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Repetitive Mild Traumatic Brain Injury in a Perinatal Nicotine Exposure Mouse Model of Attention Deficit Hyperactivity Disorder

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Keywords
Attention deficit hyperactivity disorder · Traumatic brain injury · Concussion · Attention · Working memory · Depression

Abstract
Attention deficit hyperactivity disorder (ADHD) increases the risk for concussion or mild traumatic brain injury (mTBI). At the same time, recommendations for the management of ADHD include participation in sports and other organized physical activities, including those that carry an increased risk of mTBI. Very little work has been done to determine the extent to which untreated ADHD adversely impacts behavioral outcomes of repeated mild concussions. Here, we used a perinatal nicotine exposure (PNE) mouse model of ADHD combined with a closed-head, repetitive mTBI model. The PNE mouse model carries significant construct, face, and predictive validity as a preclinical model of ADHD. Two-month-old PNE and control mice were subjected to closed-head repetitive mTBI or sham procedure once daily for 5 days. Object-based attention, novel object recognition memory, spatial working memory, and depression-like behavior were analyzed 1 day and 2 weeks following repeated mTBI. Consistent with our previous reports, mice in the PNE group showed significant deficits in object-based attention and working memory prior to mTBI. These deficits persisted following the repeated mTBI. Repeated mTBI produced a transient attention deficit in the control group but did not exacerbate the attention deficit that is characteristic of the PNE group. Although neither PNE nor repetitive mTBI alone influenced immobility in the tail suspension test, when PNE mice were subjected to mTBI, there was a transient increase in this measurement suggesting a synergistic effect of ADHD and mTBI on depression-like behavior. Thus, our data using the PNE mouse model suggest that ADHD may be a risk factor for transient depression following repeated mTBI and that repeated mTBI may be a risk factor for transient attention deficit.

Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder that affects 8–10% of children [1–3]. It is characterized by attention deficit, hyperactivity, and impulsivity that can disrupt development or function in social, academic, and occupational settings [4–6]. As described by the American Psychiatric Associa-

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tion's Diagnostic and Statistical Manual, Fifth edition (DSM-5), the symptoms, which can range from mild to severe, begin before age 12 but can last through adolescence and into adulthood [7].

While pyschostimulants methylphenidate (MPH) or amphetamines remain the first line of ADHD treatment [8–10], there is a growing appreciation for the beneficial role of nonpharmacological therapies. These include the use of physical activities such as exercise and sports [11–13]. A recent meta-analysis of 30 studies showed that moderate to intense aerobic exercise can be effective in the management of the cognitive and behavioral symptoms of ADHD [14]. However, physical activity that includes participation in contact sports increases the likelihood of concussion or mild traumatic brain injury (mTBI). In fact, regular participation in contact sports, particularly American football, rugby, hockey, soccer, and basketball, carries a high risk for repetitive concussion [15].

There is strong evidence to suggest that athletes with ADHD have a significantly higher risk of mTBI compared to non-ADHD athletes [16–20]. The risk of repetitive concussion is also higher in athletes with ADHD [19]. Furthermore, there is evidence that the symptoms of concussion, particularly verbal memory impairment [21], fatigue, and poor concentration [18], are worse in student athletes with ADHD compared to athletes without ADHD who sustain an mTBI. However, there are conflicting data on the impact of ADHD on mTBI outcomes, as baseline symptoms may mimic some of the symptoms of mTBI [19, 22, 23]. In any event, the degree to which untreated ADHD impacts the behavioral outcomes following mTBI is only now beginning to be examined [22, 24–26]. A recent meta-analysis reported that only 2 small studies to date have reported statistically significant links between ADHD and poorer clinical outcomes following mTBI [26]. Other studies found no such association leading to the conclusion that the exact association between ADHD and mTBI outcomes was unclear, in large part due to the underpowering of the available studies [9].

To address this gap in knowledge, we used a mouse model to determine the extent to which repetitive mTBI produces significant changes in behavioral outcomes in a perinatal nicotine exposure (PNE) mouse model. We used the PNE mouse model because it displays neuroanatomical, neurochemical, and behavioral phenotypes consistent with ADHD, and the behaviors in this mouse model respond to treatment with the classic stimulant drug MPH [27–30]. This model enabled us to examine whether closed-head repetitive mTBI exacerbates the attention and working memory deficits present in the PNE mouse model or produces deficits in novel object recognition memory or depression-like behaviors that are associated with traumatic brain injury [31–36] but not with ADHD.

Materials and Methods

PNE Model

C57BL/6 mice were purchased from Charles River Laboratories (Kingston, NY) and housed in the Florida State University Laboratory Animal Resources facility. The facility is a temperature- and humidity-controlled environment maintained on a 12-h light-dark cycle (lights off at 7:00 a.m. and on at 7:00 p.m.). The mice had food and water available ad libitum. Breeding age (8–12 weeks old) female mice were randomly assigned to one of the 3 experimental groups based on the type of drinking water supplied: PNE group was provided with water containing nicotine (100 μg/mL (–)-nicotine; Sigma Chemical Co., St. Louis, MO; Cat# N3876) and 2% saccharin (Alfa Aesar, Heysham, UK; Cat# A15530); the saccharin group was provided with water containing 2% saccharin (Fig. 1a). To control for the potential effects of saccharin, a third group of mice received plain water [27–29, 37]. After 3 weeks, female mice in each group were bred with drug-naïve male mice. Based on the presence of a vaginal plug, the day of successful mating was designated embryonic day 0 (E0) and the day of birth postnatal day 0 (P0). The litter size was standardized to contain 6–8 offspring on the day of birth. The 3 types of water were continued until the pups were weaned on P21. Throughout pregnancy, each female mouse was singly housed. Upon weaning, same sex offspring were housed 2–4 per cage.

Closed-Head Repetitive mTBI

The procedure was performed on male mice from the PNE and control groups at approximately P60 (Fig. 1b). An electromagnetically driven stereotaxic device designed to control the site, depth, and velocity of impact was used (Impact One Stereotaxic Impactor; Leica, Buffalo Grove, IL). Blunt force impact was achieved by enclosing the tip of the metal piston in a customized rubber cap (9 mm in diameter) [38, 39]. A custom-made foam platform was used to position the mice during impact. The rubber cap on the metal piston and the foam platform helped mimic the concussion and head rotation in humans. Anesthesia (2.5% isoflurane) was maintained throughout the procedure using a nose cone. The center of the impactor was positioned approximately 1 mm anterior to the bregma and 4 mm lateral to the longitiudinal midline [38]. The depth of the impact was adjusted to 3 mm from the surface of the skin. The impact occurred at 5 m/s with a dwell time of 100 ms. To control for the possible effects of anesthesia [40, 41], a parallel set of sham mice which received the same anesthesia were positioned in the stereotaxic frame, but were not subjected to mTBI. After impact (or sham procedure), the mice were placed on a heating pad until mobile and then returned to the home cage. The procedure was repeated once daily for up to 5 consecutive days.

Behavioral Tests

Recognition memory was analyzed using the novel object recognition (NOR) test and spatial working memory using the Y-maze. Attention was assessed using an object-based attention (OBA) test, while animals were monitored for depression-like behavior using the tail suspension test (TST). At approximately P60,

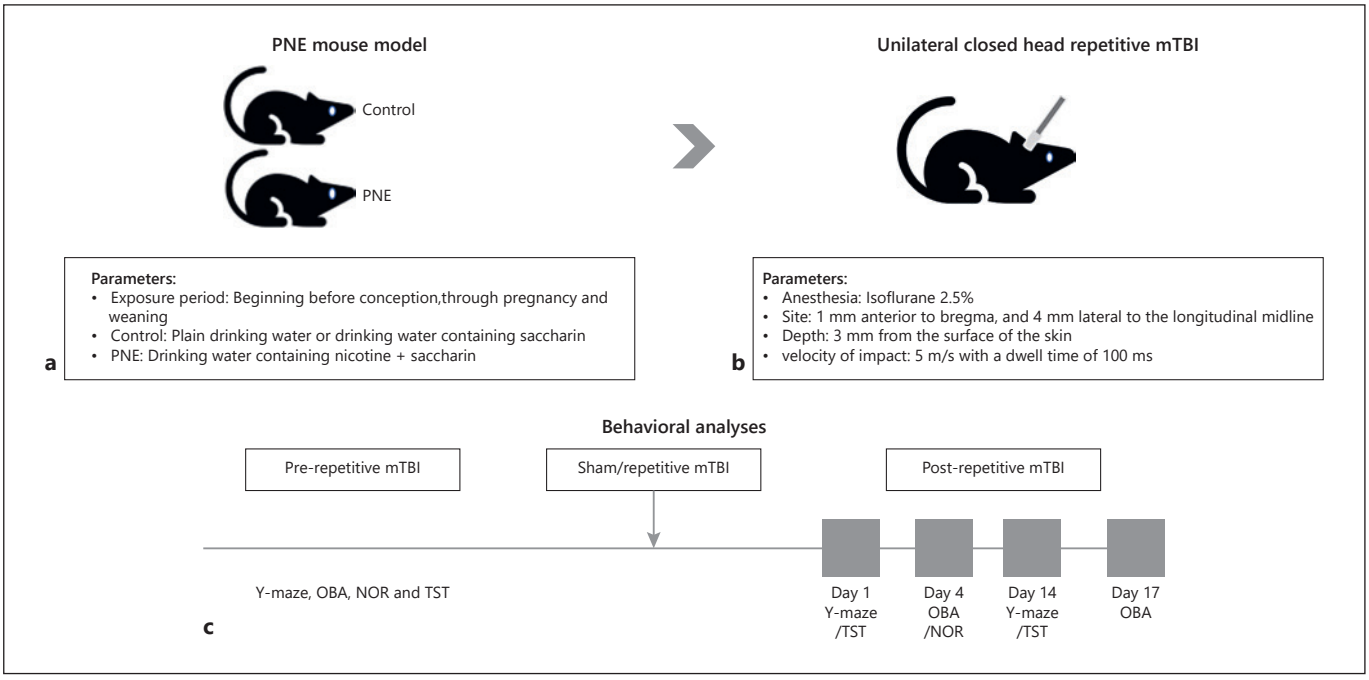

Fig. 1. An outline of the experimental design. Female mice were exposed to plain drinking water and drinking water containing saccharin or nicotine plus saccharin beginning 3 weeks before conception, throughout pregnancy, and until the offspring were weaned. **a** The mice exposed to plain drinking water and saccharin were combined into a control group, and the mice exposed to nicotine plus saccharin were the PNE group. **b** At approximately 60 days of age, the mice in the 2 groups were subjected to unilateral closed-head mTBI or sham procedure daily for 5 consecutive days. **c** The following behavioral assays were performed prior to and on 2 occasions following the mTBI or sham procedure: Y-maze test, object-based attention (OBA) test, novel object recognition (NOR) test, and tail suspension test (TST). PNE, perinatally nicotine exposed; mTBI, mild traumatic brain injury.

each mouse was handled by the experimenter for 5 min per day every day for 3 days so that the mouse became familiar with the process of being picked up and handled by the experimenter. The sequence of these behavioral assays was designed to minimize the potential impact of repeated testing. They were divided into 2 groups. Group 1 underwent Y-maze and OBA test, whereas group 2 underwent NOR and TST. The tests were performed during the lights-off period under dim lighting. Mouse behavior was recorded using a low-light video camera. The mice were habituated to the testing room for 30 min before the behavior analyses began. Upon completion of the tests, the mice were randomly assigned to mTBI or sham groups. Following the mTBI or sham procedure, within each of these 2 groups, the mice were divided again into 2 groups: group 1 underwent Y-maze and OBA test, whereas group 2 underwent TST and NOR. Y-maze assay and TST were performed 1 day after the final mTBI or sham procedure (designated as day 1). The OBA and NOR tests began on day 2 following the final mTBI or sham but were completed on day 4. Two weeks after the first round, a second round of behavioral analysis began. Y-maze, OBA, and TST but not NOR test were performed during the second round.

Novel Object Recognition (NOR)

The assay consisted of 2 days of habituation followed by a test session on the third day [28–30]. On days 1 and 2, the mice were individually placed in the test chamber (32 × 28 × 30 cm) for 20 min for habituation to the chamber. On day 3, the mice were returned to the same chamber to explore 2 identical objects: an unopened and unmarked can of food (3.14 × 3.6 × 11 cm) or a Lego object (6.4 × 6.4 × 11 cm). Time spent exploring the object placed on the left versus right hand side, as well as total time spent exploring the 2 objects, was recorded using an overhead video camera. These data were used to evaluate object or side bias. A counterbalance design was used to address potential bias [29]. Following this step, the mouse was returned to its home cage for 10 min. The mouse was reintroduced to the same chamber, which now contained one of the 2 objects it had explored earlier (the familiar object) and an object to which the mouse had not been exposed previously (the novel object). The mouse explored these 2 objects for 5 min, and its activity was recorded. An investigator blinded to the identity of the mouse analyzed the video recordings. The novel object recognition index was calculated using the formula: time spent with the novel object/the time spent with both the objects (novel object + familiar object) × 100.

Spatial Working Memory (Y-Maze)

A Y-maze was used to evaluate spatial working memory [28, 29, 37]. Unique visual cues were placed on the exterior of the walls of each of the 3 arms of the Y-maze to facilitate recognition of each arm as unique by the mouse. The mouse was placed at the center of the Y-maze to begin a 6-min trial, and its behavior was recorded using an overhead video camera. An investigator blinded to the identity of the mouse analyzed the video recordings to calculate the number

of entries into each arm and the sequence of arm entries. An arm entry was considered to have occurred when all 4 limbs of the mouse entered an arm. A "spontaneous alternation" is defined as a set of 3 consecutive arm choices without a repeated entry (e.g., ABC, BCA, and CAB). A spontaneous alternation score was calculated using the formula: number of alternations/(number of entries − 2) × 100.

Object-Based Attention (OBA)

The assay consisted of 2 days of habituation, followed by a testing session on day 3. The test apparatus consisted of an exploration chamber (40 × 40 × 25 cm) and a testing chamber (40 × 20 × 25 cm) separated by a sliding door [28, 29, 42, 43]. On day 1 (habituation), the mouse was habituated to the empty apparatus. On day 2 (habituation), the mouse was habituated to 5 objects, each made of the same wooden material, of the same size, but different shapes, in the training chamber for 5 min. Next, on the same day, the mouse explored 2 of these objects selected randomly, in the test chamber for 5 min. Day 3 began with an additional shorter habituation period (3 min in each chamber), followed immediately by exploration of the 5 objects used on day 2 in the training chamber for 3 min. Following a 10-s interval, the door separating the chambers was opened allowing the mouse to enter the test chamber and explore 2 objects, one of which was a familiar object that was selected randomly from the 5 objects used in the training chamber. The second object was a "novel" object to which the mouse had never been exposed. The familiar object was placed along the wall of the test chamber in a position analogous to its original position in the training chamber, while the novel object was placed near the wall opposite. The behavior of the mouse was recorded with an overhead video camera for 3 min. An investigator blinded to the experimental conditions analyzed the video recordings to calculate the length of time spent with each of the 2 objects (novel and familiar). A recognition index was calculated and expressed as percentage using the formula: TN/(TF + TN) × 100, where TF and TN represent the time spent during the test session exploring the familiar and the novel objects, respectively. We included in the analysis only those mice that spent at least 18 s with both objects in the test chamber, to minimize variability in the data.

Tail Suspension Test (TST)

This is a test of depression-like behavior in rodents [44, 45]. The test apparatus consisted of a custom-built rectangular box (opaque Plexiglass, 39 cm length × 13 cm width × 60 cm height) with a metal suspension rod. The mouse was secured using adhesive tape (TimeMed Labeling Systems, Inc, Burr Ridge, IL, 1.9 cm width) to the rod by its tail and suspended upside down by its tail for a period of 6 min. The tail was threaded through polycarbonate tubing (9.68 cm length × 1.1 mm diameter) to prevent the mouse from climbing its tail during the test. During suspension, the approximate distance between the mouse's nose and the bench top was 25 cm. The test is based on the observation that mice will exhibit bouts of mobility and immobility in a futile attempt to escape the stress of tail suspension. Mouse behavior was video recorded throughout the 6-min test period. A mouse was considered immobile when it hung passively and completely motionless. Duration of immobility was calculated from the video recordings.

Statistical Analysis

Three-way ANOVA with repeated measures in 1 factor was used to test the main effects of repetitive mTBI, perinatal treatment, and time after mTBI and the interaction between these factors. For statistical comparison, control mice with access to plain drinking water and controls with access to saccharin were combined into a single group because, consistent with our previous work [27–29], there were no significant behavioral differences between these 2 groups. When a significant main effect or interaction ($p < 0.05$) was found by ANOVA, the Bonferroni multiple comparison post hoc test was performed. In all cases, a p value of <0.05 was considered statistically significant. Prism 9.0 Software (GraphPad Prism 9.0 Software Inc., San Diego, CA) was used for the statistical analyses.

Results

Baseline Behavioral Analyses

We examined the effects of perinatal treatment on spatial working memory, object-based attention, novel object recognition, and learned helplessness (TST) to establish a baseline for these phenotypes prior to mTBI. We found that PNE produced significant deficits in spatial working memory ($F_{(2, 30)} = 21.75$, $p < 0.0001$) and object-based attention ($F_{(2, 35)} = 32.41$, $p < 0.0001$). However, PNE did not produce a significant deficit in novel object recognition ($F_{(2, 26)} = 0.1263$; $p > 0.05$) and did not increase immobility time in the TST for depression-like behavior (one-way ANOVA; $F_{(2, 32)} = 0.1934$; $p > 0.05$). The same behavioral tests were performed again after repeated mTBI or sham procedure.

Spatial Working Memory

Use of the Y-maze and a repeated measure 3-way ANOVA to evaluate spontaneous alternation as a measure of spatial working memory showed that there was a significant main effect of PNE ($p < 0.0001$). Post hoc comparisons showed that PNE produced a significant impairment in working memory ($p < 0.001$; Fig. 2a; Table 1). However, there was no significant main effect of mTBI ($p > 0.05$). Furthermore, there was neither the main effect of time after mTBI ($p > 0.05$) nor were the significant interactions between mTBI, perinatal nicotine treatment, or time after mTBI ($p > 0.05$; Table 1).

Object-Based Attention (OBA)

A repeated measure 3-way ANOVA showed significant main effects of mTBI and perinatal nicotine treatment ($p < 0.0001$) in the object-based attention test. The main effect of time after mTBI was not significant ($p > 0.05$). All interactions among the 3 factors were significant ($p < 0.05$). Post hoc comparisons showed significant differences between control sham and mTBI groups on day 4 and control sham and PNE sham groups on days 4 and 17 ($p < 0.0001$; Fig. 2b; Table 1).

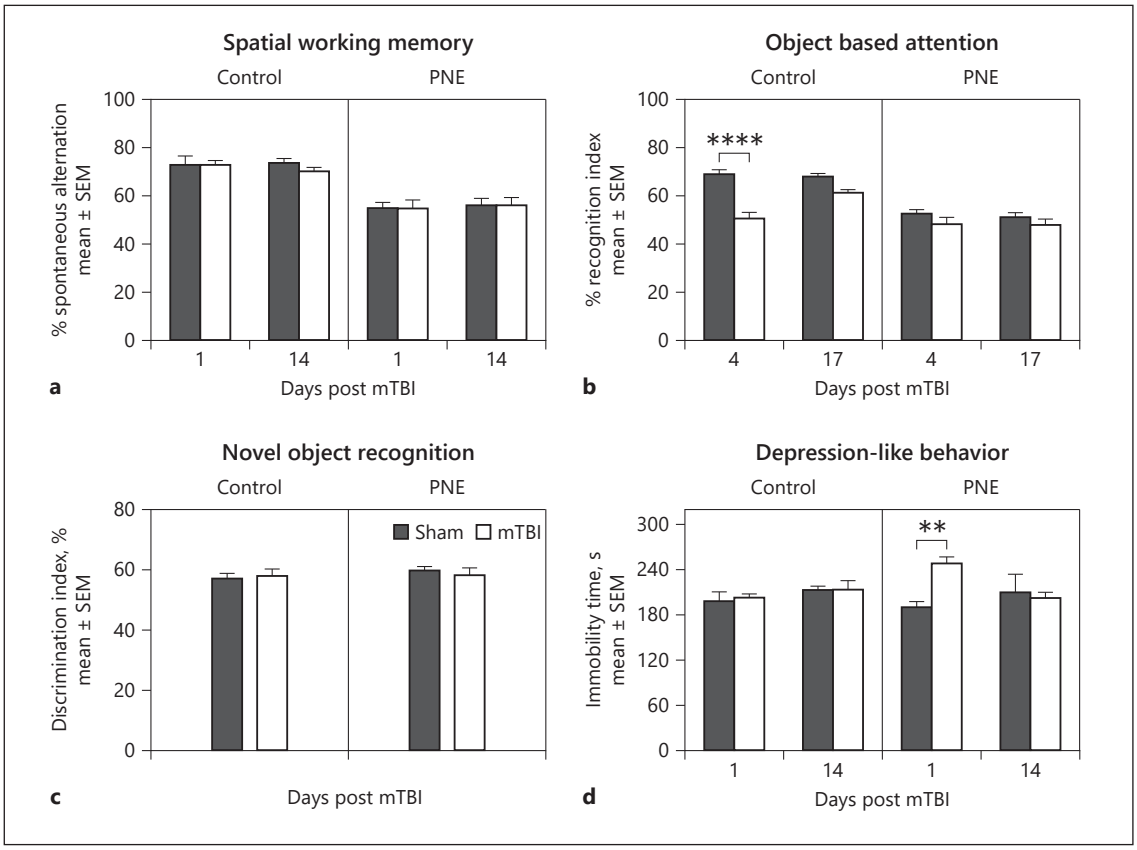

Fig. 2. Analysis of spatial working memory (**a**), object-based attention (**b**), recognition memory (**c**), and depression-like behavior (**d**) in control and PNE mice following repeated mTBI or sham procedure. Among the behaviors analyzed, there was a significant decrease in recognition index in the control group 4 days following repeated mTBI compared to the sham procedure (**b**) and a significant increase in immobility in the PNE group 1 day after repeated mTBI compared to the sham procedure (**d**). Bonferroni multiple comparison test following two-way ANOVA. $^{**}p < 0.01$; $^{****}p < 0.001$. PNE, perinatally nicotine exposed; mTBI, mild traumatic brain injury.

Novel Object Recognition (NOR)

Two-way ANOVA showed that there were no significant effects of perinatal nicotine treatment ($F_{(1, 25)}$ = 0.7148; $p > 0.05$), mTBI ($F_{(1, 25)}$ = 0.02567; $p > 0.05$), or perinatal treatment × mTBI interactions ($F_{(1, 25)}$ = 0.5118; $p > 0.05$) on recognition memory as measured in the NOR test (Fig. 2c).

Tail Suspension Test (TST)

A repeated measure 3-way ANOVA showed a significant main effect of mTBI ($p < 0.05$) in the TST for depression-like behavior. There were no significant main effects of PNE treatment ($p > 0.05$) or time after mTBI ($p > 0.05$). There was, however, a significant interaction between mTBI and time after mTBI ($p < 0.05$) as well as between PNE treatment, mTBI, and time after mTBI ($p < 0.05$). Post hoc comparisons showed significant differences between the sham PNE group and mTBI-PNE groups on day 1 after mTBI ($p < 0.05$; Fig. 2d; Table 1).

Discussion

We examined the effects of mTBI on ADHD-like behaviors using the PNE mouse model of ADHD because it shows neuroanatomical, neurochemical, and behavioral characteristics consistent with ADHD. For example, volume of the cingulate cortex is reduced in the PNE mice [27] as well as in human subjects with untreated ADHD [46]. The PNE mice show significant reductions in frontal cortical dopamine content [27, 30] and exhibit locomotor hyperactivity, attention deficit, and motor impulsivity [27–30], all of which are consistent with ADHD. Further-

Table 1. Summary of 3-way ANOVA and Bonferroni multiple comparison data for Y-maze, object-based attention test, and tail suspension test

Y-maze	
ANOVA	*F* (DFn, DFd)
mTBI	$F(1, 29) = 0.1619$
Perinatal treatment	$F(1, 29) = 110.90$****
Time after mTBI	$F(1, 29) = 0.0036$
mTBI × perinatal treatment	$F(1, 29) = 0.1875$
mTBI × time after mTBI	$F(1, 29) = 0.3117$
Perinatal treatment × time after mTBI	$F(1, 29) = 0.5792$
mTBI × perinatal treatment × time after mTBI	$F(1, 29) = 0.4006$
Bonferroni multiple comparisons test	*t*; DF
Control: sham day 1 versus PNE: sham day 1	5.213; 58.00****
Control: mTBI day 1 versus PNE: mTBI day 1	6.542; 58.00****
Control: sham day 14 versus PNE: sham day 14	5.099; 58.00****
Control: mTBI day 14 versus PNE: mTBI day 14	5.033; 58.00****
Object-based attention test	
ANOVA	*F* (DFn, DFd)
mTBI	$F(1, 34) = 33.52$****
Perinatal treatment	$F(1, 34) = 83.77$****
Time after mTBI	$F(1, 34) = 2.515$
mTBI × perinatal treatment	$F(1, 34) = 10.55$**
mTBI × time after mTBI	$F(1, 34) = 6.907$*
Perinatal treatment × time after mTBI	$F(1, 34) = 5.247$*
mTBI × perinatal treatment × time after mTBI	$F(1, 34) = 4.594$*
Bonferroni multiple comparisons test	*t*; DF
Control: sham day 4 versus PNE: sham day 4	6.067; 68.00****
Control: sham day 4 versus control: mTBI day 4	7.634 68.00****
Control: mTBI day 4 versus control: mTBI day 17	4.953 34.00***
Control: mTBI day 17 versus PNE: mTBI day 17	5.511 68.00****
Control: sham day 17 versus PNE: sham day 17	6.200 68.00****
Tail suspension test	
ANOVA	*F* (DFn, DFd)
mTBI	$F(1, 31) = 4.556$*
Perinatal treatment	$F(1, 31) = 0.6626$
Time after mTBI	$F(1, 31) = 0.00008$
mTBI × perinatal treatment	$F(1, 31) = 2.486$
mTBI × time after mTBI	$F(1, 31) = 6.072$*
Perinatal treatment × time after mTBI	$F(1, 31) = 3.358$
mTBI × perinatal treatment × time after mTBI	$F(1, 31) = 4.629$*
Bonferroni multiple comparisons test	*t*; DF
Control: mTBI day 1 versus PNE: mTBI day 1	3.444 62.00*
PNE: sham day 1 versus PNE: mTBI day 1	4.085 62.00**
PNE: mTBI day 1 versus PNE: mTBI day 14	3.492 31.00*

PNE, perinatally nicotine exposed; mTBI, mild traumatic brain injury. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

more, the PNE mouse model shows working memory deficits, a condition comorbid with ADHD [47–49]. The behavioral changes and the frontal cortical hypodopaminergic state in the PNE mice are alleviated by a single administration of the classic stimulant drug MPH [28, 30], consistent with therapeutic effects of MPH in ADHD. Thus, the PNE mouse model is an experimental model of ADHD with significant face and predictive validity. The PNE mouse model also has construct validity for ADHD because cigarette smoking during pregnancy significantly increases ADHD risk for the offspring [50, 51]. Thus, among the experimental animal models of ADHD reported thus far [52–54], the PNE mouse model is the only one with demonstrated construct, face, and predictive validity as an ADHD animal model [28]. We used only male mice in the present study because in our PNE model, only the male mice show attention and working memory deficit and motor impulsivity [28, 29]. Additionally, we used a closed-head mTBI paradigm or sham procedure repeated daily for 5 consecutive days to mimic repetitive concussive injuries suffered due to participation in sports [35, 38–41, 55].

Consistent with previously reported data [29, 30], PNE impaired spatial working memory. These deficits were not exacerbated by repetitive mTBI. Similarly, mTBI did not cause deficits in recognition memory. In contrast, previous work using controlled cortical impact [32] and the closed-head weight drop method [36] to induce brain injury both reported impairments in recognition memory as measured by the novel object recognition test. These results suggest that the approach used here, while repetitive, is less severe than the previously reported approaches and represents a truly mild impact of the type that has been shown to be underreported clinically [56]. However, whether the repetitive mTBI model used here can produce deficits in recognition memory or spatial working memory at survival periods of 2–6 months that were used in the studies mentioned above, and well beyond the 2-week survival period used here, remains to be seen.

Consistent with previous work [29, 57], this study found that PNE induced significant impairments in object-based attention. While mTBI did not exacerbate the attention deficits in the PNE mice, the repetitive injury did result in a transient impairment of attention in the non-PNE control mice. Of particular note is the finding that mTBI transiently impaired attention such that it was indistinguishable from PNE-induced inattention, suggesting that even mild injury that does not impair working memory may be sufficient to induce transient attention deficit.

A major finding from this study is that repeated mTBI produces transient depression-like behavior in the PNE mouse model. Not only did PNE not induce immobility in the tail suspension test, the repetitive mTBI in the non-PNE mice was subthreshold with respect to this depression-like behavior. The behavior emerged only as a consequence of repetitive mTBI in the PNE mice. Thus, it appears that depression-like behavior in the present study may be the result of synergistic interactions between PNE and mTBI.

Clinical studies show an inconsistent association between concussion and depression. Some studies report a nearly 75% comorbidity between depression and concussion [58], whereas others do not report an association [59]. ADHD and depression also do not show an association. We reported that mood disorders are not more prevalent in populations with ADHD than in the general population [60]. Therefore, whether treatment-naïve ADHD subjects are at an increased risk for depression, even if only transiently, following repeated concussions remains to be determined.

Our findings should be viewed within the context of the following limitations. The mice were anesthetized with isoflurane during the mTBI procedure. We recognize that anesthesia may affect behavioral outcomes [35, 40, 55]. Although we included a sham control group to address this possibility, using unanesthetized mice could address this issue directly. We used only male mice because only males show ADHD-like behaviors in the PNE mouse model. However, we cannot rule out the effect of sex on behavioral outcomes following repeated mTBI in the PNE or control groups. Although the PNE mouse model carries significant construct, face, and predictive validity for ADHD, it would be useful to examine if our findings can be replicated in other preclinical models of ADHD [52, 61, 62]. We found that the attention and working memory deficits present in the PNE mice prior to mTBI were not exacerbated by the repeated mTBI. It is possible that the effects of PNE on these behaviors led to a "ceiling" effect such that further decrease was not possible. Alternatively, increasing the "severity" of the repeated mTBI may exacerbate these deficits. Another possibility is that the behavioral assays used here (Y-maze and object-based attention test) may not be "sensitive" enough to detect the changes. Future studies could address these possibilities.

In conclusion, the data presented here suggest that repeated bouts of mTBI may increase the risk of attention deficit and that untreated ADHD may be a risk factor for transient mTBI-induced depression-like behavior.

Whether stimulants may be effective treatments for depression that results from the synergy between mTBI and ADHD remains an open question [63]; it is not known whether individuals who are being treated for ADHD carry the same risk for mTBI-induced depression as those that are not receiving the treatment. Together, these data call for a re-examination of the current recommendation that participation in contact sports is beneficial for the management of ADHD and support the recommendation that depression screening should be a routine part of post-concussion management for all individuals with mTBI [64] and particularly those with a diagnosis of ADHD.

Statement of Ethics

The studies reported here were approved by Florida State University's Institutional Animal Care and Use Committee (IACUC) and conform to US National Institutes of Health guidelines.

Conflict of Interest Statement

Lin Zhang: no conflict of interest to declare. Cathy W. Levenson: no conflict of interest to declare. Valentina Cea Salazar: no conflict to declare. Deirdre McCarthy has financial interest in Avekshan, LLC, which is disclosed to and is managed by the Florida State University Research Foundation. She is an inventor of the following intellectual property through Florida State University: US patent (#10245271 B2) and a pending US patent application 16/369,748. Joseph Biederman is currently receiving research support from the following sources: AACAP, Feinstein Institute for Medical Research, Genentech, Headspace Inc., NIDA, Pfizer Pharmaceuticals, Roche TCRC Inc., Sunovion Pharmaceuticals Inc., Takeda/Shire Pharmaceuticals Inc., Tris, and NIH. He receives honoraria from the Medlearning Inc and MGH Psychiatry Academy for tuition-funded CME courses. Through MGH corporate licensing, Dr. Biederman has a US patent (#14/027676) for a non-stimulant treatment for ADHD, a US patent (#10245271 B2) on a treatment of impaired cognitive flexibility, and a patent pending (#61/233686) on a method to prevent stimulant abuse. Dr. Biederman and his program have received royalties from a copyrighted rating scale used for ADHD diagnoses, paid by Biomarin, Bracket Global, Cogstate, Ingenix, Medavent Prophase, Shire, Sunovion, and Theravance; these royalties were paid to the Department of Psychiatry at MGH. In 2020, Dr. Biederman received an honorarium for a scientific presentation from Tris and research support from the Food and Drug Administration. In 2019, Dr. Biederman was a consultant for Akili, Avekshan, Jazz Pharma, and Shire/Takeda. He received research support from Lundbeck AS and Neurocentria Inc. Through MGH CTNI, he participated in a scientific advisory board for Supernus. In 2018, Dr. Biederman was a consultant for Akili and Shire. Ross Zafonte received royalties from (1) Oakstone for an educational CD-Physical Medicine and Rehabilitation a Comprehensive Review; (2) Springer/Demos publishing for serving as a coeditor of the text Brain Injury Medicine. Dr Zafonte serves on the Scientific Advisory Board of Myomo, Oxeia Biopharma, ElMINDA, and Biodirection. Pradeep Bhide has financial interest in Avekshan, LLC, which is disclosed to and is managed by the Florida State University Research Foundation. He is an inventor of the following intellectual property through Florida State University: US patents 14/027676 and 10245271 B2 and pending applications 61/233686 and 16/369748. He was an expert witness on behalf of the State of Florida in the case National Rifle Association of America v Swearingen, Commissioner of the Florida Department of Law Enforcement, in the United States District Court for the Northern District of Florida.

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Author Contributions

L.Z., C.L., and V.C.S. designed and performed the experiments; D.M.C.C., J.B., R.Z., and P.G.B. designed the experiments. L.Z., C.L., V.C.Z., D.M.C.C., J.B., R.Z., and P.G.B. made substantial contributions to the conception and design of the study; acquisition, analysis, and interpretation of the data; drafting and revising the manuscript; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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