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New method to induce mild traumatic brain injury in rodents produces differential outcomes in female and male Sprague Dawley rats

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HIGHLIGHTS

- New method to produce mTBI propel rats' head toward stationary impact zone.
- Female mTBI rats show widespread effects on recovery, activity, and spatial memory.
- Male rats exhibit only spatial memory deficits in comparison to sham-injured rats.
- Female, but not male, mTBI rats show reduced hippocampal neurogenesis.
- Female, but not male, mTBI rats shows reduced BDNF levels in sera.

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ABSTRACT

Background: Mild traumatic brain injuries (mTBI) are an increasing health concern due to persistent behavioral and neurological effects. To better understand these effects, researchers frequently rely on animal injury models. Existing models, however, may not adequately reproduce the mechanism of injury as it occurs in humans.
New method: Our new model for inducing mTBI in rodents entails acceleration of the animal toward a stationary impact zone to produce rapid rotational movement of the head. The aim of the present experiment was to characterize the effects of this injury in female and male rats on behavior, cognition, and neural plasticity.
Results: mTBI produced the most widespread effects in females: they were more active during recovery within minutes of mTBI and more active in the center of the open field 4 days after mTBI. Spatial learning deficits in the water maze were mild but persistent and accompanied by reduced numbers of immature neurons in the hippocampus along with reductions in sera levels of the neurotrophin, BDNF. By contrast, male mTBI rats mainly exhibited mild spatial learning deficits, with no other observed effects.
Comparison with existing methods: Our model induced effects on behavior and biology in rats that aligned with existing models. However, new patterns were observed, particularly when comparing females and males.
Conclusions: Taken together, these findings confirm the validity of this model and point to key differences between females and males in symptom severity and type. Additionally, our model adds a novel injury mechanism that complements existing rodent models.

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1. Introduction

Traumatic brain injuries (TBIs) are a mounting health concern resulting in millions of emergency department visits annually (NCIPC, 2014) and a growing national economic burden estimated at nearly 80 billion dollars in direct healthcare costs and indirect costs, such as lost productivity at work (Ma et al., 2014). Approximately 75% of TBIs are classified as mild (mTBI), commonly referred to as a concussion (NCIPC, 2003). While media attention towards the occurrence of concussions in contact sports has increased dramatically in recent years, the incidence and consequences of concussions in other domains may be underdiagnosed and overlooked. Whether sustained in sports or other ways, of particular concern is that even mTBI results in serious, long-term consequences (Ling et al., 2015; McKee et al., 2013; Stern et al., 2011) including emotion dysregulation and deficits in cognitive functioning, such as memory loss, learning deficits, and trouble concentrating (Fujimoto et al., 2004; McAllister et al., 2006; Walker and Tesco, 2013). Unfortunately, mTBI is difficult to study as there are a wide range of criteria for diagnosis, many definitions, and a range of symptoms that may not fit into existing standards. Accordingly, developing and validating animal models is also a challenge and at present few experimental models exist that adequately and consistently reproduce the kinds of events, damage, and outcomes that are seen in human patients. Nonetheless, valid models are vital for advancing our understanding of symptoms and their underlying biological basis as well as the efficacy of treatment options and neuroprotective factors (O'Connor et al., 2011). Additionally, these models allow researchers to learn more about the ways in which mild injury may differentially impact an individual based on age, biological sex, and past injury history. The aim of the present experiment was to assess a new animal model of mTBI for use with rodents that addresses limitations with and complements existing models.

Understanding the range and kinds of outcomes following mTBI in humans is challenging due to the difficulties in gathering data. Consequently, there is a dearth of information regarding injury mechanisms in current diagnostic coding, inconsistencies in diagnostic criteria, and a lack of gross anatomical findings using neuroimaging that leave critical gaps in mTBI research (Gao and Chen, 2011; Sharp and Jenkins, 2015; Walker and Tesco, 2013). Another difficulty lies in understanding the complex biomechanical forces that produce a secondary injury cascade that lead to persistent neurological deficits. Researchers rely on animal models to address these issues and advance our knowledge on the mechanisms of injuries (Morales et al., 2005). Animal models enable researchers to control for the injury mechanism, thus allowing characterization of specific components of TBI that include the primary and secondary injury cascades. Many such models have been developed and are used extensively in the field. Current models of non-penetrating impact TBIs, such as weight drop and controlled concussion paradigms, generally have the animal's head constrained and a projectile or impactor inflicts a brain injury (Cernak et al., 2010; Morales et al., 2005). The acceleration and deceleration forces can be delivered along many axes, resulting in highly variable biomechanical injuries and associated outcomes (Eucker et al., 2011). Absent or minimal in many models, however, is the rotational acceleration of the animal's head, which results in more frequent concussions (Walker and Tesco, 2013). Further, forces delivered along the sagittal plane lead to worse physiological outcomes, with horizontal and sagittal components leading to the worst brain pathology (Eucker et al., 2011). To incorporate rotational forces in our model, we used a beveled impact block that allows for rotational acceleration and deceleration primarily along the sagittal axis, with components of horizontal motion.

Another limitation of current models is that each attempts to isolate specific aspects of the injury induced, rarely capturing the entire profile of highly variable head injuries (Morganti-Kossmann et al., 2010). Controlled cortical impact (CCI) and weight drop (WD) are widely accepted models for focal injuries and such are good replications of the mechanism of injury of that type as it may occur in humans. However, existing models of diffuse brain injury, including modified WD and lateral fluid percussion (LFPI), are not as good at replicating the mechanisms of injury comparably to that as it occurs in humans (DeWitt et al., 2013). In the case of LFPI in which exposed brain tissue is subjected to a stream of fluid, the head is immobilized in a stereotactic device and unable to accelerate or decelerate after impact. Several WD models exist, but most share the feature of head immobilization, or limited motion at the time of impact.

The novel device used in the present study was designed to bring the animal's head into motion toward a stationary object and allow for recoil on the impact surface after the initial impact until the head came to rest. Accordingly, characterizing the behavioral and neural features arising from our novel model was the goal of the present study. Also examined was the extent to which these effects were sexually dimorphic. Many studies of mTBI only include male subjects and fail to explore the impact of biological sex on mTBI outcomes. Interestingly, little information is available on the differential effects of sex as a biological variable in animal models or gender in humans and what is known remains largely inconclusive (Bazarian et al., 2010; Cancelliere et al., 2016). In some cases of TBI, studies report greater neuroprotection in females possibly due to hormonal differences (Bramlett and Dietrich, 2001; Djebaili et al., 2005). Others report that gender is not a good predictor for mTBI outcome, but females more frequently report post-concussive syndrome associated with pain (Bazarian et al., 2010; Cancelliere et al., 2016). The purpose of our study was to design an apparatus that could deliver reproducible, precise, and equitable injuries across females and males and lay a foundation for future work to address these open questions.

A key component in animal models is assessing the validity of the injury mechanism and the behavioral outcomes, such that the animal's symptomatology gives some predictive value to the human condition (O'Connor et al., 2011). Immediately following the injury, we measured the latency to right and videotaped recovery to observe post-injury activity levels. In the subsequent days, we assessed locomotor activity and behavior in an open field test and spatial learning and memory using a water maze. These tests are well established in the literature, and provide a sensitive and reliable measure of rat emotion and cognition. We also sought to investigate underlying neural pathology that may align with behavioral findings. To evaluate hippocampal function and plasticity we examined the effects of injury on adult hippocampal neurogenesis. Based on previous reports, neurogenesis may be disordered following traumatic brain injury, though there are mixed findings on the specific nature of the outcome (Ibrahim et al., 2016; Robinson et al., 2016). Using the marker for immature neurons, doublecortin, we tested the hypothesis that mTBI may lead to disrupted neuronal growth and migration. Additionally, we sought evidence that levels of neurotrophic factors may be altered in the injury cascade and specifically focused on brain-derived neurotrophic factor (BDNF), which plays a critical role in neural plasticity especially as it pertains to learning, memory, and emotion. Here too, we tested the hypothesis that neurotrophic response to injury is disrupted following mTBI.

2. Materials & methods

2.1. Subjects

The subjects were 32 female and male Sprague-Dawley rats (CD strain, Charles River Laboratories, Wilmington, MA; n = 16 of