**Repetitive mild traumatic brain injury in a perinatal nicotine exposure mouse model of attention deficit hyperactivity disorder**

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Running title: Repetitive concussion and ADHD

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Funding: This work was supported by the Spaulding Rehabilitation Research Foundation, The Jim and Betty Ann Rodgers Chair Funds and an FSU Planning Grant.

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. This work used a perinatal nicotine exposure (PNE) mouse model model shares neuroanatomical, behavioral and neurochemical characteristics with ADHD, combined with a closed head, repetitive mTBI model. Two-month-old mice from PNE and control mice were subjected to closed-head repetitive mTBI or sham procedure once daily for five days. Object based attention, novel object recognition memory, spatial working memory, and depression-like behavior were analyzed one day and two 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. Depression-like behavior was not observed in either the PNE or mTBI mice. However, when PNE mice were subjected to mTBI, there was a transient increase in immobility in the tail suspension test, suggesting a synergistic effect of ADHD and mTBI. Thus, ADHD may be a risk factor for depression following repeated mTBI.

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neurobehavioral disorder that affects 8-10% of children [[1-4](#_heading=h.30j0zll)]. It is characterized by attention deficit, hyperactivity and impulsivity that can disrupt development or function in social, academic, and occupational settings. As described by the American Psychiatric Association’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 (American Psychiatriac Association 2013).

 While pyschostimulants methylphenidate (MPH) or amphetamines remain the first line of ADHD treatment (Caye et al., 2019), there is a growing appreciation for the beneficial role of non-pharmacological therapies. These include the use of physical activities such as exercise, and sports [Nowak et al., 2020]. 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 [Ng et al., 2017]. 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 [Harmon et al., 2013].

There is strong evidence to suggest that athletes with ADHD have a significantly higher risk of mTBI compared to non-ADHD athletes [[5](#_heading=h.tyjcwt), [6](#_heading=h.3dy6vkm), Adeyemo et al., 2014; Iverson et al. 2016; Iverson et al., 2020]. The risk of repetitive concussion is also higher in athletes with ADHD [Iverson et al., 2016]. Furthermore, there is evidence that the symptoms of concussion, particularly verbal memory impairment [Kaye et al., 2019], fatigue, and poor concentration [6], are worse in student athletes with ADHD compared to athletes without ADHD who sustain an mTBI. However, the degree to which untreated ADHD impacts the behavioral outcomes following mTBI is only now beginning to be examined [[7-10](#_heading=h.1t3h5sf)]. A recent meta-analysis reported that only two small studies to date have reported statistically significant links between ADHD and poorer clinical outcomes following mTBI. 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 methylphenidate [[11-14](#_heading=h.3rdcrjn)]. 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 [[15-20](#_heading=h.1ksv4uv)] 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-hr light-dark cycle (lights off at 7 AM and on at 7 PM). The mice had food and water available *ad libitum*. Breeding age (8-12 week-old) female mice were randomly assigned to one of three experimental groups based on the type of drinking water supplied: PNE group was provided with water containing nicotine [100 µg/ml; (-)-nicotine, Sigma Chemical Company, St. Louis, MO; Cat# N3876] and 2% saccharin (Alfa Aesar, Heysham, England; Cat# A15530); Saccharin group was provided with water containing 2% saccharin. To control for the potential effects of saccharin, a third group of mice received plain water [[1-4](#_heading=h.30j0zll)]. After 3 weeks, female mice in each group were bred with drug naïve male mice. Based on the presence of 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 three 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. All of the experimental procedures were in full compliance with institutional guidelines at the Florida State University and the NIH *Guide for the Care and Use of Laboratory Animals.*

*Closed head repetitive mTBI*: The procedure was performed on male mice at approximately P60. An electromagnetically driven stereotaxic device designed to control the site, depth, and velocity of impact was used (Impact One Stereotaxic Impactor, Leica, Bufalo Grove, IL). Blunt force impact was achieved by enclosing the tip of the metal piston in a customized rubber cap (9 mm in diameter) [22, 23]. 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 mimick 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 bregma, and 4 mm lateral to the longitiudinal midline [[5](#_heading=h.tyjcwt)]. The depth of the impact was adjusted to 3 mm from the surface of the skin. The impact occurred at 5 meters/sec with a dwell time of 100 msec. To control for the possible effects of anesthesia [37, 39], a parallel set of sham mice 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 for up to five consecutive days.

*Behavioral Tests:* Following the final bout of mTBI all animals were subjected to a series of behavioral tests to examine learning and memory using the Novel Object Recognition test (NOR) and the Y-maze as a measure of spatial working memory. Attention was assessed through the use of an Object-Based Attention test (OBA), while animals were monitored for the development of depression-like behaviors via the Tail Suspension Test (TST). The sequence of these behavioral assays was designed to prohibit the tests from interfering with one another. At approximately P60, each mouse was handled by the experimeter for 5 min per day every day for three days so that the mouse became familiar with the process of being picked up and handled by the experimenter prior to commencement of the mTBI or sham proceedures. After mTBI mice were divided into two 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. The mice were habituated to the testing room for 30 min before the behaviors analyses began. Upon completion of the tests, the mice were randomly assigned to mTBI or sham groups. Within each of these two groups the mice were divided again into two groups: Group 1 underwent Y-maze and OBA test whereas Group 2 underwent TST and NOR. Y-maze assay and TST were performed one day after the final mTBI or sham procedure (designated as day 1). The OBA and NOR tests began on day 2 followng the final mTBI or sham, but were completed on day 4. The second round of behavioral analysis began two weeks after the first round. NOR test was not performed during the second round.

*Novel Object Recognition (NOR)*: The assay consisted of two days of habituation followed by a test session on the third day [[10](#_heading=h.17dp8vu), [14](#_heading=h.35nkun2), [23](#_heading=h.1ci93xb)]. 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 returned to the same chamber to explore 2 identical objects: An unopened and unmarked can of food (3.14 X 3.6 X 11 cm3) or a Lego object (6.4 X 6.4 X 11 cm3). Time spent exploring the object placed on the left versus right hand side, as well as total time spent exploring the two objects was recorded using an overhead video camera. These data were used to evaluate object or side bias. A counter-balance design was used to address potential bias (refer here to your PLoS One paper). Following this step, the mouse was returned to its home cage for ten minutes. The mouse was re-introduced to the same chamber, which now contained one of the two 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 two 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) X 100.

*Spatial Working Memory (Y-maze):* A Y-maze was used to evaluate spatial working memory [[1-3](#_heading=h.30j0zll)]. Unique visual cues were placed on the exterior of the walls of each of the three 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 four limbs of the mouse entered an arm. A “spontaneous alternation” is defined as a set of three consecutive arm choices without a repeated entry (e.g. ABC, BCA, CAB). A spontaneous alternation score was calculated using the formula: number of alternations/(number of entries - 2) X 100.

*Object Based Attention (OBA):* The assay consisted of 2 days of habituation, followed by a testing session on day 3 [[1](#_heading=h.30j0zll), [2](#_heading=h.1fob9te), [7-9](#_heading=h.1t3h5sf)]. The test apparatus consisted an exploration chamber (40 X 40 X 25 cm) and a testing chamber (40 X 20 X 25 cm) separated by a sliding door [[1](#_heading=h.30j0zll), [2](#_heading=h.1fob9te), [7-9](#_heading=h.1t3h5sf)]. 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 two of these objects selected randomly, in the test chamber for 5 min. Day 3 began with an additional shorter habituation period (3 minutes 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 second interval, the door separating the chambers was opened allowing the mouse to enter the test chamber and explore two 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 minutes. An investigator blinded to the experimental conditions analyzed the video recordings to calculate the length of time spent with each of the two objects (novel and familiar). A recognition index was calculated and expressed as percentage using the formula: TN/(TF + TN) X 100, where TF and TN represent 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 20 sec 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 [[10](#_heading=h.17dp8vu), [11](#_heading=h.3rdcrjn)]. The test apparatus consisted of a custom-built rectangular box (opaque Plexiglass, 39 cm. length x 13 cm. width x 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 minutes. The tail was threaded through polycarbonate tubing (9.68 cm. length x 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. Mousebehavior 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 one 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 [[11-13](#_heading=h.3rdcrjn)], there were no significant behavioral differences between these two groups. When a significant main effect or interaction (*p*<0.05) was found by ANOVA, Bonferroni multiple comparison *post hoc* test was performed. In all cases, a *p* value of < 0.05 was considered statistically significant. Prism 8.4.2 Software (GraphPad Prism 8.0 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 deficits 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.

*Novel Object Recognition (NOR):* Two-way ANOVA showed that there was 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 x mTBI interactions (F (1, 25) = 0.5118; *p*>0.05) on recognition memory as measured in the NOR test.

*Spatial Working Memory*: Use of the Y-maze and a repeated measure three-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; Figure 1; Table 1). However, there was no significant main effect of mTBI (*p*>0.05). Furthremore, 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).

*Object Based Attention Test (OBA)*: A repeated measure three-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 three 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 day 4 and 17 (*p*<0.0001; Figure 2; Table 1) .

*Tail suspension test (TST)*: A repeated measure three-way ANOVA showed a significant main effect of mTBI (*p*<0.05) in the TST for depression-like behavior. There was 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 sham PNE group and mTBI-PNE groups on day 1 post-mTBI (*p*<0.05; Figure 3; Table 1).

**Discussion**

ADHD increases the risk of mTBI and appears to be associated with reduced tolerance to concussion and sub-concussive impacts [Nowak et al., 2020]. Interestingly, the risks of mTBI associated with ADHD do not appear to be mitigated by ADHD treatment [Cook et al., 2017; Poysophon and Rao, 2018]. We used 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 [[11](#_heading=h.3rdcrjn)] as well as in human subjects with untreated ADHD [[29](#_heading=h.49x2ik5)]. The PNE mice show significant reductions in frontal cortical dopamine content [[11](#_heading=h.3rdcrjn), [14](#_heading=h.35nkun2)] and exhibit locomotor hyperactivity, attention deficit and motor impulsivity [[11-14](#_heading=h.3rdcrjn)], all of which are consistent with ADHD. Furthermore, the PNE mouse model shows working memory deficits, a condition comorbid with ADHD [[2](#_heading=h.1fob9te), [30](#_heading=h.2p2csry), [31](#_heading=h.147n2zr)]. The behavioral changes and the frontal cortical hypodopaminergic state in the PNE mice are alleviated by a single administration of the classic stimulant drug methylphenidate [[12](#_heading=h.26in1rg), [14](#_heading=h.35nkun2)], consistent with therapeutic effects of methylphenidate 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 [[32](#_heading=h.3o7alnk), [33](#_heading=h.23ckvvd)]. Thus, among the experimental animal models of ADHD reported thus far [[34-36](#_heading=h.ihv636)], the PNE mouse model is the only one with demonstrated construct-, face- and predictive validity as an ADHD animal model [[12](#_heading=h.26in1rg)]. 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 [[12](#_heading=h.26in1rg), [13](#_heading=h.lnxbz9)]. 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 [[19](#_heading=h.3j2qqm3), [22](#_heading=h.2xcytpi), [23](#_heading=h.1ci93xb), [37-39](#_heading=h.41mghml)].

 Consistent with previously reported data [Zhang et al., 2018; 2020], 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 [Darkazalli et al., 2016] and the closed-head weight drop method [Rachmany et al., 2013] 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 under-reported clinically (Kroshus et al., 2015).

 Deficits in attention are a hallmark of ADHD with six or more symptoms of inattention in children and five or more symptoms in adolescents and adults required for diagnosis by the DSM-5 criteria [American Psychiatric Association, 2013]. Consistent with previous work [Zhang et al., 2018; 2020], this study found that PNE induced significant impairments in object-based attention. While mTBI did not exacerbate the attention deficits, 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.

There is considerable controvery surrounding the co-morbidity of ADHD and depression [Solomon et al., 2016]. While the symptoms of depression appear to be associated with concussion [Yrondi et al., 2017] with co-morbidity rates as high as 75%, mood disordes are not more prevalent in populations with ADHD than in the general population [Biederman et al., 1991]. Even more unclear is the role that ADHD may play in the development of mTBI-induced depression.

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 sub-threshold 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. While the transient nature of the mTBI-induced depression is consistent with the finding that the presence of ADHD is not a significant risk factor for the persistence of symptoms beyond a month in human populations [Iverson et al., 2017], these data raise the possibility that ADHD may reduce the resiliency to mTBI-induced depression.

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 (Iaccarino et al., 2020), 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-examnination of the current recommendation that participation in contact sports is beneficial for the management of ADHD and supports the recommendation that depression screening should be a routine part of post-concussion management for all individuals with mTBI (Stazyk et al., 2017), and particularly those with a diagnosis of ADHD.

**Additional References added by CWL (listed alphabetically)**

Adeyemo BO, Biederman J, Zafonte R, Kagan E, Spencer TJ, Uchida M, Kenworthy T, Spencer AE, Faraone SV. Mild traumatic brain injury and ADHD: a systematic review of the literature and meta-analysis. J Atten Disord. 2014 Oct;18(7):576-84. doi: 10.1177/1087054714543371

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). <https://doi.org/10.1176/appi.books.9780890425596>

Biederman J, Newcorn J, Sprich S. Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am J Psychiatry. 1991 May;148(5):564-77. doi: 10.1176/ajp.148.5.564. PMID: 2018156

Caye A, Swanson JM, Coghill D, Rohde LA. Treatment strategies for ADHD: an evidence-based guide to select optimal treatment. Mol Psychiatry. 2019 Mar;24(3):390-408. doi: 10.1038/s41380-018-0116-3.

Darkazalli A, Ismail AA, Abad N, Grant SC, Levenson CW. Use of human mesenchymal stem cell treatment to prevent anhedonia in a rat model of traumatic brain injury. Restor Neurol Neurosci. 2016 Apr 11;34(3):433-41. doi: 10.3233/RNN-150628.

Harmon KG, Drezner JA, Gammons M, Guskiewicz KM, Halstead M, Herring SA, Kutcher JS, Pana A, Putukian M, Roberts WO. American Medical Society for Sports Medicine position statement: concussion in sport. Br J Sports Med. 2013 Jan;47(1):15-26. doi: 10.1136/bjsports-2012-091941.

Iaccarino MA, Philpotts LL, Zafonte R, Biederman J. Stimulant Use in the Management of Mild Traumatic Brain Injury: A Qualitative Literature Review. J Atten Disord. 2020 Jan;24(2):309-317. doi: 10.1177/1087054718759752.

Kaye S, Sundman MH, Hall EE, Williams E, Patel K, Ketcham CJ. Baseline Neurocognitive Performance and Symptoms in Those With Attention Deficit Hyperactivity Disorders and History of Concussion With Previous Loss of Consciousness. Front Neurol. 2019 Apr 24;10:396. doi: 10.3389/fneur.2019.00396.

Kroshus E, Garnett B, Hawrilenko M, Baugh CM, Calzo JP. Concussion under-reporting and pressure from coaches, teammates, fans, and parents. Soc Sci Med. 2015 Jun;134:66-75. doi: 10.1016/j.socscimed.2015.04.011

Ng QX, Ho CYX, Chan HW, Yong BZJ, Yeo WS. Managing childhood and adolescent attention-deficit/hyperactivity disorder (ADHD) with exercise: A systematic review. Complement Ther Med. 2017 Oct;34:123-128. doi: 10.1016/j.ctim.2017.08.018.

Poysophon P, Rao AL. Neurocognitive Deficits Associated With ADHD in Athletes: A Systematic Review.

Sports Health. 2018 Jul-Aug;10(4):317-326. doi: 10.1177/1941738117751387.

Solomon GS, Kuhn AW, Zuckerman SL. Depression as a Modifying Factor in Sport-Related Concussion: A Critical Review of the Literature. Phys Sportsmed. 2016;44(1):14-9. doi: 10.1080/00913847.2016.1121091. Epub 2015 Nov 26. PMID: 26567843

Stazyk K, DeMatteo C, Moll S, Missiuna C. Depression in youth recovering from concussion: Correlates and predictors. Brain Inj. 2017;31(5):631-638. doi: 10.1080/02699052.2017.1283533. Epub 2017 Mar 22.

PMID: 28326857

Yrondi A, Brauge D, LeMen J, Arbus C, Pariente J. Depression and sports-related concussion: A systematic review. Presse Med. 2017 Oct;46(10):890-902. doi: 10.1016/j.lpm.2017.08.013. Epub 2017 Sep 14. PMID: 28919268

**Lin’s References**

1. Faraone SV, Asherson P, Banaschewski T, Biederman J, Buitelaar JK, Ramos-Quiroga JA, et al. Attention-deficit/hyperactivity disorder. Nat Rev Dis Primers. 2015;1:15020. doi: 10.1038/nrdp.2015.20. PubMed PMID: 27189265.

2. Faraone SV, Biederman J. Neurobiology of attention-deficit hyperactivity disorder. Biol Psychiatry. 1998;44(10):951-8. PubMed PMID: 9821559.

3. Faraone SV, Biederman J, Spencer T, Wilens T, Seidman LJ, Mick E, et al. Attention-deficit/hyperactivity disorder in adults: an overview. Biol Psychiatry. 2000;48(1):9-20. Epub 2000/07/29. doi: S0006-3223(00)00889-1 [pii]. PubMed PMID: 10913503.

4. Arnsten AF. Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. CNS Drugs. 2009;23 Suppl 1:33-41. doi: 10.2165/00023210-200923000-00005. PubMed PMID: 19621976.

5. Alosco ML, Fedor AF, Gunstad J. Attention deficit hyperactivity disorder as a risk factor for concussions in NCAA division-I athletes. Brain Inj. 2014;28(4):472-4. doi: 10.3109/02699052.2014.887145. PubMed PMID: 24564766.

6. Biederman J, Feinberg L, Chan J, Adeyemo BO, Woodworth KY, Panis W, et al. Mild Traumatic Brain Injury and Attention-Deficit Hyperactivity Disorder in Young Student Athletes. J Nerv Ment Dis. 2015;203(11):813-9. doi: 10.1097/NMD.0000000000000375. PubMed PMID: 26461480; PubMed Central PMCID: PMCPMC4626306.

7. Cook NE, Huang D, Silverberg N, Maxwell B, Zafonte R, Berkner P, et al. Concussion-Like Symptom Reporting in High School Student Athletes with ADHD. PM R. 2016;8(9S):S156. doi: 10.1016/j.pmrj.2016.07.032. PubMed PMID: 27672758.

8. Cook NE, Huang DS, Silverberg ND, Brooks BL, Maxwell B, Zafonte R, et al. Baseline cognitive test performance and concussion-like symptoms among adolescent athletes with ADHD: examining differences based on medication use. Clin Neuropsychol. 2017;31(8):1341-52. doi: 10.1080/13854046.2017.1317031. PubMed PMID: 28429656.

9. Cook NE, Iaccarino MA, Karr JE, Iverson GL. Attention-Deficit/Hyperactivity Disorder and Outcome After Concussion: A Systematic Review. J Dev Behav Pediatr. 2020. Epub 2020/04/23. doi: 10.1097/DBP.0000000000000808. PubMed PMID: 32317560.

10. Cook NE, Sapigao RG, Silverberg ND, Maxwell BA, Zafonte R, Berkner PD, et al. Attention-Deficit/Hyperactivity Disorder Mimics the Post-concussion Syndrome in Adolescents. Front Pediatr. 2020;8:2. Epub 2020/03/03. doi: 10.3389/fped.2020.00002. PubMed PMID: 32117823; PubMed Central PMCID: PMCPMC7014960.

11. Zhu J, Zhang X, Xu Y, Spencer TJ, Biederman J, Bhide PG. Prenatal nicotine exposure mouse model showing hyperactivity, reduced cingulate cortex volume, reduced dopamine turnover, and responsiveness to oral methylphenidate treatment. J Neurosci. 2012;32(27):9410-8. doi: 10.1523/JNEUROSCI.1041-12.2012. PubMed PMID: 22764249; PubMed Central PMCID: PMC3417040.

12. Zhu J, Fan F, McCarthy DM, Zhang L, Cannon EN, Spencer TJ, et al. A prenatal nicotine exposure mouse model of methylphenidate responsive ADHD-associated cognitive phenotypes. Int J Dev Neurosci. 2017;58:26-34. doi: 10.1016/j.ijdevneu.2017.01.014. PubMed PMID: 28179105.

13. Zhang L, Spencer TJ, Biederman J, Bhide PG. Attention and working memory deficits in a perinatal nicotine exposure mouse model. PLoS One. 2018;13(5):e0198064. Epub 2018/05/26. doi: 10.1371/journal.pone.0198064. PubMed PMID: 29795664; PubMed Central PMCID: PMCPMC5967717.

14. Zhang L, McCarthy DM, Eskow Jaunarajs KL, Biederman J, Spencer TJ, Bhide PG. Frontal Cortical Monoamine Release, Attention, and Working Memory in a Perinatal Nicotine Exposure Mouse Model Following Kappa Opioid Receptor Antagonism. Cerebral Cortex. 2020. doi: 10.1093/cercor/bhaa238.

15. Cope EC, Morris DR, Levenson CW. Improving treatments and outcomes: an emerging role for zinc in traumatic brain injury. Nutr Rev. 2012;70(7):410-3. Epub 2012/07/04. doi: 10.1111/j.1753-4887.2012.00486.x. PubMed PMID: 22747843.

16. Darkazalli A, Ismail AA, Abad N, Grant SC, Levenson CW. Use of human mesenchymal stem cell treatment to prevent anhedonia in a rat model of traumatic brain injury. Restor Neurol Neurosci. 2016;34(3):433-41. doi: 10.3233/RNN-150628. PubMed PMID: 27080073.

17. Ellis MJ, Ritchie LJ, Koltek M, Hosain S, Cordingley D, Chu S, et al. Psychiatric outcomes after pediatric sports-related concussion. J Neurosurg Pediatr. 2015;16(6):709-18. doi: 10.3171/2015.5.PEDS15220. PubMed PMID: 26359916.

18. Iverson GL, Gardner AJ, Terry DP, Ponsford JL, Sills AK, Broshek DK, et al. Predictors of clinical recovery from concussion: a systematic review. Br J Sports Med. 2017;51(12):941-8. doi: 10.1136/bjsports-2017-097729. PubMed PMID: 28566342; PubMed Central PMCID: PMCPMC5466929.

19. Petraglia AL, Plog BA, Dayawansa S, Chen M, Dashnaw ML, Czerniecka K, et al. The spectrum of neurobehavioral sequelae after repetitive mild traumatic brain injury: a novel mouse model of chronic traumatic encephalopathy. J Neurotrauma. 2014;31(13):1211-24. doi: 10.1089/neu.2013.3255. PubMed PMID: 24766454; PubMed Central PMCID: PMCPMC4082360.

20. Rachmany L, Tweedie D, Rubovitch V, Yu QS, Li Y, Wang JY, et al. Cognitive impairments accompanying rodent mild traumatic brain injury involve p53-dependent neuronal cell death and are ameliorated by the tetrahydrobenzothiazole PFT-alpha. PLoS One. 2013;8(11):e79837. Epub 2013/12/07. doi: 10.1371/journal.pone.0079837. PubMed PMID: 24312187; PubMed Central PMCID: PMCPMC3842915.

21. Zhu J, Lee KP, Spencer TJ, Biederman J, Bhide PG. Transgenerational transmission of hyperactivity in a mouse model of ADHD. J Neurosci. 2014;34(8):2768-73. doi: 10.1523/JNEUROSCI.4402-13.2014. PubMed PMID: 24553919; PubMed Central PMCID: PMCPMC3931498.

22. Luo J, Nguyen A, Villeda S, Zhang H, Ding Z, Lindsey D, et al. Long-term cognitive impairments and pathological alterations in a mouse model of repetitive mild traumatic brain injury. Front Neurol. 2014;5:12. Epub 2014/02/20. doi: 10.3389/fneur.2014.00012. PubMed PMID: 24550885; PubMed Central PMCID: PMCPMC3912443.

23. Shitaka Y, Tran HT, Bennett RE, Sanchez L, Levy MA, Dikranian K, et al. Repetitive closed-skull traumatic brain injury in mice causes persistent multifocal axonal injury and microglial reactivity. J Neuropathol Exp Neurol. 2011;70(7):551-67. Epub 2011/06/15. doi: 10.1097/NEN.0b013e31821f891f. PubMed PMID: 21666502; PubMed Central PMCID: PMCPMC3118973.

24. Alkam T, Hiramatsu M, Mamiya T, Aoyama Y, Nitta A, Yamada K, et al. Evaluation of object-based attention in mice. Behav Brain Res. 2011;220(1):185-93. doi: 10.1016/j.bbr.2011.01.039. PubMed PMID: 21277334.

25. Alkam T, Kim HC, Hiramatsu M, Mamiya T, Aoyama Y, Nitta A, et al. Evaluation of emotional behaviors in young offspring of C57BL/6J mice after gestational and/or perinatal exposure to nicotine in six different time-windows. Behav Brain Res. 2013;239:80-9. doi: 10.1016/j.bbr.2012.10.058. PubMed PMID: 23142610.

26. Alkam T, Kim HC, Mamiya T, Yamada K, Hiramatsu M, Nabeshima T. Evaluation of cognitive behaviors in young offspring of C57BL/6J mice after gestational nicotine exposure during different time-windows. Psychopharmacology (Berl). 2013;230(3):451-63. doi: 10.1007/s00213-013-3175-9. PubMed PMID: 23793357.

27. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl). 1985;85(3):367-70. Epub 1985/01/01. doi: 10.1007/bf00428203. PubMed PMID: 3923523.

28. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. The tail suspension test. J Vis Exp. 2012;(59):e3769. Epub 2012/02/09. doi: 10.3791/3769. PubMed PMID: 22315011; PubMed Central PMCID: PMCPMC3353516.

29. Makris N, Seidman LJ, Valera EM, Biederman J, Monuteaux MC, Kennedy DN, et al. Anterior cingulate volumetric alterations in treatment-naive adults with ADHD: a pilot study. J Atten Disord. 2010;13(4):407-13. doi: 10.1177/1087054709351671. PubMed PMID: 20008822; PubMed Central PMCID: PMCPMC3746768.

30. Alderson RM, Kasper LJ, Hudec KL, Patros CH. Attention-deficit/hyperactivity disorder (ADHD) and working memory in adults: a meta-analytic review. Neuropsychology. 2013;27(3):287-302. doi: 10.1037/a0032371. PubMed PMID: 23688211.

31. Brown A, Biederman J, Valera E, Lomedico A, Aleardi M, Makris N, et al. Working memory network alterations and associated symptoms in adults with ADHD and Bipolar Disorder. J Psychiatr Res. 2012;46(4):476-83. doi: 10.1016/j.jpsychires.2012.01.008. PubMed PMID: 22272986; PubMed Central PMCID: PMCPMC3686289.

32. Wickstrom R. Effects of nicotine during pregnancy: human and experimental evidence. Current neuropharmacology. 2007;5(3):213-22. Epub 2007/09/01. doi: 10.2174/157015907781695955. PubMed PMID: 19305804; PubMed Central PMCID: PMC2656811.

33. Pagani LS. Environmental tobacco smoke exposure and brain development: the case of attention deficit/hyperactivity disorder. Neurosci Biobehav Rev. 2014;44:195-205. doi: 10.1016/j.neubiorev.2013.03.008. PubMed PMID: 23545330.

34. Sagvolden T, Russell VA, Aase H, Johansen EB, Farshbaf M. Rodent models of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005;57(11):1239-47. PubMed PMID: 15949994.

35. Russell VA. Neurobiology of animal models of attention-deficit hyperactivity disorder. J Neurosci Methods. 2007;161(2):185-98. doi: 10.1016/j.jneumeth.2006.12.005. PubMed PMID: 17275916.

36. Russell VA. Overview of animal models of attention deficit hyperactivity disorder (ADHD). Curr Protoc Neurosci. 2011;Chapter 9:Unit9 35. doi: 10.1002/0471142301.ns0935s54. PubMed PMID: 21207367.

37. Petraglia AL, Plog BA, Dayawansa S, Dashnaw ML, Czerniecka K, Walker CT, et al. The pathophysiology underlying repetitive mild traumatic brain injury in a novel mouse model of chronic traumatic encephalopathy. Surg Neurol Int. 2014;5:184. doi: 10.4103/2152-7806.147566. PubMed PMID: 25593768; PubMed Central PMCID: PMCPMC4287910.

38. Petraglia AL, Dashnaw ML, Turner RC, Bailes JE. Models of mild traumatic brain injury: translation of physiological and anatomic injury. Neurosurgery. 2014;75 Suppl 4:S34-49. doi: 10.1227/NEU.0000000000000472. PubMed PMID: 25232883.

39. Semple BD, Sadjadi R, Carlson J, Chen Y, Xu D, Ferriero DM, et al. Long-Term Anesthetic-Dependent Hypoactivity after Repetitive Mild Traumatic Brain Injuries in Adolescent Mice. Dev Neurosci. 2016;38(3):220-38. Epub 2016/08/23. doi: 10.1159/000448089. PubMed PMID: 27548472.

40. King D, Brughelli M, Hume P, Gissane C. Assessment, management and knowledge of sport-related concussion: systematic review. Sports Med. 2014;44(4):449-71. Epub 2014/01/10. doi: 10.1007/s40279-013-0134-x. PubMed PMID: 24403125.

41. Zuckerman SL, Lee YM, Odom MJ, Solomon GS, Forbes JA, Sills AK. Recovery from sports-related concussion: Days to return to neurocognitive baseline in adolescents versus young adults. Surg Neurol Int. 2012;3:130. Epub 2012/12/12. doi: 10.4103/2152-7806.102945. PubMed PMID: 23227435; PubMed Central PMCID: PMCPMC3513851.