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Juvenile stress exerts sex-independent effects on anxiety, antidepressant-like behaviours and dopaminergic innervation of the prelimbic cortex in adulthood and does not alter hippocampal neurogenesis

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ABSTRACT

Stress, particularly during childhood, is a major risk factor for the development of depression. Depression is twice as prevalent in women compared to men, which suggests that biological sex also contributes to depression susceptibility. However, the neurobiology underpinning sex differences in the long-term consequences of childhood stress remains unknown. Thus, the aim of this study was to determine whether stress applied during the prepubertal juvenile period (postnatal day 27–29) in rats induces sex-specific changes in anxiety-like behaviour, anhedonia, and antidepressant-like behaviour in adulthood in males and females. The impact of juvenile stress on two systems in the brain associated with these behaviours and that develop during the juvenile period, the mesocorticolimbic dopaminergic system and hippocampal neurogenesis, were also investigated. Juvenile stress altered escape-oriented behaviours in the forced swim test in both sexes, decreased latency to drink a palatable substance in a novel environment in the novelty-induced hypophagia test in both sexes, and decreased open field supported rearing behavior in females. These behavioural changes were accompanied by stress-induced increases in tyrosine hydroxylase immunoreactivity in the prefrontal cortex of both sexes, but not other regions of the mesocorticolimbic dopaminergic system. Juvenile stress did not impact anhedonia in adulthood as measured by the saccharin preference test and had no effect hippocampal neurogenesis across the longitudinal axis of the hippocampus. These results suggest that juvenile stress has long-lasting impacts on antidepressant-like and reward-seeking behaviour in adulthood and these changes may be due to alterations to catecholaminergic innervation of the medial prefrontal cortex.

1. Introduction

Stress, particularly during childhood, is a major risk factor for the development of psychiatric disorders including depression [1,2]. Preclinical research has also shown that stress during the prepubertal juvenile period, a developmental window similar to late childhood in humans, impacts anxiety and depressive-like behaviours and increases sensitivity to the adverse effects of adult stress [3,4]. These studies indicate that the juvenile period of development is a sensitive window during which stress may predispose individuals to develop psychiatric illness in later life. Stress-related psychiatric disorders are twice as prevalent in women compared to men [5,6]. However, until recently many studies in rodents have focused solely on males or did not include sex as a biological variable [7,8]. As a result, the neurobiological changes occurring in response to juvenile stress, particularly in the female brain, are incompletely understood.

Brain development during the juvenile period is marked by the maturation and substantial remodeling of various corticolimbic areas involved in regulating the stress response, emotional processing and novelty-seeking and reward, such as the medial prefrontal cortex

Abbreviations: BrdU, 5-bromo-2'-deoxyuridine; DCX, doublecortin; dHi, dorsal hippocampus; DMS, dorsomedial striatum; FST, forced swim test; GCL, granule cell layer; IL, infralimbic area; ML, molecular layer; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; NIH, novelty-induced hypophagia; OFT, open field test; PND, postnatal day; PrL, prelimbic area; SPT, saccharin preference test; TH-ir, tyrosine hydroxylase immunoreactivity.

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(mPFC), amygdala, and hippocampus [9]. The mPFC is also an important brain region regulating decision making and risk-taking behaviour which undergoes dramatic maturation during the prepubescent juvenile period, decreasing in overall size due to synapse pruning [10]. In parallel, this period is also very important for the development of mesocorticolimbic dopaminergic brain regions including the dorsal striatum and nucleus accumbens (NAc), both of which also play a role in decision making and goal-directed behaviours [11–13]. These rapid structural changes in the juvenile brain may bring about unique vulnerability, as these brain areas may be more susceptible to negative external factors like stress during their maturation [14].

The hippocampus is one of the main structures affected in psychiatric illness and is a key regulator of the stress response. Human neuroimaging studies report volumetric reductions in the hippocampus in the brains of adult patients with depression [15–17]. In parallel, studies in rats have shown that chronic stress in adulthood adversely affects hippocampal volume and neuronal morphology [18,19]. Adult hippocampal neurogenesis, the birth of new neurons in the dentate gyrus of the hippocampus, has been implicated in stress resilience and the mechanisms of antidepressant drugs [20–23]. Chronically high levels of the stress hormone corticosterone can reduce the proliferation and differentiation of new neurons in the adult rat hippocampus [24,25]. Sex hormones can also modulate hippocampal activity and stress has been shown to affect hippocampal neuronal plasticity in a sex-dependent manner [26,27]. Accumulating evidence suggests that the adult hippocampus is functionally segregated along its longitudinal axis into anterior and posterior regions in primates and analogous ventral (vHi) and dorsal region (dHi) regions in rodents, whereby the ventral hippocampus plays a predominant role in the regulation of anxiety while the dorsal hippocampus is predominantly involved in spatial learning and memory [28–32]. In parallel, it has been reported that chronic stress reduced adult neurogenesis preferentially in the vHi rather the dHi [21,23,33,34] and that neurons derived from the vHi are more sensitive to the deleterious effects of corticosterone than those derived from the dHi in vitro [35]. However, these investigations have largely been carried out in male animals only and have not focused on the impact of stress during the prepubescent juvenile period.

It is well established that hippocampal neurogenesis occurs at a much higher rates in the young brain [36], but less is known about the impact of stress on hippocampal neurogenesis during the juvenile period when the brain is particularly sensitive to stressful events [14]. The neuroendocrine stress response of juveniles is heightened and exaggerated compared to adults [37] due to immature hypothalamic pituitary adrenal axis feedback mechanisms [38]. Therefore, activation of the stress response in the juvenile period could potentially disrupt important neurodevelopmental processes like hippocampal neurogenesis and maturation of the medial prefrontal cortex resulting in long-term dysregulation of behaviours in later life. However, only a limited number of studies have examined the impact of juvenile stress, prior to pubertal onset, on anxiety and depressive-like behaviours in adulthood in both males and females, and little is known about the neurobiology underpinning potential sex differences in the long-term behavioural consequences of juvenile stress and whether they include alterations in mesocorticolimbic dopaminergic maturation and alterations in neurogenesis along the longitudinal axis of the hippocampus that persist to adulthood [39–41]. Thus, the aim of this study was to determine whether juvenile stress applied at postnatal days 27–29 induces sex-specific changes in anxiety-like behaviour, anhedonia, and antidepressant-like behaviour in adulthood in males and females and whether these changes were associated with alterations in neurogenesis along the longitudinal axis of the adult hippocampus and the innervation of mesocorticolimbic tyrosine-hydroxylase positive fibres.

2. Materials and methods

2.1. Animals

Rats were bred in the Biological Services Unit at University College Cork from mating pairs of adult Sprague-Dawley rats obtained from Envigo. Upon weaning, all offspring were housed in same-sex group housing under standard laboratory conditions (22 ± 2 °C, 12-h light/dark cycle with lights on at 07:00 a.m. and ad libitum access to food and water). Behavioural experiments took place during the light phase of the cycle (between 09:00 and 17:00). All experiments were conducted in accordance with international standards of animal welfare as outlined by European Directive 2010/63/EU and approved by the Animal Experimentation Ethics Committee of University College Cork. All experimenters had individual authorisations from the Health Products Regulatory Authority (HPRA) affiliated with HPRA Project Authorisation AE19130/P084.

2.2. BrdU injection and juvenile stress paradigm

The experimental design is summarised in Fig. 1A. On postnatal day (PND) 26, all rats were given a single intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdU, 150 mg/kg) to label newly generated cells. Male and female rats were then randomly assigned to stress or control groups (Control males n = 9, stress males n = 10, control females n = 9, stress females n = 10) and housed in same-sex, same-treatment groups of 3–4. No more than two littermates of the same sex were assigned to the same group. Stressed rats underwent a daily stressor on PND 27–29 [41]. On PND 27, rats were individually exposed to 10 min of swim stress in a cylindrical glass water tank (21-cm diameter) filled to 30-cm with water (22 ± 1 °C). On PND 28, rats were placed on an elevated platform (platform dimensions: 12 × 12 cm, 70 cm above floor level) for three 30-min trials separated by 60-min inter-trial intervals in the home cage. On PND 29, rats were restrained in a clear acrylic rodent restrainer for two hours. Control rats were left undisturbed during this period. Upon reaching adulthood (PND 66), all rats underwent a battery of behavioural tests (Fig. 1A).

2.3. Novelty-induced hypophagia (NIH) test

Novelty-induced hypophagia is a behavioural test of anxiety which measures hyponeophagia, the latency to approach palatable food in a novel environment, which has been adapted from a protocol previously used in mice [42,43]. All rats were singly housed for the duration of the test. In the habituation phase, each animal was presented with a highly palatable solution of diluted sweetened condensed milk (1:4 in water) in their home cage for 30 min per day for 3 days. On days 4 and 5 (test days), the rats were brought to a testing room to habituate for 90 min prior to testing. On the test days, each animal was presented with the milk solution during a 10-min trial and their latency to drink the milk was measured. The test on day 4 was conducted in their home cage in the testing room under low illumination (~50 lux); whereas on day 5, the rats were in a novel, anxiogenic context [a new cage with no bedding and bright illumination (~1000 lux)]. Following the test on day 5, all rats were returned to their original group housing. Two subjects (both control females) did not drink the milk within 10 min in either the familiar nor novel context and were therefore excluded from the analysis.

2.4. Saccharin preference test (SPT)

All rats were singly housed in a cage with two water bottles for the four days of the test. Following two days of habituation to single housing and the presence of two bottles in the cage, the contents of one bottle was replaced with a 0.01% saccharin solution [44]. The volume of water and saccharin solution in both bottles was recorded every 12 h for 48 h.

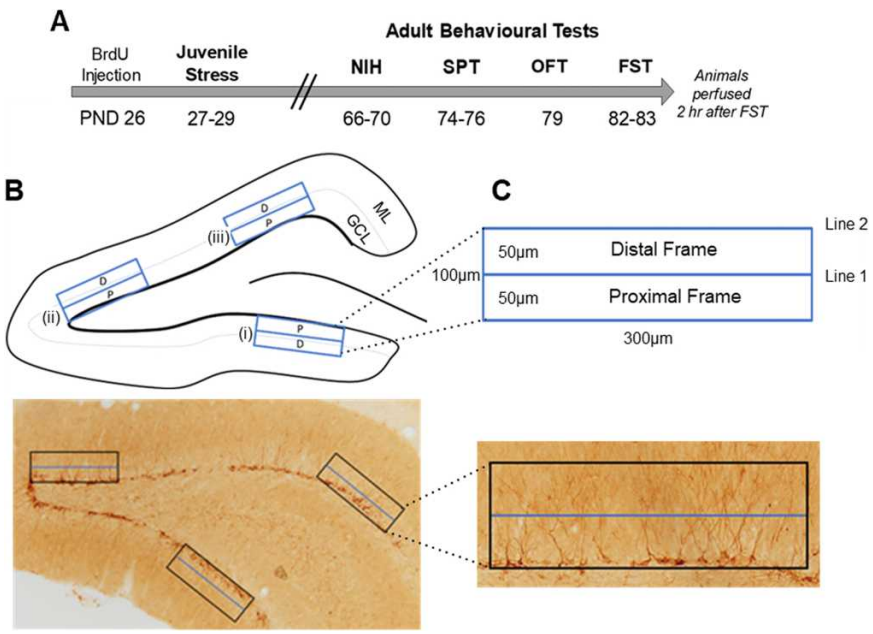


Fig. 1. Experimental design and doublecortin (DCX) imaging frames (A) Timeline of procedures and behaviours; (B) Three pairs of frames were overlaid onto the dentate gyrus of each hemisphere; one at the lateral end of the lower blade (i), one at the medial tip (ii) and one at the lateral end (iii) of the upper blade. The proximal frame encompassed the granule cell layer (GCL), and the distal frame encompassed the molecular layer (ML); (C) The dimensions of each frame. (P = proximal frame, D = distal frame). Abbreviations: Forced swim test (FST), novelty-induced hypophagia (NIH), open field test (OFT), postnatal day (PND), saccharin preference test (SPT).

The positions of the two bottles were switched after each measurement to reduce any confound produced by a side bias. Saccharin preference was calculated as a percentage of the volume of saccharin intake over the total volume of fluid intake and averaged over the second 24 h period. After the last measurement, the rats were returned to their original group housing.

2.5. Open field test (OFT)

Rats were brought to the testing room to habituate for one hour before the test. After habituation, rats were introduced to the brightly lit open field arena (90 cm diameter, 1000 lux) for 10 min. Rats were then removed and placed back into their home cage. The arena was cleaned between subjects with 70% ethanol to remove olfactory cues. Each test was video recorded, and the total distance travelled, number of entries into the centre, and time spent in the centre of the arena were scored using Ethovision XT 13. An observer blind to the experimental groups also scored rearing in the arena (when the animal briefly stands on hind limbs to explore an environment). Both supported (where the forelimbs touch the wall of the area) and unsupported rearing was measured. Behaviours were analysed for the entire 10-min duration (Fig. 3) as well as in 2 × 5-min time bins (Fig. S1).

2.6. Forced swim test (FST)

Rats were individually placed in a glass tank (21-cm diameter) filled to 30-cm mark with water (24 ± 1 °C) [45]. The forced swim test consisted of two sessions separated by 24 h: 15 min for the pre-swim and 5 min for the test swim. Following both swims each rat was gently towel-dried then returned to its home cage. The test was recorded by a video camera. An observer blind to experimental groups used the time-sampling technique to score the predominant behaviour (immobility, climbing, or swimming) for each 5-sec period of the 5-minute test (Fig. 4). Passive (immobility) and active (climbing and swimming) behaviours were defined as described by Slattery and Cryan [46] and developed by Detke et al. [47]. The observer also scored head shaking, a sexually dimorphic behaviour defined as a rapid side-to-side movement of head above water [48]. Males typically exhibit significantly more head shaking than females, a behaviour which is regulated by testosterone and sensitive to antidepressants [48–50]. Due to a video recording error during the trial, the behaviours of one control female could not be analysed. The 15-min pre-swim is not typically scored in the modified forced swim test, however, we scored behaviours during the first 5-min of the pre-swim (Fig. S2).

2.7. Perfusion and brain sectioning

On PND 83, two hours following the FST, the rats were deeply anaesthetised with an intraperitoneal injection of sodium pentobarbital and perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were removed, post-fixed in 4% PFA overnight, cryoprotected in 30% sucrose, then frozen on dry ice and stored at − 80 °C until sectioning. Brains were sectioned into 35 µm coronal sections using a Leica CM1950 cryostat and collected as free-floating sections in a series of eight and stored in cryoprotectant solution (30% ethylene glycol + 25% glycerol in PBS) at − 20 °C until immunohistochemistry.

2.8. Immunohistochemistry

The impact of juvenile stress on the survival of recently born hippocampal neurons was investigated using BrdU/NeuN immunohistochemistry as previously described [42,51,52]. Free-floating sections containing the entire rostrocaudal length of the hippocampus were washed in PBS (3 ×5 min) placed in 10 mM sodium citrate buffer (pH 6.0) at 80 °C for 20 min. Sections were washed in PBS and incubated in 2 N HCl in a water bath at 37 °C for 15 min followed by two washes in sodium tetraborate buffer (pH 8.5). Following PBS washes, sections were blocked in 10% donkey serum diluted in 0.3% Triton-X-100 in PBS (0.3% PBS-T) for 60 min at room temperature. Sections were incubated in the first primary antibody (rat anti-BrdU; Abcam, AB6326, 1:100) at 4 °C overnight. All antibodies were diluted in 2% donkey serum in 0.3% PBS-T. Sections were washed in PBS and incubated in secondary antibody (Alexa Fluor 594-conjugated donkey anti-rat, Invitrogen, A21209, 1:200) at room temperature for 90 min. Following PBS washes, sections were incubated in the second primary antibody (anti-mouse NeuN, Merck, MAB377, 1:100) at 4 °C overnight. Sections were washed and incubated in secondary antibody (Alexa Fluor 488-conjugated donkey anti-mouse, Invitrogen, A21202, 1:200) at room temperature for 90 min. Sections were washed in PBS and mounted onto Superfrost Plus slides and cover-slipped using Dako fluorescent mounting media. The slides were sealed and stored in the dark at 4 °C until imaging.

Sections were stained for doublecortin (DCX), a marker of immature

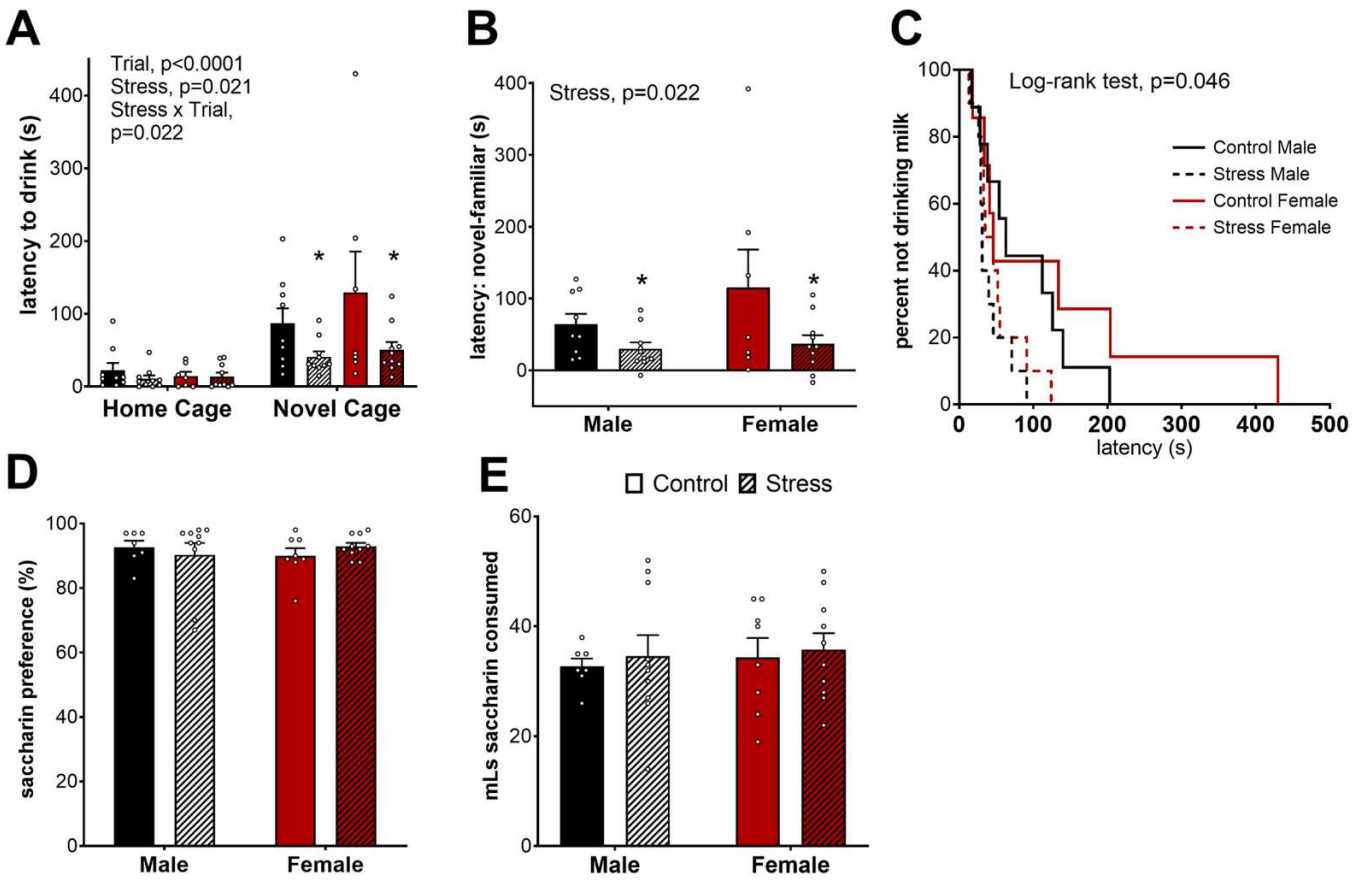


Fig. 2. Juvenile stress decreased latency to drink in females in the novelty induced hypophagia test and did not impact saccharin preference (A) Latencies to drink sweetened milk in the home cage and novel cage (B) Net latency to drink sweetened milk in [novel cage – familiar cage] (C) Kaplan-Meier survival curve of latency to drink in novel cage (D) Preference for a saccharin solution (saccharin intake / total volume of fluid intake x 100%) (E) Millilitres of saccharin consumed. n = 7–10 per group. Significant main effect of stress: * $p < 0.05$,.

neurons, to assess the impact of juvenile stress on the production of new hippocampal neurons in adulthood and on their morphology. Sections were washed in a 1% hydrogen peroxide in methanol solution for 10 min at room temperature to block endogenous peroxidase activity, washed in PBS (3 × 10 mins), and then blocked in 10% rabbit serum in 0.3% PBS-T for 2 h at room temperature. Sections were incubated in polyclonal goat anti-DCX primary antibody (Santa Cruz; SC-8066, 1:100) diluted in 5% rabbit serum in 0.3% PBS-T at 4 °C for 48 h. Sections were washed in PBS and incubated with rabbit anti-goat IgG biotinylated secondary antibody (Vector Labs; PK6105 1:200) diluted in 2% rabbit serum in PBS-T. Following PBS washes, sections were incubated in horseradish peroxidase-streptavidin-ABC complex (Vector labs; PK6105) for 2 h at room temperature. The biotin signal was amplified using an avidin-biotin complex (ABC; Vector Labs, PK6105) for 2 h and developed in 3,3′ diaminobenzidine activated by 0.3% hydrogen peroxide (DAB substrate kit, Vector, SK-4100). Sections were mounted on charged slides, dehydrated, and coverslipped with DPX mounting medium.

Sections were stained for tyrosine hydroxylase (TH), the rate-limiting enzyme of dopamine synthesis to quantify the impact of juvenile stress on dopaminergic fibres within the mPFC and its associated subcortical areas, the NAc and dorsomedial striatum (DMS) [53,54]. The protocol for immunohistochemistry was the same as above except that horse serum was used for blocking, the primary antibody was mouse anti-TH (MAB318, 1:500) and the secondary antibody was biotinylated horse anti-mouse (Vector Labs, BA 2000, 1:200).

2.9. Microscopy and image analysis

The DG in each section was imaged using an Olympus BX53 Upright Research Microscope at 100 X magnification (BrdU/NeuN) or 200 X magnification (DCX). The number of BrdU+ and NeuN+ /BrdU+ cells in the granule cell layer and subgranular zone was counted using ImageJ. The dHi was defined as AP: Bregma − 1.8 to − 5.2 and vHi as AP: − 5.2 to − 6.7 [24,51]. On average, 12–14 sections were analysed per brain: approximately 75% dorsal and 25% ventral sections. It should be noted that when the rat brain is sectioned coronally, the most rostral sections primarily contain only dHi, while the more caudal sections that contain vHi can also include parts of the dorsal and intermediate hippocampus [55,56].

The number of DCX-positive cells and their dendritic development was measured in the DG using ImageJ and counting frames (adapted from [57] and others [58,59]). Counting frames were applied at the medial and lateral ends of the upper blade and the lateral end of the lower blade of the DG. The counting frames were designed such that each pair of distal/proximal frames were aligned on top of the other with the proximal frame encompassing the GCL and the distal frame encompassing the ML (Fig. 1B). Each of these frames measured 50 μm x 300 μm. To determine the number of DCX-positive cells, the number of DCX+ cell bodies present in the proximal frame were counted. As new neurons mature, their morphology becomes more complex, therefore the morphological complexity of these DCX+ new neurons was then quantified by counting the number of dendrites that crossed from the proximal to distal frame (line 1, proximal dendritic crossing) and the number of dendrites that emerged from the distal frame (line 2, distal dendritic crossing; Fig. 1C).

For analysis of tyrosine hydroxylase immunoreactivity (TH-ir), four sections containing the DMS (AP: Bregma 1.7–0.48 mm) and three sections containing the NAc core and shell were imaged at 2X magnification

(AP: Bregma 1.6–1.0 mm). Two sections containing the mPFC were imaged at 20X magnification (AP: Bregma 3.2–2.7 mm). For the mPFC, three fields of view were imaged per hemisphere encompassing the PrL and IL were analysed for pixel density of TH-positive fibres. Image J was used to quantify the TH-ir using optical density (OD) calculated from background-subtracted mean grey values for sections containing DMS and NAc core and shell. The obtained OD values were then averaged across the 4 DMS sections, and across the 3 NAc sections. Optical density measurements take into account gradation of signal as well as the background staining on the section. This method is preferable when there is diffuse staining and individual fibres cannot be distinguished, as is the case for DMS and NAc. In the mPFC, TH-ir in individual fibres can be readily distinguished and thus pixel density measurements were used for this brain area, as described by others [60]. The layers of the cortex were analysed separately based on their morphology. Layer 1 contains largely vertically oriented fibres, whereas layer 2/3 has lateral oriented fibres, and layer 5/6 contains fibres oriented in several directions [61, 62]. Each field of view was converted to a binary image, and regions of interest were placed over each layer to determine the density of black pixels in each cortical layer and this was averaged across the two mPFC sections of each brain.

2.10. Statistical analysis

Data were analysed by two-way ANOVA with significance set at $\alpha = 0.05$. All data are shown as mean + standard error of the mean with individual data points except for the Kaplan-Meier survival curve analysis and subsequent Mantel–Cox log-rank test performed on novelty induced hypophagia data. A three-way ANOVA with repeated measures was used to analyse familiar versus novel cage latency in the novelty-induced hypophagia test (with trial as the within-subjects variable) as well as TH-ir in the mPFC (with brain region as the within-subjects variable). *Post hoc* comparisons used Fisher's LSD tests when interactions were significant. A *priori* comparisons used Bonferroni's multiple comparisons tests. Statistical analysis was conducted using GraphPad Prism 8.

3. Results

3.1. Juvenile stress decreased latency to drink in the novelty-induced hypophagia test

In the novelty-induced hypophagia test, a three-way ANOVA with repeated measures (Fig. 2A) revealed a main effect of trial (home cage versus novel cage) [$F(1,32) = 27.8$, $p < 0.0001$] as well as a main effect of juvenile stress on latency to drink the sweetened milk [$F(1,32) = 5.84$, $p < 0.05$]. Additionally, there was an interaction between trial and juvenile stress [$F(1,32) = 4.81$, $p < 0.05$; Fig. 2A]. Separate two-way ANOVAs for the home cage and novel cage latencies revealed an effect of juvenile stress in the novel cage [$F(1,32) = 6.12$, $p < 0.05$] but not the home cage [$F(1,32) = 0.86$, $p = 0.36$] Furthermore, two-way ANOVA revealed a significant main effect of juvenile stress on the net latency (novel cage latency - home cage latency) to drink [$F(1,32) = 5.79$, $p < 0.05$; Fig. 2B]. There was no effect of sex [$F(1,32) = 1.53$; $p = 0.23$] nor a stress x sex interaction [$F(1,32) = 0.90$; $p = 0.35$]. Kaplan-Meier survival curve analysis of latency to drink in the anxiogenic context was also performed. A Mantel–Cox log-rank test showed that these latency curves were significantly different from each other [χ^2

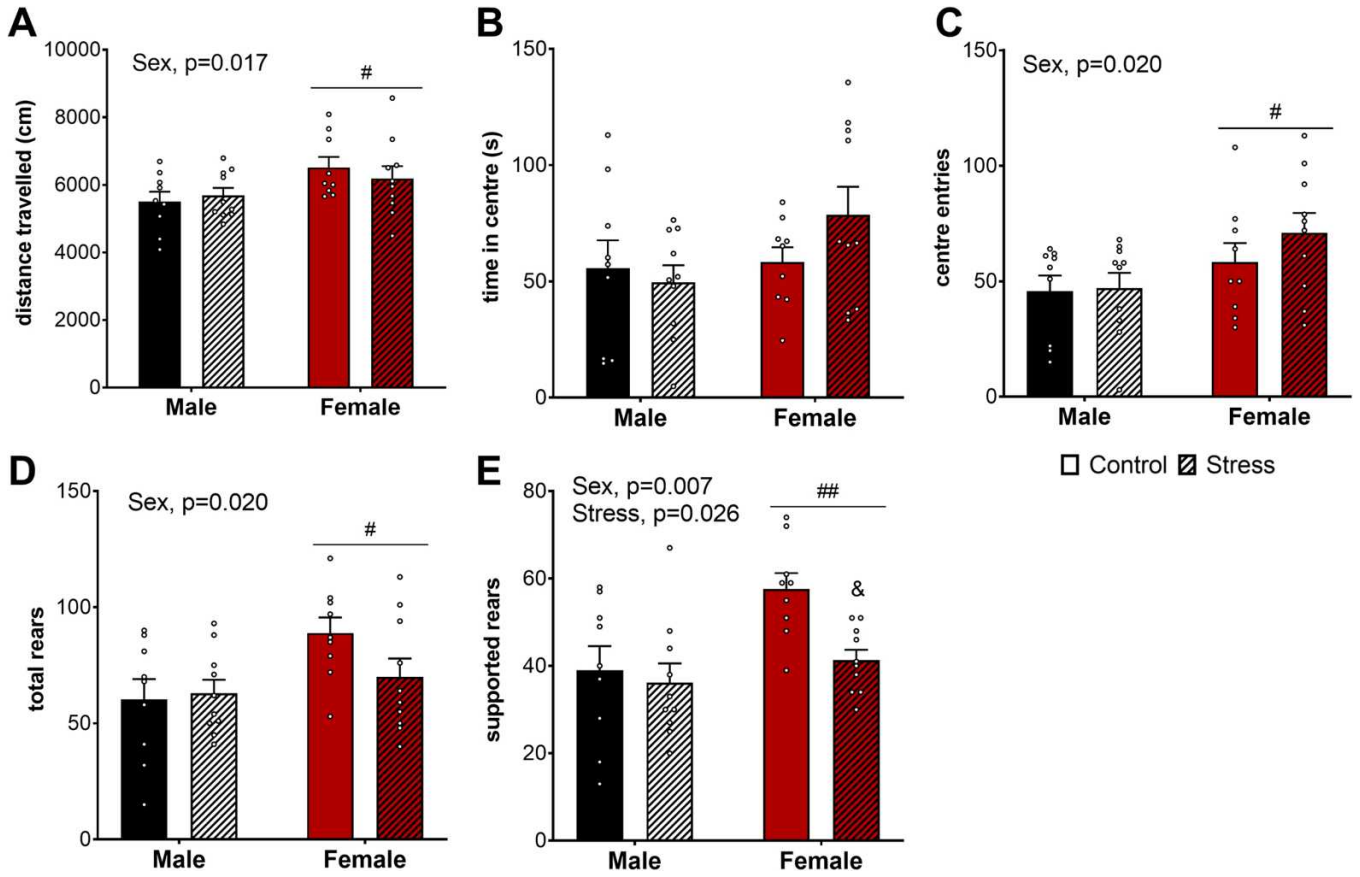


Fig. 3. Effects of juvenile stress on locomotor activity and exploratory activity in the open field test (A) Total distance travelled in open field (cm) (B) time spent in the centre of the arena (s), (C) number of entries into the centre of the arena, (D) total number of rearing behaviours, (E) number of supported rearing behaviours. Significant main effect of sex: # $p < 0.05$, ## $p < 0.01$. Significant Bonferroni's multiple comparisons test of stressed females versus non-stressed female: & $p < 0.05$. n = 9–10.

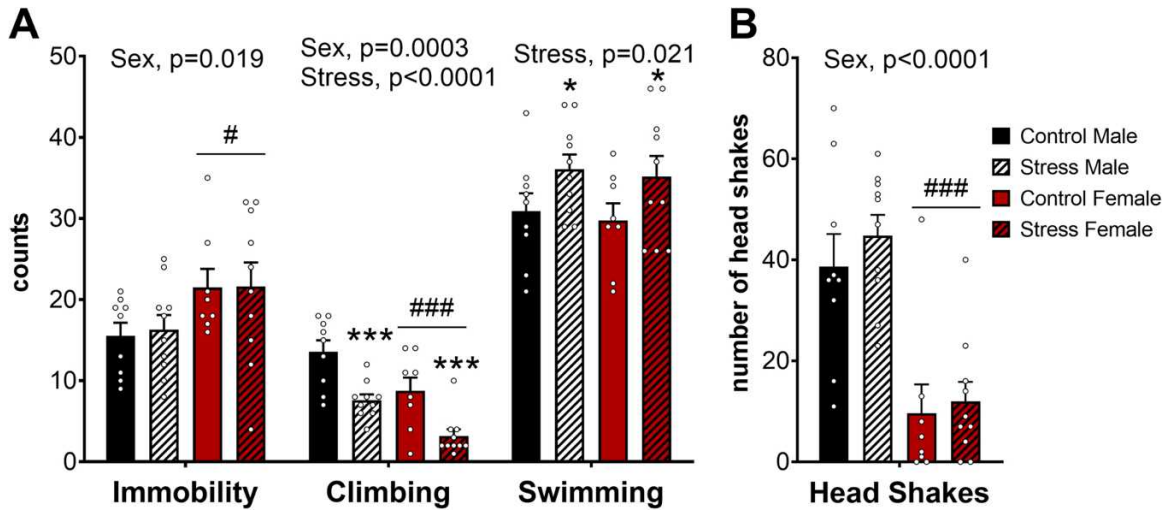


Fig. 4. Juvenile stress decreased climbing behaviour and increased swimming behaviour in the forced swim test (A) Counts of immobility, climbing, and swimming behaviours during the 5-min forced swim test (B) Number of head shake behaviours. n = 8–10 per group. Significant main effect of stress: ** $p < 0.01$, *** $p < 0.001$; Significant main effect of sex: # $p < 0.05$, ### $p < 0.001$.

(3, n = 36) = 7.99, $p < 0.05$; Fig. 2C].

3.2. Juvenile stress did not impact anhedonia in either sex as measured in the saccharin preference test

Preference to drink a saccharin solution over water was not affected by biological sex, juvenile stress, nor their interaction [Sex: F(1,31) = 0.0005, p = 0.98; Stress: F(1,31) = 0.001, p = 0.93; Sex x Stress: F (1,31) = 1.03; p = 0.32, Fig. 2D]. Likewise, the amount of saccharin solution consumed did not differ between any groups [Sex: F(1,31) = 0.19, p = 0.67; Stress: F(1,31) = 0.25, p = 0.62; Sex x stress: F(1,31) = 0.002, p = 0.95; Fig. 2E].

3.3. Increased supported rearing in the open field by females was attenuated by juvenile stress

Females were more active in the open field test (Fig. 3) as measured by overall distance travelled [F(1,34) = 6.29, $p < 0.05$; Fig. 3A] but there was no significant effect of stress [F(1,34) = 0.06; p = 0.81] nor a sex x stress interaction [F(1,34) = 0.72; p = 0.41]. There was no effect of biological sex [F(1,34) = 2.59, p = 0.12], stress [F(1,34) = 0.52; p = 0.48] nor a significant stress x sex interaction on time spent in the centre [F(1,34) = 1.82; p = 0.18]. The number of entries into the centre of the arena was higher in females compared to males [F(1,34)= 5.73, p = 0.022] but was not affected by stress [F(1,34) = 0.85, p = 0.36] nor the interaction of stress x sex [F(1,34) = 0.57, p = 0.46; Fig. 3C].

The total number of rears was greater in females compared to males [Sex: F(1,34) = 5.928, $p < 0.02$; Fig. 3D]. However, there was no effect of stress nor a stress x sex interaction [F(1,34) = 1.23, p = 0.27; F (1,34) = 2.12, p = 0.15, respectively]. Females exhibited increased supported rears [Sex effect: F(1,34) = 8.40, $p < 0.01$] and juvenile stress decreased the number of supported rears [F(1,34) = 5.45, $p < 0.05$]. There was no significant stress x sex interaction [F(1,34) = 2.72, p = 0.11; Fig. 3E]. Based on a previous report, we anticipated that juvenile stress would impact rearing behaviours [63]. Thus, planned comparisons were conducted and revealed that revealed that juvenile stress decreased the number of supported rears specifically in females (Bonferroni's multiple comparisons test, $p < 0.05$) but not in males ($p > 0.05$).

3.4. Juvenile stress decreased climbing and increased swimming behaviour in the forced swim test irrespective of biological sex

Immobility in the forced swim test (Fig. 4A) was higher in females [Sex: F(1,33) = 6.1 $p < 0.02$] than males. There were no effects of stress [F(1,33) = 0.03, p = 0.85], nor a stress x sex interaction [F(1,33) = 0.02, p = 0.88] on immobility. We observed that exposure to juvenile stress altered the escape-oriented behavioural strategies used by rats in the forced swim test. Juvenile stress significantly decreased climbing in both sexes [Stress: F(1,33) = 25.0, $p < 0.0001$] and correspondingly increased in swimming behaviour in both sexes [Stress: F(1,33) = 5.9, $p < 0.03$]. There was a sex difference in climbing behaviour but not in swimming behaviour whereby counts of climbing behaviour were lower in females than males [Sex: F(1,33) = 16.0, $p < 0.001$], while there was no sex difference in swimming behaviour [Sex: F(1,33) = 0.22, p = 0.65]. There were no significant stress x sex interactions in either climbing [(F(1,33)= 0.03, p = 0.86] or swimming [F(1,33) = 0.003, p = 0.96] behaviours. Finally, males exhibited more head shakes (Fig. 4B) during the FST than females [Sex: F(1,33) = 37.7, $p < 0.0001$] but juvenile stress had no effect on head shakes [F(1,33) = 0.72, p = 0.40] and there was no stress x sex interaction [F(1,33) = 0.14, p = 0.71].

Similar to the 5-min FST, during the first 5-min of the 15-min pre-swim session (Figure S2, Supplementary material), juvenile stress altered escape-oriented behavioural strategies in a sex-independent manner. Like the 5-min test, juvenile stress decreased climbing behaviour in both sexes in the pre-swim session. In contrast to the 5-min FST, however, juvenile stress increased immobility and had no effect on swimming behaviour in the pre-swim. In both swim sessions there was no effect of juvenile stress on headshakes, and the same sex difference in headshakes was observed in both swims. Interestingly, sex differences in immobility and climbing behaviour were only apparent in the 5-min test and not the pre-swim.

3.5. Juvenile stress increased TH-ir in layer 1 of the prelimbic cortex and had no significant effect on TH in the infralimbic cortex, dorsomedial striatum, or nucleus accumbens

There were no effects of biological sex or juvenile stress on TH-ir in the dorsomedial striatum [Sex: F(1,19) = 2.3, p = 0.14; Stress: F (1,19) = 1.58, p = 0.22; Interaction: F(1,19) = 0.23, p = 0.64; Fig. 5F]. Likewise, biological sex and juvenile stress did not affect TH-ir in the

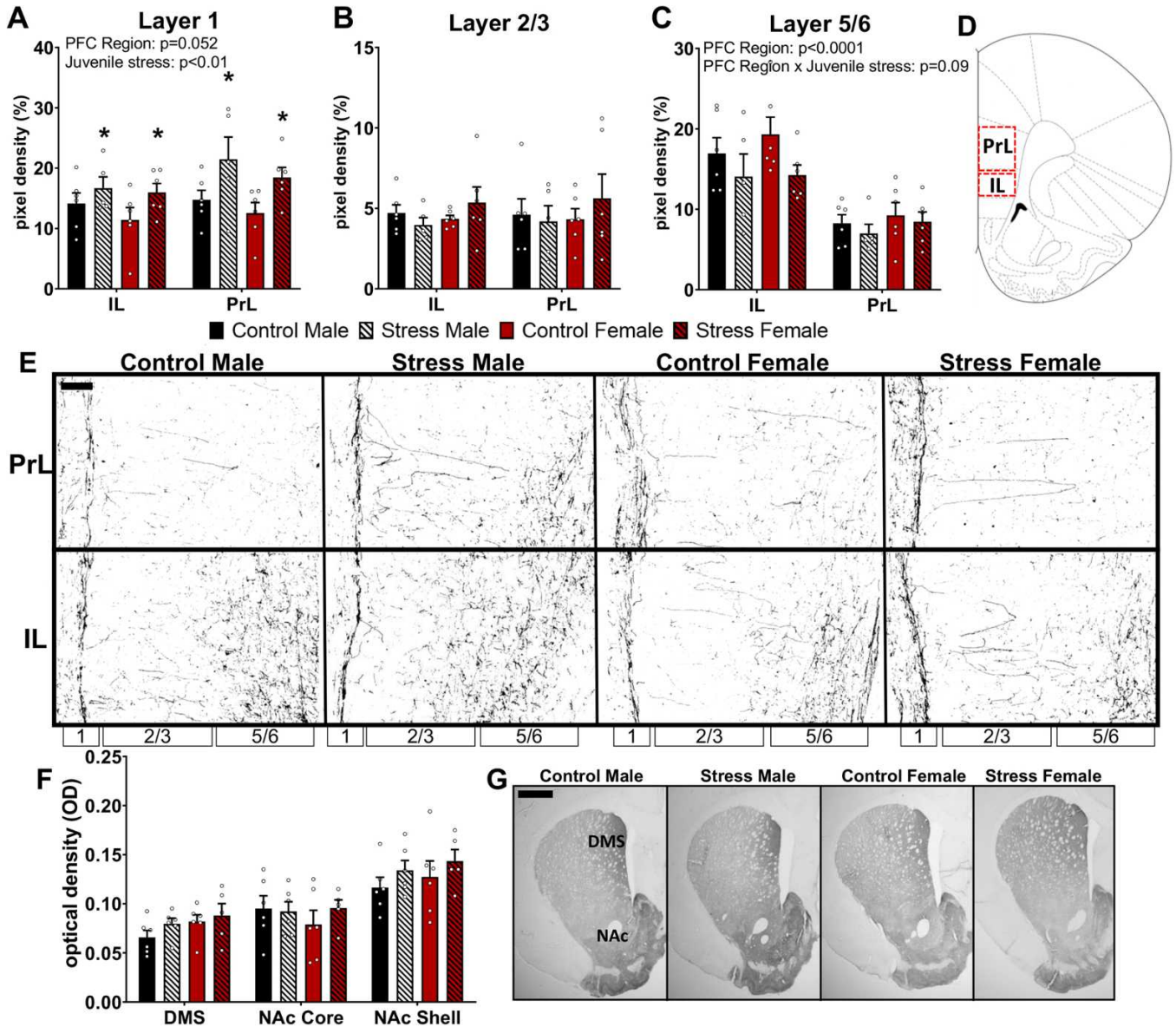


Fig. 5. Juvenile stress increased TH-ir in layer I of the prelimbic cortex and did not affect TH in the dorsomedial striatum or nucleus accumbens A) Pixel density of mPFC TH fibres in layer 1; B) layer 2/3; C) layer 5/6; D) Schematic drawing of coronal brain section containing mPFC, boxes indicate fields of view imaged for PrL and IL (adapted from [112]); E) Representative binary images of PrL and IL mPFC, numbers below correspond to cortical layers, scale bar = 100 μm; F) Average optical density values for DMS and NAc; B) Representative images containing DMS and NAc, scale bar = 1 mm. Significant main effect of stress: * $p < 0.05$. n = 5–6. Abbreviations: dorsomedial striatum (DMS), nucleus accumbens (NAc), prelimbic (PrL), infralimbic (IL), medial prefrontal cortex (mPFC).

nucleus accumbens core [Sex: $F(1,19) = 0.29$, $p = 0.59$; Stress: $F(1,19) = 0.33$, $p = 0.57$; Interaction: $F(1,19) = 0.66$, $p = 0.43$] or nucleus accumbens shell [Sex: $F(1,19) = 0.65$ $p = 0.42$; Stress: $F(1,19) = 1.8$, $p = 0.20$; Interaction: $F(1,19) = 0.003$, $p = 0.96$ Fig. 5F].

We analysed the density of TH-ir pixels within the different layers of two subregions of the mPFC, the prelimbic area (PrL) and infralimbic area (IL). Within each layer, the density of black pixels was analysed separately by three-way ANOVA (Biological sex x juvenile stress x mPFC area). In layer 1, there was a trend for mPFC area to affect TH-ir [$F(1,19) = 4.3$, $p = 0.052$]. There was also a main effect of juvenile stress on TH-ir [$F(1,19) = 8.6$, $p = 0.0085$; Fig. 5 A, 5E] whereby stressed males and stressed females both had higher density of TH-ir than the corresponding control groups. There was no main effect of biological sex [$F(1,19) = 1.6$, $p = 0.21$], nor any significant interactions between other variables.

In layer 2/3, there were no effects of juvenile stress [$F(1,19) = 0.13$, $p = 0.7$], biological sex [$F(1,19) = 0.45$, $p = 0.5$], mPFC region [$F(1,19) = 0.06$, $p = 0.8$], nor any of their interactions (Figs. 5B and E). In layer 5/6 (Fig. 5 C and E), there was a main effect of mPFC region [$F(1,19) = 89.8$, $p < 0.0001$] where TH-ir pixel density was higher in the IL compared to the PrL (Fig. 5E). We also found a trend towards an interaction effect between mPFC region and juvenile stress [$F(1,19) = 3.1$, $p = 0.093$], but this did not reach significance. There were no other significant main effects or interactions [Sex: $F(1,19) = 0.7$, $p = 0.4$; Stress: $F(1,19) = 2.8$, $p = 0.11$].

3.6. The survival of juvenile-born hippocampal neurons into adulthood was not impacted by juvenile stress or biological sex

The number of surviving BrdU+ /NeuN+ cells per section within the whole DG of the adult hippocampus (Fig. 6A) was not affected by biological sex [$F(1,19) = 0.25$, $p = 0.61$], juvenile stress [$F(1,19) = 0.27$, $p = 0.62$], nor the interaction of biological sex x stress [$F(1,19) = 0.05$, $p = 0.83$]. Likewise, when the dHi and vHi were considered separately,

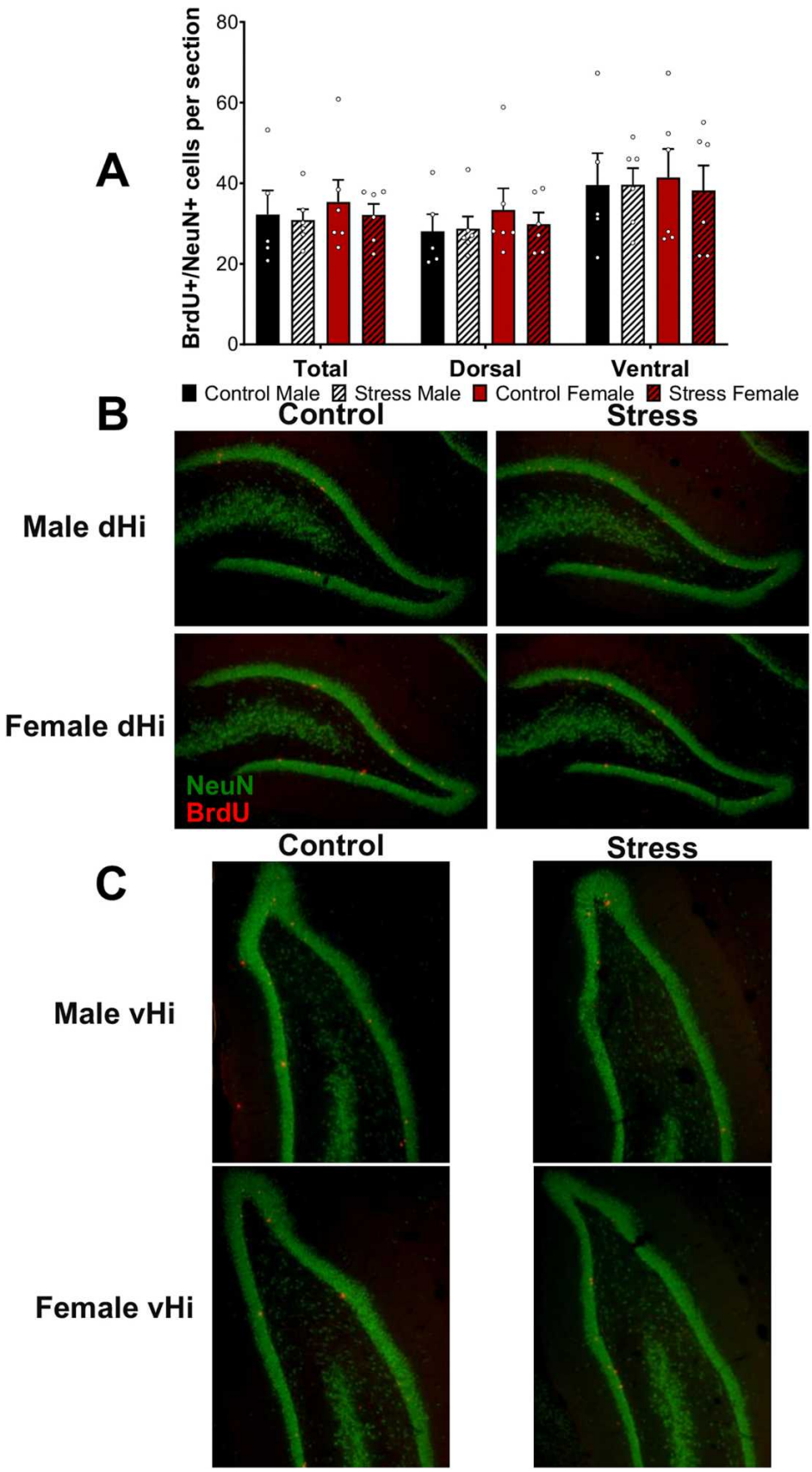


Fig. 6. Juvenile stress did not impact the survival of newly hippocampal neurons into adulthood (A) The number of surviving BrdU+ /NeuN+ cells per section in the total dentate gyrus, dorsal hippocampus (dHi), and ventral hippocampus (vHi). Representative images from dHi (B) and vHi (C). NeuN+ cell bodies = green, BrdU+ nuclei = red. Scale bar = 200 μm. n = 6.(For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

we found no statistically significant effects. [*dHi*: Sex: F(1,19) = 0.64, p = 0.43; Stress: F(1,19) = 0.13, p = 0.72; Sex x stress: F(1,19) = 0.27, p = 0.61; Fig. 6B] [*vHi*: Sex: F(1,19) = 0.001, p = 0.96 Stress: F (1,19) = 0.06, p = 0.79; Sex x stress: F(1,19) = 0.07, p = 0.81; Fig. 6C].

3.7. The number of adult-born immature hippocampal neurons was lower in females than males but was not affected by juvenile stress in either sex

There was a significant effect of biological sex whereby females had fewer immature (DCX+) new neurons in adulthood compared to males [F(1,20)= 5.412, p = 0.0306; Fig. 7A]. Juvenile stress did not affect the number of DCX+ neurons [F(1,20) = 0.18, p = 0.68] nor was there an interaction of stress x biological sex [F(1,20)= 0.3017, p = 0.59] on the number of immature neurons in the whole hippocampus.

Upon segregation along the longitudinal axis of the hippocampus, there was no significant effect of juvenile stress nor sex x stress interaction in either the dHi or vHi. [*dHi*: Stress: F(1,20) = 0.35, p = 0.56; Sex x Stress: F(1,20) = 0.68, p = 0.42] [*vHi*: Stress: F(1,20) = 0.005, p = 0.95; Sex x stress: F(1,20) = 0.46, p = 0.51; Fig. 7A]. In both the dHi and vHi, there was a trend for an effect of sex where females had fewer DCX+ cells than males, but this did not reach statistical significance [*dHi*: Sex: F(1,20) = 3.6, p = 0.077] [*vHi*: Sex: F(1,20) = 3.52, p = 0.079].

3.8. The average number of dendritic crossings per adult-born immature neuron was lower in females than males and was not affected by prior juvenile stress in either sex

As a measure of dendritic complexity and morphological maturation of newly born neurons, we quantified the average number of dendrites per DCX+ neuron which crossed into the GCL (proximal dendritic crossings) or into the ML (distal dendritic crossings, see Fig. 1). In the whole hippocampus, the number of proximal dendritic crossings per DCX+ neuron was significantly lower in females compared to males [Sex: F(1,20) = 4.58, p = 0.045]. When dHi and vHi were considered separately, the significant effect of biological sex persisted in the dHi [Sex: F(1,20) = 4.58, p = 0.045], but did not reach statistical significance in the vHi [Sex: F(1,20) = 3.58, p = 0.073]. There was no significant effect of stress nor a stress x biological sex interaction on proximal dendritic crossings per DCX+ neuron in the total hippocampus, dHi, or vHi [*Total:* stress: F(1,20) = 0.006, p = 0.94; stress x biological sex: F(1,20) = 0.26, p = 0.62] [*dHi:* Stress: F(1,20) = 0.06, p = 0.80; Stress x sex: F(1,20) = 0.0003, p = 0.98] [*vHi:* Stress: F (1,20) = 0.55, p = 0.47; Stress x sex: F(1,20)= 1.0, p = 0.32, (Fig. 7B)].

The number of distal dendritic crossings per neuron in the total hippocampus was also lower in females than males [Sex: F(1,20) = 4.855, p = 0.040; Fig. 7C]. Upon segregation of the longitudinal axis of the hippocampus, although there was a trend towards a sex effect in both dHi and vHi, but these did not quite reach statistical significance [*dHi*: F

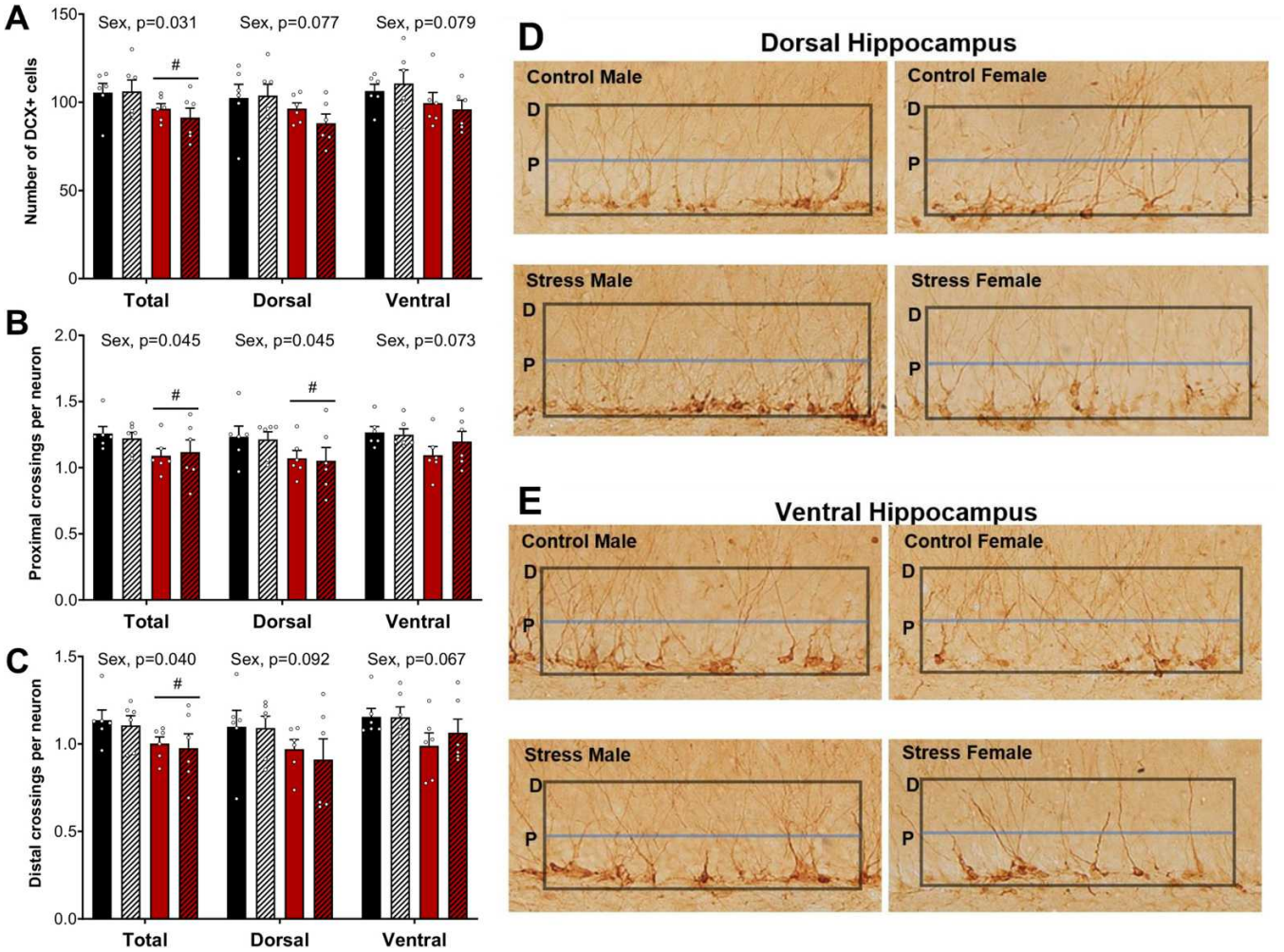


Fig. 7. Adult females had fewer immature neurons and fewer dendritic crossings than adult males A) The number of DCX+ cells in the total dentate gyrus (DG), dHi, and vHi. The number of proximal dendritic crossings (B) or distal dendritic crossings (C) per DCX+ neuron in the total DG, dHi, and vHi. Representative frames from dorsal (D) and ventral (E) DG. Significant main effect of sex: # $p < 0.05$. Frame dimensions = 300 × 100 μm. (P = Proximal frame, D=distal frame). n = 6.

(1,20) = 3.12, p = 0.092] [*vHi:* F(1,20)= 3.77, p = 0.067]. There was no significant effect of stress nor a stress x biological sex interaction on distal dendritic crossings per neuron in the total, dorsal or ventral hippocampus. [*Total*: Stress: F(1,20) = 0.22, p = 0.64; Stress x sex: F(1,20) = 0.008, p = 0.98] [*dHi:* Stress: F(1,20) = 0.14, p = 0.70; Stress x sex: F(1,20) = 0.09, p = 0.76] [*vHi:* Stress: F(1,20) = 0.31, p = 0.58; Stress x sex: F(1,20) = 0.35, p = 0.56].

4. Discussion

The aim of this study was to determine whether juvenile stress induces sex-specific changes in anxiety-like behaviour, anhedonia, and antidepressant-like behaviour in adulthood and whether these changes were associated with alterations in adult hippocampal neurogenesis and tyrosine hydroxylase expressing fibres in mesocorticolimbic structures. We found that short-term juvenile stress from PND 27–29 altered escape-oriented behaviours in the FST regardless of sex, decreased latency to drink in the novel environment in the NIH test, and decreased open field exploratory supported rearing behaviour specifically in females. Juvenile stress increased TH-ir in the mPFC of both sexes specifically in layer 1 and did not affect TH-ir in the DMS or NAc. However, juvenile stress did not impact anhedonia in adulthood as measured by the saccharin preference test and had no effect on the survival of juvenile-born hippocampal neurons into adulthood or on the number of adult-born immature neurons in the hippocampus. Lastly, sex differences were observed under baseline conditions in the open field and forced swim test and in the number of adult-born immature neurons in the hippocampus.

4.1. Exploratory behaviours and anhedonia

In the open field test, we observed a significant effect of biological sex on locomotor activity and exploratory behaviours, whereby females were more active and exploratory than males, a finding supported by previous studies [64–67]. A sex-specific effect of juvenile stress on supported rearing behaviours in the open field was also observed whereby juvenile stress decreased supported rears in females but not males. In a similar study, male rats exposed to short-term juvenile stress from PND 27–33 exhibited less rearing behaviour but unlike our study this was in a dimly lit open field and females were not examined [63].

We found no main effect of juvenile stress on either adult anxiety-like behaviour (as measured by time/entries in the open field centre) or on locomotor activity in the open field. This result is in agreement with several studies that report no effect of juvenile stress on anxiety-like behaviour nor locomotor activity in the open field in adulthood in male and female mice [39] and rats [68–70]. In contrast, some studies have reported that short-term juvenile stress (for 3–4 days prior to PND 33) decreased locomotor activity in the open field in adult rats [63,71–73] and that this occurred specifically in adult male rats but not females [74]. A subset of these studies additionally report that juvenile stress increased anxiety-like behaviour as measured by the number of crosses in the centre of the open field in male rats (females not studied) [73] and in both male and female rats [74]. Various factors may impact the discrepancies between these studies such as time during light cycle, time since stressor cessation, rodent species/strain, and lighting conditions during the test. The above-mentioned studies that report an effect of juvenile stress on open field behaviour were conducted in a dimly lit arena, whereas the open field test in the current study was conducted in an anxiogenic, brightly lit arena, which would typically result in lower locomotor activity and less time spent in the centre overall.

One of the hallmarks of depression in humans is anhedonia, the loss of pleasure in normally rewarding stimuli [75]. The saccharin preference test is designed to quantify a loss of preference for a sweet solution as a behavioural read-out for anhedonia. We found no effect of juvenile stress on saccharin preference as measured over 48 h in adult males and females. In contrast, a study using the same stress paradigm showed a female-specific decrease in saccharin preference in adults exposed to juvenile stress [41]. However, in that study, saccharin preference was assessed during a short, 5-min test following 30-min water restriction with a much higher concentration of saccharin (0.606%) which might explain our conflicting findings. However, in a somewhat similar testing paradigm to Horovitz et al. (2-hr test following 18 h of food and water derivation), another study found no effect of juvenile stress on sucrose preference in male rats [63].

4.2. Forced swim test (FST)

In the FST, juvenile stress altered escape-oriented behavioural strategies (climbing and swimming) in both males and females. Juvenile stress reduced climbing and concomitantly increased swimming behaviour independently of sex. This result agrees with one study which showed that repeated foot shock stress from PND 21–25 in male Wistar rats decreased climbing behaviour in the FST [76]. Only one study has examined the effect of juvenile/early adolescent stress on active, escape-oriented behaviours in the forced swim test in both sexes. Social isolation during a broader period of adolescence (PND 30–50) in rats lead to a female-specific *increase* in climbing behaviour in the FST in adulthood accompanied by a corresponding decrease in immobility [77]. However, this is a much more prolonged stressor which extends through puberty into mid-adolescence. By contrast, we found no effect of prepubescent juvenile stress on immobility likely because the stress-induced decrease in climbing was counteracted by a stress-induced increase in swimming behaviour. In agreement, juvenile stress (PND 27–29) did not impact immobility time in the FST in adult male Wistar rats [68]. However, females were not studied in that experiment and the authors did not report results for active behaviours such as climbing and swimming [68]. Likewise, peripubertal stress (variable stressors between PND 28–42) in rats did not induce a change in time spent "floating" in FST in stressed males and females. However, in that study the rats were tested in adolescence, just one week following stress and swimming and climbing behaviors were not measured [78].

Consistent with previous reports of sex differences in immobility in the FST, we found that females exhibited greater immobility than males [66,77,79–82] and females spend less time engaged in climbing behaviour than males [77,80,83]. We also quantified head shaking behaviour, as this is a sexually dimorphic behaviour in the FST [48,81] and confirmed that males performed more head shakes than females, but we found no effect of juvenile stress on this behaviour. In the modified FST, behaviours are typically analysed in the 5-min test and not during the 15-min pre-swim, however, we also examined behaviours during the first 5-min of the pre-swim. We found that juvenile stress decreased climbing behaviours in both the 5-min and pre-swim tests. Likewise, headshaking behaviours were higher in males compared to females in both swim sessions. However, while juvenile stress increased swimming behaviour in the 5-min test, stress increased immobility during the pre-swim, which aligns with previous reports that proportions of active and passive behaviours may differ between the pre-swim and FST [84,85]. Nevertheless, in both swim sessions, juvenile stress exerted sex-independent alterations in escape-oriented strategies.

4.3. Novelty-induced hypophagia

In the novelty-induced hypophagia test (NIH), juvenile stress decreased latency to drink the sweetened milk in the anxiogenic context. At first glance, it may appear that juvenile stress decreased anxiety-like behaviour, however, we found no effect of juvenile stress on adult locomotor activity or anxiety-like behaviour in the open field test. Exposure to variable short-term stressors prior to puberty has previously been shown to increase anxiety-like behaviour in the elevated plus maze in both mice and rats [39,63,68,74,86,87]. The contrasting data in the NIH vs the open field test may potentially be related to the presence of rewarding stimulus in the NIH. In contrast to the open field test and the

EPM, the NIH test is a conflict-based anxiety test in which the rat must decide between actions with simultaneous rewarding and aversive outcomes. Thus, we propose that the NIH data might suggest that juvenile stress may have induced increased reward seeking behaviour in a risky environment thus suggesting that juvenile stress may have affected risk-taking, decision-making, and/or rewarding processes. In agreement, it has been reported that male and female rats stressed as juveniles were faster than non-stressed controls to make a choice when presented with an ambiguous stimulus in a cognitive bias test in adulthood, thus suggesting that juvenile stress altered decision making [86]. The same group also found an increase in compulsive behaviour (perseverative responding in nose-poke task) in adult female, but not male, rats exposed to juvenile stress [88]. This suggests that juvenile stress might impact neural circuits underlying decision making, reward-related behaviour, and risk-taking behaviour.

4.4. Mesocorticolimbic dopaminergic system

Since the mesocorticolimbic dopaminergic system is key circuit that controls these behaviours and undergoes significant structural remodeling during the prepubescent juvenile period [11], we next examined the impact of juvenile stress on TH-ir within this system. The length of TH fibres in the male and female rat mPFC increases significantly between PND25–35 [62], which coincides with increased dopamine innervation in this region during the juvenile period [13] and the time period in which we applied stress in our experiment. Thus, we hypothesized that increased motivation to obtain a reward in the risky, novel environment of the NIH test may have been associated with stress-induced alterations in the development of the mescocorticolimbic dopaminergic system. We found that neither juvenile stress nor biological sex affected TH-ir in the DMS and NAc. However, we noted a significant effect of juvenile stress on the TH-ir the mPFC, which was specific to layer 1. Female rats undergo a significant increase in layer 1 TH fibre density between the ages of PND 25–35 [62] and a corresponding female-specific increase in synapse number in layer 1 of the mPFC [10], which coincides with the time during which our juvenile stress was applied. Axons continue to grow through the Nac to the mPFC during adolescence in mice, guided by the cue receptors within dopamine neurons [89]. The overall number of mPFC neurons and synapses gradually decreases between the juvenile period and adulthood and this drop in number has been shown to be more drastic in female rats compared to male rats [10,90,91]. We measured TH-ir as a proxy for dopaminergic fibres, however, it should be noted that TH-positive neurons may co-release both dopamine and noradrenaline [92]. Thus, we cannot rule out the possibility that noradrenaline also plays a role in the observed behaviours, and therefore the effects on of juvenile stress on noradrenergic signaling will need to be addressed in future studies. Nevertheless, juvenile stress appears to have disrupted the normal maturation of catecholaminergic neurons in the mPFC, as shown by increased TH-ir in layer 1 of the mPFC in stressed rats irrespective of biological sex. Stress during the juvenile/childhood period has been shown to impact various aspects of mPFC development and functioning in mice, rats, non-human primates, and humans [reviewed in 12]. However, the current study is the first to report layer-specific effects of juvenile stress on TH-ir in the mPFC of adults.

In preclinical studies of antidepressant drugs, climbing behaviours in the FST have been linked to catecholamine neurotransmitter systems in the brain [47,93] whereas swimming behaviour has been linked to serotonergic neurotransmission [47,94,95]. Therefore, the stress-induced changes in climbing and swimming behaviour observed in the FST might potentially be associated with the stress-induced increase in catecholaminergic fibres in the mPFC. Climbing behaviour is particularly affected by antidepressant drugs with selective effects on noradrenergic [95–97] and dopaminergic signaling [98,99]. Evidence further suggests that dopamine signaling specifically within the mPFC is important for climbing behaviour. Depletion of mPFC dopamine attenuated the antidepressant actions of desipramine to increase active behaviours in the FST in male rats [100]. Intra-mPFC microinjection of D2R dopamine receptor antagonist, haloperidol, abolished the antidepressant-like effect of wheel running on active behaviours in the FST in male rats [101]. Although this was not directly tested, the juvenile stress-related increase in catecholaminergic TH-positive in the layer 1 of the mPFC may partially explain decreased climbing behaviours in the FST. However, no studies have yet examined the layer-specific impact of TH-positive fibres on FST behaviours.

Other research has shown that stress during the juvenile period can disrupt dopaminergic circuits with long-lasting consequences. Juvenile stress (PND 27-33) has previously been shown to increase dopamine concentrations in the mPFC of adult male rats [63]. Chronic social isolation beginning from PND 28 in male rats increased dopamine release in the NAc accompanied by increased TH protein measured by Western blot [102]. Adolescent social defeat in male rats (PND 35–40) has been shown to increase dopamine transporter binding in the mPFC, increase D1 receptor binding in the striatum in adulthood [103], and increase mPFC dopamine release [104]. Peri-pubertal stress (PND 28–42) in male rats increased expression of monoamine oxidase A (MAOA), an enzyme that degrades dopamine, in the PFC both in male rats [105]. Additionally, pharmacological inhibition of dopamine receptor D1-D2 heteromers via intracerebrovascular injection TAT-D1 peptide in rats was shown to decrease latency to drink in NIH suggesting that modulation of dopaminergic signalling influenced behaviour in the NIH, a test we found was impacted by juvenile stress [106]. Taken together, these studies suggest a potential role for the mesocorticolimbic dopamine system in mediating the juvenile stress-induced behavioural changes in the NIH test, which supports our finding that juvenile stress increased TH-ir in layer 1 of the mPFC in adulthood.

4.5. Adult hippocampal neurogenesis

The ability of some antidepressants to decrease hyponeophagia has been shown to be dependent on adult hippocampal neurogenesis [107] and many studies have reported that chronic stress in adulthood decreases adult hippocampal neurogenesis [108,109]. However, we found that neither juvenile stress nor biological sex impacted the survival of new neurons born just prior to juvenile stress as measured by BrdU+ /NeuN+ cells in the dorsal and ventral hippocampus. Similarly, juvenile stress did not impact the number of newly born DCX+ neurons or the number of dendritic crossings per neuron across the longitudinal axis of the adult hippocampus suggesting that despite affecting behaviour in the NIH test, juvenile stress did not affect hippocampal neurogenesis. One recent study reported that juvenile stress (PND 25–27) decreased proliferation of adult-born neurons specifically in the vHi of males (BrdU+/DCX+), but not females, aged PND 60–65 [40]. This study differs from ours in that these rats were injected with BrdU as *adults* 24 h before perfusion, and thus measured proliferation, rather than the survival of neurons born during the juvenile period. That study also found an increase the total number of DCX+ cells/mm^3 specifically in the vHi of males, but not females exposed to juvenile stress [40]. However, in agreement with our current findings, juvenile stress did not impact any measure of adult hippocampal neurogenesis in females [40]. Similarly, three weeks of social isolation stress during adolescence (PND 28–49) in male mice did not impact the survival of newly born neurons (BrdU+/NeuN+) [110].

Irrespective of juvenile stress, adult females had fewer adult born DCX+ cells and these DCX+ cells exhibited fewer proximal and distal dendritic crossings than those of males. This is in agreement with a study which found that the number of newly born DCX+ hippocampal neurons born in adulthood is lower in female rats compared to males [109]. Similarly, a recent study reported that sex differences exist in the maturation and attrition of adult born hippocampal neurons in rats [111] whereby cell proliferation was greater in the male dentate gyrus and adult-born hippocampal neurons matured faster in male rats

compared to females [111]. However, we found no difference in the resulting number of juvenile-born hippocampal neurons between adult male and female rats, suggesting that the sex difference we observed in DCX+ cell numbers may be transient in nature.

5. Conclusion

In conclusion, our results showed that juvenile stress altered antidepressant-like escape-oriented behavioural strategies independent of biological sex. Juvenile stress increased reward-seeking behaviour in a risky environment and decreased supported rearing behaviour. Irrespective of stress, we also found significant sex differences in behaviour across tests and in adult hippocampal neurogenesis, further emphasizing the importance of studying sex as a biological variable. Juvenile stress-induced behavioural changes were not associated with alterations in hippocampal neurogenesis, but juvenile stress increased TH-ir in layer 1 of the mPFC in adulthood, offering a possible explanation for our observations in the NIH test and FST. Taken together, these data reinforce the conclusion that stress during the juvenile period has enduring neurobehavioural consequences in both males and females. This underlines the importance of further uncovering the neurobiology underlying stress-induced changes in the juvenile brain to develop new treatments for psychiatric illness.

CRediT authorship contribution statement

Erin Harris: Methodology, Formal Analysis, Investigation, Writing – original draft preparation, Visualization. **Andrew McGovern:** Investigation, Formal analysis. **Thieza Melo:** Investigation, Validation. **Aaron Barron:** Investigation, Formal analysis. **Yvonne Nolan**: Supervision, Resources, Writing – review & editing. **Olivia O'Leary**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2021.113725.

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