second group of six animals received the same anesthesia and scalp incision as the MTBI group; however, they did not receive the head injury. Behavioral testing of all 12 animals resumed the day after surgery. The staples were removed one week post-incision. Post-injury data was collected for six weeks.
Behavioral Assessment

Two behavioral tasks were run each week. The first test assessed the escape behavior of animals in response to a thermal stimulus delivered to the feet (either 36°C or 45°C). The shuttle-box apparatus used in this test consisted of an aluminum thermal plate measuring 18 X 29 X 2 cm and an escape platform measuring 12.5 X 15.3 cm, tilted at an angle of 12° toward the dark enclosure (Figure 2A). The plate and platform were separated by a swinging divider, which served to isolate an aversive 50 W halogen light on the side of the escape platform from the thermal plate. Both compartments were enclosed within a heat-absorbing, tinted, Plexiglas box. Rats were first acclimated to the box using 36°C in the absence of the light. Gradually the plate temperature was raised and the light implemented to allow the rats to learn the difference between the two chambers. After training, baseline data was collected four days per week for three weeks prior to MTBI. The trial time for each session was 10 minutes, during which the time spent on the platform (escape duration) was measured. Each rat was tested at both temperatures each day, varying the order of temperature. In testing escape behavior, the 45°C temperature was chosen because it is slightly higher than a temperature required to activate thermal nociceptors and because it did not cause tissue damage. The 36°C stimulus was a neutral temperature used as a control for learned avoidance of the thermal plate.
The second outcome measure, assessed once per week, used a two-sided box separated by a small passageway that was accessible over a raised platform. This test, referred to as the Dark Box test, served as a motor control for the operant escape test (Figure 2B). Three walls of both chambers and the translucent floor were equipped with florescent lights that served to elicit escape from one side of the box to the other. A computer program automatically conducted a test consisting of 10, 140-second-long trials. After an initial 10-second lights out period, both chambers were illuminated. When the animal moved from one compartment to the other, the lights in the occupied compartment shut off, but the other side stayed lit for a total of 70 seconds. The computer recorded the animal’s escape latency, which was the time between the presentation of light and its movement to the other side. After 70 seconds, the light in the original compartment shut off and a 70-second time-out with no light followed. This process was repeated for a total of ten trials.

RESULTS

Performance data on the two behavioral tasks during post-injury testing were compared to
the respective baselines of each group. Data collected on the operant escape task revealed a significant difference in escape behavior between the incision-only group and the incision + MTBI group during the first week post-injury (Fig 3). No significant differences between the groups were seen after the first week. The platform duration of the incision-only group, using a plate temperature of 45ÉC, decreased compared to baseline (Fig 3), indicating a decrease in sensitivity to the thermal stimulus. The amount of time spent on the platform by the incision + MTBI group at 45ÉC did not significantly differ from that of the pre-injury period suggesting no change in sensitivity to the thermal stimulus. Differences between the respective baseline and post-injury platform times for the two groups were calculated to be approximately 60 seconds. The results of the Dark Box test for both groups revealed no differences in escape latencies from pre- to post-injury. These results show that the experimental conditions did not affect the motor performance of the animals.

**Figure 3.** Diagram showing the longer platform time of the MTBI+ incision group than the incision-only group during week 1. Escape behavior returned to normal by week 2.

**DISCUSSION**

The results from the escape test showed that the incision-only group spent less time on the platform, meaning they spent a longer duration on the thermal plate at 45ÉC. This demonstrates a decrease in sensitivity among the incision-only group. In contrast, the platform time of the MTBI group did not significantly differ from baseline, suggesting their sensitivity to the noxious thermal stimulus did not decrease as in the incision-only group. The escape latencies in the Dark Box test were not changed from pre- to post-injury, indicating that any change in behavior noted in the operant escape task was not due to changes in motor behavior. One possible explanation for the decrease in escape duration for the incision-only group is that the incision, staples, or stress from the experimental condition activated an endogenous inhibitory system that decreased
thermal sensitivity. This change was not seen in the incision + MTBI group presumably because the head trauma interfered with the activation of an intrinsic inhibitory system and, therefore, this group was incapable of showing the same decrease in thermal sensitivity. The results of this study are the first to show an effect of MTBI on the processing of sensory information. The results support the conclusion that diffuse damage to multi-synaptic inhibitory pathways in the brain caused by the injury resulted in a “disabling” of pain inhibitory systems. MTBI may have damaged descending inhibitory pathways or circuitry responsible for their activation, thereby decreasing the influence of these pathways on thermally sensitive neurons in the spinal cord. Regardless of the exact mechanisms responsible for the behavioral effect, it is clear that there was an absolute change in the processing of thermal information in the incision + MTBI group.

In the incision-only group, it is believed that a pain inhibitory system was activated by the tissue damage from the incision conditions, from the stress of having staples in the skin, and/or from a combination of these conditions. The fact that this difference lasted only one week suggests that removal of the staples and recovery of the incision no longer provided the stimulus required for activation of the inhibitory pathways. Further studies that may elucidate the source of the pain-induced or stress-induced changes are necessary to gain a better understanding of the neurological changes produced by MTBI. The logical progression from these data will be to study the gross histology as well as to search for biochemical markers that signify a change in the normal functioning of the brain after MTBI. Pharmacological studies that would help identify the mechanism responsible for the effect in the incision-only group would involve trying to block this effect with the opiate antagonist naloxone. Previous studies support a role of endogenous opiates in the modulation of pain pathways. In an effort to evaluate the persistence and time course of the MTBI effect and the reproducibility of the incision effect, it will be important to repeat the incision protocol in both groups of animals. Biochemical assays at the time-point of maximal effect should provide further insight into the mechanism responsible for the behavioral differences between the two groups. Continued research related to the impact of acute head injury on sensory processing will allow for a more comprehensive assessment and treatment of brain injury patients as well as a better understanding of the varying severity of head injuries and the functional deficits experienced clinically.

REFERENCES