Sex-related differences in cognition after severe murine traumatic brain injury: A Morris water maze study evaluating learning and memory

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BACKGROUND: A number of sex-related outcomes following severe traumatic brain injury (TBI) appear to principally favor females. However, sex-related differences in post-TBI learning and memory remain underexplored. We hypothesized that females realize greater cognitive recovery than males following severe TBI.

METHODS: CD1 male (n = 12) and female (n = 12) mice were randomized to controlled cortical impact (severe TBI: impactor tip diameter, 3 mm; impact velocity, 6 m/s; depth, 1 mm; dwell time, 100 milliseconds) or sham craniotomy and followed for 14 days. Body weight loss recovery was measured daily as a surrogate of neuroclinical recovery. Mice underwent Morris water maze testing to evaluate learning (locating submerged escape platform) with cued and spatial trials and to recall (remembering platform location after it was removed) with probe trials.

RESULTS: Compared with uninjured male mice, male mice with TBI failed to recover lost weight for the first 7 postinjury days (i.e., day 5: MTBI: −3.7% ± 1.5% vs. MSh: +4.1% ± 1.4% body weight; $p < 0.01$), while female mice with TBI recovered the same lost weight and at the same rate as sham female mice (FTBI: −1.6% ± 1.0% vs. FSh: −1.8% ± 0.9%, −0.02% ± 0.01%; $p > 0.9$). Learning (cued and spatial) after TBI was significantly worse in males but not in females. In probe trials, impaired memory after injury was only observed in females.

CONCLUSION: Severe TBI worsens cued and spatial learning and impairs weight loss recovery in male but not female mice. Female, but not male, mice sustain memory impairment after identical severe TBI. While the mechanism(s) that underpin these observations remain unclear, sex-related neurocognitive outcome differences question the universal applicability of trial-based evidence for clinical care. (*J Trauma Acute Care Surg.* 2025;00: 00–00.

KEY WORDS: TBI; female; CCI; Morris water maze; learning; mice.

A wide array of acute care-relevant and time-sensitive conditions appear to demonstrate sex-related outcome differences including injury, stroke, and myocardial ischemia and infarction.[1,2] While civilian data suggest some outcome advantages for females, military registry data find sex agnostic outcomes.[3,4] Putative outcome advantages may potentially be tied to specific circumstances or injuries. When postinjury analysis is limited to those who require massive transfusion, no survival advantage accrues to the female sex.[5] While blunt or penetrating multicavity injury introduces great heterogeneity into study populations, a more narrowly focused population is those with isolated traumatic brain injury (TBI).

Postinjury outcomes across sexes may be influenced by the specific TBI mechanism, injury severity, and injury frequency such as the chronic traumatic encephalopathy identified in professional athletes.[6] There is some suggestion that repetitive injury magnifies the impact of sex on outcomes after mild TBI with females faring worse than males.[7] Outcome data are also influenced by the prevalence and intensity of patient-level metrics. Females appear to experience a greater neuropsychiatric symptom burden after TBI and thereby seem to report diminished quality of life metrics across their lifespan.[8,9] On the other hand, women older than 45 years required shorter intensive care unit (ICU) and hospital lengths of stay compared with males with similar injury.[10] In animal studies, the vast majority of research investigating TBI outcomes and response to treatment have been conducted in male subjects only, assuming identical responses in females, thus indicating an urgent need to clarify effect of sex in TBI.[11]

Collectively, these data fail to provide clarity regarding the impact of sex on the spectrum of outcomes after TBI. Furthermore, neurocognitive recovery after TBI rests on the interlinked components of memory, learning, and spatial awareness for

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adaptive daily functioning. It is therefore relevant to explore those specific aspects within a controlled setting, such as a reproducible murine model of severe TBI, where individual contributions to neurocognitive outcome may be measured. Based upon the plausibility of female sex-related outcome advantages across different settings, we hypothesized that females would demonstrate enhanced neurocognitive recovery after severe TBI compared with identically injured male counterparts.

MATERIALS AND METHODS

Experimental Design and Study Groups

All experimental procedures were approved by the Institutional Animal Care and Use Committee. CD1 male and female mice (25–30 g) (Charles River Laboratories, Wilmington, MA) were acclimated using regular light and dark cycles in standard husbandry facilities, with chow and water ad libitum. On day 0, all mice underwent a first craniotomy, which was then either followed by controlled cortical impact (CCI) or left untouched (sham) (Fig. 1).

Twenty-four mice were assigned to one of four groups (n = 6 for each): (1) female–sham craniotomy (FSh), (2) female–CCI (FTBI), (3) male–sham craniotomy (MSh), and (4) male–CCI (MTBI). Animal Research: Reporting of In Vivo Experiments guidelines were entirely fulfilled (Supplemental Digital Content, Supplementary Data 2, http://links.lww.com/TA/E604).

Severe TBI Model: CCI

Severe TBI was replicated by CCI in a validated murine model that has been extensively used in preclinical research. At time 0, mice were anesthetized with an intraperitoneal injection of ketamine (Mylan Institutional, Rockford, IL), xylazine (Akorn, Lake Forest, IL), and acepromazine (Beohringer Ingelheim, Duluth, GA) (Ketamine, Xylazine, and Acepromazine: 100, 10, and 1 mg/kg, respectively). Extended analgesia was then provided through subcutaneous injection of bupivacaine (Fresenius Kabi USA, Lake Zurich, IL; 0.5 mg/mL) and buprenorphine-XR (Ethiqa XR; Fidelis Animal Health, North Brunswick, NJ; 1.3 mg/mL). Mice were secured prone in a stereotactic device; a left-sided, 4-mm diameter circular transcortical craniotomy between the sagittal, bregma, and lambda sutures was created with a dental drill (Henry Schein, Melville, NY), with care to avoid dural injury. After bone flap removal, in TBI animals, a controlled cortical impactor (AMS201; AmScien Instruments, Richmond, VA) was used to injure the left parietotemporal cortex, using standard validated parameters that replicate severe TBI (impactor tip diameter, 3 mm; impact velocity, 6 m/s; cortical deformation depth, 1.0 mm; dwell time, 104 milliseconds).[12] Sham animals underwent the identical craniotomy with bone flap removal but were not subjected to CCI. The skin was closed over the exposed dura in all cases.

Body Weight Change and Neurological Recovery

Body weight loss is a marker of severity of injury, and extent and rate of weight loss recovery are clinical indicators of neurological recovery after TBI. Body weight was recorded before the first craniotomy (W0) and daily (W1, W2, W3, etc.) until completion of experiments on day 14. Body weight change is expressed as the ratio $([W0 - Wx]/W0) \times 100\%$, where x = day after craniotomy. Greater and faster weight loss recovery indicates better neuroclinical recovery.

Neurological recovery was also evaluated using the Garcia Neurological Test (GNT), which scores motor, sensory, reflex, and balance ability to a maximum sum of 18 points. For each group, the mean GNT scores were compared daily.[13]

Brain Water Content

On day 14, upon completion of water maze trials, animals were sacrificed, and the injured cerebral hemisphere was procured and the wet weight (WW) noted immediately following procurement. The organ dry weight was measured after 70°C dehydration for 72 hours. Tissue water content (edema) was calculated using a wet-to-dry ratio (% tissue water content = [(WW − dry weight)/WW] × 100%).

Morris Water Maze

On days 6 to 14 post-CCI/sham craniotomy, mice performed different daily exercise trials in a Morris water maze (MWM) (Supplemental Digital Content, Supplementary Data 3, http://links.lww.com/TA/E605). The MWM is a circular plastic tub (100-cm diameter, 50-cm high) filled with 22°C water and divided into four quadrants with a 10-cm diameter platform in the center of the north quadrant (Zone [Z] 1) (Supplemental

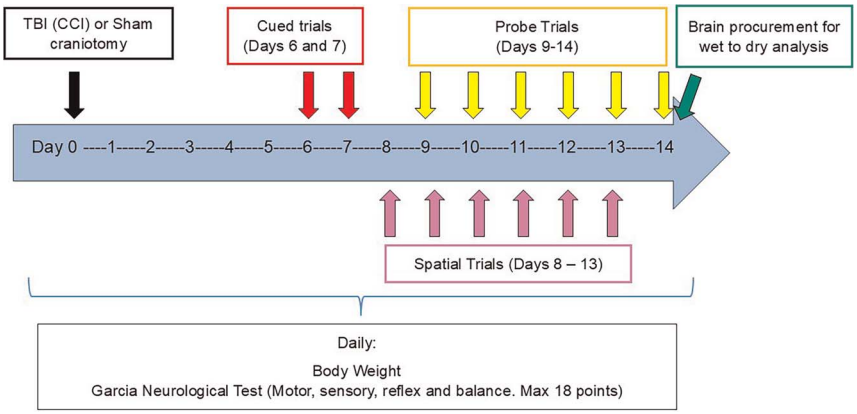

Figure 1. Experimental timeline.

Digital Content, Supplementary Data 3, http://links.lww.com/TA/E605). Zone 5 is the platform area once the platform itself is removed (probe trials). Additional concentric zones around the platform exist with greater distance from the center point: Z6 (18 cm) and Z7 (24 cm) (Supplemental Digital Content, Supplementary Data 3, http://links.lww.com/TA/E605). Daily exercises included cued, spatial, and probe trials. All the MWM trials were conducted and scored by a single operator.

Cued Learning Trials

Cued trials were conducted on days 6 and 7 post-CCI to habituate mice with the maze and teach them that platform arrival was the objective. In cued trials, the platform was placed in a random quadrant 1 cm above water level and marked with a colorful flag. Other than the visible flag on the above-water platform, there were no other visual cues in these trials. Mice were randomly placed facing outward, in different positions: north, east, south, and west and were allowed 60 seconds to reach the platform. Then, trial ended when the animal reached the platform or after 60 seconds (whichever came first). After this, animals who did not reach the platform were placed on the platform to learn that it was the goal. After all animals had spent 15 seconds on the platform, they were removed from the maze, placed under a heat lamp and dried off, and given 10 minutes to rest before the next trial. Each animal underwent four cued trials per day for days 6 and 7 post-CCI.

Spatial Learning Trials

Spatial trials were carried out on days 8 to 13 (24 exercises in total) to see if animals could learn to use visual cues to navigate to a goal. The platform was placed 1 cm below the waterline and the flag removed. Distinctive pictures cues were placed on the tub inner walls at each cardinal point (north, south, east, west) to assist animals in using visual cues to localize and swim to the now hidden platform (Supplemental Digital Content, Supplementary Data 3, http://links.lww.com/TA/E605). Each mouse underwent four spatial trials per day, each time starting in a different quadrant of the pool. If the mouse did not find and mount the platform in 60 seconds, it was mounted unto the platform by the operator and allowed to stay there for 15 seconds. After each trial, mice were again warmed and allowed to rest as above.

Probe Memory Trials

On days 9 to 14, probe trials were conducted (nine exercises in total) to evaluate the animals' long-term memory. The platform was completely removed from the pool, but the wall cues remained in the same location as they had been for spatial trials. Animals started in different quadrants but had only 30 seconds to swim after which they were removed from the water. Probe trials occurred once daily after spatial trials on days 9 to 13, while, on day 14, four probe trials were conducted without any spatial trial. Again, mice were allowed to warm up, dry, and rest between trials.

All MWM trials were recorded by a digital camera mounted above the maze and equipped with video tracking software (Ethovision, Noldus, Leesburg, VA) that recorded and analyzed each animal's trajectory and traveled parameters (distance, time, quadrant crossings, etc.). Parameters analyzed in each trial included the following: time to arrive at a given zone (latency in seconds), distance traveled to reach the zone (in centimeter), percent time duration in a zone (only in spatial and cued trials), absolute duration spent in Z5 (in seconds, only in probe trials), and frequency of crossing into a given zone. Swimming velocity was measured in centimeter per seconds. All results were reported and compared as a mean summation of all days tested unless otherwise specified.

Statistical and Power Analysis

All continuous data are presented as means ± SEM. Graphic illustrations were created in Prism software (GraphPad Software, San Diego, CA). A sample size of six animals per group was used, as previous studies[14] using the same MWM exercises demonstrated that five to six animals per group were sufficient in this model to elicit significant differences in both swimming distance to Z5 and probe trial Z1 latency. For all outcomes measured, four post hoc pairwise comparisons were conducted using analysis of variance with Bonferroni's correction to determine significance between group means (corrected $\alpha = 0.008$). p Values of <0.05 were considered statistically significant.

RESULTS

Body Weight Change and Neurological Recovery

Animal body weights upon the start of experiments did not differ significantly between groups (FSh, 27.7 ± 0.6 g; FTBI, 27.2 ± 0.6 g; MSh, 28.2 ± 0.5 g; MTBI, 28.7 ± 0.5 g; all comparisons $p > 0.99$). Significant weight loss occurred in all animals after surgical procedures (sham craniotomy or CCI) but was greatest in injured males after 48 hours (Fig. 2). Traumatic brain injury resulted in significantly greater weight loss only in males for the first 5 days. For example, on day 5, MTBI (−3.7% ± 1.5%) animals were significantly behind in weight recovery as compared with MSh (+4.1 ± 1.4, $p = 0.001$). Injured females regained lost weight similarly to FSh.

Garcia Neurological Test scores were similar among sham animals or among injured animals regardless of sex (Fig. 3). For the first week, male and female TBI animals had lower total scores than Sh counterparts, after which all animals consistently reached maximal scores.

Cerebral Water Content

Injured brain hemisphere water content at 15 days was similar in all groups, irrespective of sex.

Morris Water Maze

In cued learning trials, male TBI mice traveled a significantly longer distance to reach the platform after TBI, while injured FTBI and FSh traveled similar distances (Fig. 4). Traumatic brain injury did not significantly worsen any other cued learning parameter in males or females.

In spatial learning trials, two of the studied parameters demonstrated significant differences between sham and TBI, but this difference again only existed among male animals (Fig. 5). The MTBI animals crossed into Z6 (area surrounding the platform, 18-cm diameter) 25% less frequently than MSh ($p = 0.03$), while there was no difference in FTBI and FSh animals in this parameter (Fig. 5*A*). The same male-only effect of TBI on Z7 crossings (area surrounding the platform, 24-cm

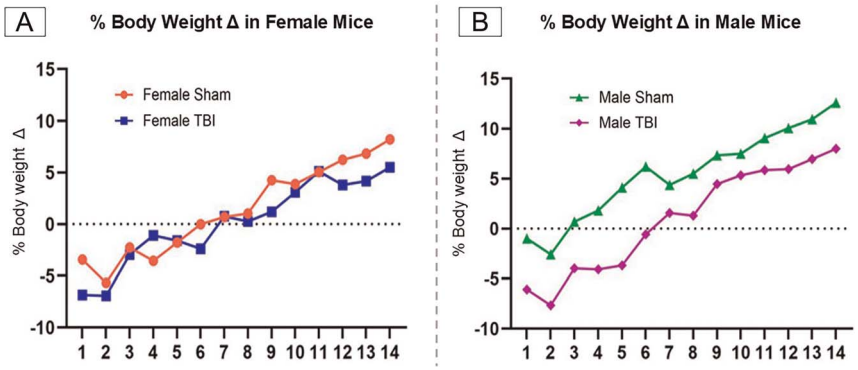

Figure 2. Mean daily percentage change in animal body weight over 14 days after either CCI or sham craniotomy (*A*) in females and (*B*) in males. Note persistent effect of TBI in males that is not seen in females.

diameter) was seen but failed to reached significance. Animals took longer to reach the zones around the platform after TBI. For example, latency to Z1 in MTBI and FTBI was greater than their sham counterparts (MTBI: 13.0 ± 1.1 seconds vs. MSh: 8.3 ± ;0.9 seconds, $p < 0.01$; FTBI: 12.6 ± 1.1 seconds vs. FSh: 6.5 ± 0.6 seconds, $p < 0.01$). However, in tighter proximity to the platform, in Z7, TBI only worsened latency in males, by more than fivefold (MTBI: 9.8 ± 1.5 seconds vs. MSh: 1.4 ± 0.5 seconds, $p < 0.01$) not affecting female Z7 latency (FTBI: 3.2 ± 0.7 seconds vs. FSh: 5.3 ± 0.9 seconds, $p = 0.79$) (Fig. 5*B*). No other spatial learning parameters demonstrated significant differences between males and females.

In memory probe trials (platform removed), TBI worsened performance in both sexes but with a slight preponderance in females. Both male and female TBI animals spent less time in Z5 than their sham counterparts (MTBI: 0.2 ± 0.0 seconds vs. MSh: 0.4 ± 0.1 seconds, $p < 0.01$; FTBI: 0.2 ± 0.0 vs. FSh: 0.5 ± 0.0, $p < 0.01$). Similarly, TBI reduced crossing frequency into Z1 in both sexes (MTBI: 2.6 ± 0.2 vs. MSh: 3.6 ± 0.3, $p = 0.04$; FTBI: 2.8 ± 0.2 vs. FSh: 4.2 ± 0.2, $p < 0.01$). However, for latency to Z1, the quadrant containing the platform, TBI only increased latency in females, not males (Fig. 6; FTBI: 11.6 ± 1.1 seconds vs. FSh: 5.1 ± 0.6 seconds, $p < 0.01$; MTBI: 11.3 ± 1.2 seconds vs. MSh: 9.8 ± 1.4 seconds, $p = 0.9$).

DISCUSSION

Traumatic brain injury describes a spectrum of injury mechanisms and consequences, as well as care and recovery trajectories. While some injuries rapidly lead to death, others are

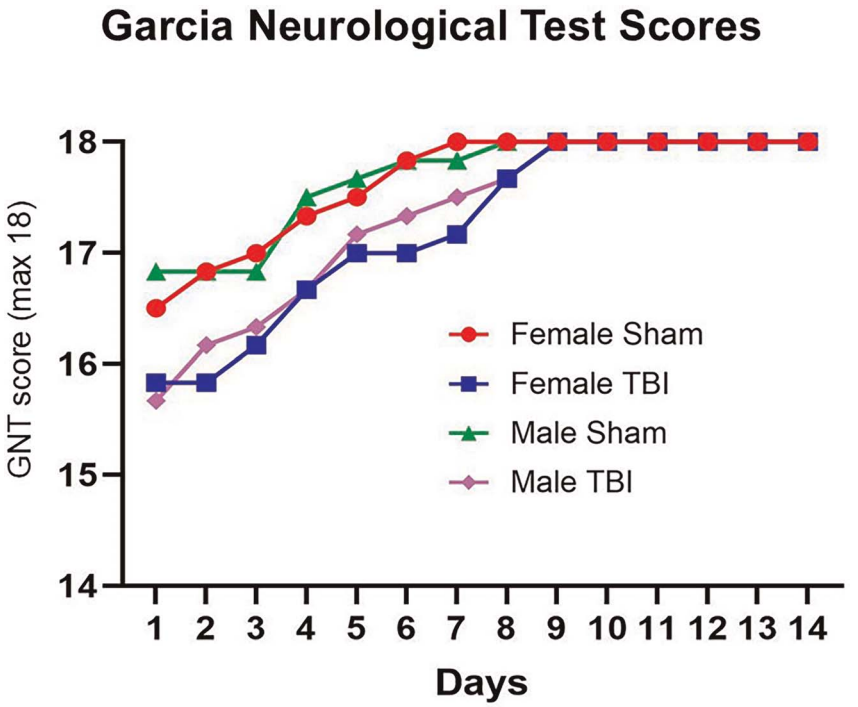

Figure 3. Functional animal neurological recovery over 14 days as evaluated by the GNT, which scores motor, sensory, reflex, and balance ability to a maximum sum of 18 points.

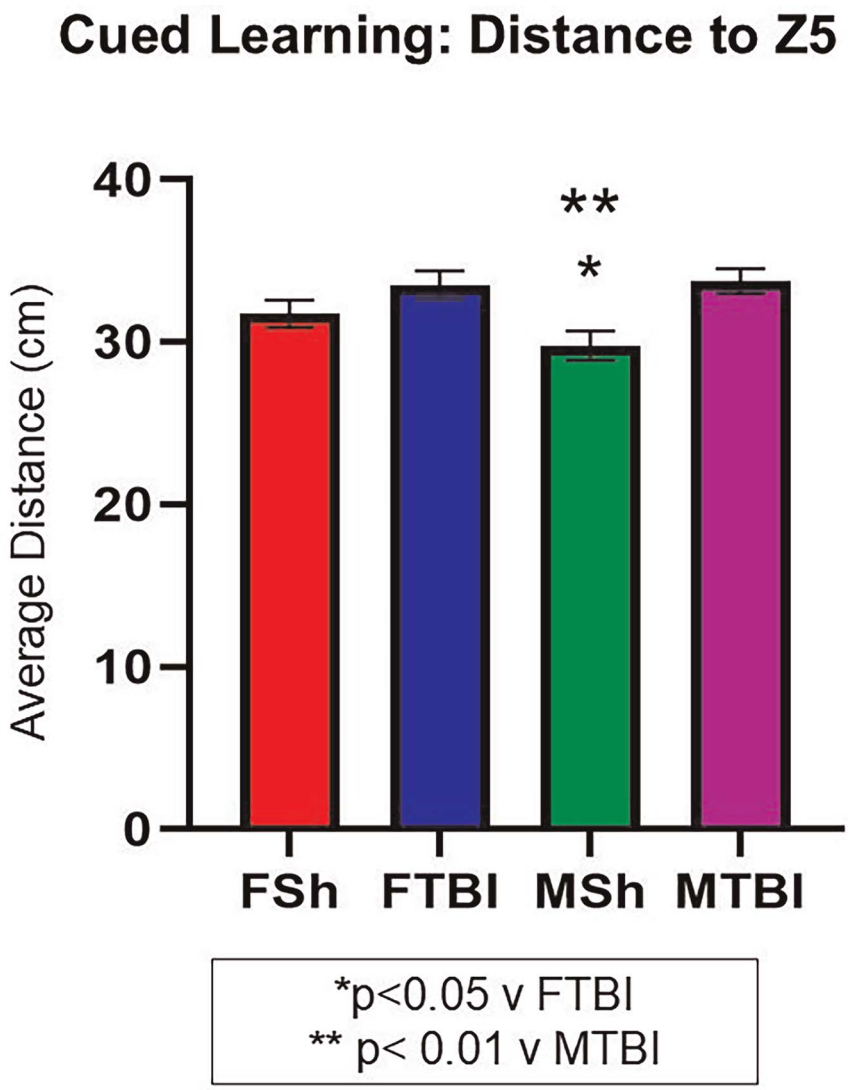

Figure 4. Mean distance needed to reach the platform (Z5) in cued learning trials.

characterized by survival accompanied by durable life-altering impediments—outcomes that are not solely linked to severe TBI.[15] Moderate and repetitive brain injuries lead to disability and impaired quality of life with evolving overlaps with neurologic conditions such as Parkinson's disease and dementia.[16] Traumatic brain injury care costs include prehospital, emergency department, critical and acute care, and operative and interventional undertakings in addition to monitoring within an ICU or specialty (i.e., neuro) ICU.[17] Postdischarge care includes rehabilitation and postrehabilitation support during convalescence, which may be problematic in resource-limited settings.[18,19] Depending on recovery quality, some brain-injured patients lose independence and require full-time care, often relying on family members as uncompensated caregivers.[20] It is therefore relevant to understand recovery mechanisms that beneficially influence outcome including the role of sex in post-TBI recovery. Relatedly, if sex-based differences direct specific kinds of post-TBI care, care timing, or therapeutic agent timing, that knowledge supports providing precision therapies that personalize care.[21]

This study helps delineate acute post-TBI outcomes that are linked to sex-based differences. Importantly, this study's data suggest that not only does TBI have different physiologic effects but it also has different effects on learning and memory in male mice as compared with in female mice. Despite identical injuries, initial weight loss recovery was worse in injured males (physiologic effect). Spatial and cued learning was also worse in injured males (learning). In contrast, females after TBI did significantly worse in probe trials than did males (memory).

Spatial and cued learning influences how one interfaces with the environment including the ability to master new tasks that require three-dimensional navigation. This model evaluated establishing and then using new memories in a short-term fashion; long-term memory was not assessed. Impaired new memory acquisition and integration influence the ability to work within a new environment that may span a rehabilitation center, work environment, or kitchen in a new residence. If long-term memory is also impaired, it may influence one's sense of self and other derailing psychosocial dynamics including those that

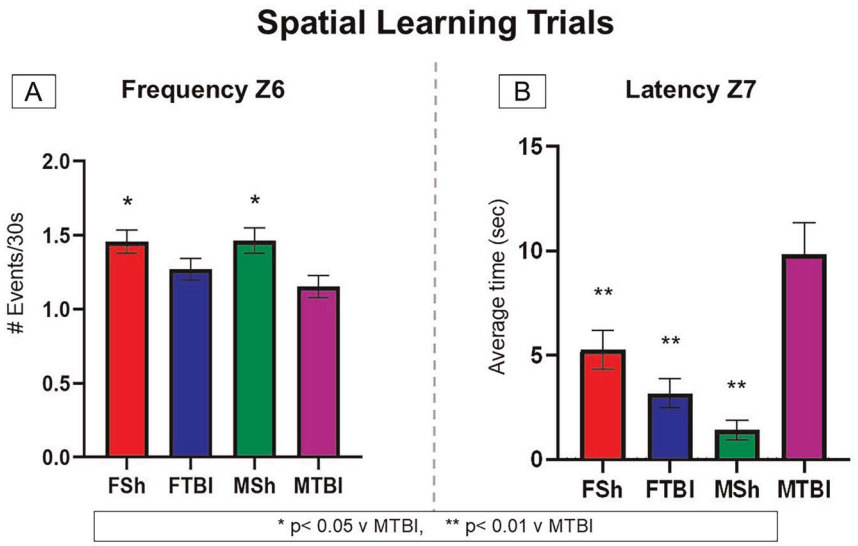

Figure 5. Spatial learning trials. (*A*) Mean frequency of mice crossing into Z6 during the 60 seconds they are allowed to swim. (*B*) Mean time required for mice to reach Z7 for the first time during 60 seconds of swimming.

define the postintensive care syndrome.[22] Traumatic brain injury influences on learning and memory as well as neurocognitive and neuropsychologic outcomes have been assessed in human TBI survivors.[23] Despite interspecies differences, some animal and human outcome metrics demonstrate broad overlap. These include new memory development and adaptive use, situational anxiety or fear, and threat responses (righting reflex in animals).[24] Humans can undergo detailed neurologic and cognitive testing, while animal evaluation relies on MWM performance and other metrics such as the Neurological Severity Score or the GNT.[25–27] Morris water maze though remains the most widely used test to evaluate cognitive impairment in rodents, focusing specifically on hippocampal function with diverse applications examining defined aspects of learning and memory after TBI.[26]

Multiple preclinical studies assessing the role of post-TBI pharmacotherapy document that both learning and memory may be enhanced by certain therapeutic agents, some of which are currently deployed for inpatient or outpatient human care.

For example, quetiapine, a sedative commonly used in critical care and psychiatry, significantly improved spatial learning and memory following severe blunt TBI for a period of 14 days with data suggesting dose-dependent effects.[14] Antithrombin III, which may be useful to reduce hypercoagulability and maladaptive clotting following TBI, was administered to a similar murine model exposed to the MWM, and it was found to enhance learning but not memory, identifying differential effects between therapeutic agents and suggesting that optimal therapy may require more than one agent.[28] Tranexamic acid, an antifibrinolytic agent useful within a short time frame after blunt TBI[29] improved neurocognitive MWM performance in mice but only when administered early (1 hour post-TBI), not late (24 hours) when tested in the same model supporting its

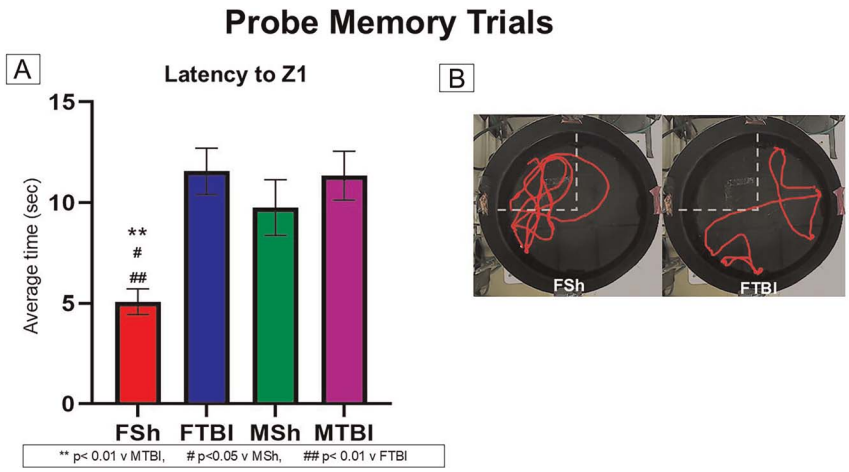

Figure 6. Probe memory trials. (*A*) Mean time required for mice to reach Z1 (where platform had been in spatial and cued learning trials) for the first time during 60 seconds of swimming. (*B*) Representative top view images of typical path taken during 30 seconds of swimming with platform absent for each of the four groups.

clinical use timeframe.[30] However, the vast majority of these and other studies, only used male subjects and assumed de facto extrapolation in females.[31] The number of investigations involving females is sparse not only in preclinical studies but also in clinical studies. In recent years, however, it has become increasingly clear that, while certain agents are beneficial or display neuroprotective effects in males after TBI, the extrapolation that these agents also benefit females cannot be assumed. Indeed, both human and animal data support sex dysmorphisms in outcomes and response to treatment after TBI.

While sex is not a modifiable feature, its influence on many of the other pathways that drive secondary brain injury, as well as those that enhance recovery, is similarly relevant to explore. Major mechanisms of injury include inflammation, edema formation, and neural degeneration that leads to motor failure and behavioral or cognitive failure. Injury leads to inflammation that triggers cytokine-directed inflammatory cell trafficking. That process drives neutrophil endothelial capture and diapedesis with subsequent microglial activation. Ongoing neuroinflammation is followed by macrophage, T-cell, and natural killer cell trafficking. While cell trafficking is key when responding to pathogen incursion, its occurrence in the intracranial space may lead to auto-antibody generation with long-term maladaptive sequelae.[32,33] Increased blood brain barrier permeability seems a central element in the transit of brain-specific antigens into the systemic circulation and the augmentation of cerebral edema formation.[33] Indeed, abnormal BBB integrity and leukocyte-endothelial interaction have been investigated in severe TBI and have defined the "neuroendothelial axis" mechanistically linking injury and cerebral as well as systemic endotheliopathy.[34]

Human studies exploring sex dimorphisms in TBI are contradictory; some reporting lower mortality and fewer complications in females,[35] yet in others, mortality rates similar in both sexes.[36] Preclinical studies appear more consistent and appear to favor females. For example, one study documents more rapid proinflammatory cell trafficking in males.[37] A potential mechanism that may drive worse post-TBI outcomes in males is differential rates of leukocyte capture and subsequently degraded BBB permeability. Recent data support these findings in a head-to-head comparison between male and female mice using a pial intravital microscopy model and revealing that, compared with sham, only TBI males, not females, demonstrated increased 48-hour penumbral BBB leukocyte mobilization and permeability resulting in greater brain edema.[38] These findings were also correlated with degraded post-TBI neurocognitive performance (GNT) in males. These findings support those of others who demonstrated that only male rats had injury-induced neuroinflammation and astrocytosis compared with sex-matched shams[39] with only male rats demonstrating increased BBB permeability (Evans blue extravasation) compared with female rats after neurologic injury.[40] Nonetheless, these findings are not universal, with a mouse study using brain magnetic resonance imaging after CCI and reporting no sex-related differences in cerebral lesion volume, neurodegeneration, and blood-brain barrier alterations, perhaps because of a longer post-TBI evaluation at 7 days.[41] Another study compares male and female rats subjected to CCI and reports similar microglia, splenocyte, and cytokines levels in both sexes but higher mean BBB permeability by fluorescent infrared imaging 72 hours after injury in females.[42] It is clear that the timeframe of histopathologic changes after TBI is particularly affected by sex with evidence of more rapid cortical and hippocampal neurodegeneration in males at 72 hours, while, in females, pronounced degeneration is not seen until 14 days after injury.[43]

There is some evidence that differences in hormone levels may drive some of these outcome differences. Estrogen and progesterone appear to attenuate microglial activation and neuroinflammation in a way that is neuroprotective.[37] Estrogen's impact maybe related to nitric oxide production enhanced cerebral blood flow and consequent vasodilation.[37] Progesterone stabilizes membranes, inhibits lipid peroxidation, and reduces excitotoxicity, effects that are anticipated to reduce reactive oxygen species damage.[43,44] Progesterone, which has an important role throughout pregnancy, appears to impart a degree of neuroprotection when accompanied by TBI. Certain studies demonstrate reduced cerebral edema and downregulation of hippocampal receptors in pregnant mice, while human data suggest lower TBI mortality in pregnant women as compared with nonpregnant women.[44,45] Penumbral alterations in BBB leukocyte trafficking where female sex or female sex hormones administered to males after TBI appear to align a preponderance of penumbral BBB hyperpermeability in untreated males only. Using pial intravital microscopy, our group previously demonstrated how progestins administered to male mice after severe TBI reduced cerebral swelling, BBB polymorphonuclear neutrophil sticking to endothelium, and microvascular permeability.[46] However, a large National Institute of Neurological Disorders and Stroke-led trial in humans receiving progesterone after TBI failed to confirm any benefit and was halted prematurely for futility reasons.[47] Nonetheless, the animal data may explain why female rats with regular estrous cycles demonstrate less post-TBI cerebral edema or elevated intracranial pressure than similarly injured males or ovariectomized females.[48] Not all effects appear directly hormone driven, as only male mice seem to be dependent on interleukin (IL)-10 for cerebral edema, microgliosis, and astrogliosis mitigation as well as successful neurofunctional recovery after moderate TBI; female mice appear unimpacted by induced IL-10 deficiency.[49] On the other hand, proinflammatory cytokine (tumor necrosis factor α and IL-1) levels 4 hours after TBI appear significantly greater in females than males, while males demonstrate much higher transforming growth factor β at 24 hours.[47]

Despite using a well-controlled murine model, there are important limitations to the current study that should be recognized. Designed as a short-term investigation of sex dimorphism after severe TBI, it cannot represent likely long-term outcomes. Only short-term memory acquisition and use were assessed, leaving implications for long-term memory unassessed. Nonetheless, our study spanned 14 days, and as murine lifecycles are not equivalent to those of humans, this may represent a time frame closer to weeks or even months after injury as 1 human year is equivalent to 9 mice days.[50] Similarly, because it was designed as a controlled cortical TBI model, it may not reflect outcomes of more diffuse injury, especially bilateral injury, which may also lead to survival with substantial disability. Correspondingly, the findings may not represent outcomes following penetrating injury or in the wake of blunt TBI coupled with polytrauma. Conversely, we did not include a noncraniotomy

(blank) group of both males and females, and as such, we cannot determine if and to what extent the craniotomy itself may have acted as an injury in the sham animals. We also only evaluated one strain of mice (CD1), and as such, findings in this study may not apply to other stains nor be extrapolatable to humans. Finally, animals were not critically ill during their assessment period, and therefore, the influence of concomitant organ failure, infection, or other commonly used therapeutic agents that would impact cerebral blood flow or intracranial pressure cannot be determined.

CONCLUSION

Our study found that severe TBI worsens cued, spatial learning, and weight loss recovery in male but not female mice. Weight loss recovery as a surrogate of neurocognitive recovery is significantly worse only in males and only early in recovery. Memory impairment after TBI appears to solely impact females in this model. Mechanisms that explain how sex-related outcome differences arise after TBI are essential to explore both mechanisms of injury and recovery after TBI and to better target therapies to improve outcomes.

AUTHORSHIP

P.S.C., G.A.B., and J.L.P. contributed in the conception and study design. P.S.C. and J.L.P. contributed in the literature review. P.S.C., P.B., M.C., M.C., A.G., C.L.J., and P.M.Q. contributed in the data acquisition. P.S.C., L.J.K., and J.L.P. contributed in the data analysis and interpretation. P.S.C., J.L.P., and L.J.K. contributed in the drafting of the manuscript. P.S.C., M.C., P.B., M.C., A.G., C.L.J., P.M.Q., D.M., L.J.K., A.K., D.S., G.A.B., and J.L.P. contributed in the critical revision.

DISCLOSURE

Conflicts of Interest: Author Disclosure forms have been supplied and are provided as Supplemental Digital Content (http://links.lww.com/TA/E606).

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