



Pre-pubertal stress and brain development in rodents

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Exposure to adversity early in life is associated with the development of a range of psychiatric disorders in adulthood. Accumulating evidence suggests that pre-puberty is a time of enhanced vulnerability to environmental insults, and that pre-pubertal stress may alter normal brain maturation. In this review, I consider the long-term consequences of pre-pubertal stress on brain and behaviour in rodent models. Recent studies support the notion that pre-puberty is a time of enhanced vulnerability to stress, with particular consequences for the limbic system. Alterations in epigenetic mechanisms are likely to be responsible for the maintenance of enduring modifications in brain and behaviour after experience of pre-pubertal stress.

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Introduction

Early life adversity

Exposure to adverse events early in life is associated with an increased risk of developing neuropsychiatric disorders in adulthood [1–3]. Several reviews address the consequences of stress experienced in perinatal [4–6] and adolescent [7–9] phases, however, until recently, comparatively less was known about the effects of stress experienced in the childhood or pre-pubertal phase [10]. The pre-pubertal brain displays several functional and structural differences to the perinatal, adolescent and adult brain and is predicted to be extremely sensitive to environmental perturbations as it undergoes significant developmental changes [11–13]. The clinical importance of pre-pubertal stress (PPS) is borne out in epidemiological studies: childhood adversity is associated with the development of disorders including anxiety, post-traumatic stress disorder, psychosis and depression in adulthood [1–3,14].

Underlying mechanisms linking PPS with increased risk for psychiatric disorders are not well understood. It has

been hypothesised that stress during early life alters brain development, enhancing vulnerability for disorders later in life [7]. Here I review recent studies in rodent models on the enduring effects of PPS on brain and behaviour, which provide support for this hypothesis. I discuss the mechanisms through which PPS may programme behaviour, before exploring associations between PPS and alterations in the limbic system and prefrontal cortex.

Pre-puberty — a vulnerable phase?

Rodents are often utilised to model the effects of early life stress on brain and behaviour. These basic models allow us to investigate underlying mechanisms with appropriate experimental control, in a manner that is not ethically possible with human participants. Numerous attempts have been made to equate developmental time-points between humans and rodents [15], and based on several considerations (including neuroanatomy, gross morphology, developmental milestones and behavioural phenotypes) the comparison seen in [Figure 1](#) is commonly used.

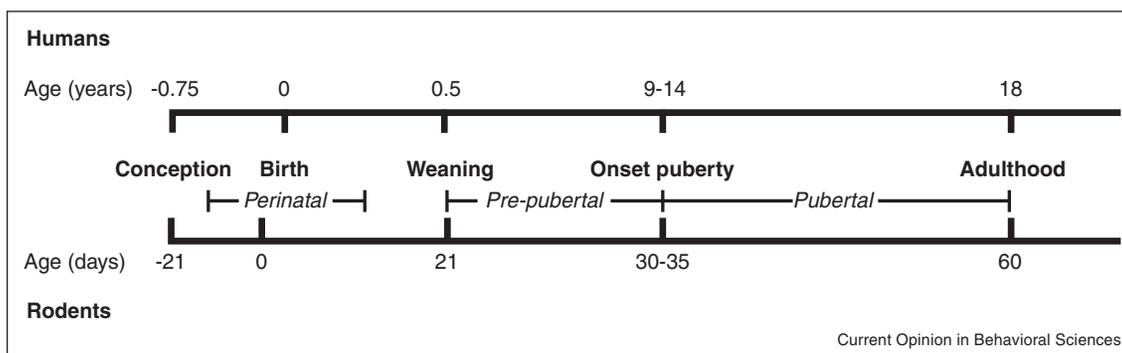
As the brain develops throughout early life, plasticity and maturation rates differ across brain regions [1]. Therefore different regions and processes may be more or less sensitive to environmental insults at any given time. During the pre-pubertal phase and continuing into adolescence, the limbic system (notably the hippocampus and amygdala) and cortical regions undergo structural and functional maturation [13,15]. These structures also play a central role in stress reactivity: they contain high densities of corticosteroid receptors, which detect glucocorticoid stress hormones and regulate the hypothalamic–pituitary–adrenal (HPA) axis [16] ([Figure 2](#)). As the HPA axis displays heightened reactivity to physical and psychological stressors in the pre-pubertal phase [17], it may be predicted that developing limbic and cortical regions are especially vulnerable to stress during this time.

Pre-pubertal stress — mechanisms of action

Stress system

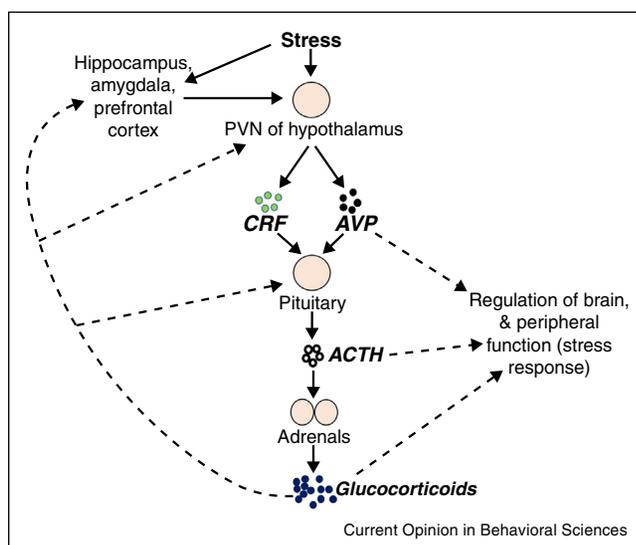
Over the last decade, PPS has been modelled in rodents using a variety of acute and chronic stress protocols. Stressors are either physical or social in nature. Social stressors are often applied over pre-pubertal *and* adolescent phases and have been considered in a recent review [18]. This review will focus on physical stressors specifically in the pre-pubertal phase. Typically, physical stressors including forced swim, restraint, footshock and elevated platform exposure are administered to animals between PND21 and 35 in a variable manner over a number of days [10,19]. Circulating levels of stress hormones have been measured in adults exposed to PPS,

Figure 1



Developmental milestones in humans and rodents [72].

Figure 2

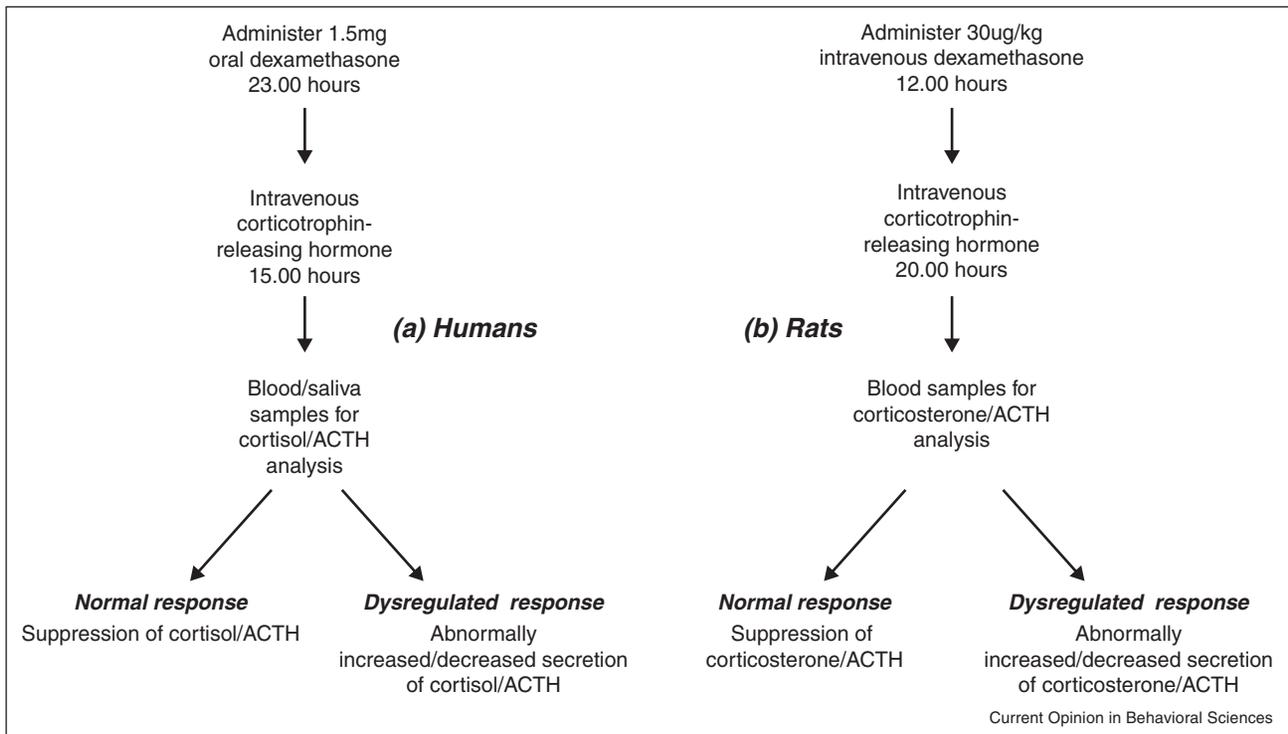


Stress and the limbic system. Physical and psychological events (or 'stressors') can disturb homeostasis, resulting in adaptive physiological and behavioural responses. Stressors may be negative or positive in nature. A major effector of the stress response is the hypothalamic–pituitary adrenal (HPA) axis. Perception of stress causes release of corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus (PVN) of the hypothalamus. This results in release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the blood system, which promotes synthesis and secretion of glucocorticoid stress hormones (mainly cortisol in humans, corticosterone in rats) from the adrenal cortex. These circulating hormones cross the blood brain barrier and are detected throughout the limbic system by mineralocorticoid and glucocorticoid receptors. The corticosteroid receptors in the limbic system contribute to a number of cognitive and affective behaviours. In a healthy system, responses are effectively terminated once the stressor is removed. Excessive or prolonged activation of stress responses early in life may interfere with normal limbic system development, leaving individuals vulnerable to psychiatric disorders [2,16,23,73].

with equivocal results [20]. Animals exposed to forced swim, elevated platform and restraint stress between PND27 and 29 displayed increased basal corticosterone levels as adults [21**], whereas those exposed to foot-shock, cat odour and forced swim over PND23–28 did not [20]. However, these animals did show a flattening of circadian rhythm of adrenocorticotropic hormone (ACTH) [20]. In humans, results are equally unclear, with some studies finding increased/decreased cortisol levels following childhood maltreatment, others finding no difference [22,23]. However, comorbidity with psychiatric disorders such as depression or anxiety or exposure to stress challenges result in altered ACTH and cortisol levels in these populations [24]. This is particularly apparent after administration of the dexamethasone/corticotrophin-releasing hormone (CRH) challenge test (Figure 3) [22–24]. This test is widely accepted as the most sensitive measure of HPA axis dysregulation in humans, and can be applied to animals [25,26], but has yet to be utilised in rodent models of PPS.

One study using mice found that PPS re-programmes corticosteroid receptor expression in the hippocampus, further suggesting dysregulation of stress responses [27*]. Animals exposed to PPS over PND25–27 showed decreased expression of mineralocorticoid receptors (MR), and altered balance of glucocorticoid receptor (GR) to MR ratios. In agreement with these findings, male suicide victims with a history of childhood abuse display altered glucocorticoid receptor expression [28,29**]. Here, decreased levels of the glucocorticoid receptor (GR) NR3C1 and corresponding increases in cytosine methylation of an NR3C1 promoter were observed in the hippocampus [28,29**]. This suggests that sustained epigenetic modifications controlling gene expression may be responsible for maintaining alterations in the HPA axis induced by early life stress. It will be important to more fully characterise the stress system after PPS in rodent models, in particular, responses to the dexamethasone suppression/CRH challenge should be investigated.

Figure 3



The dexamethasone/corticotrophin-releasing hormone (CRH) challenge in (a) humans, (b) rats. Dexamethasone (a synthetic glucocorticoid) is administered, and provides a negative feedback signal to the pituitary in healthy individuals, suppressing normal diurnal adrenocorticotropic hormone (ACTH) and cortisol/corticosterone release (the dexamethasone suppression test). To obtain a more sensitive measure of hypothalamic-pituitary adrenal (HPA) axis function, CRH can be administered the following day, and in healthy individuals pre-treatment with dexamethasone prevents substantial release of cortisol/corticosterone and ACTH [25,26].

Epigenetics

Stressful experiences are likely to programme lasting changes in brain and behaviour through epigenetic mechanisms [30]. Epigenetic modifications are mitotically heritable alterations in gene expression which occur without changes in the underlying DNA sequence, and result in increased or decreased gene expression [31]. Variation in maternal care early in life results in persistent alterations in hippocampal GR expression, and these alterations are mediated through epigenetic mechanisms [32,33]. In particular, modifications in DNA methylation in promoter regions and histone acetylation accompany alterations in gene expression in these models [32,33]. Epigenetic alterations are also found in humans exposed to childhood adversity, including repeated demonstrations of methylation changes in the GR promoter and corresponding alterations of GR expression [28,29,34]. Delineating epigenetic alterations after stress is desirable as they may provide novel targets for therapeutic intervention. To date, the epigenetic consequences of physical PPS have not been explored in animal models, and should be a target of future research.

Pre-pubertal stress – vulnerable brain regions Hippocampus

The hippocampus plays a crucial role in learning and memory processes and emotional behaviour [35]. In adulthood, acute stress (seconds to minutes) facilitates hippocampal dependent processes (improving learning and memory mechanisms), whereas more chronic exposure negatively impacts hippocampal structure and function [36]. Childhood maltreatment associates with decreased hippocampal volume in adulthood [37] (but see [23]), and there is some evidence of impaired hippocampal function [38]. Exposing rats to a 4-week variable physical and social stress protocol over the pre-pubertal and pubertal phase inhibited growth in CA1, CA3 and dentate gyrus areas of the hippocampal formation [39]. However, the consequences of physical stressors applied solely in the pre-pubertal period on hippocampal volume are currently unknown. Regarding hippocampal function, PPS impaired performance on one type of hippocampal-dependent task (contextual fear conditioning), but had no impact on another (spatial reference memory in a standard Morris Water Maze task) in male rats [40]. Conversely, in stressed females, contextual fear responses

remained intact, but these animals showed superior reference memory in the Morris Water Maze task [40]. The hippocampal formation is structurally complex and functionally dissociable, with dorsal regions showing enhanced connectivity to cortical areas, and ventral regions to subcortical structures like the amygdala. Consequently, dorsal lesions impair performance in a range of more cognitively demanding spatial tasks (including the Morris Water Maze), whereas ventral lesions alter performance on tasks with a higher affective or emotional component, including contextual fear and elevated plus maze [35]. Dorsal and ventral regions of the hippocampus display divergent developmental trajectories and development is not identical for males and females [41]. This suggests that PPS may have specific consequences for the development of dorsal and ventral hippocampal regions, and this may differ between the sexes. A series of elegant experiments by Grigoryan *et al.* [21**] provide support for this hypothesis. Here, in males, PPS impaired and facilitated long-term potentiation (LTP) in the dorsal and ventral hippocampus respectively, through regionally altered noradrenergic mechanisms [21**]. PPS also alters GABAergic modulation of granule cells in the ventral dentate gyrus specifically through serotonergic mechanisms [42*]. Due to the intimate associations between stress, noradrenaline and GABAergic mechanisms [43,44], long-term modifications in these systems may partly underlie the altered responses to emotional challenges in adult animals exposed to PPS.

PPS also has consequences for the expression of genes implicated in risk for psychiatric disorder. Brydges *et al.* [45] found increased mRNA expression of disrupted-in-schizophrenia-1 (DISC1) and decreased expression of glycogen synthase kinase beta (GSK3 β) and neuregulin 1 (NRG1) (specific to the type III isoform) in the hippocampus of stressed males and females in adulthood [45]. These genes have independently been implicated in risk for mental disorder [46,47]. Interestingly, changes in DISC1 and NRG1 were observed in adolescence, 7 days after the administration of stress, whereas alterations in GSK3 β were not apparent until adulthood [45]. This suggests that PPS alters expression of some genes in an acute yet sustained manner, whereas others develop over time.

Amygdala

The amygdala facilitates the encoding of emotional memories by working in concert with other brain areas, particularly hippocampal and cortical regions [48]. In connection with hypothalamic regions, the amygdala is especially important for fearful and threat-related behaviours [49,50]. Enhanced amygdala and hypothalamic activity is observed in PPS male rats during retrieval of a cued fear memory [51*], suggesting that PPS intensifies cued fear responses. PPS also results in mild increases in aggression, although this effect is greatly enhanced in

animals exposed to a stress protocol extending through the pre-pubertal *and* pubertal periods (PND28–42) [52–54]. Increased aggression was associated with alterations in expression of molecular markers of excitatory and inhibitory neurotransmission (including the NR1 subunit of the N-methyl D-aspartate receptor and vesicular glutamate transporter 1, glutamic acid decarboxylase 67 and vesicular GABA transporter) in the central nucleus of the amygdala in extendedly stressed animals only, with no changes observed in PPS animals [52]. PPS also affects other domains of social behaviour: stressed adult males display decreased social exploration of unfamiliar adults [55] and juveniles [56] (but see [52]).

The amygdala plays a central role in anxiety-like behaviour on the elevated plus maze, with inactivation producing anxiolytic effects [57]. PPS animals exhibit increased anxiety-like behaviour on the elevated plus maze [27*,45,55,58,59*,60*]. These results mirror responses in human populations, where childhood adversity is strongly associated with the development of anxiety disorders in adulthood [61]. In humans, the effects of childhood adversity on amygdala structure are currently unclear [62]. However, altered function is observed, with increased amygdala responses to threatening stimuli [63]. These populations also demonstrate increased anxiety and aggression, further suggesting abnormal amygdala function [64,65].

Prefrontal cortex

Through connections with other cortical and subcortical regions, the prefrontal cortex subserves executive control, decision-making and emotion regulation [66]. Childhood adversity is associated with alterations in the PFC, including cortical thinning and increased grey matter [14,64,67]. Deficits in PFC activation and executive functioning are also observed in these populations [67,68]. Attentional set shifting tasks (ASST) can be used to investigate cortical function in rodents. Animals are trained to discriminate between stimuli in one domain (e.g. two distinct odours), before learning a new discrimination between either (i) stimuli in the same domain (*intra-dimensional* shift) or (ii) stimuli in another sensory domain (e.g. tactile, *extra-dimensional* shift). Using an ASST, Luo *et al.* [59*] found no evidence that PPS impaired ability on either intra-dimensional or extra-dimensional set shifting, and correspondingly, found no alterations in the PFC monoaminergic system (specifically, noradrenaline and 5-HT, which are involved in set shifting behaviour). However, PPS increased dopamine in the prefrontal cortex, which correlated with increased anxiety behaviour in an open field task [59*]. Limited data thus far suggests that PPS alters prefrontal function but this is restricted to emotional regulation. Indeed, PPS involving early weaning and 12 days of variable stress produced anxiety-like behaviours, decreased neuronal activity in the medial PFC, increased activity in the

amygdala, and produced longer excitatory latencies in mPFC neurons after amygdala stimulation [60*]. As the prefrontal cortex exerts an inhibitory influence on the amygdala, dysfunction in this circuitry after PPS is consistent with the enhanced anxiety phenotype observed in PPS models. Further studies are needed to confirm this.

Sex differences

Although previous research in animal models of PPS has included females as well as males [10,19], the majority of studies reviewed here have focussed on male animals. When explored, sex differences are often found in response to PPS, including divergent responses in hippocampal-dependent behaviour and perseveration [10,19,40,71]. Sex differences exist in the age of onset, prevalence and symptomatology of many neuropsychiatric disorders [69,70]. This is perhaps not surprising when we consider that several brain regions display sex differences in development [70]. Future studies should address this issue, and strive to include females whenever possible.

Conclusion

Hippocampal, amygdaloidal and cortical regions work together to integrate information and produce appropriate behavioural responses. Due to their central role in stress reactivity and developmental progression throughout childhood, they are predicted to be extremely vulnerable PPS. This is especially true when coupled with the fact that pre-puberty is a time of enhanced reactivity to stress. Building on a body of research over the last decade [10], recent work in animal models of PPS provides further support for this hypothesis, and demonstrates that PPS induces alterations throughout the limbic system. Whether these alterations constitute adaptation, with early-life stressors programming resilience to adversity later in life, or simply dysfunction and increased risk for neuropsychiatric disorder, remains to be unravelled. Future studies should focus on elucidating the precise neurobiological mechanisms responsible for behavioural and molecular alterations after PPS, and special attention should be given to potential epigenetic mechanisms. Increasing our understanding of the biological mechanisms linking early-life stress with increased risk for psychiatric disorders will enable the development of targeted interventions in clinical populations with a history of childhood adversity.

Conflict of interest statement

Nothing declared.

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