J Neurosurg 121:1342–1350, 2014

Chronic gliosis and behavioral deficits in mice following repetitive mild traumatic brain injury

Laboratory investigation

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Object. With the recent increasing interest in outcomes after repetitive mild traumatic brain injury (rmTBI; e.g., sports concussions), several models of rmTBI have been established. Characterizing these models in terms of behavioral and histopathological outcomes is vital to assess their clinical translatability. The purpose of this study is to provide an in-depth behavioral and histopathological phenotype of a clinically relevant model of rmTBI.

Methods. The authors used a previously published weight-drop model of rmTBI (7 injuries in 9 days) in 2- to 3-month-old mice that produces cognitive deficits without persistent loss of consciousness, seizures, gross structural imaging findings, or microscopic evidence of structural brain damage. Injured and sham-injured (anesthesia only) mice were subjected to a battery of behavioral testing, including tests of balance (rotarod), spatial memory (Morris water maze), anxiety (open field plus maze), and exploratory behavior (hole-board test). After behavioral testing, brains were assessed for histopathological outcomes, including brain volume and microglial and astrocyte immunolabeling.

Results. Compared with sham-injured mice, mice subjected to rmTBI showed increased exploratory behavior and had impaired balance and worse spatial memory that persisted up to 3 months after injury. Long-term behavioral deficits were associated with chronic increased astrocytosis and microgliosis but no volume changes.

Conclusions. The authors demonstrate that their rmTBI model results in a characteristic behavioral phenotype that correlates with the clinical syndrome of concussion and repetitive concussion. This model offers a platform from which to study therapeutic interventions for rmTBI.
(http://thejns.org/doi/abs/10.3171/2014.7.JNS14272)

Key Words • **closed head injury** • **concussion** • **traumatic brain injury** • **behavior** • **gliosis**

Repetitive mild traumatic brain injury (rmTBI) is a significant public health problem, with as many as 25% of nonprofessional athletes in sports such as soccer, football, and cheerleading reporting multiple concussions.[5,27] In recent years, there has been increasing media attention and scientific inquiry into the long-term chronic effects of rmTBI. Clinically, rmTBI has been associated with long-term neurological impairment, including memory disturbances, parkinsonism, behavioral abnormalities, personality changes, speech irregularities, and gait abnormalities.[3,8,11,26,36] Gross pathological changes of rmTBI have also been reported, including long-term persistent brain volume loss, as well as histological changes, including tau-immunoreactive neurofibrillary tangles, the hallmark of chronic traumatic encephalopathy (CTE),[30] and amyloid beta (Aβ) deposition, the hallmark of Alzheimer's disease.[11,41] Given that the majority of these clinical and pathological reports have occurred in the setting of retrospective case series of professional athletes and military veterans, it is unclear whether any

Abbreviations used in this paper: CTE = chronic traumatic encephalopathy; LOC = loss of consciousness; MWM = Morris water maze; rmTBI = repetitive mild traumatic brain injury; ROI = region of interest.

* Dr. Mannix and Ms. J. Berglass contributed equally to this work.

This article contains some figures that are displayed in color online but in black-and-white in the print edition.

particular set of pathological or neurobehavioral changes can be attributed solely to repetitive head trauma and what role other potential contributing factors may play.[38]

The development of preclinical models of rmTBI offers the opportunity to investigate the neurobehavioral and pathological effects of rmTBI by studying the injury under controlled conditions. We have previously reported an rmTBI model with persistent Morris water maze (MWM) deficits in the absence of gross ultrastructural changes such as skull fracture, contusion, or intracranial bleeding.[32] Other models of rmTBI have suggested that deficits in balance and coordination, increased locomotor activity, and impaired cognitive function occur.[19,35] Each of these experimental models has used different modes of delivering the rmTBI injury, sometimes associated with prolonged loss of consciousness (LOC)[18,19,35] as well as high rates of associated skull fractures.[18] Most rmTBIs occurring in sports, however, are not associated with LOC or gross structural or bony injuries.[31] Thus, the clinical relevance of these models to sport-related brain injury may be limited. In addition, most studies, including our previous work, have used a relatively small number of behavioral tests, leaving the full effects of rmTBI only partially characterized.

In the present study, we assessed the performance of mice subjected to rmTBI on a variety of behavioral tasks assessing learning, memory, balance, behavior, and sensorimotor function. After behavioral testing, we evaluated histopathological outcomes, including brain volume, astrocytosis, and microgliosis, using stereological techniques to assess GFAP and Iba1 load. This approach provides a more complete characterization of the phenotype of our rmTBI model and offers a clinically relevant platform to study histological outcomes, molecular mechanisms, and therapeutic interventions.

Methods

All experiments were approved by the Boston Children's Hospital institutional animal care and use committee and complied with the NIH *Guide for the Care and Use of Laboratory Animals*. Male C57BL/6 mice were obtained from Jackson Laboratories.

Repetitive Mild TBI

The mouse rmTBI model was used as previously described.[32] Briefly, male mice (2–3 months old) were anesthetized for 45 seconds using 3% isoflurane in a 70:30 mixture of oxygen. Anesthetized mice were placed on a delicate task wiper (Kimwipe, Kimberly-Clark Corp.) and positioned such that the head was placed directly under a hollow guide tube 28 inches in length. The mice were grasped by the tail. A 54-g metal bolt was used to deliver an impact to the dorsal aspect of the skull, resulting in a rotational acceleration of the head through the Kimwipe.

Mice were randomized to undergo injury (n = 32) or sham injury (n = 21). Injured mice underwent 7 concussive injuries over 9 days. Sham-injured mice underwent anesthesia but not concussive injury. All mice recovered in room air. Loss of consciousness was defined as the time from removal of anesthesia to spontaneous righting. Anesthesia exposure for each mouse was strictly controlled to 45 seconds. LOC times reflected the effects of anesthesia as well as the effects of rmTBI.

For all behavioral testing, experimenters were blinded to injury status, using color coding stored in a password-protected computer.

Assessment of Motor Function

Motor ability and function were assessed on Days 1–3 and again at 3 months after the last injury using a rotarod. The rotarod test has been described previously.[23] In brief, the rotarod consists of a rotating drum, 4 cm in diameter, that completes 4 revolutions per minute, on which a test mouse is placed. The time(s) between placement on the rotarod and fall off from the rotarod was recorded as a measure of motor function. The first day of rotarod comprised training. During training mice learned to walk on the rotating rod for 5 minutes. If mice fell off the rod during training, they were placed back on the rod without interruption of the rotations. The 2nd and 3rd days comprised testing. On testing days, mice were placed on the rod at 4 rpm for 10 seconds to acclimate to the rod speed. After the 10-second acclimation period, the rod accelerated at 0.1 rpm/sec. Each mouse completed 4 trials on the testing days with a minimum of 5 minutes rest between each trial.

Assessment of Spatial Learning and Memory

Spatial learning and memory were assessed using a Morris water maze (MWM) paradigm on Days 6–9 and again at 3 months after injury as previously described.[33] A white pool (83-cm diameter, 60 cm deep) was filled with water to a 29-cm depth. Water temperature was maintained at approximately 24°C. Several highly visible intra- and extramaze cues were located in and around the pool. The target platform (a round, clear, plastic platform 10 cm in diameter) was positioned 1 cm below the surface of the water. During hidden and visible platform trials, the mice were randomized to 1 of 4 starting quadrants. Mice were placed in the tank facing the wall and were given 90 seconds to find the platform, mount the platform, and remain on it for 5 seconds. The mice were then placed under a heat lamp to dry before their next run. The time until the mouse mounted the platform (escape latency) was measured and recorded. Mice that failed to mount the platform within the allotted time (90 seconds) were guided to the platform by the experimenter and were allowed 10 seconds to become acquainted with its location. Each mouse was subjected to a maximum of 2 trials per day, each consisting of 4 runs, with a 45-minute break between trials. For visible platform trials, a red reflector was used to mark the top of the target platform. For probe trials, mice were placed in the tank with the platform removed and were given 60 seconds to explore the tank. Noldus Ethovision 9 software tracked swim speed, total distance moved, and time spent in the target quadrant where the platform was previously located.

Assessment of General Locomotor Activity and Anxiety

The open field test, a longstanding, well-studied paradigm,[42] was used to study locomotor activity and anxiety

in mice confined to a novel arena on Day 15 and again at 3 months after injury. The arena consisted of a 45-cm-diameter opaque, plastic circle with walls 20 cm high. The arena was placed inside a plastic transparent box with a tracking system mounted to the top, and the box was placed in an enclosed chamber to prevent distraction. Each mouse was placed in the same part of the edge of the arena facing the wall to begin its trial. The arena was virtually divided into 3 concentric circular sections: an "inner" circle 20 cm in diameter (area of 314 cm^2); a surrounding "neutral" ring, inner diameter 20 cm wide, outer diameter 40 cm (area of 932 cm^2); and the "outer" ring, inner diameter 40 cm, outer diameter 60 cm (area of 1570 cm^2). Mice were given 10 minutes to explore the arena. Time spent in each of the 3 regions was recorded and assessed for anxiety behavior. Time spent in the inner ring constituted the least anxious behavior, while time spent in the outer ring, by the perimeter of the arena, constituted anxious behavior.

Assessment of Exploratory Activity

Exploratory activity was assessed in the elevated plus maze on Day 17 and again at 3 months after injury. The elevated plus maze consists of 2 open and 2 closed arms (30 × 5 cm) extended out opposite from each other from a central platform (decision zone) to create a plus shape. The entire apparatus is raised 85 cm above the floor (Lafayette Instruments). The mice were placed on the center platform of the maze, facing a closed arm, and were allowed to explore the apparatus for 5 minutes. The maze was cleaned between subjects with a weak ethanol solution and dried. A computer-assisted video-tracking system (Noldus Ethovision) recorded the total time spent in the open center (decision zone) and closed compartments. The percent time spent in open arms is used as a surrogate measure of exploratory behaviors; mice with lower levels of exploratory behaviors spend less time in the open arms.[4]

On Day 21 after injury, the hole-board test was used to assess normal mouse behavior in a novel environment. The apparatus is a Perspex box with a floor and 4 walls (40 cm × 40 cm × 30 cm). A metal floor stands 2 cm above the Perspex floor. The metal floor contains 9 closed holes evenly spaced apart that are 1 cm deep. As the holes are shallow with a closed metal bottom, the mouse cannot escape down or fall through the holes and injure itself. Mice were placed in the box for 30 minutes. Gross (e.g., walking and running) and fine (e.g., active grooming but not moving from one position) motor activity pertaining to location in the box and proximity to the 9 holes was recorded through interruption of infrared beams located in the walls of the arena (for horizontal activity and fine movements) and in the holes to measure exploratory head-dips. The number and accuracy of nose pokes is used as a measure of normal, exploratory behavior.[43]

Immunohistochemistry

Mice were perfused with paraformaldehyde 3 months after injury, and their brains were collected for histopathological examination. Serial 20-μm coronal frozen sections from sham-injured (n = 3) and injured (n = 5) brains were cut on a cryostat (Leica) from the anterior frontal lobes through the posterior extent of the dorsal hippocampus. Every 10th section was collected and mounted on slides. After hydrogen peroxide treatment and incubation in a blocking solution containing 10% normal goat serum, sections were incubated overnight at 4°C in anti-GFAP for astrocytes (dilution 1:500, Dako) and anti-Iba1 for microglia (dilution 1:1000, WAKO) antibodies. The following day, the sections were washed and incubated sequentially with appropriate secondary antibodies, Vectastain (Vector), and diaminobenzadine (DAB), and were mounted with Permount.

Stereological Estimates

All sections were coded prior to analysis, and a Leica microscope with a motorized stage and electronic microcator was used with Stereologer software (Stereology Resource Center) to perform the analyses. Estimates of the number of astrocyte and microglia cells were obtained in the fimbria and CA1 of sham-injured and injured mice using a thin section modification of the optical fractionator method to determine cell load using object area fraction and region point counting.[12,34] Briefly, an operator blinded to treatment quantified the total volume of the fimbria and CA1, using the Calvalieri-point counting method, and the total volume of GFAP and Iba1 (GFAP and Iba1 load, respectively).[34] The fimbria was defined for this analysis as the area of white matter ventral to the stratum lucidum of the hippocampus. The anterior margin of the region of interest (ROI) was congruent with the anterior hippocampal formation, bregma –1.22 mm. The most posterior margin was congruent with the dorsal extent of the medial hippocampus, bregma –2.30 mm. The CA1 was defined as the most dorsal peak of the hippocampus. The medial border of the CA1 was congruent with the dorsal peak of the cingulum, and the lateral border was the lateral border of the molecular layer of the dentate gyrus. The anterior CA1 was defined consistent with bregma –1.94 mm, and the posterior margin was defined consistent with bregma –2.46 mm.

On every 10th section the software superimposed a lattice of regularly spaced plus signs over the ROI, and the ROI was outlined. Then, under high magnification the number of cells within each systematically spaced, unbiased sampling frame was counted. At the completion of the stereological analyses, the samples were decoded, and the mean and standard error of the mean (SEM) of the fimbria and CA1 volumes and the number of astrocyte and microglia cells were calculated. Multiple unbiased sampling frames were counted on an average of 4 sections containing the ROI/group. All coefficients of error (CE) values for the stereological estimates were less than 10%.

Brain Volume Analysis

Slides were stained with hematoxylin (Surgipath) to distinguish pathology. Using the measure tool on ImageJ (version 1.44), a blinded investigator calculated brain and hippocampal volume from serial, equally spaced brain sections.

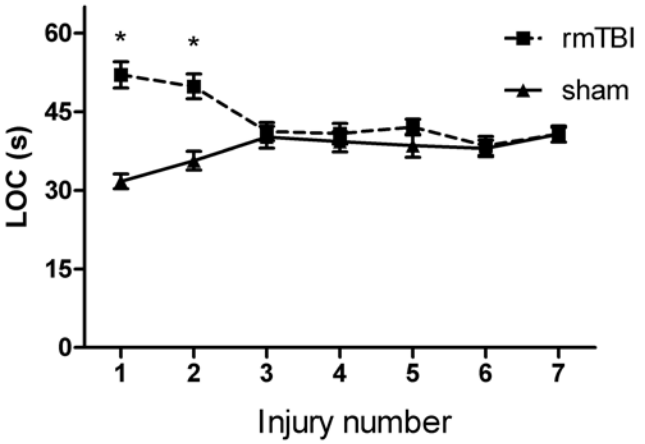

Fig. 1. Loss of consciousness (LOC) in seconds (s) in mice after rmTBI or sham injury, as measured by time to righting reflex after anesthesia. Injured mice had increased LOC compared with sham-injured mice on Days 1 and 2 of injury (*p < 0.05 for both days) but no difference in LOC for the remainder of the injury days (p > 0.1).

Statistical Analyses

Data are presented as the mean ± SEM. Continuous variables were compared between injured and sham-injured mice using the Student t-test. MWM and rotarod latencies were analyzed by repeated-measures ANOVA (group × time). To evaluate the effect of time on performance in each of the behavioral tests, we performed linear regression with time as a covariate, using clustered standard errors to account for repeated measures. Statistical significance was considered at $p < 0.05$. All analyses were performed using Stata (version 11.2, StataCorp).

Results

There were no convulsions after injury, and all mice survived. Injured mice (n = 32) had prolonged LOC compared with sham-injured mice (n = 21) on Day 1 (52.0 ± 2.9 vs 31.7 ± 1.4 seconds, $p < 0.001$) and Day 2 (49.8 ± 2.4 vs 35.7 ± 1.8 seconds, $p < 0.001$) of injury but there were no significant differences on Days 3–7 of injury (Fig. 1).

Assessment of Motor Function

Injured mice (n = 32) showed impaired performance in rotarod testing on Days 1–3 after the last injury, with significantly decreased latency to fall compared with sham-injured mice (n = 21) on Day 1 and Day 2 of testing (Fig. 2 left). Three months after the last injury, injured mice (n = 20) had persistently decreased latency compared with sham-injured mice (n = 13) (Fig. 2 right). There was no time-dependent difference in rotarod performance 1–3 days after injury compared with performance 3 months after injury (p = 0.5).

Assessment of Spatial Learning and Memory

Injured mice (n = 32) performed similarly to sham-injured mice (n = 21) on the first and second runs of the first hidden platform trial (Fig. 3a), but they performed worse overall on hidden platform trials of the MWM 6–9 days after injury (Fig. 3b). Injured mice performed similarly to sham-injured mice on Day 1 of probe trials (25.6 ± 2.7 vs 25.5 ± 2.2 seconds, p = 1.0; data not shown) but demonstrated worse performance on Day 2 (18.3 ± 0.9 vs 23.1 ± 1.2 seconds, p = 0.002).

Three months after the last injury, injured mice (n = 20) performed worse than sham-injured mice (n = 13) (Fig. 3c). Injured mice also performed worse than sham-injured mice on Day 1 of probe trials (19.0 ± 1.4 vs 28.9 ± 1.8 seconds, $p < 0.001$) and on Day 2 (17.1 ± 1.3 vs 27.7. ± 2.3 seconds, $p < 0.001$; data not shown). Compared with spatial memory performance 6–9 days after injury, there was no time-dependent difference in MWM performance 3 months after injury (p = 0.2).

Assessment of General Locomotor Activity and Anxiety

Injured mice (n = 31) showed no significant difference in time spent in the outer zone of the open field compared with sham-injured mice (n = 21) on Day 15 after injury (342.1 ± 14.5 vs 345.3 ± 10.0 seconds, p = 0.9). Similarly, there were no significant differences between injured and sham-injured mice in time spent in the neutral (213.1 ± 10.8 vs 213.1 ± 8.1 seconds, p = 1.0) or inner (41.7 ± 5.3 vs 41.5 ± 3.7 seconds, p = 1.0) zones. Three months after the last injury, injured mice (n = 20) spent less time in

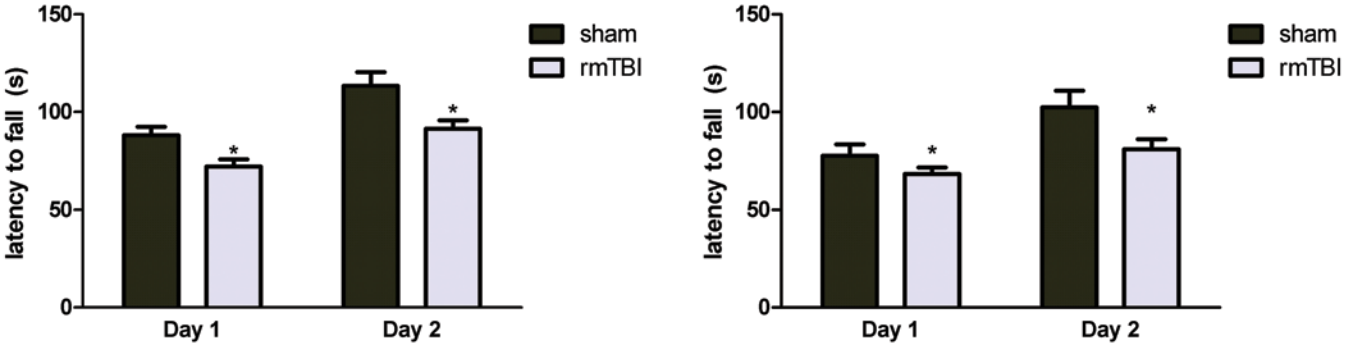

Fig. 2. Rotarod testing after rmTBI. **Left:** Compared with sham-injured mice, injured mice showed decreased latency to fall on rotarod testing on both trials 1–3 days after the last injury (72.1 ± 3.6 vs 88.0 ± 4.5 seconds on Day 1, 91.4 ± 4.3 vs 113.3 ± 7.1 seconds on Day 2, *p < 0.001 for group effect). **Right:** Three months after the last injury, injured mice showed decreased latency to fall on Day 2 of testing (80.8 ± 5.2 vs 102.4 ± 8.5 seconds, *p = 0.03).

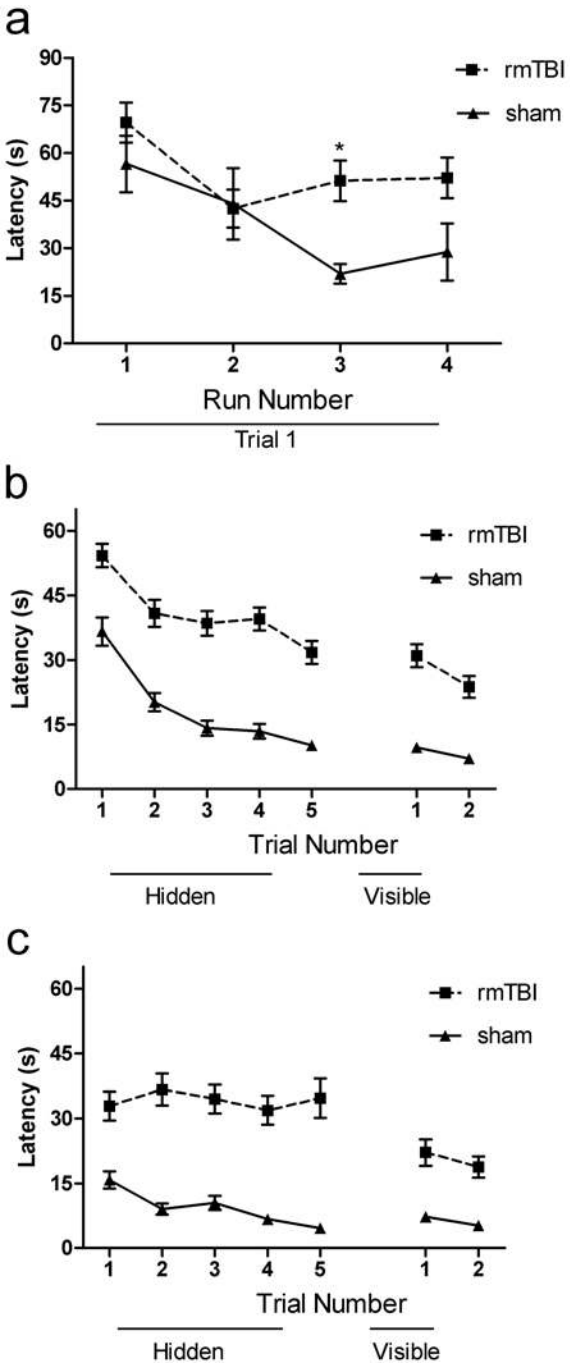

Fig. 3. Morris water maze performance in injured mice that underwent 7 rmTBIs in 9 days versus sham-injured mice. **a:** On the first and second runs of the first hidden trial, injured mice performed similarly to sham-injured mice ($p = 0.3$ and 0.9 for Run 1 and Run 2, respectively). Injured mice performed significantly worse than sham-injured mice on the third (*$p = 0.01$) run and overall on hidden trial 1 ($p = 0.03$). **b:** At Days 6–9 after the last injury, all mice demonstrated time-dependent learning ($p < 0.001$ for time). Injured mice had worse performance on hidden platform than sham-injured mice ($p < 0.001$ on repeated-measures ANOVA) and worse performance on visual platform testing ($p = 0.01$ on repeated measures ANOVA). **c:** At 3 months after the last injury, sham-injured mice ($p < 0.001$) but not injured mice ($p = 0.8$) showed time-dependent learning of the paradigm. Injured mice had worse performance than sham-injured mice on hidden platform and visual platform testing ($p < 0.001$).

the outer zone compared with sham-injured mice (n = 13) (346.1 ± 22.1 vs 419.8 ± 21.6 seconds, $p = 0.03$) and more time in the neutral zone (216.0 ± 19.8 vs 147.4 ± 18.0 seconds, $p = 0.02$), and traveled more total distance (3308.3 ± 137.3 vs 2753.7 ± 108.9 seconds, $p = 0.007$).

Seventeen days after the last injury, injured mice spent significantly more time in the open arms and significantly less time in the closed arm of the plus maze compared with sham controls (Fig. 4 left). There was no difference between injured versus sham-injured mice in distance traveled in the closed arm of the plus maze (1409.9 ± 43.4 vs 1421.1 ± 58.8 cm, $p = 0.9$). Three months later, injured mice (n = 20) continued to spend increased time in the open arm compared with sham-injured mice (n = 13) (Fig. 4 right), although there was no difference in distance traveled between the 2 groups (1139.9 ± 46.3 vs 1095.4 ± 65.2 cm, $p = 0.6$) or change in injured mice performance from 17 days to 3 months after injury ($p = 0.2$).

Assessment of Exploratory Activity

In hole-board testing on Day 21 after injury, injured mice (n = 27) showed increased exploratory activity compared with sham-injured mice (n = 21), as the number of hole pokes was significantly higher in injured mice (Fig. 5). Injured mice also showed increased basic movements compared with sham-injured mice (5748.4 ± 162.6 vs 5234.7 ± 177.9, $p = 0.04$), showing an increase in general horizontal activity, though there were no differences in fine movements between the 2 groups (3127.5 ± 79.9 vs 2891.0 ± 102.9, $p = 0.07$) indicating no change in grooming/stereotypic-like activity.

Stereological Estimates of Microglia and Astrocytes and Assessment of Brain Volume

Three months after injury, stereological estimates showed markedly increased Iba1 immunolabeling in injured versus sham-injured mice in the fimbria ($4.4 \pm 0.2 \times 10^6$ μm^3 vs $2.8 \pm 0.2 \times 10^6$ μm^3, $p = 0.003$) and CA1 (17.1 ± 0.9 vs $11.3 \pm 1.2 \times 10^6$ μm^3, $p = 0.008$) consistent with chronic microgliosis (Fig. 6). Injured mice also demonstrated increased GFAP+ astrocyte immunolabeling in the fimbria ($5.7 \pm 0.2 \times 10^6$ μm^3 vs $4.1 \pm 0.3 \times 10^6$ μm^3, $p = 0.02$) and CA1 compared with sham-injured mice ($11.5 \pm 0.4 \times 10^6$ μm^3 vs $7.9 \pm 0.2 \times 10^6$ μm^3, $p = 0.001$) indicative of chronic reactive astrocytosis (Fig. 6). Assessment of brain and hippocampal volume using Image J revealed no difference between injured and sham-injured mice (145.9 ± 2.0 vs 145.8 ± 5.0 mm^3, $p = 1.0$, and 7.9 ± 0.7 vs 8.6 ± 0.2 mm^3, $p = 0.6$).

Discussion

Here, we report a more complete complement of behavioral and histopathological testing in a previously published model of rmTBI in young adult mice. These data extend our prior reports of a prolonged MWM deficit after rmTBI[25,32] and offer a clinically relevant platform from which future studies can be conducted. We demonstrate acute, subacute, and chronic deficits in balance and spatial memory performance as well as increased explor-

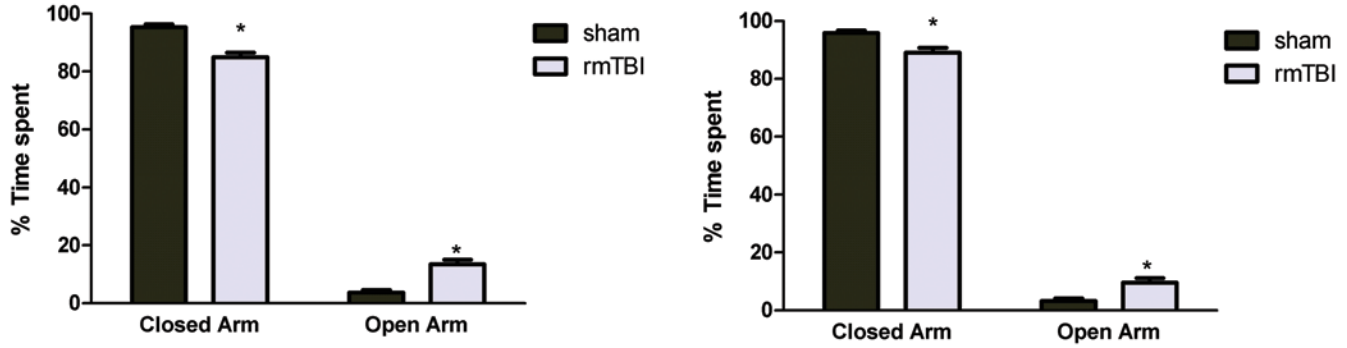

Fig. 4. Elevated plus maze testing at 17 days and 3 months after the last injury. **Left:** Seventeen days after the last injury, injured mice spent more time in the open-arm zone than sham-injured mice (13.5% ± 1.6% vs 3.6% ± 1.0%, *p < 0.001) and less time in the closed arm (84.9% ± 1.6% vs 95.4% ± 1.0%, *p < 0.001). **Right:** Three months later, injured mice spent more time in the open-arm zone than sham-injured mice (9.6% ± 1.6% vs 3.2% ± 0.9%, *p = 0.004) and less time in the closed arm (89.1% ± 1.6% vs 95.9% ± 0.9%, *p = 0.003).

atory behavior after rmTBI, offering a behavioral phenotype that has correlates in the clinical entity of concussion. In this model, behavioral deficits are associated with chronic changes in histopathology, including astrocytosis and microgliosis.

Mice exposed to repeated head impacts demonstrate rotarod deficits that correlate with balance and coordination deficits described in athletes who have experienced concussive injuries.[14] Our findings corroborate prior experimental models that have demonstrated impaired rotarod and beam balance performance after repetitive injury[18,19,22,35] but do so in a model with minimal LOC. It is vital to establish the effect of repetitive injury on balance, here using rotarod testing, for any experimental model of rmTBI, as postural instability is an important feature of human concussion[15,39] and features prominently in clinical assessments of acute and subacute injury.[28] Extending our findings to even milder models of rmTBI (fewer injuries, greater time between injuries, and no LOC) will further enhance the clinical relevance of our model.

Numerous clinical studies have demonstrated acute cognitive impairment after single mild TBI[7,28,29] with worse outcomes after repetitive injury.[9,13,18] We confirm our prior reports,[25,32] and those of other rmTBI models,[6,18,40] demonstrating visuospatial deficits and impaired MWM performance after rmTBI. We show deficits in both hidden and visual trial platform testing that correlate with clinical reports of impaired memory[28] and visual processing after mild TBI.[7]

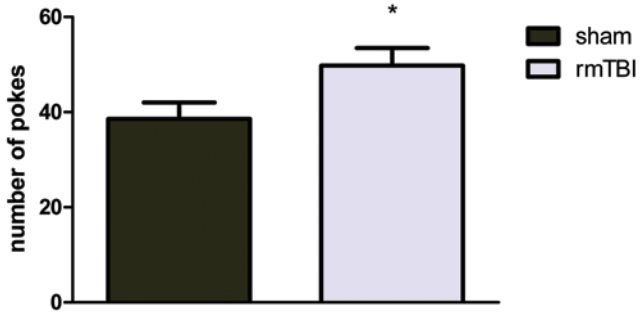

Fig. 5. Exploratory activity of injured versus sham-injured mice in a hole-board, 21 days after the last injury. Data are shown as mean values ± SEM. Injured mice had increased exploratory activity (mean number of pokes) compared with sham-injured mice (49.8 ± 3.7 vs 38.6 ± 3.4, *p = 0.02).

In addition to impairments in balance and memory tests, we demonstrate that mice undergoing rmTBI show increased locomotor activity and exploratory behavior in the open field and hole-board tests. Prior experimental models of TBI have also demonstrated postinjury hyperactivity.[17,37] Clinically, TBI, particularly in children, may result in attentional deficits, response inhibition, and hyperactivity.[21] The increased locomotor and exploratory activity also correlates with frontal disinhibition, a prominent feature in CTE.[2]

The clinically relevant behavioral deficits we report in our model were associated with robust increases in astrocyte and microglial load, a representation of total volume of Iba1 and GFAP immunolabeling in both the hippocampus and fimbria. Prior studies have demonstrated astrocyte proliferation[1,16,20,25] and microglial activation[10,24] after brain injury. It is uncertain whether proliferation of astrocytes and microglia after injury has any mechanistic relationship to the behavioral deficits we found in our model, or merely represents a reaction to neuronal injury. Further studies are needed to assess the role of glial cells after rmTBI.

Conclusions

We report here for the first time a battery of behavioral and histopathological outcomes in a clinically relevant model of rmTBI. In our model of rmTBI, we demonstrate that injured mice show impaired balance and spatial memory and increased locomotor activity and exploratory behavior, suggestive of hyperactivity and inattention. Injured mice also demonstrate astrocyte and microglial proliferation, offering a potential therapeutic target after rmTBI. By establishing the phenotype of our rmTBI model, we offer translational and clinical researchers an opportunity to use this model to test interventions that may be beneficial to patients with concussive injuries.

Disclosure

Dr. Andrews receives salary support from the Children's Hos-

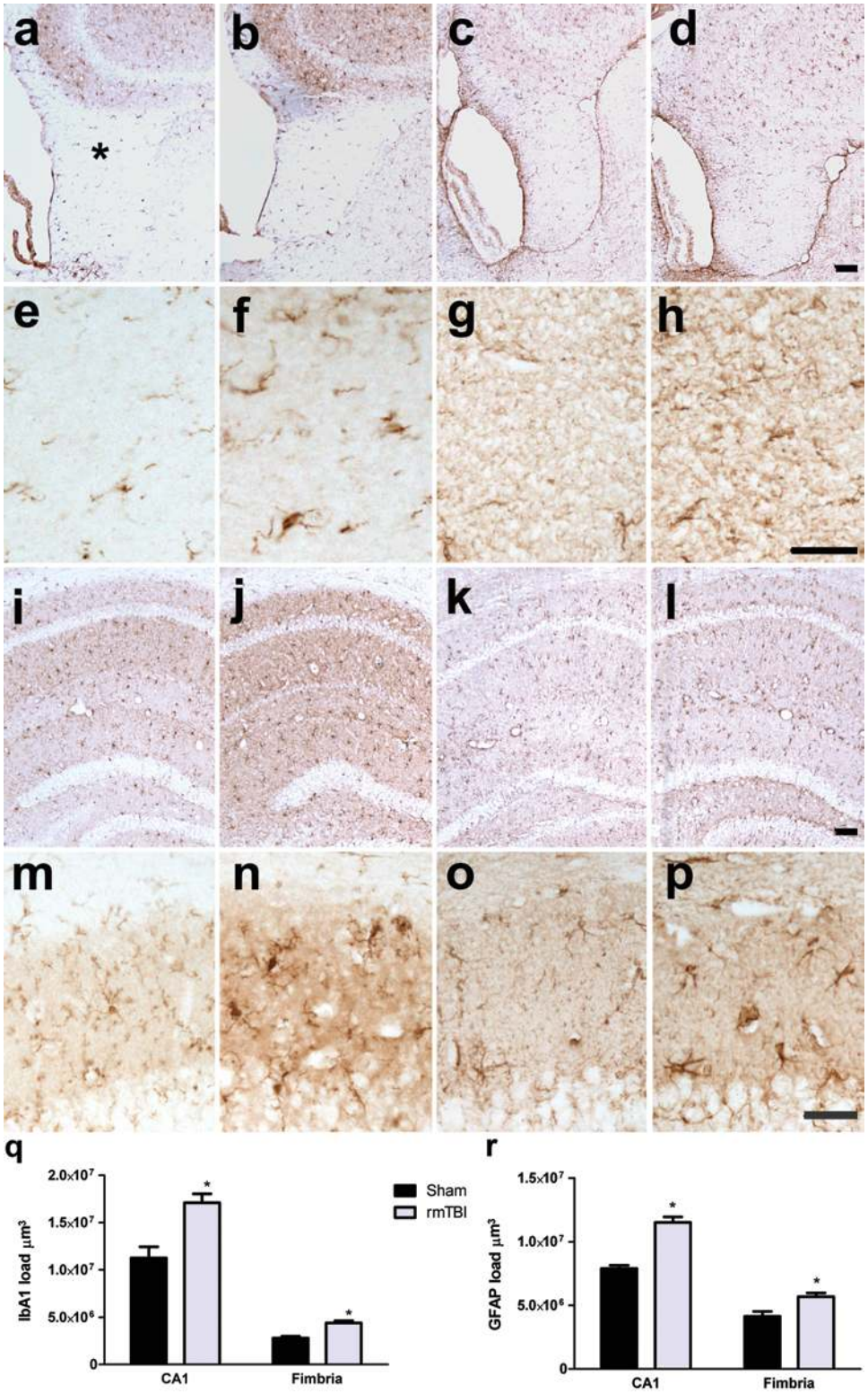

Fig. 6. a–p: After 3 months rmTBI caused increased Iba1-positive immunolabeling of microglia in the fimbria (the area indicated in panel a by the *asteri*sk) (b and f) and hippocampal CA1 (j and n), compared with controls (a, e, i, and m). Similarly, rmTBI also induced chronic astrogliosis in the fimbria (d and h) and CA1 region (l and p), compared with controls (c, g, k, and o). Bars = 100 μm. **q and r:** Stereological estimates showed markedly increased microglial load (q) in injured versus sham-injured mice in CA1 and the fimbria (*p = 0.003). Injured mice also demonstrated increased GFAP-positive astrocyte load (r) in CA1 and the fimbria compared with sham-injured mice (*p = 0.001).

pital Boston Intellectual and Developmental Disabilities Research Center (CHB IDDRC, P30 HD18655). The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Mannix, J Berglass, Berkner, Moleus, Qiu, Andrews, Gunner, Jantzie, Robinson, Meehan. Acquisition of data: all authors. Analysis and interpretation of data: Mannix, J Berglass, Berkner, Qiu, Andrews, Gunner, L Berglass, Jantzie, Robinson, Meehan. Drafting the article: Mannix, J Berglass, Berkner, Moleus, Qiu, Andrews, Jantzie, Robinson. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Mannix. Statistical analysis: Mannix. Administrative/technical/material support: Mannix, Berkner, Moleus, Andrews, Gunner, J Berglass, Jantzie, Meehan. Study supervision: Mannix, J Berglass, Berkner, Moleus, Qiu, Andrews, Gunner, L Berglass, Jantzie.

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Manuscript submitted February 4, 2014.
Accepted July 22, 2014.
Please include this information when citing this paper: published online September 30, 2014; DOI: 10.3171/2014.7.JNS14272.
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