Environmental enrichment rescues survival and function of adult-born neurons following early life stress

Running title: early life stress, hippocampus and enrichment

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Abstract

Adverse experiences early in life are associated with the development of psychiatric illnesses. The hippocampus is likely to play pivotal role in generating these effects: it undergoes significant development during childhood and is extremely reactive to stress. In rodent models, stress in the pre-pubertal period impairs adult hippocampal neurogenesis (AHN) and behaviours which rely on this process. In normal adult animals, environmental enrichment (EE) is a potent promoter of AHN and hippocampal function. Whether exposure to EE during adolescence can restore normal hippocampal function and AHN following pre-pubertal stress (PPS) is unknown. We investigated EE as a treatment for reduced AHN and hippocampal function following PPS in a rodent model. Stress was administered between post-natal days (PND) 25-27, EE from PND35 to early adulthood, when behavioural testing and assessment of AHN took place. PPS enhanced fear reactions to a CS following a trace fear protocol and reduced the survival of 4-week-old adult-born neurons throughout the adult hippocampus. Furthermore, we show that fewer adult-born neurons were active during recall of the CS stimulus following PPS. All effects were reversed by EE. Our results demonstrate lasting effects of PPS on the hippocampus and highlight the utility of EE during adolescence for restoring normal hippocampal function. EE during adolescence is a promising method of enhancing impaired hippocampal function resulting from early life stress, and due to multiple benefits (low cost, few side effects, widespread availability), should be more thoroughly explored as a treatment option in human sufferers of childhood adversity.

Introduction

Childhood adversity is associated with an increased risk of developing psychiatric illnesses[1]. Epidemiological studies have repeatedly shown that stressful early life experiences such as abuse and neglect are associated with higher rates of schizophrenia, depression, borderline personality disorder, anxiety and post-traumatic stress disorder. The hippocampus is a key target of the stress response, being enriched for corticosteroid receptors, particularly in CA1 and dentate granule cells[2]. Coupled with significant post-natal maturation during childhood and adolescence, the hippocampus is predicted to be especially vulnerable to the effects of early life stress (ELS). In support of this, meta-analyses report significant associations between childhood adversity and reduced hippocampal volume and impaired hippocampal function[3-5]. Animal models of ELS similarly report changes in hippocampal-dependent learning and memory and associated molecular changes[6-9]. This has relevance for psychiatric illness: the hippocampus is involved in cognitive and emotional functions, and smaller hippocampal volumes and abnormal hippocampal-dependent behaviours are prevalent in post-traumatic stress disorder, schizophrenia, anxiety and depression[10-13].

On a neuronal level, rodent models demonstrate that stress early in life adversely affects the generation of adult-born neurons in the dentate gyrus of the hippocampus (adult hippocampal neurogenesis (AHN))[8]. These adult-born neurons are implicated in emotional regulation and hippocampal-dependent behaviours, including trace and contextual fear conditioning, spatial navigation, pattern separation and cognitive flexibility[13-17]. There is also evidence that AHN and dentate gyrus volume are decreased in schizophrenic and depressed patients[18,19]. Lasting impairments in AHN resulting from ELS may therefore negatively affect behaviour and leave individuals vulnerable to developing psychiatric illnesses.

Neuropsychiatric disorders are predicted to be the second highest cause of global disease burden by 2020[20]. Current treatments are ineffective in up to 30% of cases, often accompanied by significant side effects, and many individuals relapse[13]. There is therefore a pressing need to develop

novel and improved treatments. Given the recent emphasis on links between dysregulated AHN and psychiatric illness, this is a promising process to target, especially considering increased AHN is postulated as one mechanism through which antidepressants exert their effects[13,14,19,21]. Environmental enrichment (EE) is a robust method for improving AHN and hippocampal-dependent cognition in adult animals[22]. In rodents, EE is a housing manipulation which increases exposure to social and physical stimuli (e.g. larger social groups, toys and tunnels), promoting exploration, social interaction and physical activity. During adolescence, EE has been shown to rescue social function, attention and anxiety behaviours following early life stressors such as maternal separation[23-27]. Interestingly, a recent study by Ardi *et al*. demonstrates that exposure to EE directly after pre-pubertal stress, but not after an additional adult stressor, prevents stress vulnerability and normalises anxiety-like behaviour after adult traumatic stress[28]. However, the impact of EE in adolescence is not yet fully explored or understood, and the potential benefits for rescuing ELS induced deficits in AHN and hippocampal-dependent behaviour are unknown[29]. This is a crucial area of exploration as EE holds great potential for enhancing brain function in human populations, as highlighted in a recent review (Kempermann, 2019)[22], particularly in the context of neuropsychiatric or neurodegenerative diseases. Cognitive, mental, nutritional, physical and social stimulation in humans have been categorised as EE which may circumnavigate or compliment pharmaceutical treatments[30]. EE based interventions are particularly appealing due to low-risk of side effects, low cost and widespread availability, and have already shown promise as an adjunct treatment for promoting functional recovery in stroke patients[31].

The first aim of this study was to investigate how early life stress given in the pre-pubertal phase (pre-pubertal stress (PPS), post-natal days 25-27, a time-point akin to human childhood[32]) impacted upon AHN and hippocampal-dependent behaviour. Secondly, we sought to determine whether EE could reverse the effects of PPS on AHN and hippocampal dependent behaviour. We also investigated whether PPS altered the number of adult-born neurons that were active during recall of a conditioned stimulus (CS) following a trace fear protocol, and subsequent modulation by EE.

Materials and Methods

Animals

Experiments were approved by Cardiff University's Animal Welfare and Ethical Review Body and adhered to UK Home Office Animals (Scientific Procedures) Act 1986 and European Regulations on animal experimentation. Lister hooded rats were bred in house from 16 adult pairs (Charles River), weaned at postnatal day (PND) 21 and housed in same-sex cages with littermates. Light was maintained on a 12:12-h light/dark cycle, and food and water were provided *ad libitum.* Male rats were used as PPS does not alter trace fear responses or neurogenesis in female animals[8].

Pre-pubertal stress & enrichment

Based on previous experiments in our laboratory[8] twenty-two animals served as controls and twenty-four animals were given a short-term PPS between PND 25-27, originally described by Jacobson-Pick and Richter Levin[33]. Litters were allocated to treatment groups (control or stressed) based on order of birth. Animals were given a 10 min swim (25±1^{o}C) in an opaque swimming tank (25cm high, 34cm diameter) on PND 25, restraint stress in plastic restraint tubes (15cm length, 5cm diameter) for 3 sessions of 30 minutes, separated by 30 minute breaks in the home cages on PND 26 and exposure to an elevated platform (15x15cm, 115cm high) for three 30 minute sessions, separated by 60 minutes in the home cage on PND 27. Animals were then returned to the holding room and housed in regular cages (32cm x 50cm x 21cm, lined with wood shavings and a wooden stick, cardboard tube and shredded paper provided as standard enrichment) in groups of three or four. On PND 35 half of the animals (PPS and control) were moved into larger enriched cages (74cm x 59cm x 40cm) in groups of six or seven (EE groups). The enriched cages contained a deep layer of sawdust bedding, platforms, wooden sticks and a variety of toys including tubes, tunnels and hammocks, which were rotated

between cages every week. The remaining animals remained in regular cages described above (control housed, or CH groups), and animals were left undisturbed, aside from cage cleaning, until early adulthood.

BrdU administration & behaviour

Between PND 57-66 animals were given a single intraperitoneal injection of bromodeoxyuridine (BrdU, 200mg/kg in 0.9% sterile saline solution), to label dividing neurons in the dentate gyrus. Four weeks later (PND 83-97) animals were trained in a trace protocol. By the third week of life, 90% of adult-born neurons express the mature neuronal marker NeuN and demonstrate electrophysiological features of maturity, and by 4 weeks immature markers such as b-III-tubulin and doublecortin are no longer detectable[34-36].

Apparatus: Two modular test chambers (32cmx25.5cmx27cm, Sandown Scientific UK) with grid floors (19 stainless steel rods, 1cm apart) and a stainless-steel pan were used for testing. Side walls were stainless steel, ceiling, front and back walls clear plexiglass. Each chamber resided inside a sound attenuating box, to which a speaker was attached on the inside. A ventilation fan provided a background noise of 63dB and a video camera was attached to the inside of the door. A shock generator was attached to the grid floor. Video recording, light, sound and shock were controlled by computer interface. The two boxes provided distinct contexts, C1 and C2. C1 was illuminated by a house light, the pan was filled with wood shavings, ceiling and walls decorated with black stars on a white background. C2 was dark, an IR light bar allowed video recording, and was scented with a drop of lavender oil in the pan. Between animals each box was cleaned with ethanol wipes, and lavender scent/sawdust replaced. Half of the animals from each group (control and PPS) and treatment (EE and CH) were trained in C1, half in C2. The unconditioned stimulus (US) was a 0.5s, 0.5mA scrambled footshock, the conditioned stimulus (CS) a 15s, 75dB white noise.

Protocol: Animals were habituated by transport to the testing room and handling every day for three days before testing began. On day 1, animals were placed into C1 or C2 for 120s. Rats then experienced 10 CS-US parings, a 30 second stimulus free trace interval separated the offset of the CS from the onset of the US. Intertrial intervals were 312s (+/-62s). This intertrial interval is optimal for producing freezing to both context and cue[8,37]. To assess contextual fear responses, twenty-four hours later animals were returned to their original training chamber for 10 minutes. Forty-eight hours after training, responses to the CS were determined by placing animals into the chamber they were not trained in (i.e. trained in C1 placed into C2 and vice versa). A plastic insert covered the bars to aid in context discrimination. After a 120s acclimation period, the CS was played for 360s, followed by a stimulus-free 240s post-CS period. Freezing was used as a measure of fear response, defined as immobility excluding movement due to respiration. It was sampled every 10s from video recordings by an observer blind to group/treatment.

Immunohistochemistry

Thirty-five minutes after the start of trace recall, animals were deeply anaesthetised and transcardially perfused with 0.01M PBS and 4% paraformaldehyde (PFA). Brains were removed and stored in PFA overnight at 4^{o}C, then transferred to 30% sucrose for cryoprotection, before being sliced coronally at 30µm on a freezing microtome (Leica RM2245). Sections were placed into cryoprotectant and stored at -20^{o}C. Sections were stained for: BrdU (labels diving cells, marking 4-week-old neurons in this study), neuronal nuclei (NeuN, mature neuronal marker) and cfos (immediate early gene, indirect marker of neuronal activity). Unless otherwise stated, sections were washed between each step for 3 x 5 minutes in 0.01M Tris-buffered saline (TBS, pH 7.4) and carried out at room temperature. One in every 12 sections throughout the entire extent of the hippocampus was denatured in 1M HCL for 30 minutes at 45^{o}C, incubated for one hour in blocking solution (0.3% Triton-X in 0.01M TBS, 2% donkey serum) then rat anti-BrdU (1:800, ab6326, abcam UK), mouse anti-NeuN (1:1000, MAB377, Merk UK)

and rabbit anti-cfos (1:5000, ABE457, Merk UK) in blocking solution for 24 hours at 4 °C, followed by Alex Fluor secondary antibodies (donkey anti-rat 488, donkey anti-mouse 647 and donkey-anti rabbit 568, 1:200, Life Technologies UK) for 2 hours in the dark. Sections were then incubated with DAPI (1:3000 in TBS, D9542, Sigma UK) for 5 minutes. Washed sections were then mounted on slides and coverslipped with mounting medium (S3023, Dako UK). Slides were imaged using Axio Scan Z1 slide scanner (Carl Zeiss Microscopy, Germany). The total number of cells double labelled with BrdU/NeuN and triple labelled with BrdU/NeuN/cfos were counted bilaterally throughout the entire infrapyramidal and suprapyramidal blades of the dentate gyrus in the dorsal (Bregma -1.72mm to -5.28mm) and ventral (Bregma -5.28mm to -6.72mm) hippocampus, according to the atlas of Paxinos and Watson (2009). As one in every 12 sections throughout the hippocampus was stained and counted, the total number of labelled cells was estimated by multiplying total counts per area by 12[38]. Counts were analysed using Zen Blue (Carl Zeiss Microscopy, Germany).

Data analysis

JMP (statistical software, SAS Institute, Cary, NC, USA) was used for all data analysis. Homogeneity of variance and normality of distribution were checked for all datasets, then data were analysed using generalised linear models, with experimental treatment (control or PPS), enrichment (EE or CH) and experimental treatment*enrichment fitted as factors. When analysing behavioural data from the training day, shock number was added as a factor, and when analysing immunohistochemical data, region (dorsal, ventral, infrapyramidal, suprapyramidal blade) was added. Where necessary, animal was nested within litter and added as a random factor to account for multiple measurements on the same animal. To account for the use of more than one animal per litter, litter was nested within group and added as a random factor. Significant interactions were teased apart using post-hoc Tukey HSD tests. Correlations between number of adult-born cells (all and active) and freezing to context and cue were explored using Pearson's pairwise correlation.

Results

Behaviour

Training day. To investigate potential differences in encoding, freezing responses post CS and post US were analysed on the training day. Freezing in the 30s post CS period was unaffected by PPS ($F_{1,13.5}$=0.03, p=0.85) or enrichment ($F_{1,31.3}$=2.9, p=0.09). Animals froze progressively more as CS-US stimuli were presented, with levels of freezing significantly higher following CS's 3-10 than 1 and 2 (shock: $F_{9,360}$=35.82, p<0.0001, Figure 1a). A similar pattern was observed following the US, with all animals freezing significantly more after US's 8-10 than 1-7 (shock: $F_{1,360}$=4.35, p<0.0001). Following US's 8-10, PPS resulted in lower levels of freezing (group*shock: $F_{9,360}$=2.9, p=0.002, Figure 1b).

Context: Results are shown for the first 120s of each period. Twenty-four hours after the trace fear protocol, all animals demonstrated robust levels of contextual freezing (Figure 2a). Levels of freezing were unaffected by PPS ($F_{1,12.75}$=0.07, p=0.8) or environmental enrichment ($F_{1,166.4}$=0.15, p=0.7).

Cue recall. Twenty-four hours after context recall, CS recall was performed. PPS enhanced freezing during the first 90s of CS presentation, this was rescued by environmental enrichment (group*enrichment: $F_{1,127.1}$=5.1, p=0.03, Figure 1b). There was no overall effect of PPS ($F_{1,14.82}$=0.55, p=0.47) or enrichment ($F_{1,127.1}$=3.92, p=0.06) on freezing to the CS. There was no effect of PPS ($F_{1,14.88}$=0.74, p=0.4) or enrichment ($F_{1,121.6}$=0.63, p=0.43) on freezing in the post-CS period. Baseline freezing was low in all groups, and unaffected by PPS ($F_{1,14.84}$=1.28, p=0.28) or enrichment ($F_{1,34.99}$=0.96, p=0.33).

BrdU/NeuN

PPS reduced the survival of adult-born neurons throughout the entire dentate gyrus, as measured by BrdU/NeuN, and this effect was abolished by enrichment (group*enrichment: $F_{1,159.3}$=11.61, p<0.001,

Figure 3a). In all groups there were significantly more adult-born neurons in the suprapyramidal blade of the dorsal hippocampus than any other region (region: $F_{3,149.7}$=8.57, p<0.0001). There was no overall effect of PPS ($F_{1,14.34}$=0.09, p=0.77) or enrichment ($F_{1,159.3}$=0.1, p=0.74).

BrdU/NeuN/cfos

PPS reduced the number of active adult-born neurons throughout the entire dentate gyrus post CS recall, as measured by BrdU/NeuN/cfos staining, and this effect was rescued by enrichment (group*enrichment: $F_{3,157}$=18.4, p<0.001, Figure 3b). In all groups there were significantly more active adult-born neurons in the suprapyramidal blade of the dorsal dentate gyrus than any other region, and more in the infrapyramidal blade of the dorsal than the ventral dentate gyrus (region: $F_{3,149.6}$=11.66, p<0.0001). There was no overall effect of PPS ($F_{1,14.45}$=0.19, p=0.67) or enrichment ($F_{1,157}$=1.05, p=0.31).

Proportion of BrdU/NeuN cells co-labelled with cfos

There was a trend for PPS to reduce the proportion of active adult-born neurons post CS recall, measured by diving the number of cells co-labelled with BrdU/NeuN by those triple labelled with BrdU/NeuN/cfos ($F_{1,22.9}$=3.18, p=0.09). Enrichment significantly increased the proportion of active adult-born neurons in the stressed group (group*enrichment: $F_{1,156.4}$=7.33, p<0.01, Figure 3c). There was a significantly higher proportion of active adult-born neurons in the dorsal compared to the ventral hippocampus across all groups (area: $F_{3,149.2}$=13.18, p<0.0001). There was no overall effect of PPS ($F_{1,13.99}$=0.33, p=0.57) or enrichment ($F_{1,156.4}$=3.52, p=0.06).

Correlations

Adult-born neurons & context

There was no correlation between freezing to context and number of adult-born neurons (con CH: r=0.36, p=0.8; PPS CH: r=0.5, p=0.6; con EE: r=0.41, p=0.81; PPS EE: r=0.4, p=0.79), number of active adult-born neurons (con CH: r=0.46, p=0.85; PPS CH: r=-0.1, p=0.49; con EE: r=0.34, p=0.78; PPS EE: r=0.42, p=0.8) or proportion of active adult-born neurons (con CH: r=0.28, p=0.77; PPS CH: r=-0.34, p=0.29; con EE: r=0.04, p=0.63; PPS EE: r=0.3, p=0.74) in any group.

Adult-born neuron & CS

There was a significant positive correlation between CS-evoked freezing and number of adult-born neurons (con CH: r=0.85, p=0.002; PPS CH: r=0.73, p=0.007, Figure 4a,b) and number of active adult-born neurons (con CH: r=0.62, p=0.05; PPS CH: r=0.73, p=0.007, Figure 4c,d), but not proportion of active adult-born neurons (con CH: r=-0.35, p=0.32; PPS CH: r=0.19, p=0.55) in control housed animals only. This relationship disappeared in animals housed in an enriched environment (BrdU/NeuN. Con EE: r=0.37, p=0.24; PPS EE: r=0.09, p=0.8, Figure 4a,b. BrdU/NeuN/cfos. Con EE: r=0.36, p=0.77; PPS EE: r=0.1, p=0.75, Figure 4c, d. Proportion active. Con EE: r=0.2, p=0.53; PPS EE: r=0.12, p=0.75).

Discussion

Early life stress (ELS) has been robustly and repeatedly associated with the development of psychiatric illnesses. ELS can impinge upon adult hippocampal neurogenesis (AHN), and disrupted AHN is postulated to play a role in psychopathology[13,16]. This process provides a novel and accessible target for improving hippocampal function and potentially preventing or alleviating psychiatric symptoms. Given that current psychiatric treatments fail in up to 30% of cases, produce significant side effects and high relapse rates, there is an urgent need to develop novel and improved treatments. Environmental enrichment (EE) is an underexplored yet promising method of improving AHN and hippocampal function in animals and humans.

We found that short-term stress exposure in the pre-pubertal phase produce decreased survival of mature adult-born neurons in the hippocampus and altered behaviour in a hippocampal-dependent task (trace fear conditioning). Early life stress (ELS) also decreased the number of adult-born neurons that were active during recall of the fear conditioned stimulus cue. Experience of an enriched environment during adolescence rescued all ELS-induced changes in adult hippocampal neurogenesis (AHN), behaviour and neuronal activation.

Stress in adulthood has long been associated with impaired learning and memory in humans and animals and with robust changes in stress-sensitive regions such as the hippocampus[39]. Evidence is mounting that stressful experiences early in life can have unique and permanent consequences for the developing brain, adversely affecting behaviour and increasing risk for psychopathology. Meta-analyses show that childhood abuse is consistently associated with smaller hippocampal volumes, and hippocampal function is also affected[40-43]. Similar changes are found in animal models, where ELS affects performance in hippocampal-dependent behaviours such as trace conditioning, pattern separation, object recognition and spatial memory, as well as producing neuronal and molecular alterations[6,8,32,44]. AHN is particularly sensitive to the effects of pre-natal and early post-natal stressors[45-50], but less is known about the post-weaning, pre-pubertal phase, a time-point more akin

to human childhood[32]. In the present study we found that pre-pubertal stress significantly decreased survival of mature (4-week-old) adult-born neurons throughout the dorsal and ventral hippocampus. In a recent study we showed that pre-pubertal stress decreased production of adult-born neurons (less than 24 hours old) and increased survival of immature adult-born neurons (birth-2 weeks, neurons with the potential to survive and be incorporated into hippocampal networks) in the ventral hippocampus only[8]. This demonstrates that different aspects of the neurogenesis process and different regions of the hippocampus can react in a unique manner to stressful early life perturbations. Future work should explore the effects of ELS on the developmental trajectories of adult-born neurons in the hippocampus.

Neither PPS nor enrichment affected contextual fear responses, confirming results from our recent study[8]. In an earlier study, we found that PPS decreased contextual fear responses in male animals[7]. However, there were crucial differences between the training protocols. The previous study administered only one shock during training, here we used 10 CS-US pairings, to ensure robust encoding of the trace protocol. It is well known that increasing the number of shocks enhances subsequent contextual freezing[51], and it is interesting to note that increasing the severity of the protocol is sufficient to overcome PPS induced deficits in contextual fear responses in our model. Contextual fear responses require an intact hippocampus in one trial studies (i.e. one shock administered) but can be acquired in the absence of a functioning hippocampus when multiple trials are given[52]. Our results therefore support the hypothesis that PPS specifically impacts upon hippocampal function in males in our model.

PPS impaired performance in a hippocampal-dependent task (trace fear). Gross hippocampal lesions selectively impair performance on trace protocols[53,54], and ablation of AHN via infusion or optogenitics has a similar effect[52,55]. PPS reduced post-US freezing on the training day and increased conditioned fear responses to the CS at recall. We recently demonstrated that PPS altered performance in trace fear and pattern separation[8], two tasks for which in-tact hippocampal function

and young adult-born neurons are crucial[53,56]. Importantly, responses to a delay protocol (10 CS-US pairings with no temporal gap, does not require intact hippocampal function[53,54,57]), were not altered. Interestingly, in a previous study we found that PPS *reduced* rather than enhanced freezing to the CS in the 48-hour recall test following a trace protocol[8]. In the present study, animals were administered BrdU in early adulthood to label mature adult-born neurons: this occurred 4 weeks before behavioural testing, so these animals were significantly older than those in the previous study at testing. This raises the intriguing possibility that age of testing in adulthood is important in determining the long-term impact of ELS. This phenomenon has already been demonstrated in pre-pubertal vs. adult animals. Perinatal stress increases hippocampal neurogenesis in pre-pubertal males yet causes a decrease in adult males. In females, this stress decreases hippocampal neurogenesis in the pre-pubertal animal, an effect that subsides in adulthood[58]. It is currently unknown how the effects of ELS change as adult animals age, and this should be the focus of further research.

In the present study, we investigated whether PPS altered the number of adult-born neurons active during CS recall. We focussed on 4-week-old adult-born neurons, as these are functionally relevant for behaviour. Once produced from dividing neural stem cells in the sub granular zone, adult-born neurons migrate to the granule cell layer and become functionally integrated into circuitry. Axonal projections extend towards the CA3 pyramidal layer, along the mossy fibre pathway, and dendrites proceed towards the molecular layer[59,60]. Before the formation of output synapses at two weeks, adult-born neurons are not thought to contribute to hippocampal function[61]. Between 4 and 6 weeks of age, adult-born neurons are functionally and morphologically mature, although they continue to develop for several months[60]. 4-week old adult-born neurons display high sensitivity to LTP induction due to high input resistance and low GABAergic inhibition[62]. Ablation of this population of adult-born neurons, but not those of other ages, disrupts hippocampal-dependent behaviour[62,63]. PPS reduced the number of adult-born neurons active during CS recall. Other studies have found changes in neuronal activity throughout the adult brain following ELS, but these have not been specific to adult-born neurons. Variations in maternal care altered neuronal activity in the hippocampus

(increased) and paraventricular nucleus and periaqueductal grey (decreased) during a fear response (shock-probe burying test)[64], and maternal separation results in higher neuronal activation throughout the brain in adults exposed to stress[65-68]. To our knowledge, the present study presents the first demonstration of decreased adult-born neuronal activity following ELS.

We also found that the number of adult-born neurons and number of active adult-born neurons was strongly correlated with freezing to the CS but not the context in all animals housed in control conditions, a relationship that was not affected by PPS. Several studies demonstrate a positive relationship between levels of AHN and performance in hippocampal-dependent tasks[69-71], yet few studies have investigated this relationship in animals exposed to ELS. Adults given limited nesting and bedding (model of ELS) show reduced survival of adult-born neurons, and AHN similarly correlated with performance in hippocampal-dependent, but not independent, tasks[72]. Unexpectedly, this relationship was disrupted by EE, suggesting that additional mechanisms aside from AHN may responsible for the beneficial effects of EE on behaviour. For example, alongside improving performance on hippocampal-dependent tasks, EE also increases glutamic acid carboxylase expression, as well as synaptic transmission and excitability in the hippocampus[73,74]. Future research should explore these potential mechanisms further.

Provision of environmental enrichment (EE) throughout adolescence rescued all ELS induced alterations in AHN and behaviour and restored the number of adult-born neurons that were active during CS recall. However, it had no effect in control animals. EE is a prominent method of improving general wellbeing and rescuing stress-induced behavioural deficits in adult animals[75]. EE also enhances AHN in adult animals[76-79]. Based on this literature, we may predict that EE during adolescence would be similarly beneficial for AHN and hippocampal-dependent behaviour. This was not the case in EE control animals, which displayed similar behaviour, levels of AHN and new-born neuronal activation to standard housed controls. The effects of EE during development, even in normal animals, are not well understood[29], and it is possible that time of exposure (e.g. adolescence vs. adulthood) may

profoundly alter the effects of EE. Hippocampal neurogenesis is considerably higher in the developing adolescent brain compared to adulthood in all species studied[80]. This naturally higher rate of hippocampal neurogenesis may be differentially affected by environmental experiences in a normally developing organism. Support for this hypothesis come from a study comparing exercise in adolescence vs adulthood. Adolescent initiated exercise increased the number of young (DCX positive) adult-born neurons, yet this was not the case in adult-initiated exercise[81]. Conversely, adult-initiated exercise enhanced fear learning, whereas adolescent-initiated exercise did not[82].

EE during adolescence was effective in restoring normal hippocampal-dependent behaviour, AHN and neuronal activation following ELS. A number of studies demonstrate the benefit of EE throughout adolescence for restoring HPA axis function, learning and memory, anxiety, fear, social performance, attention, depressive-like behaviours, amygdala activity and hippocampal LTP following maternal separation or limited nesting and bedding in the early post-natal period[23-25, 83-85]. One recent study investigated the effects of exposure to EE during adolescence, directly following PPS, and found this prevents stress vulnerability and normalises anxiety-like behaviour after adult traumatic stress. Interestingly, the same effects were not found when EE was given in adulthood, suggesting there may be an optimal window of opportunity in which to administer EE[28]. However, to our knowledge this is the first report examining the ability of EE to restore normal AHN and neuronal function following pre-pubertal stress.

AHN has been demonstrated in humans and is believed to make meaningful contributions to cognition and neural plasticity as well as contributing to hippocampal aspects of psychiatric illnesses[13, 86-89]. AHN is also implicated in the behavioural effects of antidepressants[14,21], making this an attractive process to target in the treatment of neuropsychiatric disorders. Environmental enrichment in humans (targeting cognitive, social and physical domains) may provide a novel route to improving hippocampal function, however significant translational hurdles exist, and future work should aim to more closely align pre-clinical and clinical studies[31].

In conclusion, we show that stress in the pre-pubertal phase of life results in impaired hippocampal-dependent behaviour, concomitant with a sustained decrease in survival and activity of adult-born neurons in the hippocampus. Exposure to an enriched environment throughout adolescence rescued behavioural performance and restored survival and activity of adult-born neurons to normal levels. These findings provide important insights into the neural plasticity exhibited by the hippocampus throughout development and demonstrate how environmental experiences can impair and rescue hippocampal function. Environmental enrichment may provide a novel therapeutic avenue for humans who have suffered trauma and are at elevated risk of developing neuropsychiatric disorders. Here, as with animals, a multifactorial approach may provide the most effective intervention.

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Disclosures

The authors declare no financial or other conflicts of interest.

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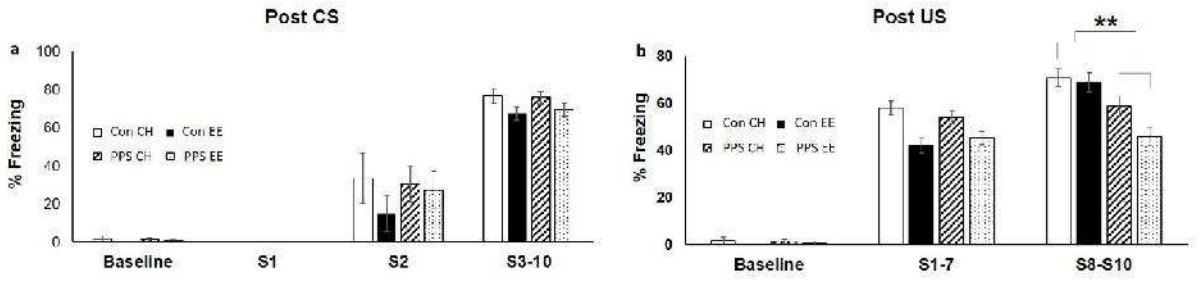
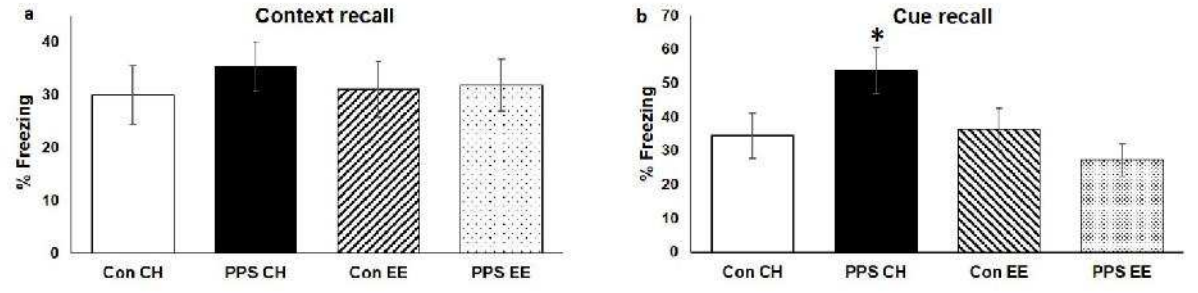
748 **Figure Legends**



749

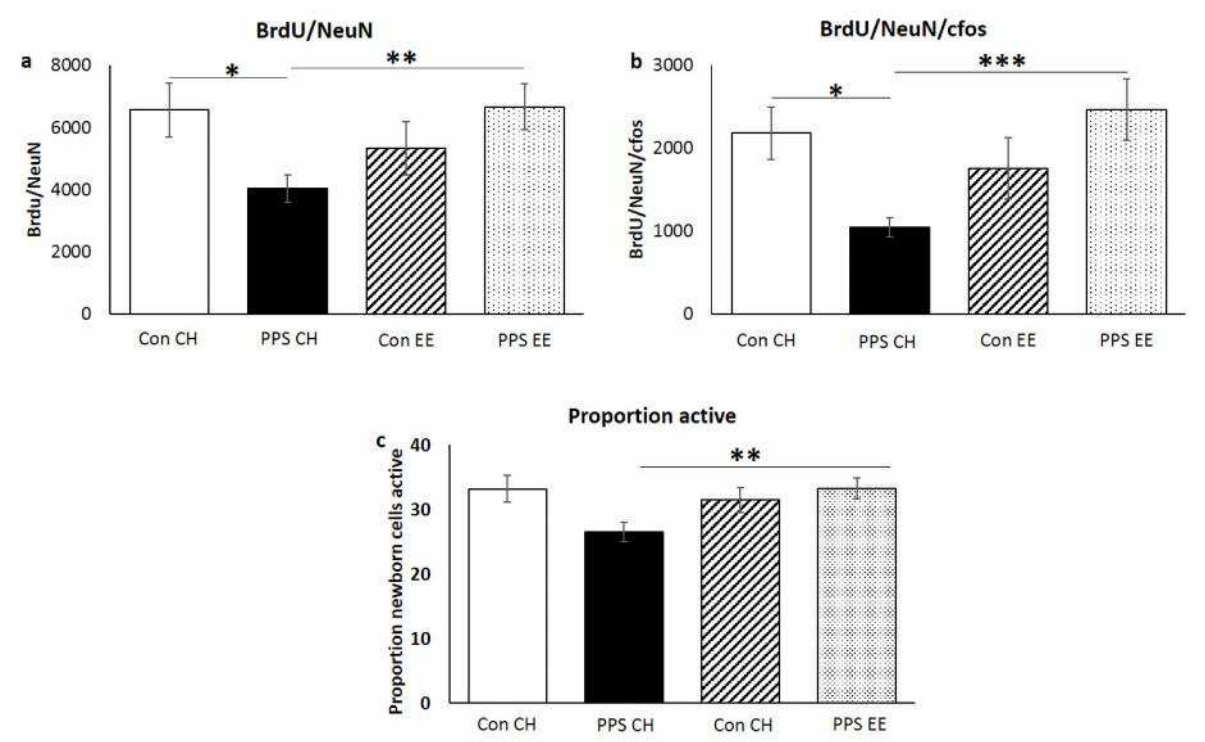
750 **Figure 1 a)** On the training day, animals froze progressively more in the post CS 'trace' interval as CS-
751 US stimuli were presented, with levels of freezing significantly higher following CS's 3-10 than 1 and
752 2. **b)** A similar pattern was observed following the US, with all animals freezing significantly more after
753 US's 8-10 than 1-7. PPS resulted in lower levels of freezing post US's 8-10. Con = control animals, PPS
754 = pre-pubertally stressed animals, CH = control housing, and EE = enriched housing. Error bars
755 represent 1 SE. **=p<0.01. Bars joined by an asterisk are significantly different to one another.

756



757

758 **Figure 2 a)** Neither PPS nor EE affected contextual freezing in the 24-hour recall test. **b)** Animals
759 exposed to PPS and housed in control conditions (PPS CH) froze significantly more to representation
760 of the CS 48 hours after conditioning. Levels of freezing in PPS animals were restored to control levels
761 following EE. Con = control animals, PPS = pre-pubertally stressed animals, CH = control housing, and
762 EE = enriched housing. Error bars represent 1 SE. *=p<0.05. Bars marked with an asterisk are
763 significantly different to all other groups.



764

765 **Figure 3.** PPS decreased **a)** survival of adult-born neurons, **b)** number of adult-born neurons active
766 during CS recall and **c)** caused a trend for reduction in the proportion of adult-born neurons active
767 during CS recall throughout the dorsal and ventral hippocampus. EE restored all measures. Con =
768 control animals, PPS = pre-pubertally stressed animals, CH = control housing, and EE = enriched
769 housing. Error bars represent 1 SE. *=p<0.05, **=p<0.05, ***=p<0.001. Bars joined by an asterisk are
770 significantly different to one another.

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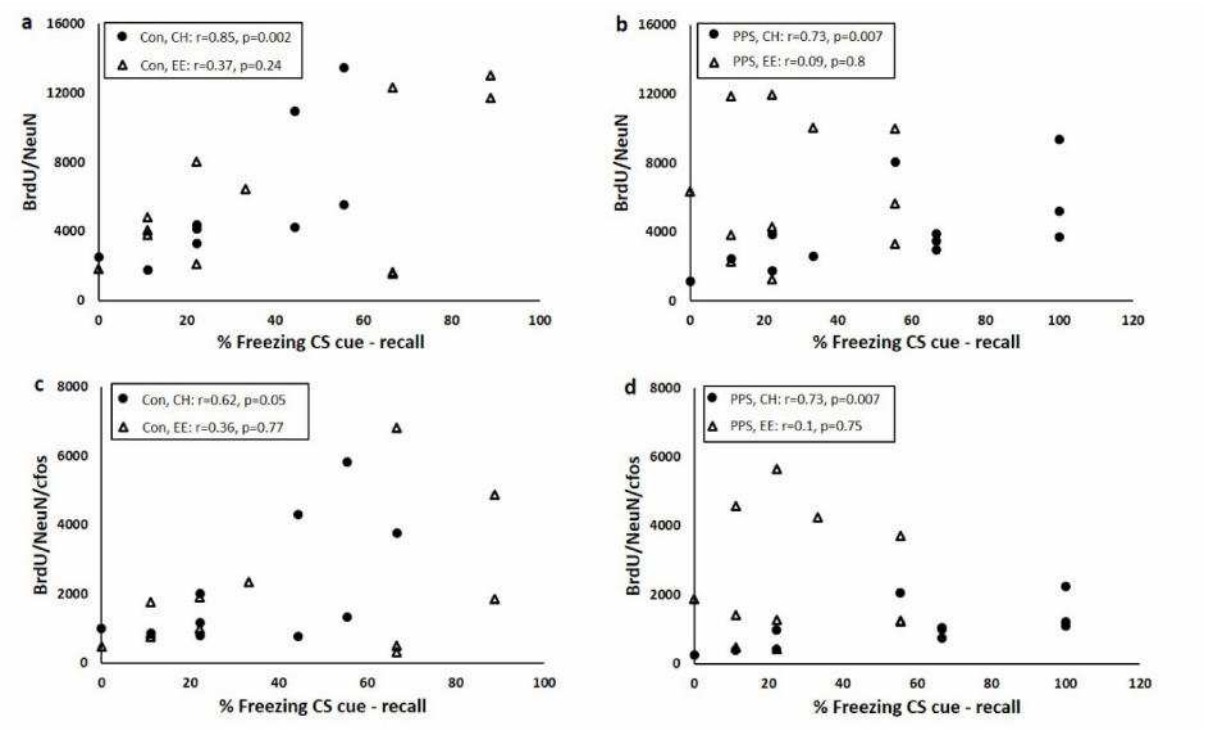

772

773 **Figure 4.** Freezing to the CS was positively correlated with number of adult-born neurons in **a)** control
774 animals (Con CH) and **b)** PPS animals (PPS CH) housed in control conditions. Freezing to the CS was
775 also positively correlated with number of adult-born neurons active during CS recall in **c)** control
776 animals (CON CH) and **d)** PPS animals (PPS CH) housed in control conditions. This relationship was not
777 observed in animals housed in enriched conditions (Con EE, PPS EE). Con = control animals, PPS = pre-
778 pubertally stressed animals, CH = control housing, and EE = enriched housing.