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Research report

Novel application of a Radial Water Tread maze can distinguish cognitive deficits in mice with traumatic brain injury

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ABSTRACT

Introduction: The use of forced-swim, rat-validated cognition tests in mouse models of traumatic brain injury (TBI) raises methodological concerns; such models are vulnerable to a number of confounding factors including impaired motor function and stress-induced non-compliance (failure to swim). This study evaluated the ability of a Radial Water Tread (RWT) maze, designed specifically for mice, that requires no swimming to distinguish mice with controlled cortical impact (CCI) induced TBI and Sham controls.
Methods: Ten-week-old, male C57BL6/J mice were randomly assigned to receive either Sham (n = 14) or CCI surgeries (n = 15). Mice were tested for sensorimotor deficits via Gridwalk test and Noldus CatWalk gait analysis at 1 and 32 days post-injury. Mice received RWT testing at either 11 days (early time point) or 35 days (late time point) post-injury.
Results: Compared to Sham-treated animals, CCI-induced TBI resulted in significant impairment in RWT maze performance. Additionally, CCI injured mice displayed significant deficits on the Gridwalk test at both 1 day and 32 days post-injury, and impairment in the CatWalk task at 1 day, but not 32 days, compared to Shams.
Conclusions: The Radial Water Tread maze capitalizes on the natural tendency of mice to avoid open areas in favor of hugging the edges of an apparatus (thigmotaxis), and replaces a forced-swim model with water shallow enough that the animal is not required to swim, but aversive enough to motivate escape. Our findings indicate the RWT task is a sensitive species-appropriate behavioral test for evaluating spatial memory impairment in a mouse model of TBI.

1. Introduction

Traumatic brain injury (TBI) represents a significant health concern, with an estimated 1.3 million individuals seeking emergency room treatment in the United States annually as a direct result of brain injury (Faul et al., 2010). Cognition and memory-related deficits are consistently ranked among the most common complaints from patients who have experienced TBI (Levin, 1995; Schretlen and Shapiro, 2003). Therefore, it is crucial that any therapeutic strategy aimed at alleviating TBI symptoms prove effective at reducing TBI-related cognitive impairments. Though attempts to replicate TBI sequela in a number of animal models have contributed greatly to our understanding of the molecular, pathophysiological, and cognitive disturbances caused by TBI, the quantification of cognitive deficits seen in humans post-injury (particularly mild cognitive deficits) using mouse models remains a challenge. Here we present some of the issues surrounding swimming-based cognitive testing protocols in mouse model controlled-cortical impact (CCI) studies of TBI, and test the efficacy of an alternative cognitive testing apparatus that ameliorates some of these limitations.

The use of mice in behavioral and cognitive research continues to increase due to several factors: (i) rapidly expanding strains of

Abbreviations: TBI, traumatic brain injury; MWM, Morris Water Maze; RWT, Radial Water Tread; CCI, controlled-cortical impact.

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genetically modified mice are available for study; (ii) murine background genetics are well understood; (iii) mice reproduce relatively quickly compared to many other mammals; (iv) maintaining and managing mouse colonies is more efficient than with many other mammalian species, including rats; and importantly, (v) mice are capable of performing a range of behavioral tasks that are considered to be relevant to the cognitive capabilities of higher organisms.

Nonetheless, many cognitive tests commonly used with mice were originally optimized and validated using rats, which differ substantially from mice in terms of genetics, cognitive capabilities, and neuroethological factors, which greatly influence how mice and rats interact with their environments (Ellenbroek and Youn, 2016; Francis et al., 2014). The most common cognitive test used to investigate spatial memory, the Morris Water Maze (MWM), was specifically developed to capitalize on instinctual behaviors found in rats (Morris, 1984; Whishaw and Tomie, 1995), not mice. As rats are well adapted to swimming (a task generally believed to be aversive to mice; Hölscher, 1999; Whishaw, 1995), it is not surprising that rats consistently outperform mice when tested in the MWM (Whishaw and Tomie, 1995), even when tank size has been accounted for (Frick et al., 2000). Non-performance and stress can additionally complicate result interpretation, as failure to complete the task may not necessarily reflect a lack of learning or memory retention (Hölscher, 1999; Koopmans et al., 2003; Francis et al., 1995; Wahlsten et al., 2003). In keeping with this, a study by Harrison et al. (2009) found that not only did mice tested on the MWM exhibit significantly greater plasma corticosterone levels than mice tested using a non-swimming-based spatial memory test, but there was also an inverse correlation between corticosterone levels and latency to escape the maze.

In TBI studies utilizing swim-based testing protocols, the complexities of carrying out cognitive testing are further compounded by the sensorimotor deficits often induced by brain injury. The results of repeated forced swimming trials using the MWM and similar swimming based tests are potentially compromised by the pronounced motor deficits exhibited post-TBI (Brody and Holtzman, 2006; Fox et al., 1999, 1998a,b). Even studies employing mild CCI have been shown to produce pronounced sensorimotor deficits in mice (Fox et al., 1998b; Yu et al., 2009). Cognitive tests which are intrinsically physically demanding can make it challenging to distinguish the effect of sensorimotor changes from changes in cognitive and/or mnemonic information processing capabilities. Such effects can potentially be minimized, however, by reducing the physical strain required in cognitive tests when testing models in which motor deficits are present.

The purpose of the current study was to evaluate the ability of a Radial Water Tread (RWT) maze spatial memory test to effectively differentiate between Sham and CCI induced TBI in a mouse model. The apparatus was designed as a mouse-specific alternative to the MWM, which capitalizes on instinctual and naturalistic behavior of mice, and does not require swimming. This RWT maze has previously been used successfully to study aged mice (Pettan-Brewer et al., 2013; Wiley et al., 2011; Enns et al., 2009), in which stress-induced test-failure has been frequently cited as a confounding factor. Currently, however, the RWT maze task has not been evaluated in a murine model of TBI.

Briefly, the RWT maze capitalizes on the natural tendency of mice to avoid open areas in favor of hugging the edges of an apparatus (thigmotaxis), and replaces the standard forced-swim paradigm of the MWM with water shallow enough that animals are not required to swim (Fig. 1A-B). The water is cold (12–14 °C) and thus is mildly aversive, providing sufficient motivation for the mice to escape the maze.

As proof of concept that measurable motor changes were present following the relatively mild CCI employed in this study, and thus validate the use of a less physically demanding cognitive task, mice were additionally assessed for the presence of sensorimotor deficits. Detailed gait analysis was conducted using the Noldus Catwalk, and contralateral limb impairment was assessed with the Gridwalk test. Both the Catwalk (Neumann et al., 2009; Cross et al., 2015) and Gridwalk (Baskin et al., 2003; Onyszchuk et al., 2007) have been used previously to successfully assess sensorimotor deficits following TBI in mice.

We hypothesized that the RWT maze would effectively distinguish between Sham and TBI models. Such a result would validate the use of this apparatus as a test of spatial memory in TBI mouse model studies employing a CCI model, and could offer a novel alternative to physically demanding swimming-based cognitive tests for TBI studies.

2. Results

2.1. Radial Water Tread maze

Maze testing consisted of 3 trials per day (maximum 180 s per trial) for 4 days (acquisition period). A 3 trial short term memory test was conducted on day 5, and long term memory test on day 12. No testing took place between days 5 and day 12. Groups were compared by standard *t*-test with data presented as mean ± standard deviation.

2.2. Early time point (11 days post-injury) – Fig. 2A

Two subjects (one Sham and one TBI) were deemed to be unmotivated by the conditions of the RWT maze, and were removed from analysis, resulting in a total of 7 Sham and 8 TBI subjects analyzed.

Data analysis revealed the Sham group displayed a significantly reduced latency to escape the maze compared to the TBI group during the short term memory test (day 5; 20.81 ± 14.68 vs. 48.58 ± 37.66, $t[13] = 1.8266$, $p = 0.045$) and long term memory test (day 12; 10.86 ± 8.10 vs. 70.54 ± 57.79, $t[13] = 2.6969$, $p = 0.0091$). There was also a significant group effect during the initial acquisition period (days 1–4), as determined by repeated measure analysis of variance ($F[1,13] = 16.823$, $p = 0.001$).

2.3. Late time point (35 days post-injury) – Fig. 2B

The Sham group had a significantly reduced latency to escape the maze compared to the TBI group 35 days post-injury on the long term memory test only (day 12; 19.89 ± 20.10 vs. 61.06 ± 31.96, $t[10] = 2.6707$, $p = 0.0117$). There was no statistically significant difference between the TBI and Sham controls on the short term memory test ($t[10] = 1.3053$, $p = 0.1105$), and no significant group effect during the acquisition period ($F[1,10] = 0.643$, $p = 0.441$).

2.4. CatWalk gait analysis differentiates between Sham and TBI mice on a variety of parameters 1 day, but not 32 days, post-injury – Fig. 3 (A–D)

Mice were assessed for sensorimotor deficits in order to confirm the CCI-induced brain insult resulted in quantifiable motor impairment. TBI and Sham controls were compared by standard *t*-test with data presented as mean ± standard deviation.

Details of the individual catwalk parameters are indicated in experimental procedures.