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A mouse model of human repetitive mild traumatic brain injury

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ABSTRACT

A novel method for the study of repetitive mild traumatic brain injury (rmTBI) that models the most common form of head injury in humans is presented. Existing animal models of TBI impart focal, severe damage unlike that seen in repeated and mild concussive injuries, and few are configured for repetitive application. Our model is a modification of the Marmarou weight drop method and allows repeated head impacts to lightly anesthetized mice. A key facet of this method is the delivery of an impact to the cranium of an unrestrained subject allowing rapid acceleration of the free-moving head and torso, an essential characteristic known to be important for concussive injury in humans, and a factor that is missing from existing animal models of TBI. Our method does not require scalp incision, emplacement of protective skull helmets or surgery and the procedure can be completed in 1–2 min. Mice spontaneously recover the righting reflex and show no evidence of seizures, paralysis or impaired behavior. Skull fractures and intracranial bleeding are very rare. Minor deficits in motor coordination and locomotor hyperactivity recover over time. Histological analyses reveal mild astrocytic reactivity (increased expression of GFAP) and increased phospho-tau but a lack of blood–brain-barrier disruption, edema and microglial activation. This new animal model is simple and cost-effective and will facilitate characterization of the neurobiological and behavioral consequences of rmTBI. It is also ideal for high throughput screening of potential new therapies for mild concussive injuries as experienced by athletes and military personnel.

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1. Introduction

Traumatic brain injury (TBI) results from a blow to the head. The severity of injury can range along a continuum from mild (e.g., brief change in mental status, somatic effects or consciousness) to severe (e.g., extended unconsciousness, coma, prolonged amnesia) to fatal. Any form of TBI can result in short- and long-term disability. On a global scale, TBI is a serious health concern and is the leading cause of mortality and disability among individuals in high-income countries. TBI is one of the most common neurological diagnoses in the US (Rutland-Brown et al., 2006) and the CDC has estimated that 1.7 million people sustain TBI on an annual basis.

Perhaps the form of TBI that has garnered the greatest scrutiny recently, in the public eye as well as within military, scientific and medical communities, is repetitive, mild TBI (rmTBI). Military operations in Iraq and Afghanistan are revealing that TBI accounts for about 28% of all combat casualties (Okie, 2005) and approximately 88% of those are closed-head injury (US Medicine, May 2006, vol. 42). The US Defense and Veterans Brain Injury Center has estimated that ~180,000 military service members have been diagnosed with mTBI from 2001 to 2010 and other estimates place this number as high as 320,000 (Tanielian and Jaycox, 2008). Persistent accounts of rmTBI suffered by athletes (amateur and professional) have also directed much needed attention to this growing and significant problem. It has been estimated that 1.6–3.8 million sports-related TBIs occur each year (Guskiewicz et al., 2000; Halstead and Walter, 2010). Epidemiological studies reveal that about 60% of retired professional football players sustained at least 1 concussion during their careers (Guskiewicz et al., 2005) and approximately 25% experienced repeat injury (Guskiewicz et al., 2005; Pellman et al., 2004).

RmTBI generally produces a constellation of symptoms (e.g., headache, dizziness, confusion) collectively referred to as post-concussive syndrome (Halstead and Walter, 2010; Pellman et al., 2003b). Reports of more serious consequences of rmTBI such as chronic traumatic encephalopathy (McKee et al., 2009, 2010;

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Omalu et al., 2010) and increased co-morbidity of neurodegenerative disorders are also emerging (Chen and D'Esposito, 2010; Guskiewicz et al., 2007a; Masel and DeWitt, 2010; Plassman et al., 2000). The situation with regard to rmTBI is made even more complex by the fact that it is extremely difficult to detect. For the most part, routine imaging approaches (e.g., CT and MRI) contribute little to the evaluation and management of mild concussion (Van Boven et al., 2009) but more advanced and specialized approaches such as diffusion tensor imaging are showing promise (Levin et al., 2010; Mac Donald et al., 2011).

The availability of an animal model of rmTBI would certainly facilitate a better understanding of the neurobiological and behavioral outcomes of rmTBI. A large number of animal models of TBI have been developed and these have been effective in characterizing the molecular and cellular bases of acute, severe (i.e., single-impact) TBI (Cernak, 2005; Finnie, 2001; LaPlaca et al., 2007; Lighthall et al., 1989; Long et al., 2009; Morales et al., 2005; Park et al., 1999; Weber, 2007). Biomechanical analyses of head impacts sustained by athletics have revealed that the most critical factors in producing mild, concussive brain injuries are high velocity impact and rapid head acceleration (Guskiewicz et al., 2007b; Meaney and Smith, 2011; Pellman et al., 2003a,b; Viano et al., 2007, 2005, 2009; Viano and Pellman, 2005). Unfortunately, most existing animal models do not achieve these factors (see (Viano et al., 2009) for discussion). The original Marmarou acceleration weight drop model (Marmarou et al., 1994) causes severe compressive deformation of the cranial vault and results in cortical injuries (e.g., contusions and bleeding) beneath the site of impact. It is also characterized by a high incidence of brainstem injury that can lead to transient hypertension, prolonged apnea, respiratory failure and mortality (Cernak, 2005; Chen et al., 1996; Flierl et al., 2009; Kilbourne et al., 2009; Schumann et al., 2008; Stahel et al., 2000). Highly effective methods for producing mild, closed-head injuries have been employed in the study of single impacts (Milman et al., 2005; Zohar et al., 2003). TBI models other than closed-head, to include lateral fluid percussion (Thompson et al., 2005) and controlled cortical impact (Lighthall et al., 1989) involve direct loading of the brain and do not impart rapid changes in head acceleration (i.e., subject heads are fixed in a stereotaxic frame) making them less than ideal to recapitulate the kinds of injury seen after rmTBI in humans. Because the vast majority of existing animal models of TBI impart such severe injuries with single impacts, it is difficult to study repetitive injuries using them. Results from those attempts to apply multiple injuries (usually 2) have been somewhat inconsistent in finding that by comparison to single injuries, multiple insults worsen (Hamberger et al., 2009; Kanayama et al., 1996; Laurer et al., 2001; Longhi et al., 2005; Uryu et al., 2002), make little difference (Creeley et al., 2004; DeFord et al., 2002) or actually improve outcome (DeRoss et al., 2002). We report presently a new mouse model that makes possible the study the human form of rmTBI.

2. Materials and methods

2.1. Apparatus and application of the method of rmTBI

The essential components and overall arrangement of the rmTBI apparatus are shown in Fig. 1. Weights (19 mm diameter) were milled from solid brass to the desired mass (e.g., 60, 75, 95, 120 g) by varying their length but the 95 g weight was the only one used presently for impacting the mouse skull. This initial weight was estimated from the ratio of mouse to rat brain weights (~0.21) to scale down from the 450 g weight used with rats in the Marmarou method (Kreipke et al., 2006). A small steel cap (2 × 10 mm) is glued to the bottom of the weight to restrict the zone of contact to the top of the mouse head between the ears. Weights are dropped vertically through a PVC guide tube (20 mm diameter × 1.5 m length). The vertical traverse of the dropped weight is limited to ~40 mm of impact displacement by Orvis Super Strong knotless tapered leader (5X), commercially available nylon fly fishing line (2.2 kg test, 0.53 mm diameter). A stage consisting of a slit piece of aluminum foil, held in place by an "H"- shaped Plexiglas frame (15 cm length × 9 cm width × 23 cm depth) holds subjects in place. In this fashion, the slit foil just supports the body weight of a mouse (22–25 g) with little or no resistance or restraint upon impact. A sponge cushion (15 cm length × 9 cm width × 13 cm depth) is located 10 cm below the aluminum foil stage to receive the falling mouse while the weight remains tethered above the free-moving body of the animal. A photograph showing the dimensions of the foil stage and placement of an anesthetized mouse on it is included as Supplementary Fig. 1. In some experiments, the new rmTBI method was compared to the original Marmarou method (Marmarou et al., 1994) by resting mice directly on the sponge cushion. Immediately upon impact, mice were withdrawn to prevent re-hits by the weight as subjects recoiled from the impact-induced compression of the cushion causing secondary injuries to the brain and spinal cord.

All procedures involving the use of animals in this study were reviewed and approved by the Wayne State University Institutional Animal Care and Use Committee. Mice are lightly anesthetized with isoflurane (i.e., until unresponsive to paw or tail pinch) and placed immediately under the vertical PVC tube. Mice are suspended chest-down on a slit piece of aluminum foil 10 cm above a foam cushion. The mouse is quickly positioned so that its head is directly in the path of the falling weight by first resting the weight on the scalp midline between bregma and lambda. Incisions in the scalp or emplacement of a protective skull helmet are not necessary. The weight is then pulled rapidly upward by an attached string to the desired drop distance and released. The downward traverse of the falling weight is restricted by the string such that upon contact, the weight travels no more than 1 cm beyond the original position of the dorsal surface of the head. Immediately upon impact, the mouse falls freely onto the foam cushion. In this arrangement, the impact-induced acceleration and fall always involves a 180° horizontal rotation of the mouse body. The mouse is moved immediately to a holding cage to recover. A short video clip of the method is included as Supplementary Material for online presentation. As part of the initial and preliminary validation of our method, mice were exposed to 1, 5 (1 per day for 5 successive days) or 10 (1 per day for 5 days with a 2 day rest after the 5th) impacts (specified below) using a single weight (95 g) dropped from 1 m. The time after exposure to injury at which outcome tests were carried out are specified below in the Section 3. A total of 327 mice were used in this study and the numbers used in each experiment are specified in the figure legends.

2.2. Neurological and behavioral assessment

2.2.1. Edema

Edema was determined as brain water content using the wet/dry method as previously described (Kawai et al., 2001; Masada et al., 2001). Mice were decapitated and brains were rapidly removed from the skull. Whole, fresh brain was weighed on aluminum foil, dried for 72 h at 80 °C in a Personal Hyb hybridization oven (Strategene, La Jolla, CA), and reweighed. Brain water content was defined as % of water calculated as (wet weight – dry weight)/(wet weight) × 100.

2.2.2. Recovery of righting reflex

Immediately following the weight-drop impact, mice were placed on their backs in a clean cage. Controls were anesthetized but not subjected to head impact. Righting reflex response was