Published in final edited form as:

Brain Res. 2017 February 15; 1657: 140–147. doi:10.1016/j.brainres.2016.11.027.

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

Marcella M. Cline^a, Josh C. Yumul^a, Lisa Hysa^a, Dalia Murra^a, Gregory G. Garwin^{a,c}, David G. Cook^{d,e}, Warren C. Ladiges^b, Satoshi Minoshima^{a,c}, and Donna J. Cross^{a,c,*}

^aDepartment of Radiology, University of Washington, Seattle, WA, USA

^bDepartment of Comparative Medicine, University of Washington, Seattle, WA, USA

^cDepartment of Radiology, University of Utah, Salt Lake City, UT, USA

^dDepartment of Pharmacology, University of Washington, Seattle, WA, USA

^eGeriatric Research Education and Clinical Center (GRECC), VA Puget Sound Health Care System, Seattle, WA, USA

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.

*Corresponding author at: University of Utah, Department of Radiology, 30 N. 1900 E. #1A71, Salt Lake City, UT 84132-2140, USA. donna.cross@hsc.utah.edu (D.J. Cross).

Declaration of interest

The authors declare no conflicts of interest.

Keywords

Traumatic brain injury; Cognitive testing; Behavioral testing; Water maze; Controlled-cortical impact

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 sequelae 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 genetically modified mice are available for study; (ii) murine back-ground 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 $\pm$ 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 $\pm$ standard deviation.

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

2.5. 1 day post-injury

The Sham group scored 17% higher than the TBI group on the parameter of cadence (12.31 ± 2.93 vs. 10.22 ± 0.91 , $t[27] = 2.6366$, $p = 0.007$), and 26% higher on the parameter of average speed (20.49 ± 7.2 cm/s vs. 15.25 ± 2.59 , $t[27] = 2.6446$, $p = 0.0065$).

Swing speed was significantly higher in the Sham group for the right front (RF) paw (14% increase, 56.03 ± 12.78 cm/s vs. 48.15 ± 6.34 , $t[27] = 2.1238$, $p = 0.0215$) right hind (RH) paw (28% increase, 61.10 ± 9.95 vs. 43.95 ± 9.64 , $t[27] = 4.7121$, $p < 0.001$), and left front (LF) paw (18% increase, 56.67 ± 13.23 vs. 45.90 ± 5.84 , $t[27] = 2.8707$, $p = 0.004$), though

not for the left hind (LH) paw (11% increase, $59.19 \pm 12.08 \pm 52.51 \pm 9.93$, $t[27] = 1.6312$, $p = 0.057$).

Swing was reduced by 24% in the Sham group for the RH only (0.1072 ± 0.0140 vs. 0.1330 ± 0.0321 , $t[27] = 2.7769$, $p = 0.005$). No significance was found for any other paw (RF: 17% decrease, 0.1280 ± 0.0250 vs. 0.1494 ± 0.0580 , $t[27] = 1.2751$, $p = 0.1065$; LF: 14% decrease, 0.1306 ± 0.0356 vs. 0.1495 ± 0.03563 , $t[27] = 1.4984$, $p = 0.073$; LH: 8% decrease, 0.1055 ± 0.0135 vs. 0.1139 ± 0.0142 , $t[27] = 1.6179$, $p = 0.0585$).

2.6. 32 days post-injury

At 32 days post-injury the CCI-treated mice did not differ significantly from Sham controls on any of the motor performance measures, with the sole exception of Swing in the RH paw, which remained 12% lower in the Sham group (0.09092 ± 0.0131 vs. 0.10153 ± 0.01748 , $t[27] = 1.8768$, $p = 0.0355$).

2.7. Gridwalk test – Fig. 4

To assess contralateral limb impairment animals were given gridwalk testing at 1 day and 32 days post-injury. Briefly, this apparatus is an elevated grid on which the mouse is placed and allowed to roam freely, while the number of foot-faults (defined as the animal attempting to place weight on a foot falling between the wire grids) committed by ipsi- and contralateral limbs are recorded. Results were analyzed by standard *t*-test and data presented as mean $\pm$ standard deviation.

Gridwalk testing revealed statistically significant group differences in percentage of contralateral foot faults on both 1 day post-injury (75.15 ± 17.42 vs. 88.12 ± 11.09 , for Sham and TBI respectively, $t[27] = 2.4076$, $p = 0.0116$), and 32 days post-injury (60.07 ± 17.59 vs. 72.69 ± 14.16 , $t[27] = 2.1361$, $p = 0.0209$).

3. Discussion

To our knowledge, this is the first study to assess cognitive deficits in a mouse model of TBI using the Radial Water Tread maze. The results reported here support the use of this apparatus as a species-appropriate alternative to swim-based cognitive tests in mouse models of TBI, but suggest that the post-injury timing of cognitive assessment might play a role in maximizing effect size.

The RWT maze protocol employed by this study required relatively minimal testing; necessitating just four days of training, a short term memory test (STM) on day five and a long term memory test (LTM) on day 12. The results indicate that CCI significantly reduced latency to escape the RWT maze, and though this result was true of both time points that were investigated, the effect was far more pronounced at the earlier (11 days post-injury) time point. Indeed, when cognitive assessment was delayed until 35 days post-injury, the RWT maze was only able to successfully distinguish between Sham and TBI models on the LTM, but not STM, testing day. Whether this is representative of the time-dependent nature of measurable deficits associated with mild TBI injuries, or simply due to the relatively small sample size, is unclear.

Though previous research using the RWT maze was successful in assessing age-related cognitive decline in multiple transgenic mouse strains (Pettan-Brewer et al., 2013; Wiley et al., 2011; Enns et al., 2009), the current study is the first to apply this cognitive test in a model of traumatic brain injury. While this makes direct comparison to previous studies more challenging, the results presented here are consistent with those reported using the MWM and circular Barnes maze to test similar injury models. The MWM has been shown to distinguish between Sham and TBI mice as little as 2 days post-injury (Smith et al., 1991, 1995); however, this testing protocol required extensive maze training prior to injury. A testing protocol employed by Fox et al. (1998b), which more closely mirrors that reported here, successfully used the MWM to distinguish between Sham and CCI induced TBI mice 7–10 days postinjury.

The circular Barnes maze appears to offer a viable alternative to swimming-based cognition testing in mouse models of TBI. The Barnes is similar to the RWT maze described here in that it capitalizes on the instinctual thigmotaxis behavior found in mice, and does not require swimming. Like the MWM, however, the Barnes was originally designed for rat models (Barnes, 1979), and only adapted for mouse use much later (Bach et al., 1995). While the Barnes maze has been used successfully in mouse models of TBI (Fox et al., 1998a), it has not been without criticism. Mice have been known to express hesitation rather than immediately entering Barnes maze escape holes (which are positioned in the floor of the apparatus), even going so far as to resist exiting in order to further explore the apparatus (Koopmans et al., 2003). Therefore, increased latency to escape can potentially reflect exploratory behavior rather than memory deficits. Researchers have also reported that increased negative stimuli (such as bright lights or fan) are often necessary to motivate escape behavior in mice (Pompl et al., 1999). The RWT maze evaluated in the current study offers a novel hybrid of these two commonly used tests by appealing both to mouse instinctual behavior, and including a stimulus aversive enough to motivate escape (shallow cold water).

As confirmation that the CCI procedure used in this study resulted in measurable sensorimotor dysfunction, two tests of sensorimotor function were conducted; the Gridwalk and Noldus Catwalk. Gridwalk testing revealed significant contralateral foot fault bias in TBI mice both 1 day and 32 days post-injury. Interestingly, despite not receiving any CCI, Sham mice displayed slight contralateral limb deficits directly following their Sham surgery, likely as a result of craniotomy associated intracranial pressure changes. Catwalk gait analysis revealed subtle differences between Sham and TBI models on a variety of parameters. Such differences, however, appear to be almost entirely time-dependent, with all but one parameter in which statistically significant differences were observed having spontaneously resolved by 32 days post-injury.

These results deserve particular attention for two reasons. Firstly, they highlight the strong effect even a relatively mild CCI induced TBI can have on acute motor function. Researchers would be well advised to consider the relative degree of physical strain required when choosing behavioral tasks to test such models, as group differences in motor function can potentially confound result interpretation. Secondly, the resolution of nearly all measurable sensorimotor deficits as measured by the Catwalk by 32 days post-injury

presents an interesting finding. This suggests the CCI-induced performance deficits on the RWT maze 35 days postinjury were unlikely due to confounding sensorimotor deficits, but rather represent solely impaired spatial memory (see Fig. 5).

While the current study offers promising data to support the use of the RWT as a species appropriate alternative to forced-swim tests of cognition following CCI induced TBI in mouse models, it is not without limitations. The removal of two subjects from RWT maze analysis due to non-motivation is concerning, though not atypical of maze testing. Non-motivation remains a persistent issue in exploratory testing (Wahlsten et al., 2003). Further optimization, including lighting and water temperature adjustment, might be useful in eliminating such issues in future testing.

Finally, though the results of swim-based cognition testing in research models with inherently impaired or altered motor function are potentially influenced by group dependent motor deficits, it should be stated that the RWT maze is not immune to similar confounding influence. As even mild TBI models can result in measurable motor deficits, as we have seen here, the results of any testing which requires movement have the potential to be influenced by such confounding factors. Researchers can reduce the possibility of confounding motor deficits affecting data interpretation by reducing the physical strain required in such tests, but cannot eliminate the possibility altogether.

4. Conclusions

Effective measurement of cognitive and memory deficits that occur as direct result of traumatic brain injury is of critical importance, both in human and animal models. The most commonly used tests of post-injury cognitive changes in mouse models of TBI, however, were designed specifically to capitalize on instinctual behaviors found in rats, not mice. Forced-swim testing paradigms such as the MWM present even further issues, as swimming-based tests of cognition are potentially confounded by anxiety and sensorimotor deficits inherent to TBI models. This paper presented a possible alternative test of spatial memory and cognition; the Radial Water Tread maze. This apparatus presents a novel alternative to rat-biased cognition tests by capitalizing on mouse instinctual behavior, and not requiring swimming. The RWT maze was able to successfully distinguish between Sham and TBI mice at both early and late time post-injury, supporting the use of this apparatus as a viable alternative to the MWM in mouse models of CCI induced TBI.

5. Experimental procedure

5.1. Subjects

All procedures and animal handling were conducted in accordance with the animal care guidelines issued by the National Institutes of Health and by the University of Washington Animal Care and Use Committee. Twenty-nine male C57BL/6J mice, ten weeks of age, were purchased from The Jackson Laboratory, Bar Harbor, ME. Animals were kept on a 12-h light/12-h dark cycle with *ad libitum* access to food and water, and were randomly assigned to one of two groups (*Sham* = 14, *TBI* = 15). Following surgery, all animals were housed individually.

5.2. Surgery

Animals were anesthetized with isoflurane (1.5–2.5% in flowing O₂) during surgery and core temperature was maintained at 37 °C via electronic heating pad. The animal was secured in a stereotaxic frame, hair removed from the scalp with topical hair removal cream (Nair), and the skin scrubbed with alternating washes of Betadine and isopropyl alcohol. An interscapular subcutaneous injection of 1.0 ml isotonic saline was given to prevent dehydration during surgery. Lidocaine and Bupivacaine (1 mg/kg) were injected subcutaneously into the scalp and a midline incision was made. Following retraction of fascia and skin, a 5 mm craniotomy was performed with a bone drill 2 mm to the right of the midline, midway between lambda and bregma. Through this opening the animal was subjected to a controlled cortical impact using a solenoid-driven cortical impactor with a 3 mm convex tip (Leica Biosystems, Richmond Illinois). The impact injury was generated using the following parameters: 6 m/s strike velocity, 1 mm depth of penetration, and 200 ms contact time. Immediately following impact, the craniotomy was covered with a sterile polypropylene disc cemented to derma with VetBond and the scalp was closed with sutures. The animal was removed from anesthesia and given a subcutaneous injection of Buprenorphine (0.5 mg/kg). Sham controls received a craniotomy, but no impact injury.

5.3. Cognitive testing

5.3.1. Radial Water Tread maze (Fig. 1A & B)—Water maze testing was conducted at two time points; 11 days (early time point) and 35 days (late time point) post-injury. As the RWT maze is a test of memory, it was undesirable to test the same animals at multiple time points, and Sham(n = 14) and TBI (n = 15) animals were further divided into early-time point testing (Sham n = 8, TBI = 9) and late-time point testing (Sham = 6, TBI = 6). Group differences in short term and long term memory days were compared using students *t*-test, and repeated measures analysis of variance was used to detect group differences during the initial four day acquisition period.

The RWT maze consists of a 32-inch galvanized steel tub, with nine holes placed at equal intervals around the device, roughly 1 ½ inch above the apparatus floor. Of these nine holes, eight terminate after roughly one inch (decoy exits), and one leads to a safety box hidden behind a 90-degree angle bend (to prevent visual confirmation of escape route). The safety box consists of a dark plastic container (30" L × 15" W × 15" H) heated via electronic heating pad. Five large unique visual cues line the sides of the apparatus.

Testing was conducted in a brightly lit room. Before each mouse was tested, the maze was sanitized and sprayed with 70% ethanol, and filled with an inch of cold water (12–14 °C). Water was changed between animals, and temperature was monitored during filling to ensure it was within the desired range. The testing protocol was as follows; once the maze was prepped, the mouse was introduced to the center of the maze, and given 180 s to find the escape tunnel leading to the safety box. If the mouse was unable to find the exit within the time period, that trial was recorded as 180 s and they were gently guided by hand to the exit. Once inside the safety box, the mouse was rewarded with a food treat and allowed to remain in the box for 60 s, after which they were removed and the safety box and maze were

sprayed with 70% ethanol to prevent olfactory cues. This testing protocol was repeated for a total of three trials per day for each mouse.

If a mouse attempted to enter a decoy hole and did not voluntarily re-enter the maze after five seconds, they were picked up and placed back in the center of the maze. If a mouse failed to complete the maze in under 180 s during any two-day period, the mouse was considered to be insufficiently motivated by the testing conditions, and was removed from analysis.

Mice were given three trials a day for four days (acquisition period). On the fifth day, mice were given a short-term memory test consisting of three trials. Mice were then unexposed to the maze until day-12 (one week after their short-term memory test), at which point they were tested for a final three trials (long-term memory test).

Hypothermia has previously been identified as a potential confounding factor in water maze testing requiring cold-water (Iivonen et al., 2003), and to address this potential issue temperature data was acquired from 5 male C57BL/6J mice, 13 weeks of age, two-hours prior to, and immediately following, RWT maze testing. Mice exhibited an average decline in temperature of 1.8 °C following maze testing, and showed no discernable signs of hypothermia.

5.4. Sensorimotor testing

5.4.1. Gait analysis—Detailed gait analysis was acquired using the CatWalk automated gait analysis system (Noldus Information Technology, Wageningen, The Netherlands) 1 day and 32 days post-injury, and TBI and Sham groups were compared by standard *t*-test. The testing device consists of a 1.3-m-long glass plate with dim fluorescent light beamed into the glass. Under low overhead lighting, the fluorescent light is reflected downward allowing images of the footprints to be recorded by a camera under the walkway as the mouse's paws came in contact with the glass surface. Mice performed 3 consecutive runs through the apparatus. Images from each trial were converted into digital signals and processed with a threshold of 179 arbitrary units (au). Following the identification and labeling of each footprint, gait data were generated for the following parameters:

- Swing (s): the duration in seconds of no contact of a paw with the glass plate
- Swing Speed (cm/s): the speed of the paw during swing
- Cadence: number of steps per second
- Average Speed (cm/s): mean speed traveled across the glass plate

5.5. Gridwalk test

To assess contralateral limb impairment animals were given gridwalk testing 1 day and 32 days post-injury (same time points as those used in Catwalk gait analysis). Animals were placed on an elevated grid surface (30 L × 35 W × 31 H cm with grid spacing of 2.5 cm), and the number of foot-faults (defined as the animal attempting to place weight on a foot falling between the wire grids) committed by ipsi- and contralateral limbs were recorded using a video camera, and later counted by a researcher blind to treatment groups. Each

assessment consisted of three trials lasting 1 min each with an inter-trial interval of 1 min. The following equation was used to calculate the percentage of contralateral limb bias; $([\text{Right foot faults}/\text{total foot faults}] \times 100)$, and groups were compared using standard *t*-test.

5.6. Statistical methods

Data were analyzed using standard *t*-test or repeated measures ANOVA where indicated. *T*-tests were one-tailed, based on a *priori* hypothesis of group differences. All calculations were performed using SPSS v. 24 (IBM Corporation, Armonk, NY, USA), with significance defined as $p > 0.05$.

Acknowledgements

This research was supported by the Institute for Translational Health Sciences pilot project grant opportunity (UL1TR000423), the University of Washington Center on Human Development and Disability, University of Washington Animal Behavior Core and Brain Imaging Core, the Department of Veterans Affairs Office of Research and Development Medical Research Service (DGC), University of Washington Friends of Alzheimer's Research (DGC), and the UW Royalty Research Fund (DGC). We also thank Toby Cole for his assistance with this project.

Abbreviations:

TBI	traumatic brain injury
MWM	Morris Water Maze
RWT	Radial Water Tread
CCI	controlled-cortical impact

References

- Bach M, Hawkins R, Osman M, Kandel E, Mayford M, 1995 Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal ltp in the range of the θ frequency. *Cell* 81, 905–915. [PubMed: 7781067]
- Barnes C, 1979 Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Compar. Physiol. Psychol* 93, 74–104.
- Baskin Y, Dietrich W, Green E, 2003 Two effective behavioral tasks for evaluating sensorimotor dysfunction following traumatic brain injury in mice. *J. Neurosci. Methods* 129 (1), 87–93. [PubMed: 12951236]
- Brody D, Holtzman D, 2006 Morris water maze search strategy analysis in PDAPP mice before and after experimental traumatic brain injury. *Exp. Neurol* 197 (2), 330–340. [PubMed: 16309676]
- Cross DJ, Garwin GG, Cline MM, Richards TL, Yarnykh V, Mourad PD, Minoshima S, 2015 Paclitaxel improves outcome from traumatic brain injury. *Brain Res.* 1618, 299–308. 10.1016/j.brainres.2015.06.006. [PubMed: 26086366]
- Ellenbroek B, Youn J, 2016 Rodent models in neuroscience research: is it a rat race? *Dis. Model. Mech* 9 (10), 1079–1087. 10.1242/dmm.026120. [PubMed: 27736744]
- Enns L, Morton J, Treuting P, Emond M, Wolf N, Mcknight G, Ellipsis Vella A, 2009 Disruption of protein kinase A in mice enhances healthy aging. *PLoS One* 4 (6), E5963. [PubMed: 19536287]
- Faul M, Xu L, Wald MM, Coronado VG, 2010 Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006 Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Atlanta (GA).
- Fox G, Fan L, Levasseur R, Faden A, 1998a Effect of traumatic brain injury on mouse spatial and nonspatial learning in the barnes circular maze. *J. Neurotrauma* 15 (12), 1037–1046. [PubMed: 9872460]

- Fox G, Fan L, Levasseur R, Faden A, 1998b Sustained sensory/motor and cognitive deficits with neuronal apoptosis following controlled cortical impact brain injury in the mouse. *J. Neurotrauma* 15 (8), 599–614. [PubMed: 9726259]
- Fox G, Levasseur R, Faden A, 1999 Behavioral responses of C57BL/6, FVB/N, and 129/SvEMS mouse strains to traumatic brain injury: implications for gene targeting approaches to neurotrauma. *J. Neurotrauma* 16 (5), 377–389. [PubMed: 10369558]
- Francis C, Natarajan S, Lee MT, Khaladkar M, Buckley PT, Sul J-Y, Eberwine J, Kim J, 2014 Divergence of RNA localization between rat and mouse neurons reveals the potential for rapid brain evolution. *BMC Genomics* 15, 883. [PubMed: 25301173]
- Francis D, Zaharia M, Shanks N, Anisman H, 1995 Stress-induced disturbances in Morris water-maze performance: interstrain variability. *Physiol. Behav* 58 (1), 57–65. [PubMed: 7667428]
- Frick K, Stillner E, Berger-Sweeney J, 2000 Mice are not little rats: species differences in a one-day water maze task. *NeuroReport* 11 (16), 3461–3465. [PubMed: 11095500]
- Harrison F, Hosseini A, McDonald M, 2009 Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. *Behav. Brain Res* 198 (1), 247–251. [PubMed: 18996418]
- Hölscher C, 1999 Stress impairs performance in spatial water maze learning tasks. *Behav. Brain Res* 100 (1–2), 225–235. [PubMed: 10212070]
- Iivonen H, Nurminen L, Harri M, Tanila H, Puoliväli J, 2003 Hypothermia in mice tested in Morris water maze. *Behav. Brain Res* 141 (2), 207–213. [http://dx.doi.org/10.1016/S0166-4328\(02\)00369-8](http://dx.doi.org/10.1016/S0166-4328(02)00369-8). [PubMed: 12742257]
- Koopmans G, Blokland A, Vannieuwenhuijzen P, Prickaerts J, 2003 Assessment of spatial learning abilities of mice in a new circular maze. *Physiol. Behav* 79, 683–693. [PubMed: 12954410]
- Levin H, 1995 Neurobehavioral outcome of closed head injury: implications for clinical trials. *J. Neurotrauma* 12 (4), 601–610. [PubMed: 8683611]
- Morris R, 1984 Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11 (1), 47–60. [PubMed: 6471907]
- Neumann M, Wang Y, Kim S, Hong S, Jeng L, Bilgen M, Liu J, 2009 Assessing gait impairment following experimental traumatic brain injury in mice. *J. Neurosci. Methods* 176, 34–44. [PubMed: 18805438]
- Onyszczuk G, Al-Hafez B, He Y, Bilgen M, Berman N, Brooks W, 2007 A mouse model of sensorimotor controlled cortical impact: characterization using longitudinal magnetic resonance imaging, behavioral assessments and histology. *J. Neurosci. Methods* 160 (2), 187–196. [PubMed: 17049995]
- Pettan-Brewer C, Touch D, Wiley J, Hopkins H, Rabinovitch P, Ladiges W, 2013 A novel radial water tread maze tracks age-related cognitive decline in mice. *Pathobiol. Aging Age-related Dis* 3, 1–4.
- Pompl P, Mullan M, Bjugstad K, Arendash G, 1999 Adaptation of the circular platform spatial memory task for mice: use in detecting cognitive impairment in the APPSW transgenic mouse model for Alzheimer's disease. *J. Neurosci. Methods* 87 (1), 87–95. [PubMed: 10065997]
- Schretlen D, Shapiro A, 2003 A quantitative review of the effects of traumatic brain injury on cognitive functioning. *Int. Rev. Psychiatry* 15, 341–349. [PubMed: 15276955]
- Smith D, Okiyama K, Thomas M, Claussen B, McIntosh T, 1991 Evaluation of memory dysfunction following experimental brain injury using the morris water maze. *J. Neurotrauma* 8 (4), 259–269. [PubMed: 1803034]
- Smith D, Soares H, Pierce J, Perlman K, Saatman K, Meaney D, McIntosh T, 1995 A model of parasagittal controlled cortical impact in the mouse: cognitive and histopathologic effects. *J. Neurotraum* 12 (2), 169–178.
- Wahlsten D, Rustay N, Metten P, Crabbe J, 2003 In search of a better mouse test. *Trends Neurosci* 26, 132–136. [PubMed: 12591215]
- Wiley J, Pettan-Brewer C, Ladiges W, 2011 Phenylbutyric acid reduces amyloid plaques and rescues cognitive behavior in AD transgenic mice. *Aging Cell* 10 (3), 418–428. [PubMed: 21272191]
- Whishaw I, 1995 A comparison of rats and mice in a swimming pool place task and matching to place task: some surprising differences. *Physiol. Behav* 58, 687–693. 4. [PubMed: 8559777]
- Whishaw I, Tomie J, 1995 Of mice and mazes: similarities between mice and rats on dry land but not water mazes. *Physiol. Behav* 60 (5), 1191–1197.

Yu S, Kaneko Y, Bae E, Stahl C, Wang Y, Loveren H, Borlongan C, 2009 Severity of controlled cortical impact traumatic brain injury in rats and mice dictates degree of behavioral deficits. *Brain Res*, 157–163 10.1016/j.brainres.2009.06.067.

Fig. 1.

Radial water tread maze (A) and schematic (B). The maze consists of a 32-inch galvanized steel tub with nine exits, each 1 ½ inch above the apparatus floor. Of these exits, eight terminate after roughly 1 in. (decoy exits), and one leads to a heated safety box hidden behind a 90 degree angle bend to prevent visual confirmation of escape route. Upon reaching the safety box, subjects received a food incentive and 1-min rest. The apparatus is filled with one inch of cold water (12–14 °C) to motivate escape behavior, and lined with five unique visual cues for spatial orientation.

Fig. 2.

Radial Water Tread Maze successfully distinguishes between Sham and TBI mice at both early and late time points. (A) 11 days post-injury Sham mice had significantly reduced latency (time to complete maze in seconds) than TBI mice on days 5 and 12. Significant group differences were also apparent during the initial acquisition period consisting of testing days 1 through 4 (B) 35 days post-injury Sham mice had significantly reduced latency (time to complete maze in seconds) than TBI mice on day 12 only. There were no group differences during the acquisition period. Data points represent group means ($\pm$ SEM). Significance is reported as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Fig. 3.

CatWalk Gait Analysis differentiates between Sham and TBI mice on a variety of parameters 1 day, but not 32 days, post-injury. Bars represent group means ($\pm$ SEM). Significance is reported as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). (A) Cadence, (B) Average Speed (cm/s), (C) Swing (s), (D) Swing Speed (cm/s). RF: right front paw, RH: right hind paw, LF: left front paw, LH: left hind paw.

Fig. 4. Significant contralateral foot fault bias in TBI compared to Sham mice 1 and 32 days post-injury. Bars represent group means ($\pm$ SEM). Significance is reported as $p < 0.05$ (*).

Fig. 5.

Cognitive deficits in long term memory retention are detectable by Radial Water Tread maze at both early and late time points following TBI, while CCI-induced sensorimotor deficits measured by Catwalk Gait Analysis returned to control levels by 32 days post-injury. (A) RWT maze long term test (day 12) at 11 and 35 days post-injury, and (B) Average speed measured by Catwalk Gait Analysis at 1 and 32 days post-injury. Data points represent group means ($\pm$ SEM). Significance is reported as $p < 0.05$ (*) and $p < 0.01$ (**).