Evaluation of Touchscreen Chambers To Assess Cognition in Adult Mice: Effect of Training and Mild Traumatic Brain Injury

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Abstract

Cognitive impairments are often experienced after a mild traumatic brain injury (mTBI). In the clinical arena, neuropsychological assessments are used frequently to detect cognitive deficits. Animal models of mTBI, however, rely on an assortment of behavioral tasks to assess cognitive outcome. Computer-based touchscreen systems have been developed for rodents and are hypothesized to offer a translational approach to evaluate cognitive function because of the similarities of tasks performed in rodents to those implemented in humans. While these touchscreen systems have been used in pre-clinical models of neurodegenerative diseases and psychiatric disorders, their use in assessing cognitive impairment after mTBI has not been investigated. We hypothesized that mTBI would result in impaired cognitive performance on touchscreen tasks, particularly those with hippocampal-based learning components, including the paired associate learning (PAL) task and the location discrimination (LD) task. Adult male, C57BL/6 mice received a single impact-acceleration mTBI. We found that training mice before injury to perform to criteria is arduous and that performance is sensitive to many environmental variables. Despite extensive optimization and training, mice failed to perform better than chance in the PAL paradigm. Alternatively, mice demonstrated some capacity to learn in the LD paradigm, but only with the easier stages of the task. The mTBI did not affect performance in the LD paradigm, however. Thus, we concluded that under the conditions presented here, the PAL and LD touchscreen tasks are not robust outcome measures for the evaluation of cognitive performance in C57BL/6 mice after a single impact-acceleration mTBI.

Keywords: animal studies; behavioral assessments; cognitive function; learning and memory; traumatic brain injury

Introduction

NEUROPSYCHOLOGICAL ASSESSMENTS are valuable clinical tools in diagnosing and tracking cognitive impairment associated with mild traumatic brain injuries (mTBI).¹ Neuropsychological assessments are considered essential diagnostic tools to identify the occurrence of mTBI, particularly in patients wherein other diagnostic methods such as neural imaging are less sensitive.^{2–4} For example, a widely used research diagnostic tool developed specifically to detect mTBI, the Sport Concussion Assessment Tool, which is now in the third iteration (SCAT3), has a large neuropsychological component.⁵ More recently, neuropsychological tests that have been administered traditionally via pen and paper have been adapted into computerized assessments.⁶ Two of the more widely used computerized neurocognitive tests for mTBI are the Automated Neuropsychological Assessment Metrics (ANAM) and the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT). Both of these computer-based tests

evaluate attention, working memory, reaction time, the ability to make decisions, and problem solving ability in an effort to determine changes in cognitive function after a suspected brain injury. The ANAM was developed by the Department of Defense and has been used frequently to assess cognitive change after injury within the military setting.^{7,8} ImPACT is commonly used in the context of sports to assess athletes after a head impact and was approved by the Food and Drug Administration in August 2016 as the first medical device to evaluate cognitive function after a suspected head injury.^{9,10}

In pre-clinical modeling of mTBI, animal models seek to mimic the clinical condition not only in terms of the pathophysiology but also replicating functional or behavioral alterations. Consequently, there are myriad behavioral assessments that pre-clinical researchers employ to evaluate cognitive functioning after mTBI (for review, see ¹¹). For example, the Morris Water Maze (MWM) is one such assessment that has served as the gold standard to evaluate hippocampal-dependent spatial learning and memory after mTBI

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in rodent models.^{11–13} In addition, the novel object location (NOL) and novel object recognition (NOR) tests have been used frequently to examine spatial and nonspatial hippocampal-dependent memory, respectively.^{14,15} Although these tests often demonstrate cognitive impairment in mTBI pre-clinical models, the severity and duration of deficits detected is highly variable and dependent on injury model.^{16–18} Thus, we hypothesize that cognitive tasks that require higher cognitive load will be more sensitive to mTBI-induced changes in cognition. In addition, we hypothesize that cognitive assessments that more closely mimic neuropsychological evaluations used clinically may proffer enhanced translational predictivity as related to the modeling of mTBI in mice.¹⁹

Recently, a touchscreen assessment of cognition was created for rats, and adapted for mice, modeled after the Cambridge Neuropsychological Test Automated Battery (CANTAB) touchscreen neuropsychological test for humans.²⁰ CANTAB also markets their product for TBI, but its application within the field has been limited compared with ANAM and ImpACT.²¹ These rodent touchscreen chambers have been used mostly to study models of neurodegenerative diseases and psychiatric disorders, but have the ability to be adapted to other fields.^{22,23}

To our knowledge, these touchscreen chambers have never been evaluated as a tool for measuring cognitive deficits in pre-clinical models of mTBI. Because much of the previous literature has focused on mTBI-induced deficits in hippocampal-dependent tasks,^{24–26} we selected two touchscreen paradigms that involve the hippocampal system: the paired associate learning (PAL) task and the location discrimination (LD) task.^{27–29} The PAL task was devised to resemble the CANTAB PAL test used in humans and assesses the ability to associate a distinct object with a specific spatial location. The LD task is similar to aspects of the pattern recognition memory (PRM), spatial recognition memory (SRM), and spatial working memory (SWM) tests within CANTAB and evaluates pattern separation by assessing the ability to discriminate between visuospatial information. In animal models, lesioning or pharmacologically inactivating the hippocampus resulted in deficits in both PAL and LD tasks.^{27,28} In contrast, pharmacological enhancement of cognitive function has been shown to improve PAL task per-

formance in rodents.³⁰ Various CANTAB tasks including PAL, PRM, SRM, and SWM have been used clinically to assess patients with TBI and found performance deficits after moderate to severe TBI and even in milder instances.^{31,32} The ANAM and ImpACT neurocognitive tests also include test batteries that resemble aspects of the PAL and LD paradigms. Specifically, patients with mTBI evaluated with ANAM exhibited poorer performance in associative learning, working memory, and visual spatial memory.³³

The overall goal of this study was to evaluate an assessment of cognitive function after mTBI in mice that more closely mirrored assessments conducted in the clinical arena. Specifically, the purpose of this study was twofold: (1) to evaluate the ability of mice to perform on hippocampal-based cognitive tasks within the touchscreen chamber and (2) to determine whether mTBI induces impairments in touchscreen assessments using a murine model that we have shown previously to result in cognitive impairment on other tasks.¹³ We first evaluated the training ability of mice on the touchscreen chambers with an array of parameters and then assessed performance on two hippocampal-dependent tasks, PAL and LD. Finally, we evaluated the effect of mTBI on LD task performance. We found that the training process for mice is long, tedious, and sensitive to many environmental variables. Under our testing conditions, C57BL/6 mice struggled to perform better than chance in the PAL task, but were able to learn the LD task. In addition, we found that a single impact-acceleration mTBI did not alter performance in the LD task.

Methods

Animals

Male C57BL/6 mice (2 or 3 months old, $n = 260$) were obtained from either Charles River Laboratories (Wilmington, MA) or Jackson Laboratory (Bar Harbor, ME). On arrival, mice were housed individually in a temperature ($23^{\circ}\text{C} \pm 3^{\circ}\text{C}$) controlled vivarium with a cotton nestlet and cardboard tube for environmental enrichment. Either a standard 12-h light/dark cycle (lights on at 7 AM) or a reversed 12-h light/dark cycle (lights on at 7 PM or 10 PM) was utilized (Table 1). Mice were allowed to acclimate to the light/

TABLE 1. ANIMALS AND EXPERIMENTAL CONDITIONS

Group	n	Age	Vendor	Light cycle	Water/ food restriction	Reward	Experiment
Cohort A	10	3 mo	Charles River Laboratories	Standard (7 AM on/ 7 PM off)	Water dep. 60 min before testing/food <i>ad libitum</i>	10% condensed milk	Pre-training
Cohort B	24	3 mo	Charles River Laboratories	Reverse (7 AM off/ 7 PM on)	Water dep. 60 min before testing/food <i>ad libitum</i>	10% condensed milk	Pre-training
Cohort C	34	3 mo	Charles River Laboratories	Reverse (7 AM off/ 7 PM on)	Water dep. 60 min before testing/food <i>ad libitum</i>	10% condensed milk + flavor	Pre-training
Cohort D	48	3 mo	Charles River Laboratories	Reverse (10 AM off/ 10 PM on)	Water dep. 60 min before testing/food dep. to 80% of baseline	10% powdered milk + flavor and sweetener	Pre-training, sPAL
Cohort E	48	2 mo	Charles River Laboratories	Reverse (10 AM off/ 10 PM on)	Water dep. 60 min before testing/food dep. to 80% of baseline	10% powdered milk + flavor and sweetener	Pre-training, sPAL
Cohort F	48	3 mo	Charles River Laboratories	Reverse (10 AM off/ 10 PM on)	Water dep. 60 min before testing/food dep. to 80% of baseline	10% powdered milk + flavor and sweetener	Pre-training, LD, mTBI
Cohort G	48	3 mo	Jackson Laboratory	Reverse (10 AM off/ 10 PM on)	Water dep. 60 min before testing/food dep. to 80% of baseline	10% powdered milk + flavor and sweetener	Pre-training, LD, mTBI

sPAL, similar paired associate learning; LD, location discrimination; mTBI, mild traumatic brain injury.

dark cycle for a week before testing during which time they were handled daily. Some mice had access to standard food *ad libitum* while others were food restricted (Table 1). The body weights of the food-restricted mice were reduced slowly over the course of five days and maintained at 80–85% of their baseline free-feeding body weights. All mice were water deprived for 60 min before testing. Testing was conducted five days per week between 10 AM–6 PM. All procedures and protocols in this study were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham in accordance with the National Institutes of Health guidelines.

Apparatus

Mice were assessed using automated touchscreen operant chambers (Bussey-Saksida Mouse Touchscreen Chamber Model 80614, Lafayette Instrument Co., Lafayette, IN) that have been described previously.^{34,35} The trapezoidal-shaped chambers consisted of black Perspex walls and a perforated metal floor creating an animal working area with the following dimensions: small base = 5.5 cm, large base = 24 cm, depth = 18 cm, height = 20 cm. Each chamber was equipped with a touchscreen at the large base, liquid reward dispenser at the small base, house light, reward chamber light, tone generator, and infrared (IR) beams with an observation system. Each chamber was placed in a cubicle (inside dimensions: 45 cm × 51 cm × 49 cm tall) that served to attenuate external sounds and light. A black plastic mask (28 cm wide × 18 cm tall) was inserted in front of the touchscreen allowing for different visual stimuli to be presented and different tasks to be utilized.

Two different masks were used in this study, a 3 × 1 (Model 80614-M2, Lafayette Instrument Co., Lafayette, IN) and a 7 × 2 (Model 80614-M3, Lafayette Instrument Co.). The 3 × 1 mask, used for the PAL task, consisted of one row with 3 three square windows (2 cm × 2 cm). The 7 × 2 mask, used for the LD task, consisted of two rows with seven windows (2.4 cm wide × 2 cm tall) with stimuli only being presented in the bottom row. A peristaltic pump (Model 80204M, Lafayette Instrument Co.) precisely delivered a drop of liquid reward. The reward was a milkshake consisting of 10% milk (condensed or powdered) in distilled water with systematic variations designed to entice the mice to perform including flavoring with Hershey's® syrup (chocolate or strawberry; Hershey, PA) and further sweetening with sucralose sweetener (Splenda®, Heartland Food Products, Westwood KS); see Table 1. The system was controlled by the Animal Behavior Environment Test (ABET) II software (Model 89505, Lafayette Instrument Co.) allowing for the experimental programming, execution, and data collection. After each session, chambers were thoroughly cleaned with chlorhexidine.

Pre-training

All mice were pre-trained in the touchscreen chambers before assessment with PAL and LD tasks. Training procedures followed those listed in the instruction manuals provided by Campden Instruments Ltd/ Lafayette Instrument Co.^{36,37} and described previously protocols, with some modification (Supplementary Table 1; see online supplementary material at www.liebertpub.com).^{34,35} Briefly, mice underwent a five-stage stepwise training paradigm as described below.

Stage 1: Habituation. Mice were acclimated to the chambers and introduced to reward. For habituation 1, mice were placed in a chamber for one 15 min session with the goal of introducing the mouse to the chamber to acclimate. During the acclimation session, all chamber lights were off, and neither stimulus nor reward was presented. Habituation 2 consisted of three sessions introducing the mice to the reward lasting 20 or 40 min (Supplementary Table 1; see online supplementary material at www.liebertpub.com). Dur-

ing these sessions the reward tray light was activated, followed by a tone and the dispensing of liquid reward. Once the mouse collected the reward, there was a 20 sec delay (reward tray light off) before the next trial. Mice were required to complete one session at each habituation step. For the mice advancing past pre-training to the PAL and LD tasks (Table 1 and Supplementary Table 2; see online supplementary material at www.liebertpub.com), 48 mice were habituated initially for each group but only the 24 best performers, as based on number of trials completed, advanced onto further training/ testing.

Stage 2: Initial Touch Training (ITT). Mice were trained to associate the reward with a stimulus on the screen. A white square (stimulus) was displayed randomly in one of the windows in the mask. For the PAL task (3 × 1 mask), the stimulus was presented in any one of the three windows. For the LD task (7 × 2 mask), the stimulus was presented in any one of the bottom row windows (positions 8–14). The image was removed after 30 sec. On removal of the image, a cued reward phase was initiated that included playing a tone, illuminating the reward tray light, and dispensing of the liquid reward. Once the mouse collected the reward, the tray light was extinguished, and there was a 20 sec delay before the next trial began. Each session concluded after 40 min or after the completion of 20 trials, which was the criterion for advancement (Supplementary Table 1; see online supplementary material at www.liebertpub.com).

Stage 3: Must Touch Training (MTT). Mice were trained to touch the stimulus on the screen to elicit the reward. A white square was displayed randomly in one of the windows as described with ITT. On touching the white square, a tone was played, the reward tray was illuminated, and a liquid reward was dispensed. There was a 20 sec delay after the reward was collected before the start of the next trial. Failure to touch the screen or touching a blank window resulted in no reward response generated. Each session concluded after 40 min or after the completion of 20 trials, which was the criterion for advancement (Supplementary Table 1; see online supplementary material at www.liebertpub.com).

Stage 4: Must Initiate Training (MIT). Mice were trained to initiate the training session. On entering the chamber, the reward tray was illuminated and primed with liquid reward. The mouse had to collect the reward to begin the session and initiate the presentation of the stimulus. Training for the remainder of the session was identical to MTT. Each session concluded after 40 min or after the completion of 20 trials, which was the criterion for advancement (Supplementary Table 1; see online supplementary material at www.liebertpub.com).

Stage 5: Punish Incorrect Training (PIT). Mice were punished for incorrect responses. PIT followed the same format as described in MIT, except mice were punished if they failed to touch the correct window containing the white square as the stimulus. Touching the wrong window (a blank window) resulted in a time-out period of 5 sec in which the stimulus disappeared, the house light was illuminated (for those trained in reversed light/dark cycle), and no reward was dispensed. Each session concluded on the completion of 20 trials or after 40 min had elapsed. Criterion for advancement was set at the completion of 20 trials in 40 min with a 60% correct response rate for two consecutive days. Successful mice advanced onto similar PAL (sPAL) or LD training.

sPAL Task

The sPAL task used the 3 × 1 mask. There were three possible stimuli images with each image having a specific correct location in one of the three windows. Images and correct locations were the

following: flower—left window, plane—middle window, and spider—right window. Similar to MIT, mice were required to initiate the training session by collecting the liquid reward from the primed and illuminated tray. The first trial then began with the presentation of two similar stimuli. One stimulus was in the correct location associated with that specific image and the other stimulus was in the incorrect location. There were six possible trial types with the three different stimuli. Touching the image in the correct location resulted in a tone being played, illumination of the reward tray, and delivery of the liquid reward. Similar to pre-training, collecting the reward served as a reset (tone and tray light off) with a 20 sec delay before the next trial began. Before sPAL testing could begin, mice were required to complete the sPAL acquisition stage where incorrect touches were ignored and there was no punishment. Each session concluded after 40 min or after the completion of 20 trials, which was the criterion for advancement.

For sPAL testing, mice were punished for incorrect responses. Similar to PIT, touching the wrong image resulted in a 5 sec time-out period in which all stimuli disappeared and the house light was turned on. Once the time-out period ended, mice were administered a correction trial in which the same stimuli were re-presented. The mice were required to complete successfully the correction trial before moving onto the next trial with new stimuli. Mice were evaluated on the number of trials they completed and correct response percentage. Correction trials did not count in overall percentage correct. Mice were given one session per day, which concluded after 40 min or the completion of 20 trials, and sPAL testing ranged from 5–14 days.

LD Task

The 7×2 mask was used with the LD task, but stimuli were only presented in the bottom row (positions 8–14). As in pre-training, the stimulus presented with this task was a white square. Unlike pre-training, however, where there was only one white square, the LD task used two white squares that were positioned equally left and right from the center window (position 11). There were three difficulty levels associated with the LD task based on the separation of the stimuli. The stimuli were located far apart (large separation) for the easy level (positions 8 and 14), a moderate distance apart for the intermediate level (positions 9 and 13), and close together (small separation) for the hard level (positions 10 and 12).

The LD task consisted of three stages: acquisition, reversal, and probe. Mice were required to initiate the training session by collecting the pre-dispensed reward from the illuminated tray, similar to MIT. For LD acquisition, the stimuli (white squares) were presented at the intermediate separation level. Each mouse had one side, either left (position 9) or right (position 13), randomly designated as correct. Touching the correct stimulus elicited a tone, illuminated the tray, and dispensed the reward. There was a 10 sec delay after the reward was collected before the next trial began. As with PIT, an incorrect response resulted in a time-out period of 5 sec where the house light was turned on and no reward was issued. Each session concluded on the completion of 20 trials or after 40 min had elapsed. Criterion for advancement was set at the completion of 20 trials in 40 min with a 60% correct response rate for two consecutive days.

LD reversal followed similar procedures as LD acquisition with some minor modifications. Again, the intermediate separation level was used with one side (left or right) initially being designated as the correct location and counterbalanced across animals. On completing seven of eight trials correctly, the correct location side was reversed to the opposite side. The training session ended after the completion of 20 trials or after 40 min had elapsed. Criterion for advancement was set at the completion of at least one reversal in three of four consecutive sessions. In addition, mice were evaluated on the number of trials they completed and the correct response percentage. The LD reversal training ranged from 3–8 days.

For LD probe sessions, both the easy and hard separation levels were used. One side was randomly designated as the correct location side (either left positions 8 and 10 or right positions 12 and 14, based on difficulty separation), and there were no reversals. Each trial, mice were randomly presented with stimuli at either the hard separation positions or easy separation positions. The stimuli were not displayed in the same positions for more than three consecutive trials. Similar to LD acquisition and reversal, touching the stimulus on the correct side resulted in reward, while touching the stimulus on the incorrect side resulted in a time-out period of 5 sec. Each session, mice were given 60 min to complete 40 trials (20 easy and 20 hard distributed randomly throughout the session). Each session ended after the 60 min time limit had expired or all 40 trials were completed. Mice were evaluated on the number of easy and hard trials they completed, as well as the correct response percentage at both the easy and hard separation levels. The LD probe testing ranged from 5–10 days.

Experimental mTBI

Mice that successfully completed all of the pre-training, LD acquisition, and LD reversal received either sham surgery or mTBI surgery before being evaluated with LD probe sessions. The mTBI model has been described previously by our laboratory.¹³ Briefly, a weight drop device was used in which a 100 g cylindrical weight was released from a height of 60 cm to impact the mouse head and create an impact-acceleration injury. Mice were anesthetized with a 4% isoflurane induction and 2% maintenance for all surgical procedures. For injury induction, the mouse rested on a foam pad (14×7 cm, 5 cm thickness), and the weight was dropped onto a metal disk (1.25 cm diameter, 1 mm thickness) that was affixed to the skull of the mouse at midline with cyanoacrylate adhesive. The duration of transient unconsciousness was determined by measuring righting reflex time, as described previously.¹³ Animals receiving the sham surgery experienced the same procedures with omission of the impact. The LD probe testing began 24 h after mTBI or sham surgery.

Statistical analysis

All data were recorded in the touchscreen session using the ABET II software (Lafayette Instrument Co.) and subsequently analyzed using Prism 7 (GraphPad Software Inc., La Jolla, CA). Data are reported as the mean ± standard error of the mean (SEM) with statistical significance set at $p < 0.05$. The Student *t* test was used to analyze pre-training trials and righting times. Repeated measures two-way analysis of variance (ANOVA) was used to analyze between-group differences across testing days with sPAL and LD tasks. To determine significant differences identified by ANOVA, the appropriate *post-hoc* analysis was performed sequentially.

Results

Pre-training completion: effect of criteria, light/dark cycle, and food restriction

Performance of the mice (Cohort A) in stage 3, MTT, was used as the outcome measure to indicate pre-training completion. Initially and based on the instruction manuals provided by Lafayette Instrument Co.^{36,37} and previously published work,^{35,38} completion criterion was set at 36 trials in 60 min for MTT. Only 40% of mice (4/10) were able to meet this criterion, however. Consequently, we reduced the criterion to 30 trials in 60 min, as described previously,^{30,34,39} and this reduction in criterion resulted in 50% of mice (5/10) reaching criterion. We noted that the mice consistently completed approximately 20 trials (average = 19.8–22.8 trials) each testing day, which demonstrated that additional testing days did not

affect trial completion. Thus, a further reduction in the completion criterion, 20 trials in 40 min, resulted in 60% of mice (6/10) able to complete MTT (Fig. 1A).

Although reducing the completion criterion resulted in an increase in the percentage of mice performing the task successfully, we next hypothesized that reversing the light/dark cycle of housing conditions, such that mice were evaluated in the touchscreen chambers during their dark-active cycle, would improve the percentage of mice that completed MTT further. Previous studies had also utilized reverse light/dark cycles with touchscreen assessments.^{27,28,30,34,35} Altering the light/dark cycle (Cohort B) had a modest improvement on the percentage of mice completing the MTT stage, because 75% of the mice (12/16) completed this stage (Fig. 1B and Supplementary Table 2; see online supplementary material at www.liebertpub.com). Next, we hypothesized that improving the palatability of the food reward may affect MTT completion.^{28,34} Thus, we added alternating chocolate or strawberry flavor to the plain milk reward (Cohort C) which resulted in 100% of mice (13/13) completing the MTT stage of pre-training (Fig. 1B and Supplementary Table 2; see online supplementary material at www.liebertpub.com).

These optimization steps—namely, reversing the light/dark cycle and flavoring the reward—substantially increased the success rate of mice at the MTT stage; however, mice exhibited poor completion on the subsequent pre-training stages with only 28% (7/25) of mice (Cohorts B and C) completing must initiate training (MIT) and 29% (2/7) completing punish incorrect training (PIT) (Fig. 1C and Supplementary Table 2; see online supplementary material at www.liebertpub.com). Consequently, in the subsequent optimization steps, mice (Cohorts D and F) were evaluated under conditions of reversed light/dark cycle (as above), additional sweetening of the flavored reward, and food restriction such that the mice were maintained at 80–85% body weight. Further, mice that were the worst performers (lowest 50% in number of trials completed) at stage 1 (habituation) of pre-training were eliminated from subsequent training. Collectively, these optimization steps resulted in a significant increase in the percentage of mice that were able to complete each pre-training stage (MTT = 48/48–100%; MIT = 47/48–98%; PIT = 44/47–94%; Fig. 1C and Supplementary Table 2; see online supplementary material at www.liebertpub.com).

Taken together, these data indicate that mice perform better when tested under a reverse light/dark cycle. Motivation to complete the

touchscreen training can be increased by making the reward more palatable and with food restriction. In addition, screening for trial completion performance at stage 1 (habituation) of pre-training to eliminate poor performers likely results in better pre-training completion overall.

FIG. 1. Manipulation of training task completion criteria, palatability of reward, and food deprivation increases the percentage of mice that complete pre-training tasks. (A) Evaluation of the must touch training (MTT) completion rate with different completion criteria. Reducing the completion criterion from the recommended 36 trials in 60 min to 20 trials in 40 min resulted in a greater percentage of mice that completed the task. $n = 10$ (Cohort A). (B) The percentage of mice that completed the task was further increased by reversing the light/dark cycle so that mice were trained/tested in their active (dark) cycle and by increasing the palatability of the reward by adding flavoring. $n = 16$ for Reverse L/D (Cohort B) and 13 for Reverse L/D + flavor (Cohort C). (C) The percentage of mice that completed training was increased further to 90–100% of the mice completing the task by food restricting the mice, eliminating the non-performers, and further increasing the palatability of the flavored reward with sweetener compared with the cohorts in the reverse light/dark cycle and flavored reward only. ITT, initial touch training; MIT, must initiate training; PIT, punish incorrect training. $n = 29$ for Reverse L/D + flavor (Cohorts B + C) and 48 for food restricted + sweetener (Cohorts D + F).

FIG. 2. Younger age at initiation of training has a modest negative effect on pre-training performance. (A) Percentage of mice completing each pre-training stage before evaluation with the similar paired associate learning task. (B) The 2-month-old mice took significantly more trials to meet criteria for must touch training (MTT). There were no significant differences between groups in the number of trials it took to meet criterion for (C) must initiate training (MIT) and (D) punish incorrect training (PIT). Initial touch training (ITT) began with $n = 24$ for 3-month-old mice (Cohort D) and 25 for 2-month-old mice (Cohort E). * $p < 0.05$ compared with 3-month-old mice.

sPAL task performance: Effect of age

Initial evaluation of pre-training completion used 3-month-old mice to be consistent with the age used in our previous mTBI studies.¹³ Published work, however, from pioneers in the field of touchscreen cognitive assessment of rodents, Drs. Bussey and Saksida,^{28,40} has demonstrated success in performance when starting with slightly younger mice, 8–9 weeks of age. Therefore, we assessed the effect of age on performance comparing mice that were either 3 months old (Cohort D) or 2 months old (Cohort E) at initiation of training. In pre-training, 96–100% of both the 2- and 3-month-old mice reached criterion for stages 1–4 including ITT, MTT, and MIT (Fig. 2A and Supplementary Table 2; see online supplementary material at www.liebertpub.com). Age-based differences in performance, however, were observed at stage 5, PIT, in that only 67% (16/24) of the 2-month-old mice met criterion compared with the 96% (23/24) of the 3-month-old mice (Fig. 2A and Supplementary Table 2; see online supplementary material at www.liebertpub.com). In addition, the 2-month-old mice took a significantly greater number of trials to reach completion criteria on the MTT stage of training ($p < 0.0001$; Fig. 2B). There were no significant differences in the number of trials for MIT ($p = 0.13$; Fig. 2C) or PIT ($p = 0.27$; Fig. 2D).

Mice (Cohorts D and E) that successfully advanced through all of the pre-training stages were then evaluated on the sPAL task (Fig. 3A). All of the mice in both groups successfully completed sPAL acquisition with both groups taking an average of one session ($p = 0.87$; 3 month, 1.22 ± 0.13 sessions; 2 month, 1.19 ± 0.14 sessions; Supplementary Table 2; see online supplementary material at www.liebertpub.com). For sPAL testing, there were no significant differences between groups in the number of trials completed

($F_{1,37} = 1.65$, $p = 0.21$; Fig. 3B) and no significant improvements across test days ($F_{4,148} = 0.11$, $p = 0.98$). As a whole, neither group was able to complete the required 20 trials per day in five days of testing. Indeed, the 3-month-old mice completed on average 14.29 ± 1.04 trials per day while the 2-month-old mice averaged slightly less, 12.28 ± 1.14 ($p = 0.21$). In addition, there were no significant differences in the percentage of correct responses between groups ($F_{1,37} = 0.26$, $p = 0.62$; Fig. 3C) with both groups performing near chance ($p = 0.62$; 3 month, 43.87 ± 2.16 % correct; 2 month, 45.59 ± 2.64 % correct) and no significant improvements across the five test days ($F_{4,148} = 0.31$, $p = 0.87$). When individual mouse performance was examined, only two mice in each group reached criterion (Supplementary Table 2; see online supplementary material at www.liebertpub.com). Extending the sPAL test period of the 3-month-old mice to 14 days to increase task exposure did not result in any significant improvements in the number of trials completed ($p = 0.10$) or the correct response percentage ($p = 0.43$; Fig. 3D). It did result, however, in three additional mice reaching criterion. An extended test period was not evaluated in the 2-month-old mice.

Taken together, these data indicate that varying training age by one month does not drastically alter performance. In addition, under these testing conditions, mice are not able to effectively learn the sPAL task and do not perform greater than chance, regardless of age.

LD task performance: Effect of mouse vendor

Other work has shown that the vendor from which mice are obtained can be a methodological concern that affects behavioral outcomes.^{41,42} In addition, previous work from the laboratories of Drs. Bussey and Saksida^{28,39,40} used mice from Harlan Laboratories

FIG. 3. Neither age nor extended testing affected performance in the similar paired associate learning (sPAL) task. (A) Diagram of the images used in the sPAL task with the six possible trial types shown. A check mark delineates the correct image location with an X mark representing the incorrect location. No significant differences were observed between groups or across the testing days in (B) the number of trials completed or (C) the percentage of correct responses. (D) Testing the 3-month-old mice for an additional nine days did not result in improved performance on the number of trials completed (left side) or the percentage correct (right side). $n = 23$ for 3-month-old mice (Cohort D) and 16 for 2-month-old mice (Cohort E).

and Jackson Laboratory, while all of our preceding touchscreen assessments had been conducted with mice purchased from Charles River Laboratories. Thus, we conducted an experiment to evaluate the effect of vendor, assessing mice obtained from Charles River Laboratories (Cohort F) compared with Jackson Laboratory (Cohort G), in 3-month-old C57BL/6 mice. In pre-training, 91–100% of the mice in both groups met criteria for all the pre-training stages (Supplementary Fig. 1; see online supplementary material at www.liebertpub.com). In addition, there were no significant differences between groups in the number of trials it took to complete each training stage (MTT, $p = 0.21$; MIT, $p = 0.19$; PIT, $p = 0.25$; Supplementary Fig. 1; see online supplementary material at www.liebertpub.com).

The 21 mice in each group (Cohorts F and G) that finished pre-training were then evaluated on the LD task (Fig. 4A). The LD acquisition and reversal training were both conducted at the intermediate level (Fig. 4A). All of the mice except one were able to

complete the LD acquisition criterion successfully (Supplementary Table 2; see online supplementary material at www.liebertpub.com). There was no significant difference ($p = 0.76$) in the number of sessions it took each group to meet criterion for LD acquisition with both groups taking an average of four sessions (Charles River, 4.05 ± 0.51 sessions; Jackson, 4.25 ± 0.42 sessions; Fig. 4B). In addition, the average correct response percentage when the mice met criterion for LD acquisition was well above chance (Charles River, 78.70 ± 1.55 % correct; Jackson, 77.00 ± 2.11 ; Fig. 4B). Similar results were observed with LD reversal training with no significant differences between groups in the number of trials completed each day ($F_{1,39} = 3.66$, $p = 0.06$; Fig. 4C) or the percentage of correct responses ($F_{1,39} = 0.007$, $p = 0.93$; Fig. 4D). Additional training days did not result in improvements in the number of trials completed ($F_{2,78} = 0.80$, $p = 0.45$) or the percentage of correct responses ($F_{2,78} = 0.84$, $p = 0.44$) for either group. While only one mouse failed to meet the LD reversal criterion (Supplementary

FIG. 4. Location discrimination (LD) task performance was affected by task difficulty but not vendor or extended testing. (A) Diagram of the LD task with the three difficulty levels depicted. Only the bottom row (positions 8–14) was used throughout. The intermediate level was used for LD acquisition and reversals, while the easy and hard levels were used for LD probes. (B) Both groups were able to meet LD acquisition training criterion in an average of four sessions (left side) with a 77–79% correct (right side). There were no significant differences between groups or across days for LD reversal training in (C) the number of trials completed or (D) the percentage of correct responses with neither group performing above chance. (E) Training the Charles River mice for an additional five days had no significant effect on either the number of trials completed (left side) or the percentage correct (right side). $n = 21$ for Charles River (Cohort F) and 20 for Jackson (Cohort G).

Table 2; see online supplementary material at www.liebertpub.com) and both groups were able to complete almost all 20 trials each day ($p = 0.06$; Charles River, 19.00 ± 0.44 trials; Jackson, 19.88 ± 0.08 trials), the percentage of correct responses remained close to chance ($p = 0.93$; Charles River, 48.89 ± 1.81 % correct; Jackson, 49.10 ± 1.72 % correct; Fig. 4D). The LD reversal training was extended for up to eight days in the Charles River group to determine whether mice could learn the task and increase correct responses with additional training sessions. The 3-month-old Charles River mice, however, did not improve in number of trials completed ($p = 0.60$) or correct response rate ($p = 0.14$; Fig. 4E) with extended training sessions.

Taken together, these data indicate that there is no effect of mouse vendor on touchscreen performance with respect to pre-training completion rate or LD training. Mice are able to learn the LD task successfully during acquisition training, performing better

than chance. In addition, they are able to meet criterion for LD reversal training by completing one reversal; but in our hands, they failed to respond correctly at a rate better than chance, thus indicating an inability to adapt to and learn the new correct location.

LD task performance: Effect of brain injury

On completing LD reversal training and before being evaluated on the LD probe test, mice underwent either a single mTBI or sham surgery. Loss of consciousness was used as an indicator of injury severity by measuring return of righting reflex.¹³ Suppression of the righting reflex was significantly increased for mice receiving mTBI compared with sham controls ($p = 0.0002$; mTBI, 291.25 ± 31.10 sec; sham, 158.90 ± 9.13 sec).

Given that there was no significant effect because of the vendor, mice from the Charles River and Jackson groups were analyzed

FIG. 5. Mild TBI (traumatic brain injury) did not affect location discrimination (LD) task performance. Mouse performance on the easy and hard LD task after mTBI (gray) or sham (black) surgery. No significant differences were observed between sham and mTBI groups in the number of trials completed in either the easy position (A) or the hard position (C). Similarly there were no significant differences observed between sham and mTBI groups in the percentage correct for the easy (B) or hard position (D). As seen in (E) for the easy position and (F) for the hard position, there were significant increases in trials completed (left side) and percent correct (right side) within groups with additional five days of testing, but no significant effect because of injury. $n=20/\text{group}$. * $p<0.05$ compared with sham 1–5; # $p<0.05$ compared with mTBI 1–5.

together after being equally distributed into mTBI and sham groups. The LD probe testing was initiated 24 h after mTBI/sham surgery and utilized both the easy and hard levels (Fig. 4A). Data from the easy and hard levels were separated to determine whether task difficulty affected performance. For the number of easy trials correct, injury had no effect on performance ($F_{1,38}=0.77$, $p=0.39$; Fig. 5A). There was, however, a significant effect with respect to test day ($F_{4,152}=8.58$, $p<0.0001$; Fig. 5A). Pairwise comparisons revealed an increase in easy trials correct with additional testing. Specifically, the mTBI group had significantly more easy trials correct on test days four and five compared with test day one and day five compared with test day two; the sham group had more trials correct on test day five compared with test days one and two. By day five, all groups were able to complete almost all 20 easy trials correctly with an average across test days of 16.34 ± 0.48 trials for the mTBI group and 17.01 ± 0.60 trials for the sham group (Fig. 5E). Similar results were observed when evaluating the percentage of easy trials completed correctly with no significant effect because of injury ($F_{1,38}=0.44$, $p=0.51$; Fig. 5B), but significant

improvements across the testing period ($F_{4,152}=7.76$, $p<0.0001$; Fig. 5B). Again, pairwise comparisons revealed that both the mTBI and sham groups were significantly improved on test day five compared with days one and two with an average percent correct across the five days of 82.83 ± 2.39 for the mTBI group and 85.37 ± 2.98 for the sham group (Fig. 5E).

Data collected from the hard level resulted in similar findings to what was observed at the easy level, except overall performance was slightly lower. There was no significant effect of injury when evaluating the number of hard trials correct ($F_{1,38}=1.07$, $p=0.31$; Fig. 5C) or the percentage of hard trials correct ($F_{1,38}=0.54$, $p=0.47$; Fig. 5D). Again, there was a significant effect because of test day for both the number of hard trials correct ($F_{4,152}=10.3$, $p<0.0001$; Fig. 5C) and percentage of hard trials correct ($F_{4,152}=8.97$, $p<0.0001$; Fig. 5D). Pairwise comparisons revealed significant increases for the following groups on the following days: mTBI on test days three to five (for # correct) and test days two to five (for % correct) compared with day one and sham on test days four and five compared with day

one (for # correct) and on day five compared with days one and three (for % correct). On day one of testing, all groups were performing at chance, or slightly above, with an average hard correct response between 50% and 73%. By day five, all groups had improved and the correct response percentage increased to well over chance at 83–95% correct (Fig. 5D).

Half of the mice in each group were evaluated for an additional five days at the easy and hard levels. Both the mTBI group and sham group exhibited increased performance from days one to five to days six to 10. Specifically, on days 6–10, the mTBI group had a significant increase in the number of trials correct at the easy ($p=0.006$) and hard ($p=0.02$) levels and a significantly higher percentage of correct responses at the easy ($p=0.01$) and hard ($p=0.02$) levels (Fig. 5E,F). Similarly, the sham group demonstrated greater performance on days 6–10 with significantly increased correct trials at both easy ($p=0.04$) and hard ($p=0.02$) levels and a higher percent correct response on the hard level ($p=0.02$; Fig. 5E,F). There were still no differences observed between the sham and mTBI groups on days six to 10, however.

Taken together, these data indicate that mice do have the ability to learn on the LD task when there are no reversals as evidenced by higher correct response percentages and improvements across test days. Under our testing conditions, however, this task was not effective in detecting deficits with this single mTBI injury model, because there were no differences in performance between sham and mTBI mice.

Discussion

The goal of this study was to determine whether mTBI in mice would affect cognitive performance on rodent touchscreen chambers, modeled after computerized neuropsychological assessments used clinically. Here, we show that with a stepwise training regimen and the appropriate experimental manipulations, mice were capable of being trained to use the touchscreen and associate touching a stimulus with reward receipt. The C57BL/6 mice, however, failed to perform better than chance when evaluated with the PAL task and the more difficult variations of the LD task, both hippocampal-based learning and memory paradigms. In addition, we found that inducing a single mTBI using an established impact-acceleration model did not result in altered performance in the LD task. While the PAL and LD touchscreen paradigms did not serve as robust indicators of cognitive performance under the conditions presented here, it is possible that more favorable results could be obtained using the touchscreen tasks under different experimental parameters.

The role of food restriction

Success in the execution of complex behavioral tasks in rodents can be increased by implementing strategies that entice mice to perform. One such strategy is controlling and limiting rodent food intake paired with the delivery of a food/fluid reward in the behavioral paradigm. Manufacturer instructions as well as other studies using these touchscreen chambers all listed food restriction as a key procedural aspect.^{27,28,30,34,37} While food restricting mice to 80–85% body weight helps to motivate mice to seek the food reward and thereby increase performance on the task, we were hesitant to implement this strategy for several reasons. First and foremost, it is well documented that caloric restriction is neuroprotective by mechanisms such as reducing pro-inflammatory factors, increasing neurotrophic factors, and improving mitochondrial function.^{43–46} Previous research has demonstrated such protective aspects of caloric restriction before TBI including: reduced

lesion volume, suppression of microglia activation, increased brain-derived neurotrophic factor (BDNF), and improvements in spatial memory.^{45,46} Because the goal of this study was to evaluate cognitive function after mTBI, we were concerned that food restricting mice would potentially confound the experimental design by conferring neuroprotection.

An additional concern was that food restriction can elicit an increased stress response in rodents as indicated by elevated corticosterone levels as well as increased anxiety and depression.^{47,48} For example, previous work that compared the effect of food restriction alone with food restriction plus touchscreen training found that food restriction alone resulted in increases in fecal corticosterone and markers associated with hippocampal learning such as BDNF, c-FOS, and zif268 similar to levels observed with touchscreen training plus food restriction.⁴⁹ Considering the multiple effects that food restriction could have on neuroprotection and learning, it becomes challenging to distinguish the effect of mTBI on cognitive performance when paired with food restriction. Ultimately in this study, we used food restriction because our data show that food restriction was necessary for training. Specifically, the manipulations of food restriction coupled with increasing the palatability of the reward yielded more than 90% of the mice advancing through PIT (stage 5) of the training. As mentioned previously, however, we did not observe an effect of mTBI on performance, and a possible reason could be the confounding variable of food deprivation.

Advancement criterion and session number considerations

An important element in the experimental setup is the criterion used for advancement to the next training stage. The initial criterion for advancement from one stage to the next was set at the completion of 36 trials in 60 min based on manufacturer recommendations and previous study protocols.^{35–38} In our experiment, however, mice struggled repeatedly to complete 36 trials. Because other studies demonstrated that mice exhibited greater success when the criterion was reduced to 30 trials in 60 min,^{30,39} we also reduced the advancement criterion. In our hands, reducing criterion to 20 completed trials in 40 min increased the success rate to 60%. This decreased exposure to the task could account for some of the lack of success in sPAL and LD testing, however. An alternative approach subdivides the training sessions in two to account for the decreased exposure caused by reducing the criterion. For example, the session criterion could be reduced to 18 trials in 30 min and expose the mice to two sessions instead of one to reach the 36 trials and 60 min.³⁵ In addition, the criterion could be increased in a graded manner as the mouse performance improves. We did not evaluate these alternative approaches to modifying the training criterion, but future work that specifically evaluates the effect of criterion and exposure would be informative.

Because the training is sequential, it is critical that mice perform well on each stage before moving on to the next and more difficult stage. We found that as early as habituation, where the task was simply collecting reward, some mice completed very few trials. To increase the probability of successfully training mice, we opted to advance the top 50% of mice based on trial completion. Although a somewhat arbitrary cutoff, this procedure resulted in a subsequent successful completion of pre-training for 90% of the mice. It may be useful in future studies to determine additional or alternative biological indicators that could better predict performance.

In addition to the advancement criterion, the number of sessions to which the mice are exposed could be an influential factor on

performance. In the current study, adult male mice did not respond at percentage correct rates greater than chance in the sPAL task or the more difficult version of the LD task that involved reversals, suggesting a lack of robust learning. Our results were similar to those in the study by Delotterie and coworkers³⁸ within the first five days of testing. Delotterie and colleagues³⁸ demonstrated, however, that the percentage correct increased to 65% after 15 sessions, 70% after 20 sessions, 75% after 25 sessions, 80% after 35 sessions, and plateauing at around 85% correct after 40–50 sessions. It has been noted that mice might require upward of 50 sessions to meet a 70% correct response.³⁵

Thus, it is plausible that with additional testing days and/or sessions, the cognitive performance of the mice may have improved. It is important to note that it is likely that the number of additional days and sessions would be substantial, because our data show that extending sPAL testing to 14 days and LD reversal training to eight days did not result in improved performance. From the perspective of evaluation of cognition after mTBI, exposing the mice to extensive testing sessions, which would follow weeks of pre-training, is highly likely to have a large effect on the age of the mouse. Because we found that training at a younger age does not improve performance and age at injury is often a modifier of TBI-induced pathology and outcomes,⁵⁰ the balance between session number and age of the mice should be considered carefully in the experimental design.

Additional variables affecting training

The automated touchscreen chambers presented here function differently than traditional rodent behavioral assessments that utilize operant conditioning. Traditional chambers are equipped with response levels or nose poke apertures that require a physical interaction (i.e., pressing or snout touch) in response to a stimulus to obtain a reward.^{51–53} In previous studies, enhanced performance has been demonstrated when there is this tactile stimulation compared with just visual cues.^{54,55} The touchscreen chambers used in the present study, however, are designed to detect mouse responses with infrared beams, and therefore the mice do not have to elicit a touch response physically. Relying on visual stimuli alone could lead to a deprived somatosensory feedback state and account for the lack of mouse motivation to respond repeatedly during training, thereby affecting their ability to learn the task. Touchscreen operant chamber experts, Drs. Bussey and Saksida,⁵⁶ specifically evaluated motivation for repetitive responding with these touchscreens, however, and demonstrated similar performances in the progressive ratio task as reported with traditional operant chambers.

Notably, we found other variables that affected training. First, we found that making the liquid/food reward more palatable by adding sweetness and flavor improved training performance. This result is consistent with findings from other operant training paradigms that show an interaction between operant behavior and highly palatable foods on reward circuitry.⁵⁷ Second, we found that reversing the light/dark cycle such that mice were tested in their dark-active cycle increased completion of the MTT stage from 60% under normal light/dark cycle conditions to 100%. These data along with the results from other behavioral studies suggests that mice perform better when evaluated during the dark-active phase of the light/dark cycle given that they are nocturnal in nature.⁵⁸ A third variable we evaluated was the effect of vendor, because other work has shown that the vendor can be a methodological concern that affects behavioral outcomes.^{41,42} Our data showed that there was not an effect of vendor on behavioral performance, with more than

90% of mice obtained from either Charles River or Jackson Laboratories advancing past the PIT training stage.

The fourth variable that we considered to some extent was age at initiation of training. It is important for our mTBI model that we evaluate young adult mice, and previously we have used mice that are 3 months old at injury¹³; thus, we assessed a narrow age difference focusing on 2-month-old versus 3-month-old mice. We found that the younger, 2-month-old mice took more sessions to complete the MTT training than their older counterparts. Also, fewer of the 2-month-old mice advanced to the PIT stage of training. Thus, while initiation of the training at a younger age could be a strategy to control for age at injury because of the long training and testing processes, initiation of training in 3-month-old mice yields better training completion, which suggests this variable should be considered carefully in the experimental design.

Some other potentially important parameters that we did not evaluate include mouse strain and single versus group housing. With regard to mouse strain, Graybeal and colleagues⁵⁹ showed that C57BL/6J mice were among the best performers with respect to the number of sessions taken to meet criteria in pre-training. Thus, although we did not evaluate strain difference, we used a strain that has been shown previously to learn in a touchscreen chamber task. With regard to housing, previously published touchscreen protocols recommend group housing mice.^{34,35} Yet the effect of single versus group housing on operant responding in mice is controversial because others report no effect.⁶⁰ As with other experimental design variables discussed above, group housing particularly in terms of environmental enrichment can affect outcome after mTBI; for review see.⁶¹ Thus, we singly housed mice before and after mTBI, but acknowledge that housing could affect performance.

sPAL performance and considerations

Mice performed at or slightly below chance in terms of percentage of correct responses in the sPAL task, which is inconsistent with previous findings.^{29,38} Similar to the variables that could affect performance in the training stages as discussed above, variables specific to the PAL task could account for variation in performance between studies. For example, the PAL task has traditionally used three distinct objects as the stimuli (Fig. 3A).^{29,30,38} These three objects have been demonstrated to not elicit a stimulus bias, meaning that the animal does not show preference for one of these objects over the others.²⁰ It has been noted, however, that the use of line patterns might be a better option for images over the traditional objects because there is less variability, although this is not a variable we evaluated here.^{28,35}

In addition, there are two versions of the PAL task, sPAL and dPAL. While sPAL uses the same image as stimuli, dPAL uses different images as stimuli relying on both spatial and non-spatial information. Although studies have implicated hippocampal involvement with the dPAL task, we did not evaluate performance on the dPAL task. Notably, dPAL generally is considered to be more difficult than sPAL for mice to learn, taking mice 40 sessions to reach 70% correct and 95 sessions to reach 80% correct.^{28,29,38} Thus, the aforementioned concerns about the duration of training also apply to the dPAL task.

LD performance and considerations

The LD task is an assessment of pattern separation and as such is characterized as a task that is dependent on hippocampal function, with the dentate gyrus (DG) being one region of the hippocampus

playing an essential role.^{62–64} Unlike sPAL, mice were able to learn the LD task as evidenced by the acquisition and probe data. When evaluated at the intermediate separation during acquisition, 41/42 mice were able to complete the trials with an average correct response of 77–78%. For the probe trials, mice also exhibited correct responses that were well above chance at both the easy (large) and hard (small) separations. Performance improved over the course of the week for both the easy separation (68–86% on day one to 88–94% on day five) and the hard separation (50–73% on day one to 83–95% on day five). Our probe data is in agreement with several others studies that have demonstrated better rodent performance when the separation is larger.^{27,39,65,66}

Importantly for our study, we did not observe an effect of injury across the five test days, suggesting that while mice are learning within the LD task, a single mTBI using the impact-acceleration model does not affect task performance. Clinically, cognitive impairments are experienced acutely after a single mTBI that often resolves in five to seven days; although 10–15% of persons report deficits that persist past seven days.^{67–70} It is important that animal models of mTBI produce similar detectable deficits, which were not demonstrated using this task during the acute time frame. To ensure that the mice were indeed injured, we measured suppression of the righting reflex after impact, a frequently used behavioral biomarker for mTBI that determines the duration of loss of consciousness. In the current study, mice that received an impact experienced a righting time of almost 5 min, which was significantly longer than their uninjured counterparts. This duration of transient unconsciousness is similar to that published previously by our laboratory using this model and in other studies using various mTBI models.^{13,16,71,72}

While other work has shown that performance on pattern separation tasks tested with the LD paradigm is impaired when the hippocampus is lesioned, no other study has evaluated the effect of TBI on LD performance, suggesting that the deficits induced by mTBI may be too subtle to detect with the LD paradigm used in the present study. One key variable to consider that is specific to the LD task is the implementation of the probe sessions. In the current study, we used the two-choice location discrimination protocol for the probe session such that separation difficulties of hard (small) and easy (large) were randomly presented with only one side designated as correct (i.e., no reversals).³⁶ An alternate protocol is to reverse the side of the correct location but only assess mice at either the hard (small) separation or easy (large) separation within one session, as done by all the aforementioned studies.³⁴ It is assumed that the reversal paradigm significantly affects performance by creating a more difficult task, which is also reflected in our data, because the mice did not perform as well during LD reversal training. Thus, is it possible that increasing the number of reversals would have resulted in a task with greater cognitive load, which may have been more challenging for mice after mTBI. This hypothesis was not evaluated in the current study, however.

Conclusions

While touchscreen operant chambers offer a translational approach to assess cognitive function in rodents, there are several caveats to their utilization for cognitive assessment after mTBI in mice—namely, the duration of the training process, the need for food restriction, and the effect of criteria and session number are all variables that should be carefully considered in assessment of cognition after injury. Importantly, as has been eloquently noted previously, experimental design and conditions can affect behavioral results profoundly in a wide variety of testing paradigms such

that evaluation of cognitive function after mTBI requires careful consideration of many factors that could influence outcome.⁶¹ The touchscreen operant chambers underscore the importance of considering and controlling even the most subtle of variables.

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