Video Article

A Mouse Model of Single and Repetitive Mild Traumatic Brain Injury

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Abstract

Mild Traumatic Brain Injury (mTBI) can result in the acute loss of brain function, including a period of confusion, a loss of consciousness (LOC), focal neurological deficits and even amnesia. Athletes participating in contact sports are at high risk of exposure to large number of mTBIs. In terms of the level of injury in a sporting athlete, a mTBI is defined as a mild injury that does *not* cause gross pathological changes, but does cause short-term neurological deficits that are spontaneously resolved. Despite previous attempts to model mTBI in mice and rats, many have reported gross adverse effects including skull fractures, intracerebral bleeding, axonal injury and neuronal cell death. Herein, we describe our highly reproducible animal model of mTBI that reproduces clinically relevant symptoms. This model uses a custom made pneumatic impactor device to deliver a closed-head trauma. This impact is made under precise velocity and deformation parameters, creating a reliable and reproducible model to examine the mechanisms that contribute to effects of single or repetitive concussive mTBI.

Video Link

The video component of this article can be found at https://www.jove.com/video/55713/

Introduction

Traumatic Brain Injury (TBI) is defined as a head injury sustained from an external physical force, resulting in the disruption of normal brain function. It represents a significant socio-economic and public health burden, with the Center for Disease Control and Prevention 2015 Report to Congress estimating that 2.5 million Americans sustain a TBI every year. This impacts not only the patient's quality of life, but also places an extremely high economic cost on the community, currently estimated at $76.5 billion annually. The amount of actual brain damage conveyed and the acute phase symptomologies is what defines mild, moderate and severe TBI.

Mild Traumatic Brain Injury (mTBI), also referred to as concussion, accounts for over 70% of TBIs reported each year[1]. It is most common among athletes who participate in high-risk contact sports including boxing and football[2]. Unlike moderate or severe forms of TBI, the immediate damage and symptoms associated with mTBI are sometimes not as pronounced[3]. By contrast, the long-term effects of mTBI can be just as debilitating as those seen in the moderate and severe forms. Those who suffer from repetitive mTBI have been shown to develop chronic traumatic encephalopathy (CTE), as well as other cognitive and degenerative illnesses[4]. Therefore, it is important to gain a greater understanding of the mechanisms that contribute to the short-term symptoms and overall long-term damage that arises following mTBI.

In humans, the definition of concussion as defined by the Fourth International Conference on Concussion in Sport (Zurich 2012)[5] states that the level of injury for sports concussion is mild, does not cause gross pathological changes, but does cause short-term neurological deficits that are spontaneously resolved. Indeed, a recent study investigated the effect of mTBI on cognitive impairment in high school football players, using head impact telemetry systems. This study showed the amount of times players sustained helmet impacts >20 g in a single season ranged from a low of 226 (average, 4.7 per session) to a high of 1855 (average, 38.6 per session)[6]. Most of these impacts did not result in the clinical diagnosis of a concussion; but evidence of functional changes in brain function could be observed using fMRI[6]. The brain changes that cause these functional changes are unknown, and therefore there is a pressing need to have a reliable and reproducible model to facilitate research into the effects of concussive and subconcussive mTBI.

Despite previous attempts to model mTBI in mice and rats[7], many report adverse effects. In particular, most rodent models are limited in their repetitive nature using fewer than five mTBIs impacts, as well as having adverse pathological events including intracerebral bleeding, skull fractures, severe axonal injury, neuronal cell death, and increased mortality[8,9,10,11,12]. Herein, we describe a mouse model of mTBI that is closer to the true definition of concussion in humans. This model recapitulates many of the symptoms observed in human mTBI, such as the mechanical force that results in the transient loss of consciousness with no overt gross brain pathology. Furthermore, it is advantageous in the fact that it can be utilised for both single impact and repetitive impact paradigms over long time periods as reported previously[13].

Protocol

These studies were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes Health. The protocol was approved by the Institutional Animal Care and Use Committee at Georgetown University. Mice were housed in a temperature controlled animal facility and were kept on a 12 h light/12 h dark cycle. Food and water were available *ad libitum*.

1. Preparation of the mTBI Apparatus

NOTE: The mTBI apparatus includes a data acquisition (DAQ) box to control impact parameters, a high-velocity pneumatic impactor to perform the impaction, and a moulded gel filled base to decelerate head after impact.

1. Turn on compressed air and initialize the high-velocity pneumatic impactor to a pressure setting of 861.85 kPa.
2. Calibrate the DAQ control system to following parameters- piston velocity of 2.35 m/s, surface dwell time of 31.5 ms.
3. Place the molded gel-filled base (compressibility 64 kPa/mm) such that the midline is perpendicular with the trajectory of impactor tip.

2. mTBI Impaction

1. Record the weights of all mice to be used.
2. Anesthetize mice with 3% isoflurane in oxygen for 120 s in an induction chamber.
3. Transfer mice to mTBI apparatus, continuing anesthesia via a pliable, non-fixed nose cone.
4. Whilst in nose cone, position mouse head on the gel pad so that the flat surface of the skull is presented perpendicular to the impactor tip.
5. Place tape over mouse head to create a flat surface and hold ears away from the impact site.
6. Lower the polytetrafluoroethylene impactor tip to align with the sagittal midline in the center of the head. The impactor tip used is 10 mm in diameter and will cover the area of the scalp from just behind the eyes to the midline of the ears. Adjust the impactor so it is just touching the surface of the mouse head.
7. Retract the impactor tip and manually dial down the required deflection depth (7.5 mm).
8. Arm the DAQ control system and depress trigger button to impact mouse head, either a singular or repetitive impacts. Repetitive impacts are delivered in succession with no delay between each impact.
9. Immediately after the final impact, remove mouse from anesthesia and the mTBI apparatus and place in supine position.
10. Using a stopwatch measure the latency of the return of righting reflex (supine to prone), to determine loss of consciousness time, as well as time to ambulation (unimpeded walking).
11. Monitor mouse and upon recovery to normal behaviour, return to its home cage.
12. Sham mice receive identical handling and anesthesia, but receive no impact.

Representative Results

The use of this novel mTBI device allows for single and repetitive mild head injuries without risk of skull fracture or structural brain damage. The model uses a custom made pneumatic Teflon impactor device to deliver a closed head mechanical energy impact. The impact is made under precise velocity and deformation parameters, creating a reliable and reproducible model to examine the mechanisms that contribute to effects of single or repetitive concussive mTBI (**Figure 1**).

The presence or absence of LOC is a useful tool in grading concussion severity in animal models. The return of the righting reflex time is an acute neurological evaluation of injury severity that we used to quantify the LOC after a single and repeat mTBIs (7.5 mm depth, **Figure 2**). During the procedure mice receive a total of 3 min of isoflurane in oxygen, and thus all mice including shams have a LOC period after anaesthesia withdrawal. Single mTBI results in significantly increased LOC compared to sham mice (36.4 ± 1.6 s v 64.2 ± 7.7 s, n = 5, **p <0.01, **Figure 2A**). This also correlated with increased ambulation times following a single mTBI (52.0 ± 4.5 s v 140.0 ± 21.1 s, n = 5, **p <0.01, **Figure 2B**). In repeat injury paradigms (a total of 30 impacts, 5 impacts per day for 6 days), there was significantly elevated LOC and ambulation times on all testing days (repeated measures two-way analysis of variance injury effect $F_{1,14}$= 22.92, p <0.0003). During the entire study, the average LOC over the 6 days was Sham: 35.5 ± 1.4 s v mTBI: 64.9 ± 1.7 s, n = 8, p <0.01, **Figure 2C**, and average ambulation times Sham: 64.3 ± 3.3 s v mTBI: 160.8 ± 5.3 s, n = 8, p <0.01, **Figure 2D**. Iba1 staining for microglia/macrophages revealed no change between sham and single mTBI mice, but extensive Iba1 immunoreactivity in the optic tract in repeat mTBI mice (**Figure 3**). Repeat mTBI mice showed no evidence of grey matter inflammation in the cortex (**Figure 3**) or other brain areas.

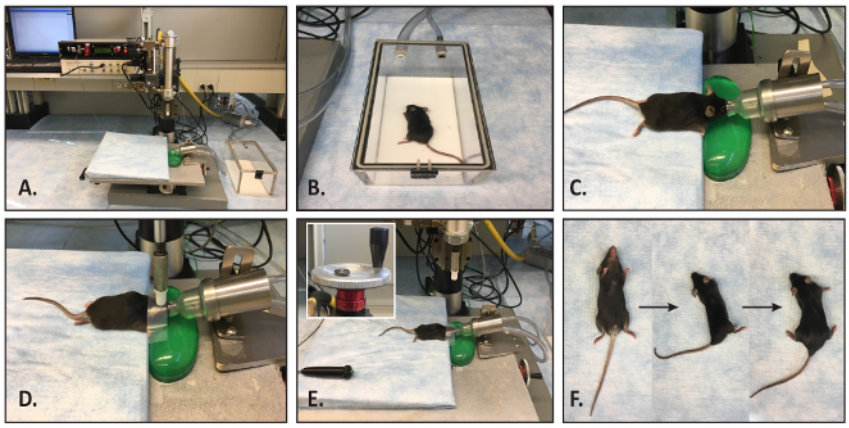

Figure 1: Illustrative Representation of the Mouse Model of mTBI. (**A**) The setup of all materials required to perform mTBI. (**B**) Mouse placed in isoflurane induction chamber for 2 min. (**C**) On the mTBI apparatus, mouse placed in a non-fixed nose cone to continue anesthesia. (**D**) Gently tape mouse head to create a flat surface and hold ears back. Impactor tip lowered to just touch the surface of the head. (**E**) Impactor tip retracted and required deflection depth is lowered using the dial (inset). mTBI performed by depressing trigger button. (**F**) Loss of consciousness measured by the time taken for the return of righting reflex (supine to prone). Please click here to view a larger version of this figure.

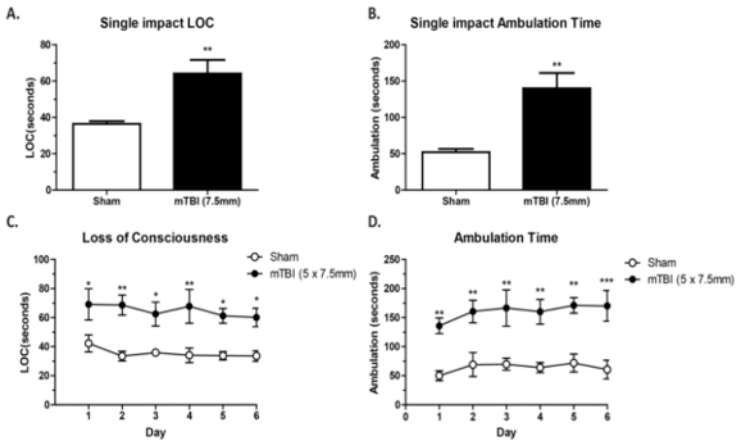

Figure 2: Single and Repeat mTBIs Increases Transient Loss of Consciousness and Return to Ambulation Times. Mice that receive a single mTBI have significantly increased (**A**) loss of consciousness (LOC) and (**B**) ambulation times compared to sham control mice (n = 5 per group, **p <0.01). (**C**-**D**) Repetitive mTBI (5 impacts per day for 6 days) significantly increases daily LOC and ambulation times compared to sham mice (n = 8 per group, *p <0.05, **p <0.01, ***p <0.001). Data expressed as mean ± S.E.M, analyzed by Two-way repeated measures ANOVA with a Bonferroni post-hoc test. Please click here to view a larger version of this figure.

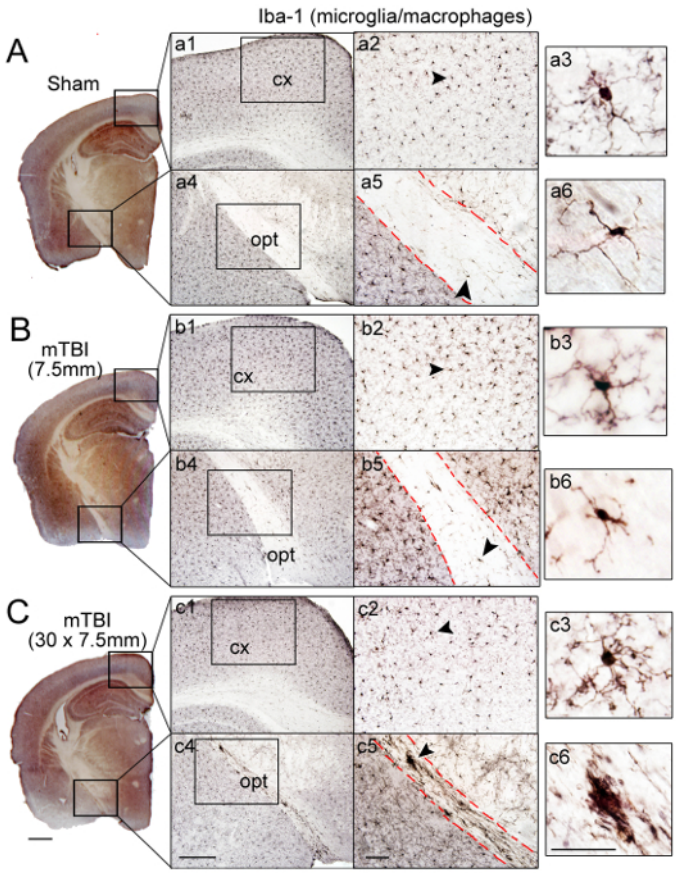

Figure 3: Repeat mTBI Induces Microglia/Macrophages Activation in the Optic Tract. (**A**) Iba1 staining in sham mice reveals quiescent microglia/macrophages in the cortex (a1 - a3) and limited staining in the optic tract (a4-a6). (**B**) Mice that receive a single mTBI (7.5 mm) have similar Iba1 staining profiles as sham mice in both the cortex (b1-b3) and optic tract (b4-b6), 24 h post-impact. (**C**) Repeat mTBI mice (5 impacts per day for 6 consecutive days) display a strong inflammatory response in the optic tract (c4-c6) but not in the cortex (c1-c3), 24 h after the last impact (Day 7). Enlarged images demonstrating the morphology of Iba-1 positive microglia/macrophages in the cortex (a3, b3, c3) and optic tract (a6, b6, c6) of sham, single and repetitive mTBI mice, respectively. Scale bars = 200 µm (A, B, C); 50 µm (a1-a5, b1-b5, c1-c5); 20 µm (a3, a6, b3, b6, c3, c6, magnified panels). Images representative of n = 6 per group. Cortex (cx), optic tract (opt), mild traumatic brain injury (mTBI). Please click here to view a larger version of this figure.

Discussion

In humans, mTBI is characterized by a functional impairment in the absence of structural injury. This can occur with, or without, a loss of consciousness[1]. Exposure to repeat concussions is currently thought to underlie the development and/or progression of neurodegenerative diseases such as CTE[4]. It is well documented that CTE is commonly found in boxers and football players and although exposure to repeat concussions (including those that do not result in a loss of consciousness) is known to be an essential element in developing CTE, we do not yet know why the mechanisms by which repeat concussion causes the distinct changes that occur in the brain.

A major hurdle to understanding these mechanisms is the difficulty in developing models that accurately recapitulate the key symptomologies observed in individuals that sustain an mTBI impact. This means the acute and chronic biological pathways underlying the concussive impacts are difficult to investigate and new treatments cannot be developed. In particular, existing animal models of repeat concussion are very severe, using only 2-3 repeat impacts that cause extensive neuronal cell loss, skull fracture and loss of brain tissue[8,10,11,12]. This extent of injury is not occurring after repeat concussion in humans, demonstrating the need for a new model of single and repetitive mild head impact.

Here we describe a novel model of mTBI, using a custom made diffuse injury device that has been specifically designed to transfer energy through the skull and brain and away from the mouse. Through both single impact and repetitive impact paradigms, the model allows for a means to begin investigating how concussion contributes neurological impairment later in life. The single hit paradigm recapitulates the hallmark characteristics of mild traumatic brain injury observed in humans, while the repetitive paradigm allows for the examination of how these seemingly mild injuries contribute to chronic and persistent degeneration over time. The device allows for repetitive mTBI head impacts without risk of skull fracture or structural brain damage.

As is the case with many techniques, certain aspects of this protocol are important to note for the generation of accurate, reliable results. During chamber induction and mTBI injury, it is essential to maintain a consistent level of anesthesia to each mouse. Due to the fact that righting and ambulation time is a key outcome measurement of this mTBI procedure, researchers should ensure that both mTBI injury and sham animals are exposed to comparable levels of anesthetic. Specifically, for the duration of the entire procedure, mice should only be under anesthesia for 3 min

total (2 min in the induction chamber, 1 min with the mTBI nose cone device). This low level and duration of anesthesia is a major advantage over many other mTBI models, and yields consistent righting reflex response times (sham mice 20-40 s, mTBI mice 50-100 s). Additionally, when utilizing the repeat mTBI paradigm, it is important to maintain a record of mouse weights every day for the duration of the study. This is to ensure consistent monitoring for stress and general animal welfare throughout the entire time course of the mTBI procedures. This procedure does not result in mice becoming withdrawn or isolated. Normal grooming, eating and drinking should be observed within the first hour post procedure.

Post-injury there is no gross morphological pathology to the brain tissue resulting from either single or multiple impacts over a 1-week time course**.** Following a single mTBI, the only cellular response observed is a transient reduction in excitatory synapses; there is no inflammation, loss of grey or white matter, axonal injury or cell death. With 30 repeat mTBI (5 per day, over 6 days) there is chronic inflammation of the optic tract, at similar levels as previously quantitated[1]. Chronic white matter inflammation has been observed many years after a single mTBI[14], and chronic inflammation can be detected in living athletes with a history of repeat mTBI[15]. The limitations of this model are that it cannot be used to study axonal injury outside of the optic tract or mechanisms of cell death, as these are absent in our model. Also based on our previously published data, this model does not induce changes in amyloid or tau pathology at 1-month post-mTBI in a mouse model of Alzheimer's disease[13]. We believe this lack of amyloid and tau pathology is related to the absence of axonal pathology in our model. Our model does provide a platform to investigate discrete changes in neuron networks, synaptic integrity and composition, and alterations in behavior following repeat concussive blows. Based on these outcomes, this novel model produces clinically relevant symptomology in a controlled, rigorous, and efficient manner. Further use of this model will allow for the investigation into the mechanisms that underlie the acute and chronic pathophysiology of mTBI and concussion.

Disclosures

The authors have nothing to disclose.

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