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Injury during adolescence leads to sex-specific executive function deficits in adulthood in a pre-clinical model of mild traumatic brain injury

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ABSTRACT

Adolescents are more likely than adults to develop chronic symptoms, such as impulsivity and difficulty concentrating, following a mild traumatic brain injury (mTBI) which may relate to disruption of pre-frontal cortex (PFC development). During adolescence the PFC is undergoing extensive remodelling, driving maturation of executive functions incorporating attention, motivation and impulse control. In part maturation of the PFC is driven by outgrowth of dopaminergic neurons to the PFC under the guidance of specific axonal targeting cues, including netrin-1. How a mTBI in adolescence may alter the expression of these axonal targeting cues, and the influence on PFC development is not yet known. As such the effects of mTBI in mid-adolescence on executive functioning in adulthood (12 weeks) were examined via the 5-choice serial reaction task in both male and female Sprague Dawley rats. Animals at p35 (n = 12–16 per group) were injured via weight drop (100 g from 0.75 m) and injury confirmed by a significant increase in righting reflex. Interestingly, while a mid-adolescence mTBI in females led to significantly higher omissions and decreased accuracy when task difficulty was high (stimulus duration 1 s), males had significantly increased premature response rate when the intertrial interval was varied. Examination of levels of TH, as a reflection of dopaminergic innervation, found no difference in either gender post-TBI in the PFC, but a significant increase in the limbic system (nucleus accumbens) in males, but not females, chronically post-TBI, suggesting an imbalance between the regions. The increase in TH was accompanied by a chronic reduction in netrin-1 within the nucleus accumbens in males only. Taken together, these results indicate that mTBI in adolescence leads to sex specific effects in different domains of PFC function in adulthood, which may relate to subtle alterations in the developmental trajectory of the mesocortical limbic pathway in males only.

1. Introduction

Adolescents have the highest rates of mild traumatic brain injury (mTBI) [1] and respond differently to mTBI than adults. A mTBI describes a mechanical insult to the head resulting in uncontrolled movement of the brain and stretching of axons, without focal structural abnormalities [2]. Interruption of neuronal communication produces symptoms including transient loss of consciousness, irritability and difficult concentrating. Symptoms normally resolve within a few weeks; however, adolescents are uniquely vulnerable, taking twice as long to recover from mTBI as adults [3–5], with emerging evidence of long-term effects, such as subtle cognitive deficits [6] and higher rates of risky behaviour associated with increased substance use [7] seen in adulthood following mTBI in adolescence. Sex also appears to play a role in outcome following mTBI, with females reporting a greater number and severity of symptoms [8–11] and requiring a longer duration to recover. Importantly reports suggest that high-school and college aged female athletes are more at risk of concussion than males when playing comparable sports [12–14].

Vulnerability to mTBI in adolescence may relate to disruption of prefrontal cortex (PFC) development, which drives maturation of higher cognitive abilities such as decision-making, behavioural inhibition and attention (executive functions) [15]. The PFC undergoes continued pruning of grey matter into the early twenties and changes in neurotransmitter systems, particularly dopamine (and its receptors, classed as $D1_R$ or $D2_R$), occur throughout adolescence [16]. Dopaminergic input to the PFC increases via growth of axons from the ventral tegmental area (VTA) [17], as controlled by axonal signalling molecules, including netrin-1, and the relative concentration of its receptors DCC and UNC-5. DCC attracts, while UNC-5 repels axons [18,19]. New research has

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shown that dopaminergic neurons reaching the PFC from adolescence onwards first pause within the limbic system (in the nucleus accumbens) before continuing [20]. The fate of the dopaminergic neurons is driven by the decreasing DCC:UNC5 ratio within VTA dopaminergic neurons throughout adolescence, driving outgrowth of neurons from the NAc to the PFC [21–23]. Indeed experimentally decreasing DCC expression in VTA dopaminergic neurons during adolescence increases dopamine innervation of the PFC [24]. Importantly, the effects of mTBI on these signalling molecules (netrin-1, DCC, UNC-5) has not been investigated, although other acute insults, such as stroke, increased levels of DCC within DA neurons for up to 14 days post-insult [25], suggesting that mTBI may similarly alter expression. Increased DCC expression would encourage DA neurons to stay within the limbic system rather than continue to project to the PFC, setting the stage for executive dysfunction in adulthood.

This study investigated whether mTBI in adolescence led to sex-specific alterations in executive function encompassing attention, behavioural inhibition, and reward salience, with a specific focus on effects of injury within sex. Furthermore we examined whether alterations in executive function were driven via alterations in DCC, UNC-5 and netrin-1 which are responsible for dopaminergic innervation of the PFC and if this led to changes in markers of dopaminergic neurotransmission within the PFC or NAc

2. Methods

All studies were performed within the guidelines established by the National Health and Medical Research Committee of Australia and were approved by the Animal Ethics Committee of the University of Adelaide. Male or female Sprague Dawley rats were group housed by sex in individually ventilated cages in a controlled temperature environment under a 12 h light/dark cycle. Rats were randomly allocated to receive either sham anaesthesia or a single mild TBI at postnatal day 35 (p35), which is described as representing mid-adolescence in humans [26]. Animals were either perfused with saline and brains fresh frozen at 24 h post-injury (n = 7 per group) or undertook a behavioural battery (n = 12–14 per group) and were perfused at six weeks post-injury, with half allocated to immunohistochemical analysis (n = 6–7 per group) and half to biochemical analysis (n = 7 per group).

2.1. Rodent model of TBI

As per Mychasuik et al, animals were subject to a closed head weight drop model of diffuse TBI [27]. A diffuse TBI describes a head injury that results from acceleration and deceleration forces associated with a blunt trauma and does not lead to any obvious focal structural abnormalities such as contusions [28]. The model combines a high velocity impact with rapid head acceleration to mimic clinical concussive injuries [29]. Animals were briefly anaesthetised with 3.5 % isoflurane in air for three minutes before being placed chest down on scored tin foil with the head directly in the path of a 100 g weight that was released from a height of 0.75 m. The weight strikes the rat's head, causing the animal to fall through the foil, undergo a 180° rotation and land on a foam bed below. Sham animals underwent the same period of anaesthesia without impact. When applied to adolescent rats, the diffuse injury model produces behavioural outcomes representative of post-concussive symptoms, including balance difficulties in the acute recovery phase [30]. Loss of righting reflex post-injury was used as confirmation of injury and was measured as the time taken for animals to return to a standing position from a prone position following removal of anaesthetic. As such the righting time is thought to be reflection of loss of consciousness [31] and is used as marker of injury severity post-TBI [32,33]. For example, Hallam et al. reported a significant correlation between injured neurons and duration of loss of righting reflex after a more moderate injury [34].

2.2. Five serial choice reaction time task (5CSRTT)

At 19 days post-injury, animals began training on the 5CSRTT task and undertook two probe trials immediately upon reaching criterion as outlined below. Behavioural testing was conducted in the Bussey-Saksida Touchscreen operant chamber (Campden Instruments Ltd.,U.K.) as previously described [31]. Animals were food restricted for five days prior to starting training (at 14 days post-injury), with males receiving 5 g/100 g of body weight and females 4 g/100 g of body weight per day. Both males and females either maintained or gradually gained weight across the testing procedure in line with continued maturation, with the degree of food restriction determined to be optimal to facilitate performance on the task in our pilot studies.

In the first day of training, sugar pellets (Dustless Precision Sugar Pellets, ASF0042, 45 mg, Able Scientific, Australia), which serve as reward for the 5CSRTT, were introduced in the home cage of the animal, as a means of habituating them to the pellets. The next day (Day 1), 10 pellets were placed in the magazine of each chamber, and the animals were allowed to habituate to their respective chamber for 30 min before returning to their home cage. Animals were required to consume all sugar pellets to move on to the next task. In the first phase of training (Initial touch), any touch on the touchscreen is rewarded with a pellet, whereas in the second phase of training (Must touch), animals must touch the correct stimulus on the screen to be rewarded with a pellet, with the criteria for success outlined in Table 1. Following successful completion, animals moved on to the 5CSRTT training where the stimulus duration was gradually decreased over time. An overall depiction of the experimental timeline can be seen in Fig. 1.

A session begins with a sugar pellet being released. Upon retrieval, the magazine light switches off and a 5 s inter-trial interval (ITI) begins prior to the presentation of the stimulus light. If the animal made a response during the 5 s ITI (premature response), the houselights remained off, but no pellet was rewarded. Following the ITI, the animal either had to nose poke the right window when the stimulus was presented or within the specified limited hold time after the stimulus light had disappeared (Table 1). If the right window was nose poked (correct response), a tone was generated, a sugar pellet was released, and the magazine light turned on. Conversely, if the wrong window was nose poked (incorrect response), the houselights turned on for 5 s (timeout) and no pellet was rewarded. If the animal did not make a response within the limited hold time (omission), there was a 5 s timeout period, with the houselights turned on and no food pellet rewarded. Following a 5 s ITI, the next trial would begin. Each training session was completed at the end of 100 trials or at 30 min, whichever came first. Animals were

Table 1
Outcome measures in the 5CSRTT.

Accuracy	the number of correct responses divided by the sum of correct and incorrect responses (# correct responses/[# correct responses + # incorrect responses] × 100). Accuracy is the main measure of attentional performance in this task.
Omissions	total number of omissions divided by total number of trials. Percent omissions reflect the proportion of trials that end with an omission.
Correct Response Latency	Time taken to register a correct response
Reward Collection Latency	Time taken to collect the reward
Premature response rate	total number of responses performed during the ITI (i.e., before presentation of the light stimulus) divided by the total number of trials. Premature responses reflect response disinhibition/impulsivity in this task.
Perseveration	total number of nosepoke responses performed after a correct response, but before collection of the reward, divided by the total number of trials. Perseverative responses are a measure of compulsivity/cognitive inflexibility in this task.

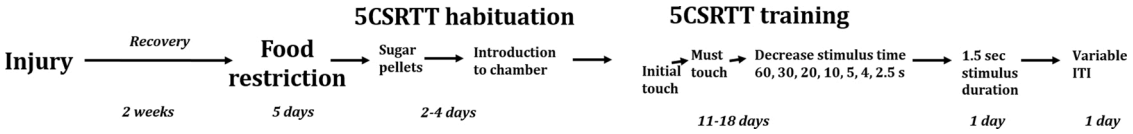

Fig. 1. Injury timeline. Timing of events with a two week rest period following induction of injury, followed by the initial period of food restriction and then training on the 5CSRTT.

required to complete each stage with >80 % accuracy and <20 % omissions before moving on to the next stage.

Once animals had reached criterion, two test days were run. In the first, stimulus duration was reduced to 1.5 s and, in the second, the ITI was pseudorandomly varied between 1, 3, 5 and 7 s, instead of being fixed at 5 s. The ITI for a given trial was drawn randomly without replacement from a list of possible ITI durations, ensuring that all ITI durations were used an equal number of times in each testing session, while remaining unpredictable for the animal. All other parameters remained equal to the standard parameters of the final training schedule.

Response outcomes were recorded by the ABETII and extracted and saved as a Microsoft Excel file. Outcome measurements include:

- Accuracy: the number of correct responses divided by the sum of correct and incorrect responses (# correct responses/[# correct responses + # incorrect responses] × 100). Accuracy is the main measure of attentional performance in the task.
- Percent Omissions: total number of omissions divided by total number of trials. Percent omissions reflect the proportion of trials that end with an omission.
- Correct Response Latency: Time taken to register a correct response
- Reward Collection Latency: Time taken to collect the reward
- Premature Response Rate: total number of responses performed during the ITI (i.e., before presentation of the light stimulus) divided by the total number of trials. Premature responses reflect response disinhibition/impulsivity in the task.
- Perseveration: total number of nosepoke responses performed after a correct response, but before collection of the reward, divided by the total number of trials. Perseverative responses are a measure of compulsivity/cognitive inflexibility in the task.

2.3. Western blot

Brains were dissected using a rat stereotaxic atlas as a guide to collect the PFC and nucleus accumbens (NAc). The samples were homogenised in freshly prepared RIPA with 1X cOmplete™ EDTA-free protease inhibitor cocktail (Sigma) and centrifuged for 30 min at 14,000 rpm at 4 °C, before the supernatant was collected. Protein concentration was estimated with Pierce BCA Protein Assay Kit (ThermoScientific) with the absorbance read at 540 nm.

Gel electrophoresis was completed using Bolt 4–12 % Bis-Tris Plus gels (Life Technologies) with 40 ug of protein loaded per well. Gels were run at 500 V and transferred to a PVDF membrane using the iBlot 2 Dry Blotting System (Life Technologies). Membranes were washed in 1X tris-buffered saline with tween (TBST) (3 washes ×5 min), then blocked with 5% milk powder in TBST for 1 h. Membranes were incubated with primary antibodies overnight, which were used at individually optimised concentrations (see Table 2).

Secondary antibodies to the respective primary antibodies (donkey anti rabbit and donkey anti chicken, IRDye 800CW; LI−COR, Inc.) were used at 1:8000 the following day for 1 h. Western blots were imaged using an Odyssey Infrared Imaging System (model 9140) (LI−COR, Inc.) set at auto resolution for optimum visualisation. Analysis of band signals was performed using Image Studio Lite version 5.2. The same control sample was run on each gel, with expression of protein normalized to both the housekeeper and to the loading control.

Table 2
Antibodies of interest.

Antibody	Conc.	Catalogue #	Company	Purpose of marker
Rabbit anti-Tyrosine Hydroxylase (TH)	1:1000	ab112	Abcam	Catalytic enzyme for conversion of tyrosine to DA
Rabbit anti-netrin-1	1:500	ab126729	Abcam	Axonal signalling molecule
Rabbit anti-UNC-5	1:2000	AF1005	Novus Biologicus	Receptor for netrin-1
Goat anti-DCC	1:250	AF844	R&D systems	Receptor for netrin-1
Rabbit anti-Dopamine Transporter (DAT)	1:1000	ab111468	Abcam	Reuptake of DA from the synaptic cleft
Rabbit anti-Dopamine Receptor D1 (DRD1)	1:1000	ab20066	Abcam	Receptor from $D1_R$ family
Rabbit anti-Dopamine Receptor D3 (DRD3)	1:250	ab42114	Abcam	Receptor from $D2_R$ family
Rabbit anti-COMT	1:100	ab20066	Abcam	Enzyme that degrades catecholamines
Rabbit anti-Synaptophysin	1:1000	ab8049	Abcam	Synaptic vesicle protein
Mouse anti-GAPDH	1:10000	Ab108162	Abcam	Housekeeping protein

2.4. Immunohistochemistry

Formalin fixed brains were sucrose protected and then frozen in OCT. 20 μm sections were taken from the PFC (2 sections at +3.8 cm and +3.3 mm relative to bregma) and NAc (2 sections at +1.3 and 0.9 mm from bregma). For immunohistochemistry, endogenous peroxidases were blocked with methanol/hydrogen peroxide (0.5 %), followed by antigen retrieval in citrate buffer. Sections were then incubated with 30 % normal horse serum for 1 h, prior to incubation overnight at room temperature with the TH antibody (ab112, Abcam: 1:1000). The next day, the appropriate biotinylated secondary antibody (1:250, Vector) was applied for 30 min, followed by streptavidin horseradish peroxidase for 60 min, with the bound antibody detected with 3,3-diaminobenzidine tetrahydrochloride (Sigma). Sections were counterstained with hematoxylin. Slides were digitally scanned using a Nanozoomer, viewed with the associated NDP view software, with images exported for analysis with Image J (Corrigan et al., 2011; Corrigan et al., 2014). For the mPFC, 4 × 40x images were taken from the infralimbic and prelimbic regions on the left and right side of each slide. For the NAc, 4 × 40x images were taken from the left and right side of each slide. In ImageJ, the number of pixels above an automated threshold value was determined and expressed as a percentage of total pixels within the field, with results expressed as an average value across the sections for each area of interest.

2.4.1. Data analysis

All data were analysed using IBM SPSS Statistics 27. There were no

outliers removed for the final analysis, as no animals scored more than 2 standard deviations from the mean value for any measurement. To analyse acute injury characteristics and learning (i.e. number of trials to reach criterion), a two-way analysis of variance (ANOVA), with injury and sex as the between subjects factors, was conducted. Post hoc testing was conducted according to Tukey's method.

For probe trial data, a two-way multivariate analysis of variance (MANOVA) was conducted on all probe trial measures, with injury group and sex both as between groups factors. Pairwise comparisons were conducted using Bonferroni's adjustment for multiple comparisons. Effect size (partial eta squared) and observed power were both calculated. Based upon the low values for both effect size (partial eta squared = 0.153) and observed power (0.464) for sex in Probe trial 1, separate MANOVAs were conducted on all probe trial measures in males versus females in order to better probe the specific effect of injury within each group.

Biological marker data was similarly analysed by MANOVA with axonal targeting markers (netrin-1, UNC-5 and DCC) analysed separately from markers of dopaminergic neurotransmission (TH, COMT, DAT, DrD1, DrD3 and SYN). Injury group and sex were both between groups factors and pairwise comparisons were conducted using Bonferroni's adjustment for multiple comparisons. Where a significant sex*injury interaction was found, a two-way ANOVA was conducted with post hoc testing was conducted according to Tukey's method. For all testing, p values <0.05 were considered statistically significant.

3. Results

3.1. Acute injury characteristics

At day of injury, females were slightly smaller than males, regardless of group allocation (male sham 153.94 ± 25.36, male mTBI 162.13 ± 15.74 vs female sham 136.46 ± 15.9, female mTBI 139.2 ± 17.85, $p < 0.05$) (data not shown). Immediately following injury righting reflex was measured, with a significant main effect of injury ($F_{(1, 53)} = 25.63$, $p < 0.001$) but not sex ($F_{(1, 53)} = 0.15$, $p = 0.70$), with injury leading to a significant increase in righting time (333.13 ± 139.00 vs 185 ± 76.01 s, $p < 0.001$)(Fig. 2). No sex*injury interaction was found for righting reflex time ($F_{(1, 53)} = 1.9$, $p = 0.67$), suggesting injury severity was similar between males and females.

3.2. Functional outcome following mTBI in adolescence

Executive function post-TBI was evaluated using the 5CSRTT. In the training phase of the 5CSRTT there was no sex*injury effect ($F_{(1, 53)} = 1.54$, $p = 0.22$, $\eta_p^2 = 0.03$), but significant main effects of both injury ($F_{(1, 53)} = 5.6$, $p < 0.01$, $\eta_p^2 = 0.10$) and sex ($F_{(1, 53)} = 13.6$, $p < 0.05$, $\eta_p^2 = 20$) on trials to reach criterion. Injury led to a small increase in number of trials (14 (11–16) vs 15 (11–18), $p < 0.05$), with females also taking longer to complete the task than males (16 (12–18) vs 14 (11–16) $p < 0.05$) (data not shown).

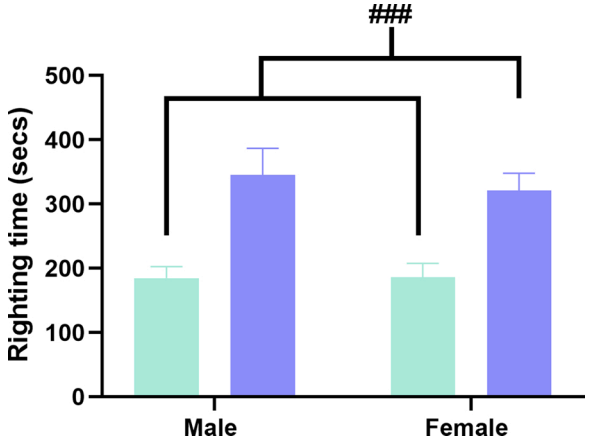

Fig. 2. Acute injury characteristics. Injury led to a significant increase in righting time (###$p < 0.001$ injured vs sham animals), which was not influenced by sex ($p = 0.70$).

Two probe trials were then conducted: one investigating the effects of decreasing stimulus time (Fig. 3) and the other on varying ITI (Fig. 4). Decreasing the duration of the visual stimulus increases attentional load, while varying the ITI increases the demand on inhibition of inappropriate responding in the absence of stimulus presentation. Thus the two probe trials were designed to increase cognitive demand and target attention (Probe 1) and inhibition (Probe 2).

Performance in the probe trials of the 5CSRTT was examined using a 2 × 2 multivariate analysis of variance across the various measures of performance. When stimulus time was decreased to 1.5 s no significant interaction was found for injury*sex (Pillai's Trace = 0.20; $F_{7,47} = 1.70$, $p = 0.13$, $\eta_p^2 = 0.20$), but there was a significant main effect of injury (Pillai's Trace = 0.42; $F_{7,47} = 4.87$, $p < 0.001$, $\eta_p^2 = 0.42$), with injured animals showing significantly lower accuracy (63.49 %±11.15 % vs 78.09 %±14.23 % $p < 0.001$) and higher latency to collect reward (1.25 ± 0.23 s vs 1.10 ± 0.25 s, $p < 0.05$) than shams. Additionally, TBI animals made more omissions than sham animals (50.61 ± 17.35 vs 40.15 ± 16.98, $p < 0.05$). No differences were found between sham and mTBI animals for premature response rate ($p = 0.08$), perseveration (0.88), correct response latency (0.14) or incorrect response latency ($p = 0.92$).

While no main effect of sex was found (Pillai's Trace = 0.15; $F_{7,47} = 1.22$, $p = 0.31$, $\eta_p^2 = 0.15$), the observed power was low (0.46), indicating that our study was not sufficiently powered to detect subtle effects due to sex. Given this, as well as the fact that previous papers have reported sex specific alterations in performance on a similar task, the go-no go task, following injury (Hehar et al., 2015), we probed this further in our dataset using multivariate analyses of variance to compare performance on all measures between sham and injured animals in males and females separately (Fig. 3). In males, no effect of injury was found (Pillai's Trace = 0.29; $F_{7,18} = 1.05$, $p = 0.43$, $\eta_p^2 = 0.29$), whereas, in females, a significant effect of injury was found (Pillai's Trace = 0.66; $F_{7,23} = 6.34$, $p < 0.001$, $\eta_p^2 = 0.66$). Injured females had significantly lower accuracy (59.69 %±10.80 % vs 82.06 %±13.67 %, $p < 0.001$), higher omissions (49.73 ± 16.04 vs 35.59 ± 15.49, $p < 0.05$), and a significant increase in reward collection latency (1.28 ± 0.21 vs 1.06 ± 0.23 s, $p < 0.01$) compared to their shams. No significant effect of injury was seen in premature response rate ($p = 0.44$), perseveration ($p = 0.56$), correct response latency ($p = 0.08$) or incorrect response latency ($p = 0.43$).

A second probe trial was then conducted assessing inhibitory function by varying the ITI (Fig. 3). In this trial a significant interaction was found for injury*sex (Pillai's Trace = 0.31; $F_{7,47} = 2.92$, $p < 0.05$, $\eta_p^2 = 0.31$, with a significant main effect of injury (Pillai's Trace = 0.28; $F_{7,47} = 2.58$, $p < 0.05$, $\eta_p^2 = 0.28$), but not sex (Pillai's Trace = 0.20; $F_{7,47} = 1.65$, $p = 0.15$, $\eta_p^2 = 0.15$). Injury was found to significantly decrease accuracy (80.24 ± 1.2 vs 84.05, $p < 0.05$), increase premature response rate (26.71 ± 2.07 vs 18.46 ± 2.23, $p < 0.01$ and decrease reward latency (1.23 ± 0.04 vs 1.39 ± 0.05, $p < 0.05$), with no effect on omissions ($p = 0.13$), perseveration ($p = 0.86$), correct response latency (0.51) or incorrect response latency ($p = 0.59$). Further probing of the dataset was then performed using multivariate analyses of variance to compare performance on all measures between sham and injured animals in males and females separately. In this task females showed no significant effect of injury (Pillai's Trace = 0.40; $F_{7,23} = 2.20$, $p = 0.07$, $\eta_p^2 = 0.40$), whereas, in males, a significant effect of injury was found (Pillai's Trace = 0.51; $F_{7,18} = 2.65$, $p < 0.5$, $\eta_p^2 = 0.51$). Injured males had significantly lower accuracy (76.64 ± 7.01 vs 84.57 ± 5.18, $p < 0.01$), higher premature response rate (27.93 ± 16.12 vs 15.12 ± 8.87, $p < 0.01$) and a significant increase in reward collection latency (1.28 ± 0.2 vs 1.06 ± 0.23 s, $p < 0.05$). No significant effect of injury was seen in omissions (p

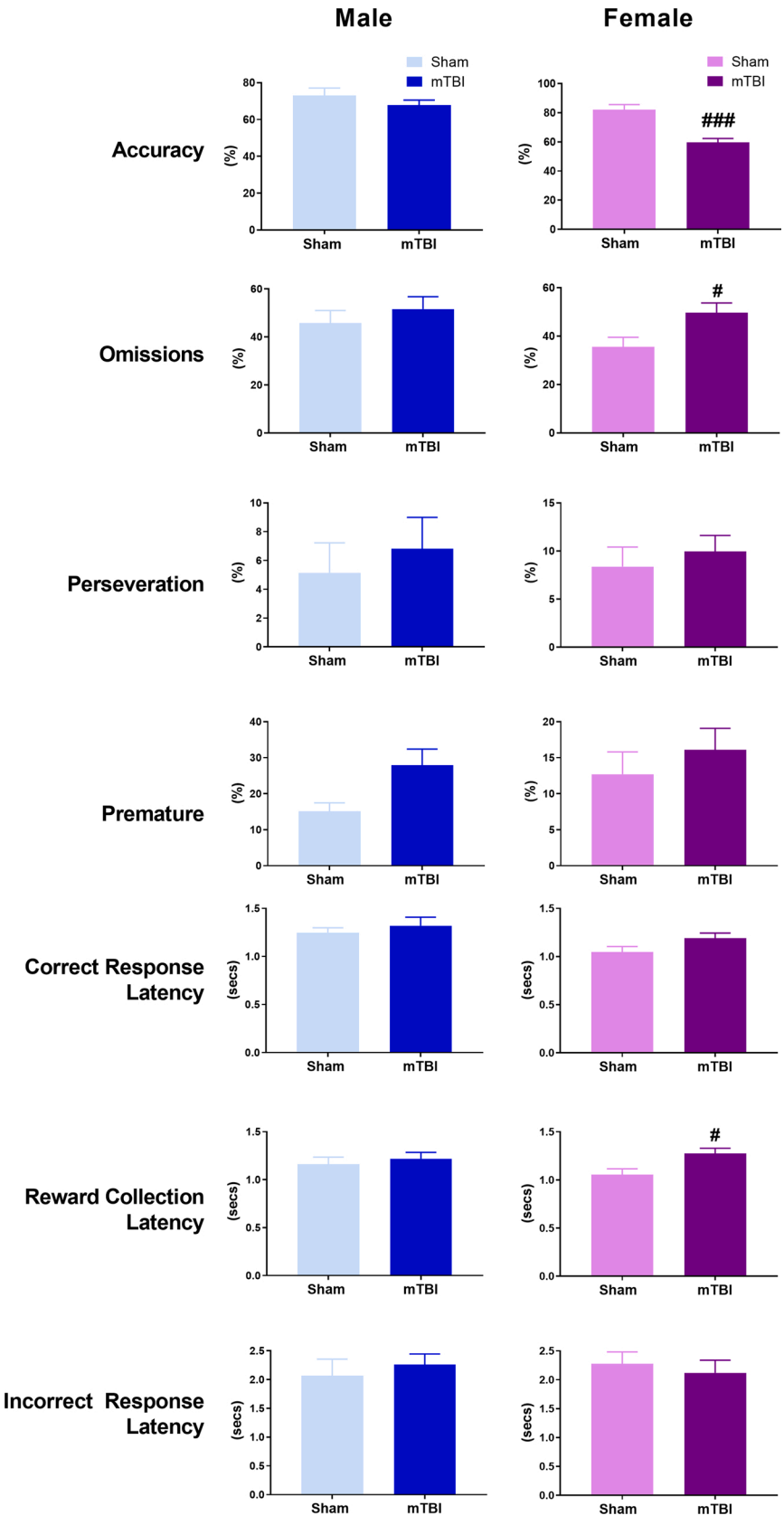

Fig. 3. Data from the first probe trial in the 5CSRTT when stimulus time was decreased to 1.5 s to increase attentional demand. Injury in females, but not males led to a significant decrease in accuracy ($p < 0.001$), increase in omissions ($p < 0.05$) and an increase in reward collection latency ($p < 0.05$). No effect of prior injury was seen in perseveration, premature response rate, correct response latency or incorrect response latency. (male shams n = 12, mTBI n = 14, female shams n = 14, female mTBI n = 16; #$p < 0.05$, ###$p < 0.001$ compared to female shams).

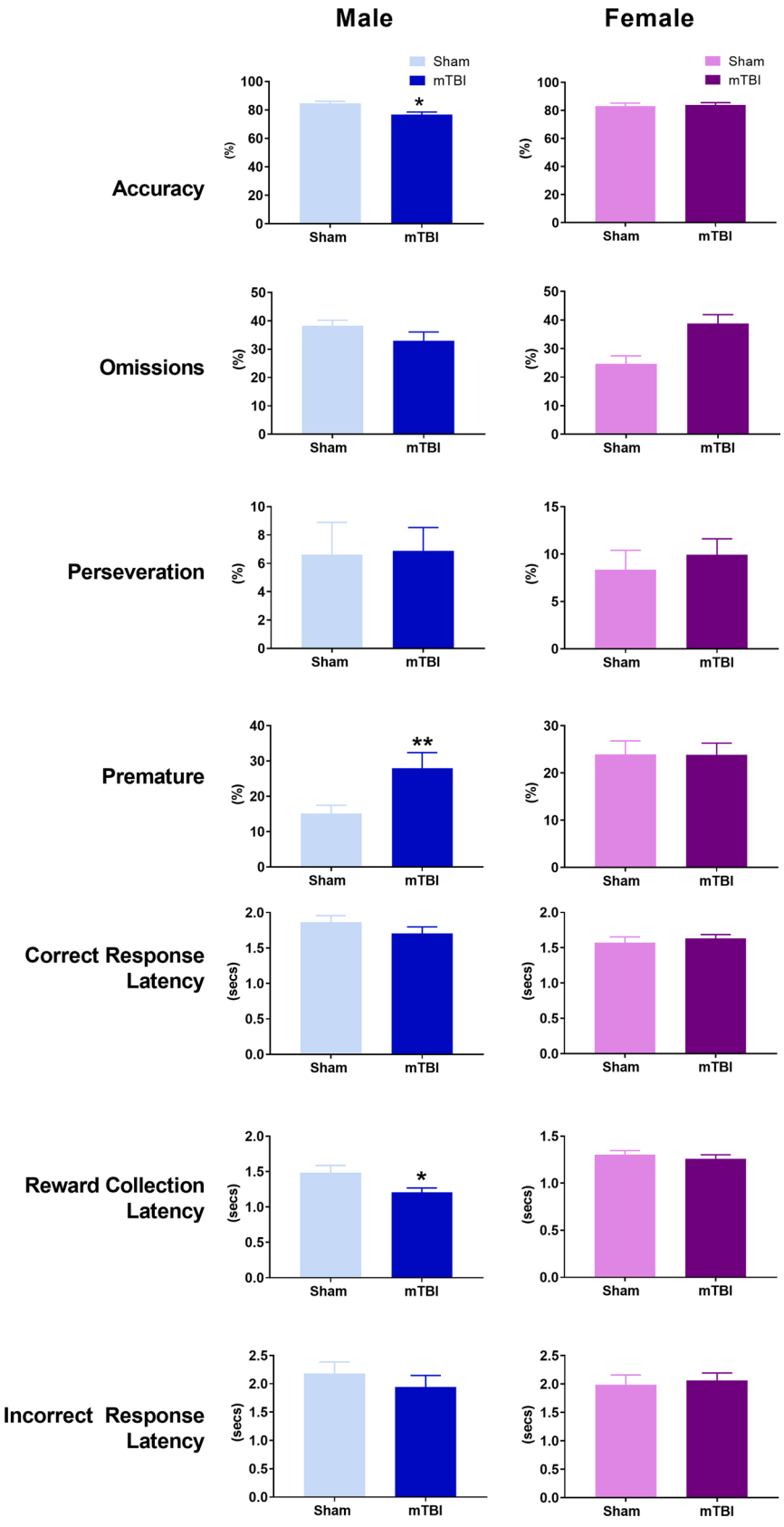

Fig. 4. Data from the second probe trial in the 5CSRTT where ITI was varied to increase inhibitory demand. Males, but not females had a significant decrease in accuracy ($p < 0.01$), increase in the premature response rate ($p < 0.01$) and decrease in reward collection latency ($p < 0.05$). No effect of prior injury was seen in omissions, perseveration, correct response latency or incorrect response latency. (male shams n = 12, mTBI n = 14, female shams n = 14, female mTBI n = 16; *$p < 0.05$, **$p < 0.01$ compared to male shams).

= 0.17), perseveration (p = 0.58), correct response latency (p = 0.16) or incorrect response latency (p = 0.43).

3.3. Effect of injury in adolescence on expression of the axonal signalling molecule Netrin-1 and its receptors UNC-5 and DCC

Within the PFC a 2 × 2 multivariate analysis of variance for markers of interest driving axonal targeting (netrin-1, UNC-5 and DCC) found no significant effect of sex*injury at either 24 h (Pillai's Trace = 0.16; $F_{3,22}$ = 1.37, p = 0.28, η_p^2 = 0.16) or 6 weeks post-injury (Pillai's Trace = 0.002; $F_{3,19}$ = 1.37, p = 0.997, η_p^2 = 0.02), with no main effect of sex or injury at either time-point (Fig. 5).

In contrast within the NAc at 24 h post-injury although no significant sex*injury effect was found (Pillai's Trace = 0.11; $F_{3,22}$ = 2.23, p = 0.11, η_p^2 = 0.23), there was a significant main effect of injury (Pillai's Trace = 0.32; $F_{3,22}$ = 3.47, p < 0.05, η_p^2 = 0.32), but not sex (Pillai's Trace = 0.05; $F_{3,22}$ = 0.42, p = 0.74, η_p^2 = 0.05) (Fig. 6A-C). Injury was found to significantly reduce netrin-1 (0.59 ± 0.04 vs 0.72 ± 0.4, p < 0.05) and UNC5 (0.45 ± 0.3 vs 0.51 ± 0.03, p < 0.05) relative expression, but not DCC (p = 0.17). By 6 weeks post-injury there was a significant effect of sex*injury (Pillai's Trace = 0.52; $F_{3,19}$ = 6.2, p < 0.01, η_p^2 = 0.51), with no main effect of injury (Pillai's Trace = 0.29; $F_{3,19}$ = 2.69, p = 0.08) η_p^2 = 0.30) or sex (Pillai's Trace = 0.10; $F_{3,19}$ = 0.71, p = 0.56, η_p^2 = 0.10) (Fig. 6D-F). A significant sex*injury effect was seen for netrin-1 expression (p < 0.01) and UNC-5 (p < 0.05), but not DCC (p = 0.80). Post-hoc analyses found that injured males had a relative decrease in expression of netrin-1 relative to their shams (0.95 ± 0.24 vs 1.46 ± 0.31, p < 0.01) and an increase in UNC-5 (1.90 ± 1.04 vs 0.87 ± 0.23, p < 0.05). In contrast injury in females had no effect on either marker (netrin 1: 1.18 ± 0.25 vs 1.05 ± 0.23, p = 0.66; UNC-5: 1.13 ± 0.58 vs 1.33 ± 0.35, p = 0.95).

3.4. Effect of adolescence on expression of markers of dopaminergic neurotransmission within the PFC and NAc chronically

Given the alterations noted in proteins driving dopaminergic outgrowth within the NAc we then performed analysis of proteins involved in dopaminergic transmission at 6 weeks post-injury within both the PFC and NAc to determine whether this had any effect on dopamine signalling. These markers included TH, the enzyme involved in dopamine production, DAT, a dopamine transporter, COMT which is involved in enzymatic degradation of dopamine, the dopamine receptors DrD1 and DrD3 and synaptophysin as a general marker of synapses.

In the PFC a 2 × 2 multivariate analysis of variance across the various markers of interest found no effect of sex*injury (Pillai's Trace = 0.35; $F_{7,15}$ = 1.27, p = 0.33, η_p^2 = 0.37), nor main effects of sex (Pillai's Trace = 0.35; $F_{7,15}$ = 1.27, p = 0.33, η_p^2 = 0.27) or injury (Pillai's Trace = 0.21; $F_{7,15}$ = 0.55, p = 0.78, η_p^2 = 0.21) (Fig. 7),

Within the NAc a 2 × 2 multivariate analysis of variance across the various markers of interest found a significant effect of sex*injury (Pillai's Trace = 0.57; $F_{7,15}$ = 2.89, p < 0.05, η_p^2 = 0.57), with a main effect of sex (Pillai's Trace = 0.59; $F_{7,15}$ = 3.08, p < 0.5, η_p^2 = 0.59), but not injury (Pillai's Trace = 0.39; $F_{7,15}$ = 1.35, p = 0.29, η_p^2 = 0.39) (Fig. 8). Examination of the main effect of sex found a significant decrease in DAT levels in females (1.00 ± 0.21 vs 1.89 ± 0.21, p < 0.01) and MBCOMT (0.65 ± 0.05 vs 0.84 ± 0.05, p < 0.05), but no effect on TH (p = 0.79), SCOMT (p = 074), DrD1 (p = 0.91), DrD3 (p = 0.29) or SYN (p = 0.05) In contrast a significant sex*injury effect was found for TH (p < 0.05), MBCOMT (p < 0.05) and synaptophysin (p < 0.05), but not for DAT (p = 0.26), SCOMT (p = 0.67), DrD1 (p = 0.11) or DrD3 (p = 0.72). Post-hoc analyses found a significantly higher relative expression of TH in males with a previous mTBI relative to male shams (2.30 ± 0.86 vs 1.23 ± 0.78, p < 0.05), an effect not seen in females (1.72 ± 0.77 vs 1.96 ± 0.54, p = 0.86). Similarly higher relative expression of synaptophysin

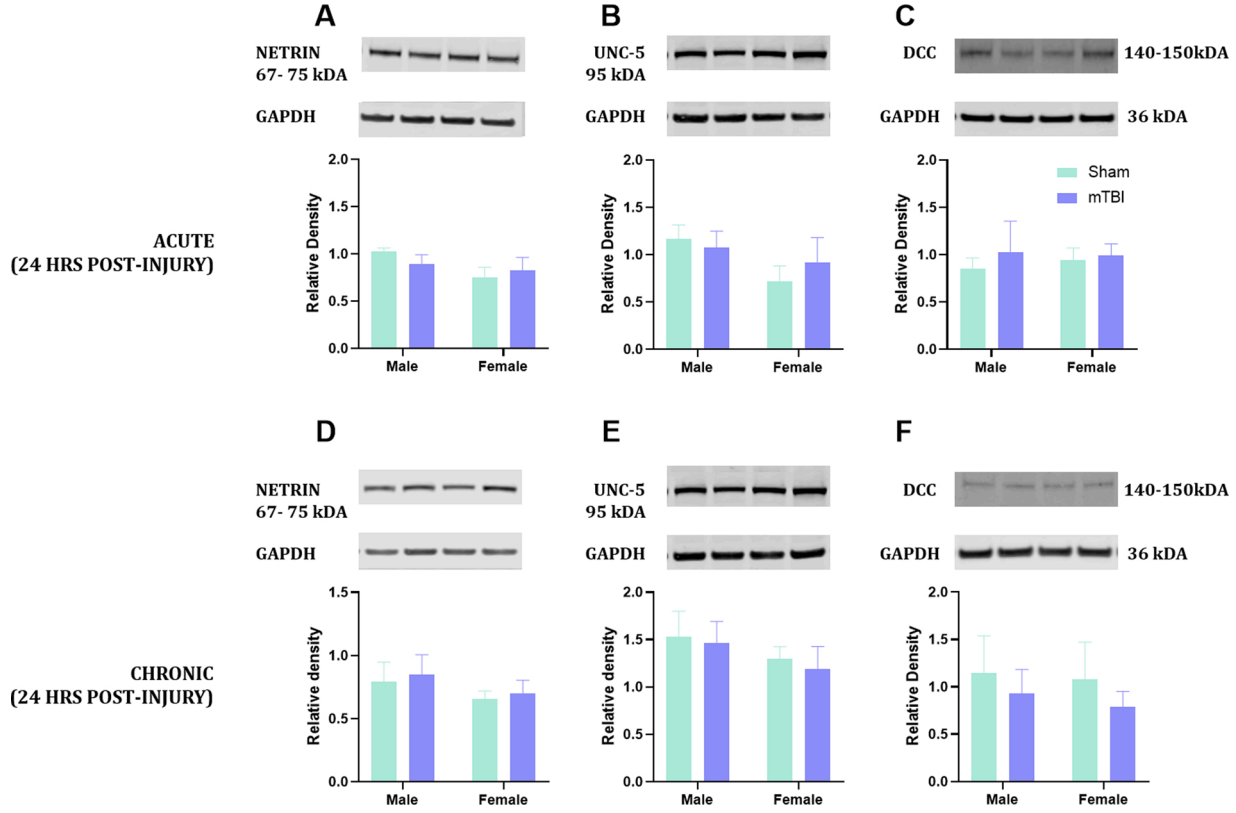

Fig. 5. Analysis of the axonal targeting molecules netrin-1, UNC-5 and DCC within the PFC found no effect of sex*injury, sex or injury at 24 h (AC) or 6 weeks (DF——) post-injury. (n = 5-7 per group).

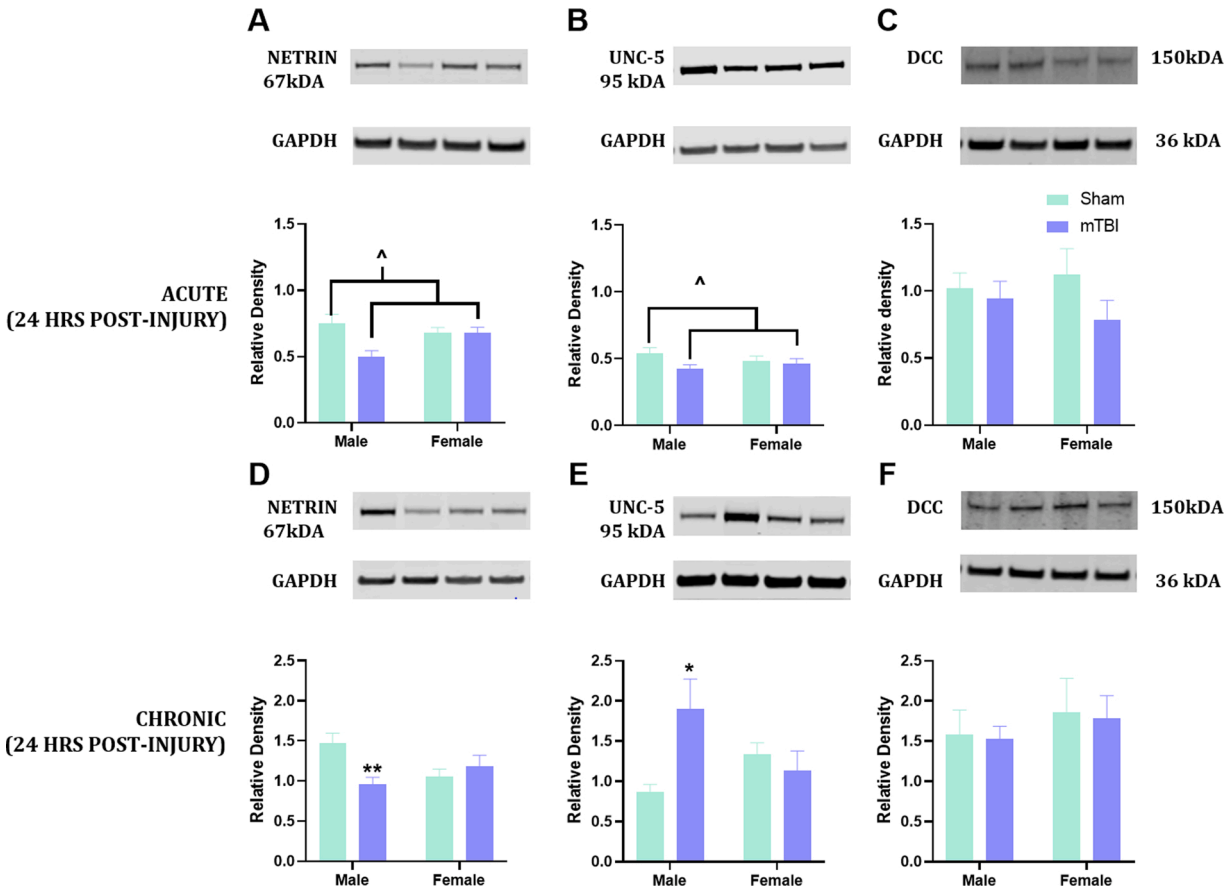

Fig. 6. Analysis of the axonal targeting molecules netrin-1, UNC-5 and DCC within the NAc acutely (AC—) found a significant effect of injury on the relative expression of netrin-1 (A) and UNC-5 (B), but not DCC (C). At 6 weeks post-injury a significant sex*injury effect was found, with no main effect of injury nor sex. Further analyses found significant decreases in netrin-1 expression following injury in males, but not females (D) and an increase in UNC-5 (E) with no differences in DCC (F) (^$p < 0.05$ compared to shams, *$p < 0.05$, **$p < 0.01$ compared to male shams, n = 5-7 per group).

was seen following injury in males (2.24 ± 0.81 vs 1.32 ± 0.39, $p < 0.05$), but not females (1.34 ± 0.40 vs 1.30 ± 0.43, $p = 0.99$). In contrast MBCOMT expression was not significantly different between male injured and sham animals ($p = 0.06$), but was significantly higher following injury in males compared to injured females (0.97 ± 0.23 vs 0.53 ± 0.20, $p < 0.05$), with no difference in male and female shams (0.69 ± 0.10 vs 0.76 ± 0.14, $p = 0.74$).

To support changes noted via western blot, TH immunostaining was also performed within the NAc and mPFC (Fig. 9). Two-way ANOVA of %TH staining within the mPFC found no interaction between sex*injury ($F_{(1, 24)} = 0.004$, $p = 0.94$, $n_p^2 = 0.006$) nor main effects of either sex ($F_{(1, 24)} = 0.05$, $p = 0.81$, $n_p^2 = 0.81$) nor injury ($F_{(1, 24)} = 2.28$, $p = 0.14$, $n_p^2 = 0.087$). In contrast within the NAc a significant interaction of sex*injury was found ($F_{(1, 24)} = 7.20$, $p < 0.01$, $n_p^2 = 0.16$), with no main effects of either injury ($F_{(1, 24)} = 2.63$, $p = 0.12$, $n_p^2 = 0.16$) or sex ($F_{(1, 24)} = 0.17$, $p = 0.69$, $n_p^2 = 0.03$). Post-hoc analysis found significantly higher %TH area in mTBI males compared to sham males (40.14 ± 8.80 vs 31.79 ± 7.06, $p < 0.05$), whereas similar levels were seen in females (34.10 ± 7.12 vs 34.62 ± 3.32, $p = 0.99$).

4. Discussion

Following an mTBI in adolescence, subtle, sex-dependent alterations in executive function were seen in early adulthood on the 5CSRTT, which were dependent on the type of probe trial conducted. When ITI was varied to increase inhibitory demand, males, showed an increase in impulsivity post-TBI, decreased accuracy and decreased reward collection latency. In contrast, prior injury in adolescence in females meant that when attentional demand was increased by decreasing stimulus time, they were less motivated to perform the task, as seen by a higher omission rate both as well as reduced accuracy and increased reward collection latency. These deficits in executive function were accompanied by subtle alterations in the dopaminergic pathway within the NAc, in males, but not females. Increased TH via both western blot and immunohistochemistry was observed at 6 weeks post-injury within the NAc compared to male shams, alongside an increase in synaptophysin. Furthermore an increase in MBCOMT in injured males compared to female mTBI animals were found. No significnat alterations were seen within the PFC. These subtle alterations in dopaminergic signalling were associated with decreased expression of netrin-1 within the NAc, an axonal signalling molecule that promotes the outgrowth of dopaminergic axons from the PFC to the NAc in adolescence, alongside a chronic increase in UNC-5 which repels axons.

Here, we found that increased impulsivity in males, as detected on the 5CSRTT, following mTBI in adolescence was associated with changes in dopaminergic markers within the NAc. There are two domains of impulsivity, in impulsive choice and impulsive action [32]. Premature responding in the 5CSRTT reflects impulsive action, with deficits in inhibitory control of highly pre-potent responses when anticipating reward [33]. In particular, increased premature response rate in previously injured males was evident only in the variable ITI trial, where temporal predictability of the cue was reduced, thereby increasing the demand on inhibiting inappropriate responding. Effects seen under variable ITI conditions are thought to more likely reflect state rather than trait impulsivity. State impulsivity describes the variable momentary responses to contextual intrinsic and extrinsic triggers

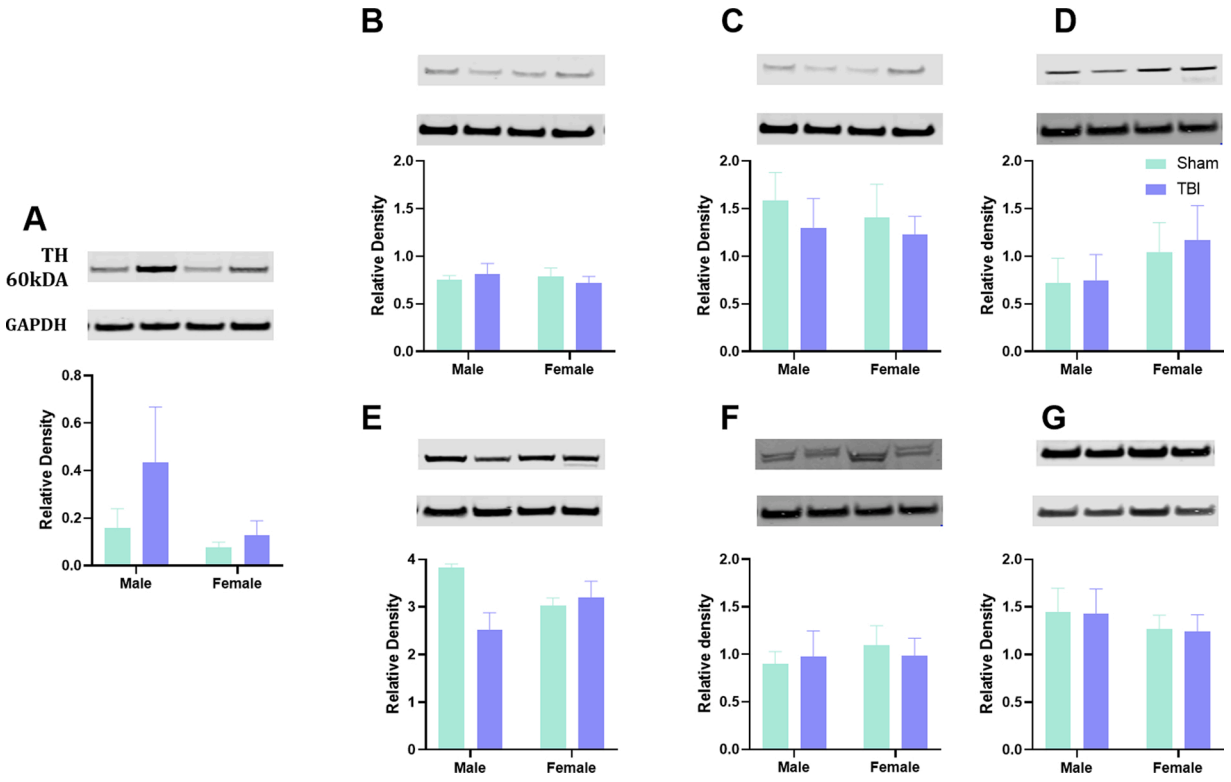

Fig. 7. Analysis of markers involved in dopaminergic neurotransmission including TH (A), MBCOMT (B), SCOMT (C), DAT (D), DrD1 (E) DrD3 (F) and SYN (G) within the PFC at 6 weeks post-injury found no overall effect of sex*injury, injury or sex.

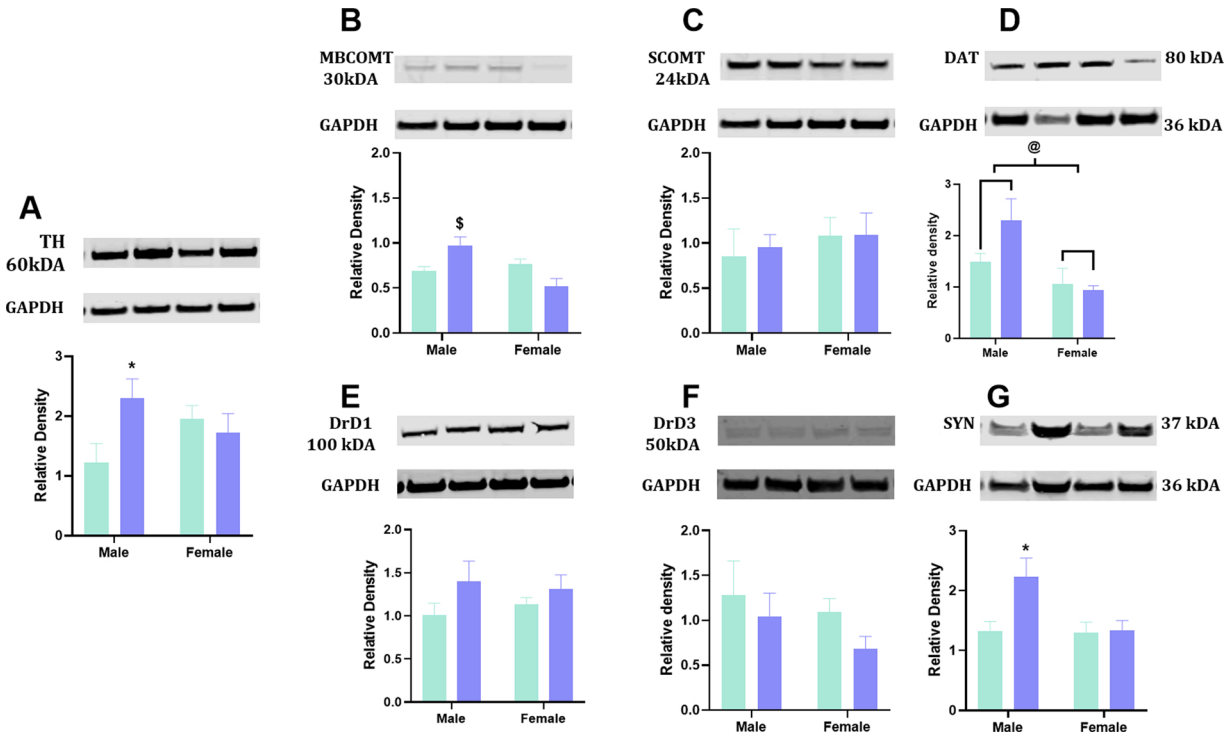

Fig. 8. Analysis of markers involved in dopaminergic neurotransmission within the NAc at 6 weeks following injury found an overall effect of sex*injury with injured males, but not females having higher levels of TH (A) and SYN (G) than their sham counterparts (*p < 0.05). Injured males also had higher levels of MBCOMT compared to injured female counterparts (B, $p < 0.05). There was also a main effect of sex with DAT relative expression higher in males than females (D, @p < 0.05). No effect was seen in relative expression of SCOMT (C), DrD1 (E) or DrD3 (F). (n = 5 = 7 per group).

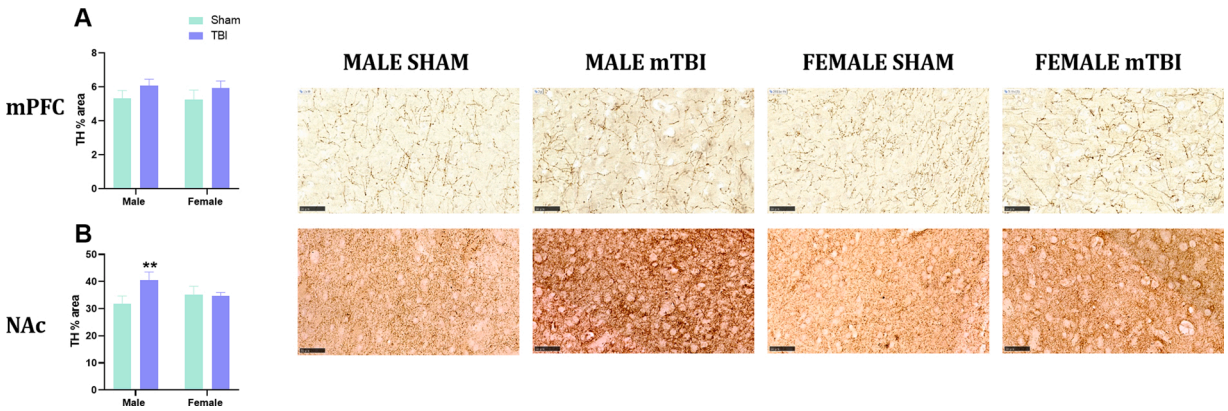

Fig. 9. Immunohistochemical analysis of TH within the mPFC (A) and NAc (B) at 6 weeks post-injury. No effect of injury or sex was seen within the mPFC, but a significant sex*injury interaction was found in the NAc, with male injured animals having a higher %TH staining than their shams (**$p < 0.01$), an effect not seen in females. (n = 7 per group, scale bar = 50 μm).

[34,35] compared to enduring personality characteristics of an individual that remain stable over time in trait impulsivity [36]. The increased impulsivity in males seen here is in accordance with previous reports with increased premature response rate in the go-no go task at two-four weeks following diffuse injury delivered at either p30 [37] or p45 [38], with impulsivity similarly more prominent in males than females. Clinical reports following TBI during childhood and adolescence also show a link to poor inhibitory control [39–41]. However, it should be noted that impaired impulse control post-TBI is not just limited to injuries during childhood or adolescence. Pre-clinically, increased premature response rate in the 5CSRTT was seen following mild, moderate or severe bilateral frontal cortex focal injury in adult rats, persisting to 14 weeks post-injury [42]. Similarly, clinically, longer latencies in the stop-signal task are noted in those with a history of moderate-severe injury in adulthood [43]. Importantly, however, these do reflect either more severe injuries, or a specific insult targeted to the PFC, compared to the diffuse injury employed here. Indeed, uncomplicated mTBI in adults has been reported to have no effect on premature response rate in the continuous performance test (similar to the 5CSRTT) [44] and impulsivity in veterans was associated more strongly with post-traumatic stress disorder than with a history of mTBI. Thus, during adolescence, even a mild TBI in males may have long lasting effects on impulse control, although further investigation is required.

These deficits in impulse control following mTBI were associated with altered markers of dopaminergic signalling, within the NAc, in males compared to females. Relative expression of TH, the rate-limiting enzyme responsible for converting the amino acid L-tyrosine to L-DOPA, L-DOPA is the immediate precursor for DA synthesis [45], the increase in TH may lead to alterations in dopaminergic signalling. Indeed, the increase in TH was accompanied by an increase MBCOMT, which regulates the levels of dopamine within the synapse its enzymatic degradation [46]. This is suggestive that increased dopamine synthesis may have led to a compensatory increase in clearance mechanisms. Notably, alterations were only seen in MBCOMT, which has been shown to have a higher affinity for dopamine than SCOMT and is thus thought to be the form of COMT most involved in dopaminergic degradation [47]. An increase in dopamine could drive increased impulsivity, given that increasing dopamine pharmacologically increases premature responses in the 5CSRTT, as seen following acute administration of amphetamine [48–50], methylprenidate [51], cocaine [50] and the selective DA reuptake inhibitor GBR 12,909 [48,50,52], with the NAc found to be critically involved. Although not specifically analysed here, the NAc consists of two functionally distinct compartments, the core and shell. In particular, dopamine within the NAc core seems to drive impulsivity within the 5CSRTT, with infusion of DA receptor antagonists directly into the NAc core reducing premature response rate (Pattij et al., 2007; Pezze et al., 2007). Indeed the DAT inhibitor, MPH, which would enhance dopaminergic signalling, only increased premature response rate when infused in the NAc core, but not the shell. It is thought that increased dopamine within the NAc core mediates increases in the salience of reward related cues in the 5CSRTT [53], and, in support, the previously injured male mTBI animals had a reduced latency to retrieve the reward. The enhanced dopaminergic transmission driving state impulsivity differs from trait impulsivity, which is characterised by low basal dopamine [54,55] and improvements with dopamine enhancing treatments [56,57]. Even in TBI models, amphetamine treatment to enhance dopaminergic output was found to improve impulsivity following severe bilateral frontal TBI, but worsen it following mild TBI [58], suggesting that underlying mechanisms may differ depending on the degree of circuit disruption. Thus, understanding how dysregulation of dopaminergic neurotransmission may promote impulsivity post-TBI is integral for determining optimal treatment strategies following different severities of injury.

Surprisingly the alterations in dopaminergic markers in previously injured males had minimal effects on attention in the task. Although, male animals showed a decrease in accuracy on the variable ITI task, it may relate to the increased premature response rate driving selection of an incorrect screen before the animal was able to register the stimulus. In contrast previously injured females showed impaired accuracy and higher omissions, unrelated to dopaminergic marker alterations. Of note dopaminergic neuron activation correlates with the detection of salient stimuli [59] and can be impaired by either hypo [60] or hyper-dopaminergic states [61]. Furthermore D1 DA receptor stimulation in the PFC is thought to be important for working memory performance [62,63] with intra-PFC infusions of DA D1, receptor antagonists producing delay-dependent impairments in spatial working memory tasks [64].

The effects on the dopaminergic system presented here differ from reports in pre-clinical adult models of TBI. Following focal TBI models, DAT levels have been found to be chronically reduced within the striatum, which incorporates the caudate, putamen and nucleus accumbens [65], frontal cortex [66] and midbrain [67]. This is supported by clinical studies, where reduced DAT levels were noted in the striatum one year following moderate-severe TBI using the PET ligand 11C-β-CFT [68]. Here no effect of injury was noted on DAT levels, although overall males had a higher level of expression than females, demonstrating no long term decrease in DAT expression post-injury in males. In the literature the decrease in DAT is thought to reflect a chronic reduction in dopamine release and evoked outflow [65,69–71], given that DAT undergoes a compensatory downregulation in response to reduced dopamine levels [72]. Specifically within the NAc, even a mild fluid percussion TBI led to suppression of DA release at 1–2 weeks post-injury within both the core

and shell, with recovery by 4 weeks post-injury [73]. These results contrast to the increase in TH found here. Evidently, further studies are needed to confirm whether there is an increase in dopamine release within the NAc, but they are suggestive of enhanced dopamine synthesis. The differences may relate to employment of a diffuse rather than a focal model, which would be expected to cause more direct disruption of dopaminergic systems due to more extensive cell loss. Alternatively, age at injury may influence the response of the dopaminergic system, given the extensive alterations occurring during adolescence.

It has been hypothesised that, in adolescence, there are two populations of dopaminergic axons within the NAc, mesolimbic dopamine axons that express DCC receptors that promote target recognition to the NAc, and mesocortical axons that lack DCC receptors and continue to grow to the PFC throughout adolescence [20,23]. Netrins can act as either attractants or repellents for growing axons depending on the expression of DCC and UNC-5. DCC results in attraction to netrins [18,19], whereas the presence of both DCC and UNC-5 repels axonal responses [74]. Alterations in the expression via haplosufficiency of either netrin-1 [75] or DCC [23] enhances dopaminergic input to the mPFC in adulthood, presumably by promotion of continued growth of mesolimbic dopamine axons. Indeed, specifically reducing netrin-1 within the NAc during adolescence via injection of Netrin-1 shRNA construct viral silencer significantly increased the volume of dopaminergic input to the mPFC [76]. In contrast here, a chronic reduction in netrin-1 expression in the NAc did not appear to increase dopaminergic input to the mPFC, at least as measured by TH immunoreactivity. This may relate to the chronic increase in UNC-5 relative expression noted within the NAc. Although acutely an overall and subtle injury effect was seen with decreased levels of UNC-5 was seen, by 6 weeks post-injury an increase was seen in injured males, but not females relative to their shams. In a hippocampal slice model, it was found that increased UNC-5 expression triggered mossy fibre sprouting by switching the netrin-1 response to repulsion [77]. Similarly, UNC-5 has been shown to induce neurite outgrowth in neuroblastoma cells in a netrin-1-dependent manner [78] and to modulate synaptic differentiation in motor neuron dendrites in C. elegans [79]. Thus, the increase in UNC-5 may have facilitated dendritic branching and synaptogenesis of the dopaminergic neurons within the NAc, as seen by the relative increase in synaptophysin and TH in this study. Synaptophysin is an integral membrane protein found in synaptic vesicles, and is a specific and sensitive marker for pre-synaptic terminals, and thus the increase in suggestive of increased synapses within the NAc [80], potentially from VTA dopaminergic neurons. Further studies utilising axon-initiated viral transduction to label neurons projecting from the VTA that are present within the NAc prior to injury could be utilised to assess whether injury affects outgrowth to the mPFC and the fate of those neurons remaining within the NAc.

It should be noted that protein changes were evaluated primarily via western blot with normalisation to GAPDH. Use of housekeeping proteins can be problematic with potential with levels to vary as an effect of injury [103]. However, following a similar closed head injury to that employed here in mice levels of GAPDH mRNA were not found to significantly differ in the acute phase (<48 h post-injury), with suggestions that this was the most suitable housekeeper [104]. GAPDH remains commonly used as a housekeeper for both western blot [105,106][107] [81] and RT-PCR [108] following TBI, but it is noted that variable alterations in expression could affect the results presented here.

Vulnerability to alterations in dopaminergic markers in males, but not females, following mTBI may relate to sex differences in maturation during adolescence. Puberty, as signalled by final maturation of the hypothalamic-pituitary-gonadal (HPG) axis with secondary sex characteristics, is earlier in female rats (p30-p42) compared to males (p42-p55) [82], in line with adolescence being defined as p22−60 in females compared to p28−70 in males [83]. Thus, injury at p35 may have had differential effects due to the impact at differing stages of development. Striatal development in particular seems to differ depending on sex, with males reaching peak striatal volume at ~15 years of age compared with 12 for girls [84]. Furthermore, there are sex differences in the profiles of DR1 and DR2 receptors within the striatum in rodent studies, with males peaking at p40 before decreasing rapidly, whereas females have constant levels. [85]. As such, dopaminergic input to the NAc may be more vulnerable to mTBI during mid-adolescence in males than females. Within the PFC, however, dopaminergic input increases during adolescence are not associated with circulating gonadal hormones, nor does the timing or pattern differ substantially between males and females [86]. Indeed no chronic effects on dopaminergic markers were seen chronically following injury regardless of sex.

Female rats did, however, show impaired performance on the 5CSRTT having a higher rate of omissions and decreased accuracy following injury, with an increase in reward collection latency. It should be noted that the overall power to detect sex specific changes was low in the decreased stimulus time trial, and we conducted further analyses within sex to compare injured versus sham animals based on previous literature reports of sex specific behavioural impairments post-TBI [38]. Thus the results presented here need to be interpreted with caution. Nonetheless it appeared that the pattern of deficits found in the females on the 5CSRTT reflected impaired attention, with decreased response accuracy and increased response omissions [87]. There was no evidence of a motor deficit, with no alteration in correct response latency, although reward collection latency was decreased. Our findings are in line with previous research that, following a concussion during adolescence (p45), females, but not males had decreased accuracy in the go-no go task, with no change in impulsivity. Clinically, females have been found to be 1.7 times more likely to experience cognitive impairment following a concussion compared to males [8]. Indeed, following a mTBI, females were found to report more symptoms than males including poorer memory, more irritability and higher rates of anxiety and depression [88]. These deficits may be long-lasting, with impaired cognitive processing speed at 6 months following a single concussion in collegiate athletes [89]. What drives the differential response to TBI in females is yet to be determined.

Attentional processes within the 5CSRTT appear reliant on the mPFC, which exerts top-down control over a number of subcortical structures with a putative role in cognitive behaviour, including the NAc and hippocampus [90]. Either over-activation [91] or excitotoxic lesions [92] of the mPFC are associated with impaired accuracy and increased omissions in the 5CSRTT. In rodent studies, females show a much more dramatic drop in both neuronal, and hence synaptic, number within the mPFC than males during adolescence, with peak synaptic numbers in the female cortex at p35, with a significant drop by p45 that then persists to adulthood [93]. Although males also show a reduction in adolescence, it is much more gradual. Studies suggest that neuronal loss in the mPFC does not relate to GABAergic interneurons and is thus more likely to reflect glutamatergic pyramidal cells [93]. Interruption in neuronal pruning via mTBI at a critical period could potentially alter the inhibitory/excitatory balance within the mPFC, driving subtle cognitive deficits independent of dopaminergic changes. Indeed the transition from adolescence to early adulthood when these processes are taking place coincides with increased capacity for a variety of cognitive tasks, particularly those that involve cognitive flexibility [94,95]

Alternatively, the impaired performance in females may not be age dependent but instead relate to intrinsic differences between the sexes. Female axons were found to be smaller, with fewer microtubules, and thus are at higher risker of breaking compared to male axons following an equivalent force from simulated TBI [96]. Furthermore, although injury was delivered prior to establishment of regular oestrous cycles (~p42) [82], it is possible that injury interrupted establishment of these cycles. Both oestrogen [97] and progesterone [98,99] when administered exogenously have beneficial effects in pre-clinical models, reducing oedema and maintaining cerebral blood flow. The production of FSH and LH have been found to be reduced following moderate-TBI clinically [100,101], which could prevent establishment of regular cycles and thus the presence of neuroprotective oestrogen and

progesterone. Furthermore, elevated cortisol levels were associated with anovulation caused by central HPG axis suppression post-TBI [102], which could negatively affect cognitive outcome. For example higher cortisol levels are associated with poorer overall cognitive performance [103,104]. Examination of the specific effects of adolescent mTBI in females on hormonal expression and relationship with cognitive symptoms is thus required.

In summary, a single mTBI during adolescence was sufficient to lead to subtle deficits in executive function in early adulthood in a preclinical model. Males were more impulsive whereas females appeared to have deficits in attention. In males only, increased impulsivity was associated with altered expression of markers of the dopaminergic system within the NAc, with these findings differing from previous results reported with injury during adulthood where dopaminergic transmission is chronically reduced. Our findings provide increased support for the heightened vulnerability of females to chronic cognitive dysfunction following TBI. There is a pressing need to further dissect the post-injury cascades in males versus females and how sex interacts with age at time of injury to identify novel treatment targets for each population, and to determine biomarkers to predict those most likely to develop chronic symptoms post-TBI.

CRediT authorship contribution statement

Lola Kaukas: Validation, Visualization, Data curation, Writing - original draft. **Joshua L Holmes:** Investigation, Methodology, Validation. **Freshta Rahimi:** Investigation, Methodology, Validation, Data curation. **Lyndsey Collins-Praino:** Supervision, Writing - review & editing. **Frances Corrigan:** Conceptualization, Methodology, Data curation, Writing - review & editing.

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References

[1] A.L. Zhang, D.C. Sing, C.M. Rugg, B.T. Feeley, C. Senter, The rise of concussions in the adolescent population, Orthop. J. Sports Med. 4 (8) (2016), 2325967116662458.
[2] A.C. McKee, D.H. Daneshvar, The neuropathology of traumatic brain injury, Handb. Clin. Neurol. 127 (2015) 45–66.
[3] M.F. Grady, Concussion in the adolescent athlete, Curr. Probl. Pediatr. Adolesc. Health Care 40 (7) (2010) 154–169.
[4] M.P. McClincy, M.R. Lovell, J. Pardini, M.W. Collins, M.K. Spore, Recovery from sports concussion in high school and collegiate athletes, Brain Inj. 20 (1) (2006) 33–39.
[5] C. Konrad, A.J. Geburek, F. Rist, H. Blumenroth, B. Fischer, I. Husstedt, V. Arolt, H. Schiffbauer, H. Lohmann, Long-term cognitive and emotional consequences of mild traumatic brain injury, Psychol. Med. 41 (6) (2011) 1197–1211.
[6] A. Tapper, D. Gonzalez, E. Roy, E. Niechwiej-Szwedo, Executive function deficits in team sport athletes with a history of concussion revealed by a visual-auditory dual task paradigm, J. Sports Sci. 35 (3) (2017) 231–240.
[7] B. Alcock, C. Gallant, D. Good, The relationship between concussion and alcohol consumption among university athletes, Addict. Behav. Rep. 7 (2018) 58–64.
[8] D.K. Broshek, J.R. Freeman, Psychiatric and neuropsychological issues in sport medicine, Clin. Sports Med. 24 (3) (2005) 663–679, x.
[9] T. Covassin, C.B. Swanik, M.L. Sachs, Sex differences and the incidence of concussions among collegiate athletes, J. Athl. Train. 38 (3) (2003) 238–244.
[10] T. Covassin, P. Schatz, C.B. Swanik, Sex differences in neuropsychological function and POST-CONCUSSION symptoms of concussed collegiate athletes, Neurosurgery 61 (2) (2007) 345–351.
[11] K.E. Ono, T.G. Burns, D.J. Bearden, S.M. McManus, H. King, A. Reisner, Sex-based differences as a predictor of recovery trajectories in young athletes after a sports-related concussion, Am. J. Sports Med. 44 (3) (2016) 748–752.
[12] T. Covassin, R. Moran, R.J. Elbin, Sex differences in reported concussion injury rates and time loss from participation: an update of the national collegiate athletic association injury surveillance program from 2004-2005 through 2008-2009, J. Athl. Train. 51 (3) (2016) 189–194.
[13] S.L. Zuckerman, Z.Y. Kerr, A. Yengo-Kahn, E. Wasserman, T. Covassin, G. S. Solomon, Epidemiology of sports-related concussion in NCAA athletes from 2009-2010 to 2013-2014: incidence, recurrence, and mechanisms, Am. J. Sports Med. 43 (11) (2015) 2654–2662.
[14] S. Tanveer, N. Zecavati, E.B. Delasobera, T.O. Oyegbile, Gender Differences in Concussion and Postinjury Cognitive Findings in an Older and Younger Pediatric Population, Pediatr. Neurol. 70 (2017) 44–49.
[15] P. Yuan, N. Raz, Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies, Neurosci. Biobehav. Rev. 42 (2014) 180–192.
[16] H. Trantham-Davidson, L.C. Neely, A. Lavin, J.K. Seamans, Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex, J. Neurosci. 24 (47) (2004) 10652–10659.
[17] A.C. Reichelt, Adolescent maturational transitions in the prefrontal cortex and dopamine signaling as a risk factor for the development of obesity and high Fat/High sugar diet induced cognitive deficits, Front. Behav. Neurosci. 10 (189) (2016).
[18] J.R. de la Torre, V.H. Höpker, G.L. Ming, M.M. Poo, M. Tessier-Lavigne, A. Hemmati-Brivanlou, C.E. Holt, Turning of retinal growth cones in a netrin-1 gradient mediated by the netrin receptor DCC, Neuron 19 (6) (1997) 1211–1224.
[19] M.S. Deiner, T.E. Kennedy, A. Fazeli, T. Serafini, M. Tessier-Lavigne, D. W. Sretavan, Netrin-1 and DCC mediate axon guidance locally at the optic disc: loss of function leads to optic nerve hypoplasia, Neuron 19 (3) (1997) 575–589.
[20] D. Hoops, C. Flores, Making dopamine connections in adolescence, Trends Neurosci. 40 (12) (2017) 709–719.
[21] C. Manitt, C. Eng, M. Pokinko, R.T. Ryan, A. Torres-Berrío, J.P. Lopez, S. V. Yogendran, M.J.J. Daubaras, A. Grant, E.R.E. Schmidt, F. Tronche, P. Krimpenfort, H.M. Cooper, R.J. Pasterkamp, B. Kolb, G. Turecki, T.P. Wong, E. J. Nestler, B. Giros, C. Flores, dcc orchestrates the development of the prefrontal cortex during adolescence and is altered in psychiatric patients, Transl. Psychiatry 3 (12) (2013) e338-e338.
[22] M. Pokinko, A. Grant, F. Shahabi, Y. Dumont, C. Manitt, C. Flores, Dcc haploinsufficiency regulates dopamine receptor expression across postnatal lifespan, Neuroscience 346 (2017) 182–189.
[23] L.M. Reynolds, A.J. Gifuni, E.T. McCrea, P. Shizgal, C. Flores, dcc Haploinsufficiency results in blunted sensitivity to cocaine enhancement of reward seeking, Behav. Brain Res. 298 (Pt A) (2016) 27–31.
[24] L.M. Reynolds, M. Pokinko, A. Torres-Berrio, S. Cuesta, L.C. Lambert, E. Del Cid Pellitero, M. Wodzinski, C. Manitt, P. Krimpenfort, B. Kolb, C. Flores, DCC receptors drive prefrontal cortex maturation by determining dopamine axon targeting in adolescence, Biol. Psychiatry 83 (2) (2018) 181–192.
[25] A. Tsuchiya, T. Hayashi, K. Deguchi, Y. Sehara, T. Yamashita, H. Zhang, V. Lukic, M. Nagai, T. Kamiya, K. Abe, Expression of netrin-1 and its receptors DCC and neogenin in rat brain after ischemia, Brain Res. 1159 (2007) 1–7.
[26] L.P. Spear, The adolescent brain and age-related behavioral manifestations, Neurosci. Biobehav. Rev. 24 (4) (2000) 417–463.
[27] R. Mychasiuk, A. Farran, M. Angoa-Perez, D. Briggs, D. Kuhn, M.J. Esser, A novel model of mild traumatic brain injury for juvenile rats, J. Vis. Exp. (94) (2014) 51820.
[28] A.C. McKee, D.H. Daneshvar, The neuropathology of traumatic brain injury, Handb. Clin. Neurol. 127 (2015) 45–66.
[29] D.C. Viano, I.R. Casson, E.J. Pellman, Concussion in professional football: biomechanics of the struck player–part 14, Neurosurgery 61 (2) (2007), 313-27; discussion 327-328.
[30] R.Au- Mychasiuk, A.Au- Farran, M.Au- Angoa-Perez, D. Au - Briggs, D. Au - Kuhn, M.J.Au- Esser, A novel model of mild traumatic brain injury for juvenile rats, JoVE (94) (2014), e51820.
[31] A. Arulsamy, J. Teng, H. Colton, F. Corrigan, L. Collins-Praino, Evaluation of early chronic functional outcomes and their relationship to pre-frontal cortex and hippocampal pathology following moderate-severe traumatic brain injury, Behav. Brain Res. 348 (2018) 127–138.
[32] N. Broos, L. Schmaal, J. Wiskerke, L. Kostelijk, T. Lam, N. Stoop, L. Weierink, J. Ham, E.J. de Geus, A.N. Schoffelmeer, W. van den Brink, D.J. Veltman, T.J. de Vries, T. Pattij, A.E. Goudriaan, The relationship between impulsive choice and impulsive action: a cross-species translational study, PLoS One 7 (5) (2012), e36781.
[33] J.L. Evenden, Varieties of impulsivity, Psychopharmacology 146 (4) (1999) 348–361.
[34] Jeffrey W. Dalley, Barry J. Everitt, Trevor W. Robbins, Impulsivity, compulsivity, and top-down cognitive control, Neuron 69 (4) (2011) 680–694.
[35] J. Wingrove, A.J. Bond, Impulsivity: A state as well as trait variable. Does mood awareness explain low correlations between trait and behavioural measures of impulsivity? Pers. Individ. Dif. 22 (3) (1997) 333–339.
[36] C.G. DeYoung, Impulsivity As a Personality Trait, Handbook of Self-regulation: Research, Theory, and Applications, 2nd ed., Guilford Press, New York, NY, US, 2011, pp. 485–502.
[37] R. Mychasiuk, H. Hehar, M.J. Esser, A mild traumatic brain injury (mTBI) induces secondary attention-deficit hyperactivity disorder-like symptomology in young rats, Behav. Brain Res. 286 (2015) 285–292.
[38] H. Hehar, K. Yeates, B. Kolb, M.J. Esser, R. Mychasiuk, Impulsivity and Concussion in Juvenile Rats: Examining Molecular and Structural Aspects of the Frontostriatal Pathway, PLoS One 10 (10) (2015), e0139842.
[39] V. Anderson, C. Catroppa, S. Morse, F. Haritou, J. Rosenfeld, Attentional and processing skills following traumatic brain injury in early childhood, Brain Inj. 19 (9) (2005) 699–710.
[40] K.J. Sinopoli, R. Schachar, M. Dennis, Traumatic brain injury and secondary attention-deficit/hyperactivity disorder in children and adolescents: the effect of reward on inhibitory control, J. Clin. Exp. Neuropsychol. 33 (7) (2011) 805–819.

[41] H.S. Levin, G. Hanten, L. Zhang, P.R. Swank, J. Hunter, Selective impairment of inhibition after TBI in children, J. Clin. Exp. Neuropsychol. 26 (5) (2004) 589–597.
[42] C. Vonder Haar, F.C.W. Lam, W.K. Adams, L.-K. Riparip, S. Kaur, M. Muthukrishna, S. Rosi, C.A. Winstanley, Frontal Traumatic Brain Injury in Rats Causes Long-Lasting Impairments in Impulse Control That Are Differentially Sensitive to Pharmacotherapeutics and Associated with Chronic Neuroinflammation, ACS Chem. Neurosci. 7 (11) (2016) 1531–1542.
[43] L. Rochat, C. Beni, J.M. Annoni, P. Vuadens, M. Van der Linden, How inhibition relates to impulsivity after moderate to severe traumatic brain injury, J. Int. Neuropsychol. Soc. 19 (8) (2013) 890–898.
[44] C.J. Chen, C.H. Wu, Y.P. Liao, H.L. Hsu, Y.C. Tseng, H.L. Liu, W.T. Chiu, Working memory in patients with mild traumatic brain injury: functional MR imaging analysis, Radiology 264 (3) (2012) 844–851.
[45] G.C. Cotzias, Metabolic modification of some neurologic disorders, Jama 210 (7) (1969) 1255–1262.
[46] L. Yavich, M.M. Forsberg, M. Karayiorgou, J.A. Gogos, P.T. Männistö, Site-Specific Role of Catechol-&em&O&/em&-Methyltransferase in Dopamine Overflow within Prefrontal Cortex and Dorsal Striatum, J. Neurosci. 27 (38) (2007) 10196.
[47] T. Lotta, J. Vidgren, C. Tilgmann, I. Ulmanen, K. Melen, I. Julkunen, J. Taskinen, Kinetics of Human Soluble and Membrane-Bound Catechol O-Methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme, Biochemistry 34 (13) (1995) 4202–4210.
[48] M. Loos, J. Staal, A.N. Schoffelmeer, A.B. Smit, S. Spijker, T. Pattij, Inhibitory control and response latency differences between C57BL/6J and DBA/2J mice in a Go/No-Go and 5-choice serial reaction time task and strain-specific responsivity to amphetamine, Behav. Brain Res. 214 (2) (2010) 216–224.
[49] T. Pattij, M.C. Janssen, L.J. Vanderschuren, A.N. Schoffelmeer, M.M. van Gaalen, Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control, Psychopharmacology 191 (3) (2007) 587–598.
[50] M.M. van Gaalen, R.J. Brueggeman, P.F. Bronius, A.N. Schoffelmeer, L. J. Vanderschuren, Behavioral disinhibition requires dopamine receptor activation, Psychopharmacology 187 (1) (2006) 73–85.
[51] R. Navarra, R. Graf, Y. Huang, S. Logue, T. Comery, Z. Hughes, M. Day, Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test, Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (1) (2008) 34–41.
[52] P.J. Baarendse, L.J. Vanderschuren, Dissociable effects of monoamine reuptake inhibitors on distinct forms of impulsive behavior in rats, Psychopharmacology 219 (2) (2012) 313–326.
[53] K.C. Berridge, T.E. Robinson, What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res. Brain Res. Rev. 28 (3) (1998) 309–369.
[54] K. Blum, A.L.-C. Chen, E.R. Braverman, D.E. Comings, T.J.H. Chen, V. Arcuri, S. H. Blum, B.W. Downs, R.L. Waite, A. Notaro, J. Lubar, L. Williams, T.J. Prihoda, T. Palomo, M. Oscar-Berman, Attention-deficit-hyperactivity disorder and reward deficiency syndrome, Neuropsychiatr. Dis. Treat. 4 (5) (2008) 893–918.
[55] J.W. Buckholtz, M.T. Treadway, R.L. Cowan, N.D. Woodward, R. Li, M.S. Ansari, R.M. Baldwin, A.N. Schwartzman, E.S. Shelby, C.E. Smith, R.M. Kessler, D. H. Zald, Dopaminergic network differences in human impulsivity, Science 329 (5991) (2010), 532-532.
[56] J.M. Swanson, N.D. Volkow, Psychopharmacology: concepts and opinions about the use of stimulant medications, J. Child Psychol. Psych. 50 (1-2) (2009) 180–193.
[57] S.H. Kollins, J.S. March, Advances in the pharmacotherapy of Attention-Deficit/Hyperactivity disorder, Biol. Psych. 62 (9) (2007) 951–953.
[58] C. Vonder Haar, F.C. Lam, W.K. Adams, L.K. Riparip, S. Kaur, M. Muthukrishna, S. Rosi, C.A. Winstanley, Frontal Traumatic Brain Injury in Rats Causes Long-Lasting Impairments in Impulse Control That Are Differentially Sensitive to Pharmacotherapeutics and Associated with Chronic Neuroinflammation, ACS Chem. Neurosci. 7 (11) (2016) 1531–1542.
[59] W. Schultz, P. Dayan, P.R. Montague, A neural substrate of prediction and reward, Science 275 (5306) (1997) 1593.
[60] S.J. Fallon, A. Hampshire, R.A. Barker, A.M. Owen, Learning to be inflexible: enhanced attentional biases in Parkinson's disease, Cortex 82 (2016) 24–34.
[61] J.D. Cohen, D. Servan-Schreiber, A theory of dopamine function and its role in cognitive deficits in schizophrenia, Schizophr. Bull. 19 (1) (1993) 85–104.
[62] A.F.T. Arnsten, Catecholamine regulation of the prefrontal cortex, J. Psychopharmacol. 11 (2) (1997) 151–162.
[63] J. Zahrt, J.R. Taylor, R.G. Mathew, A.F.T. Arnsten, Supranormal Stimulation of D&sub&1&/sub& Dopamine Receptors in the Rodent Prefrontal Cortex Impairs Spatial Working Memory Performance, J. Neurosci. 17 (21) (1997) 8528.
[64] J.K. Seamans, S.B. Floresco, A.G. Phillips, D&sub&1&/sub& Receptor Modulation of Hippocampal–Prefrontal Cortical Circuits Integrating Spatial Memory with Executive Functions in the Rat, J. Neurosci. 18 (4) (1998) 1613.
[65] A.K. Wagner, J.E. Sokoloski, D. Ren, X. Chen, A.S. Khan, R.D. Zafonte, A. C. Michael, C.E. Dixon, Controlled cortical impact injury affects dopaminergic transmission in the rat striatum, J. Neurochem. 95 (2) (2005) 457–465.
[66] H.Q. Yan, A.E. Kline, X. Ma, Y. Li, C.E. Dixon, Traumatic brain injury reduces dopamine transporter protein expression in the rat frontal cortex, NeuroReport 13 (15) (2002).
[67] R. Shimada, K. Abe, R. Furutani, K. Kibayashi, Changes in dopamine transporter expression in the midbrain following traumatic brain injury: an immunohistochemical and in situ hybridization study in a mouse model, Neurol. Res. 36 (3) (2014) 239–246.
[68] A.K. Wagner, J.M. Scanlon, C.R. Becker, A.C. Ritter, C. Niyonkuru, C.E. Dixon, Y. P. Conley, J.C. Price, The influence of genetic variants on striatal dopamine transporter and D2 receptor binding after TBI, J. Cereb. Blood Flow Metab. 34 (8) (2014) 1328–1339.
[69] S.S. Shin, E.R. Bray, C.Q. Zhang, C.E. Dixon, Traumatic brain injury reduces striatal tyrosine hydroxylase activity and potassium-evoked dopamine release in rats, Brain Res. 1369 (2011) 208–215.
[70] Y.H. Chen, E.Y. Huang, T.T. Kuo, H.I. Ma, B.J. Hoffer, P.F. Tsui, J.J. Tsai, Y. C. Chou, Y.H. Chiang, Dopamine release impairment in striatum after different levels of cerebral cortical fluid percussion injury, Cell Transplant. 24 (10) (2015) 2113–2128.
[71] E.Y.-K. Huang, T.-H. Tsai, T.-T. Kuo, J. Tsai Jr., P.-F. Tsui, Y.-C. Chou, H.-I. Ma, Y.-H. Chiang, Y.-H. Chen, Remote effects on the striatal dopamine system after fluid percussion injury, Behav. Brain Res. 267 (2014) 156–172.
[72] J.M. Gulley, N.R. Zahniser, Rapid regulation of dopamine transporter function by substrates, blockers and presynaptic receptor ligands, Eur. J. Pharmacol. 479 (1) (2003) 139–152.
[73] Y.H. Chen, E.Y. Huang, T.T. Kuo, B.J. Hoffer, J. Miller, Y.C. Chou, Y.H. Chiang, Dopamine release in the nucleus accumbens is altered following traumatic brain injury, Neuroscience 348 (2017) 180–190.
[74] K. Hong, L. Hinck, M. Nishiyama, M.M. Poo, M. Tessier-Lavigne, E. Stein, A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion, Cell 97 (7) (1999) 927–941.
[75] J.H. Kim, D. Lavan, N. Chen, C. Flores, H. Cooper, A.J. Lawrence, Netrin-1 receptor-deficient mice show age-specific impairment in drug-induced locomotor hyperactivity but still self-administer methamphetamine, Psychopharmacology 230 (4) (2013) 607–616.
[76] D.E. Vosberg, M. Leyton, C. Flores, The Netrin-1/DCC guidance system: dopamine pathway maturation and psychiatric disorders emerging in adolescence, Mol. Psychiatry 25 (2) (2020) 297–307.
[77] R. Muramatsu, S. Nakahara, J. Ichikawa, K. Watanabe, N. Matsuki, R. Koyama, The ratio of 'deleted in colorectal cancer' to' uncoordinated-5A 'netrin-1 receptors on the growth cone regulates mossy fibre directionality, Brain 133 (1) (2009) 60–75.
[78] M. Picard, R.J. Petrie, J. Antoine-Bertrand, E. Saint-Cyr-Proulx, J.F. Villemure, N. Lamarche-Vane, Spatial and temporal activation of the small GTPases RhoA and Rac1 by the netrin-1 receptor UNC5a during neurite outgrowth, Cell. Signal. 21 (12) (2009) 1961–1973.
[79] M.T. Killeen, The dual role of the ligand UNC-6/Netrin in both axon guidance and synaptogenesis in C. elegans, Cell Adh. Migr. 3 (3) (2009) 268–271.
[80] S.E. Kwon, E.R. Chapman, Synaptophysin regulates the kinetics of synaptic vesicle endocytosis in central neurons, Neuron 70 (5) (2011) 847–854.
[81] Y. Liu, Y. Zhang, M. Hu, Y.H. Li, X.H. Cao, Carnosic acid alleviates brain injury through NF-κB-regulated inflammation and Caspase-3-associated apoptosis in high fat-induced mouse models, Mol. Med. Rep. 20 (1) (2019) 495–504.
[82] M.R. Bell, Comparing postnatal development of gonadal hormones and associated social behaviors in rats, mice, and humans, Endocrinology 159 (7) (2018) 2596–2613.
[83] E.M. Lewis, J.F. Barnett Jr., L. Freshwater, A.M. Hoberman, M.S. Christian, Sexual maturation data for Crl Sprague-Dawley rats: criteria and confounding factors, Drug Chem. Toxicol. 25 (4) (2002) 437–458.
[84] A. Raznahan, P.W. Shaw, J.P. Lerch, L.S. Clasen, D. Greenstein, R. Berman, J. Pipitone, M.M. Chakravarty, J.N. Giedd, Longitudinal four-dimensional mapping of subcortical anatomy in human development, Proc. Natl. Acad. Sci. U. S.A. 111 (4) (2014) 1592–1597.
[85] S.L. Andersen, M. Rutstein, J.M. Benzo, J.C. Hostetter, M.H. Teicher, Sex differences in dopamine receptor overproduction and elimination, Neuroreport 8 (6) (1997) 1495–1498.
[86] J. Willing, L.R. Cortes, J.M. Brodsky, T. Kim, J.M. Juraska, Innervation of the medial prefrontal cortex by tyrosine hydroxylase immunoreactive fibers during adolescence in male and female rats, Dev. Psychobiol. 59 (5) (2017) 583–589.
[87] T.W. Robbins, The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry, Psychopharmacology 163 (3-4) (2002) 362–380.
[88] E. Farace, W.M. Alves, Do women fare worse? A metaanalysis of gender differences in outcome after traumatic brain injury, Neurosurg. Focus 8 (1) (2000) e6.
[89] D. Ellemberg, S. Leclerc, S. Couture, C. Daigle, Prolonged neuropsychological impairments following a first concussion in female university soccer athletes, Clin. J. Sport Med. 17 (5) (2007) 369–374.
[90] A. Luchicchi, O. Mnie-Filali, H. Terra, B. Bruinsma, S.F. de Kloet, J. Obermayer, T. S. Heistek, R. de Haan, C.P.J. de Kock, K. Deisseroth, T. Pattij, H.D. Mansvelder, Sustained Attentional States Require Distinct Temporal Involvement of the Dorsal and Ventral Medial Prefrontal Cortex, Front. Neural Circuits 10 (2016), 70-70.
[91] T.A. Paine, L.E. Slipp, W.A. Carlezon Jr., Schizophrenia-like attentional deficits following blockade of prefrontal cortex GABAA receptors, Neuropsychopharmacology 36 (8) (2011) 1703–1713.
[92] Y. Chudasama, F. Passetti, S.E. Rhodes, D. Lopian, A. Desai, T.W. Robbins, Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity, Behav. Brain Res. 146 (1–2) (2003) 105–119.

[93] J. Willing, J.M. Juraska, The timing of neuronal loss across adolescence in the medial prefrontal cortex of male and female rats, Neuroscience 301 (2015) 268–275.
[94] M.E. Andrzejewski, T.L. Schochet, E.C. Feit, R. Harris, B.L. McKee, A.E. Kelley, A comparison of adult and adolescent rat behavior in operant learning, extinction, and behavioral inhibition paradigms, Behav. Neurosci. 125 (1) (2011) 93–105.
[95] W.A. Koss, A.D. Franklin, J.M. Juraska, Delayed alternation in adolescent and adult male and female rats, Dev. Psychobiol. 53 (7) (2011) 724–731.
[96] J.-P. Dollé, A. Jaye, S.A. Anderson, H. Ahmadzadeh, V.B. Shenoy, D.H. Smith, Newfound sex differences in axonal structure underlie differential outcomes from in vitro traumatic axonal injury, Exp. Neurol. 300 (2018) 121–134.
[97] R.L. Roof, E.D. Hall, Estrogen-Related Gender Difference in Survival Rate and Cortical Blood Flow After Impact-Acceleration Head Injury in Rats, J. Neurotrauma 17 (12) (2000) 1155–1169.
[98] T.R. Espinoza, D.W. Wright, The role of progesterone in traumatic brain injury, J. Head Trauma Rehabil. 26 (6) (2011) 497–499.
[99] D.G. Stein, Progesterone exerts neuroprotective effects after brain injury, Brain Res. Rev. 57 (2) (2008) 386–397.
[100] H.J. Schneider, I. Kreitschmann-Andermahr, E. Ghigo, G.K. Stalla, A. Agha, Hypothalamopituitary dysfunction following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a systematic review, Jama 298 (12) (2007) 1429–1438.
[101] J. Wagner, J.R. Dusick, D.L. McArthur, P. Cohan, C. Wang, R. Swerdloff, W. J. Boscardin, D.F. Kelly, Acute gonadotroph and somatotroph hormonal suppression after traumatic brain injury, J. Neurotrauma 27 (6) (2010) 1007–1019.
[102] P. Ranganathan, R.G. Kumar, K. Davis, E.H. McCullough, S.L. Berga, A.K. Wagner, Longitudinal sex and stress hormone profiles among reproductive age and post-menopausal women after severe TBI: a case series analysis, Brain Inj. 30 (4) (2016) 452–461.
[103] S.J. Lupien, F. Maheu, M. Tu, A. Fiocco, T.E. Schramek, The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition, Brain Cogn. 65 (3) (2007) 209–237.
[104] S. Ouanes, E. Castelao, S. Gebreab, A. von Gunten, M. Preisig, J. Popp, Life events, salivary cortisol, and cognitive performance in nondemented subjects: a population-based study, Neurobiol. Aging 51 (2017) 1–8.