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Research report

A modified beam-walking apparatus for assessment of anxiety in a rodent model of blast traumatic brain injury

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HIGHLIGHTS

- Anxiety and motor function often suffers as a result of traumatic brain injury.
- Elevated plus maze and beam-walking tasks are used to test these traits in rodents.
- A novel task, the modified beam-walking apparatus, combines these tasks into one.
- Anxiety scores show agreement between this novel and standard tests post-injury.
- This novel task is capable of dissociating how injury affects motor versus anxiety.

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ABSTRACT

The elevated plus maze (EPM) is used to assess anxiety in rodents. Beam-walking tasks are used to assess vestibulomotor function. Brain injury in rodents can disrupt performance on both of these tasks. Developing novel paradigms that integrate tasks like these can reduce the need for multiple tests when attempting to assess multiple behaviors in the same animal. Using adult male rats, we evaluated the use of a modified beam-walking (MBW) apparatus as a surrogate indicator for anxiety. We used a model of blast-induced traumatic brain injury (bTBI). A total of 39 rats were assessed before and at 3, 6, 24, 72, and 168 h either post- bTBI ($n = 33$) or no-injury ($n = 6$) using both EPM and MBW. A novel anxiety index was calculated that encompassed peeks and re-emergences on MBW. The proposed MBW anxiety index was compared with the standard anxiety index calculated from exploration into different sections of EPM. Post- bTBI, rats had an increased anxiety index when measured using EPM. Similarly, they peeked or fully emerged less out of the safe box on MBW. It was found that this novel MBW anxiety index captured similar aspects of behavior when compared to the standard anxiety index obtained from EPM. Further, these effects were dissociated from the effects of bTBI on motor function simultaneously measured on MBW. Over the course of 168 h post-bTBI, rats gradually recovered on both EPM and MBW. The MBW apparatus succeeded at capturing and dissociating two separate facets of rat behavior, motor function and anxiety, simultaneously.

1. Introduction

Damage to the central nervous system (CNS) can be caused by injury such as stroke and head trauma. Symptoms such as reflex suppression, loss of motor function, loss of cognitive function, increased anxiety, and depression are often associated with

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brain injury [6,13,14]. Traumatic Brain Injury (TBI) continues to be a foremost cause of death and disability [12,29,35]. Physical disability due to loss of motor function can be very difficult to overcome post injury; nonetheless, psychological changes such as anxiety and depression have been described as even more debilitating comorbid conditions [29,32]. Indications of anxiety including fear and apprehension can hinder recovery and rehabilitation, often negatively impacting social outcomes such as employment, personal relationships and functional abilities of daily living [29]. Increased use of explosive devices in recent wars has led to higher incidences of closed head, blast-induced TBI (bTBI) with frequent psychiatric comorbid conditions, including anxiety dysfunction [16,19,20,22,26].

Preclinical studies on TBI and bTBI have been carried out extensively using rodents [6,13,14,19,20,22,24,25,34]. Since brain injury often induces a variety of symptoms, different behavioral tests need to be conducted to compare changes pre- and post-injury. Effective, repeatable behavioral assessments are critical for assessing the severity of induced injury in laboratory settings. However, problems exist with running batteries of different assessments. Many neuropsychiatric behavioral assessments performed in rodents require extensive time and training to complete; and exposing animals to different environments can introduce increasingly confounding factors that become difficult to control for. Two behavioral assessments in particular are routinely used in brain injury experiments: the elevated plus maze (EPM) and the beam-walking apparatus. Combining these two tests into a single test would reduce the number of assessments to behaviorally profile animals. Rodents have a natural fear of open, exposed areas and often prefer to hide in dark, enclosed areas [3]. However, they are also inclined to explore open areas when not under conditions of threat. Hence, the EPM takes advantage of this conflict and is commonly used to test anxiety in rodents, as indexed by characterizing innate hesitancy to want to explore open spaces [4,6,27]. Beam-walking on the other hand is often used for assessing balance and motor function in rodents after injury. Deficits observed on the beam-walking apparatus are strongly correlated with severity of TBI [13,14,25,]. Thus, there is novel utility in designing a combined paradigm that carries discriminatory power in being able to dissociate in space and time motor function from anxiety-related behaviors while still only requiring a single apparatus and thus a single behavioral assessment.

Therefore, the goal of this study is to evaluate, in a novel model of bTBI that has recently been developed in our laboratory [8], the validity of a modified beam-walking (MBW) apparatus for assessing dissociable levels of anxiety and motor coordination, as compared to the standard EPM or standard beam-walking apparatus that is routinely used for anxiety or motor assessments alone. Our two-in-one behavioral assessment uses a MBW apparatus that could potentially be useful for assessing and dissociating motor function deficits from anxiety in a single task not previously demonstrated.

2. Materials and methods

The study was approved and conducted according to the guidelines set by the Institutional Animal Care and Use Committee at the University of Minnesota. For this study, 39 male Wistar rats (Charles River Laboratory, Wilmington, MA) were used to assess performance on the EPM and on a new test using the MBW apparatus. Animals were ordered to arrive to our laboratory six days prior to the start of training to allow for acclamation and elimination of any induced stress due to transportation. Animals were kept in pairs in each cage under 12 h light/dark cycle with free access to food and water. For the study, we divided the animals into two groups: one group ($n = 33$) with induced bTBI and another ($n = 6$) as non-injured yoked-controls. Assessment of the effects of the newly developed bTBI method, brain damage, and behavioral effects has been recently characterized in our laboratory [8].

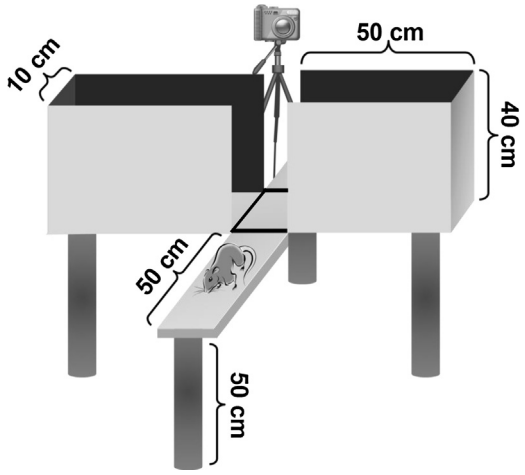

Fig. 1. Standard elevated plus maze (EPM) apparatus. Rats traversed an elevated plus maze with two enclosed and two open arms. Conflict to explore open arms versus remaining in safe enclosed spaces captures anxiety-related behaviors.

2.1. Brain injury protocol

The injury (bTBI) was induced immediately after obtaining baseline assessments of all animals on the two apparatuses (EPM and MBW). Briefly, after each animal was anesthetized with 2.5% isoflurane, the right side of the animal's head was shaved and placed atop the lens on a lithotripsy machine (STS-T system, Medstone International, Inc., Austin, TX). Positioning of the rat's skull on the lens was performed by aligning the back of the left eye to a laser pointer mounted on a micromanipulator. Once the proper position of the skull was confirmed, the lithotripsy machine was used to deliver 5 shockwave pulses (24 kVp at 1 Hz) to the frontal lobe area of the brain. To alleviate pain, buprenorphine (0.05 mg/kg) was given prior to injury. After inducing the injury, animals were returned to their cage to recover from anesthesia with free access to food and water. To control for the effect of anesthesia on animal's behavior (particularly at 3 and 6 h after the baseline), animals in the yoked-control group were also anesthetized and shaved, but not subjected to the shockwave pulses.

2.2. Elevated plus maze

An EPM apparatus was constructed (shown in Fig. 1) based on the dimensions routinely used for rats [4,6,23,27]. Although the EPM is often used for assessment of initial conflict in a novel context during a single testing session, for our purposes we aimed to demonstrate that anxiety-related behaviors reached a steady-state that is consistent over time and across apparatuses with enough training. Time spent on each region of the EPM (open arm, closed arm, central platform) as well as entries into each region were measured. Additionally, other qualitative observations were noted, including freezing behavior, head rearing, stretched-out postures, and defecation. The EPM apparatus was wiped clean between trials. In order to obtain a single value that integrates all of these anxiety-related behaviors, an anxiety index value was calculated as follows [5,33]:

$$\text{EMP anxiety index} = 1 - \frac{\frac{\text{Open arm time}}{\text{Total time in all areas}} + \frac{\text{Open arm entries}}{\text{Total enteries in all areas}}}{2}$$