Programming the brain and behaviour by early life stress: A focus on neuroactive steroids.

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Programming the brain and behaviour by early life stress: A focus on neuroactive steroids

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ABSTRACT

Animal studies have amply demonstrated that stress exposure during pregnancy or in early post-natal life can adversely influence brain development and have long-term 'programming' effects on future brain function and behaviour. Furthermore, a growing body of evidence from human studies supports the hypothesis that some psychiatric disorders may have developmental origins. Here the focus is on three adverse consequences of early life stress: dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis, heightened anxiety behaviour and cognitive impairments, with review of what is known about the underlying central mechanisms.

Neuroactive steroids modulate neuronal activity and play a key role in neurodevelopment. Moreover they can negatively modulate activity of the HPA axis, exert anxiolytic actions and influence cognitive performance. Thus neuroactive steroids may provide a link between early life stress and the resultant adverse effects on the brain and behaviour. Here a role for neuroactive steroids, in particular the 5α-reduced/3α-hydroxylated metabolites of progesterone, testosterone and deoxycorticosterone, is discussed in the context of early life stress. Furthermore, the impact of early life stress on the brain's capacity to generate neurosteroids is considered and the evidence for an ability of neuroactive steroids to over-write the negative effects of early life stress on the brain and behaviour is examined. Enhanced understanding of the influence of early life stress on brain neurosteroid systems could aid the identification of new targets for developing treatments for stress-related conditions in humans.

INTRODUCTION

The perinatal period is a time of active neuroplasticity when the developing brain undergoes complex processes (e.g. neurogenesis, synaptogenesis, dendritic and axonal arborisation, programmed cell death and myelination) and as such brain development and neuronal organisation is particularly vulnerable to insults at this time. Insults such as stress during the perinatal period can detrimentally 'programme' the infant's brain leading to profound alterations in neuroanatomy, physiological and neuroendocrine function and behaviour in later life. The phenomenon of 'early-life programming' of the brain and behaviour is well established in rodents (1), and a growing body of evidence supports the idea that various childhood/adulthood disorders in humans have their origins in early life. For example, in women maternal stress exposure during pregnancy is associated with an increased incidence of neurodevelopmental disorders (e.g. attention deficit disorder, autism,

schizophrenia), affective disorders (e.g. anxiety, depression), cognitive deficits and emotional/behavioural problems in their children later in life (2). The neuroendocrine stress axis, the hypothalamo-pituitary-adrenal (HPA) axis is particularly vulnerable to early life programming by stress (1, 3) and the resultant HPA axis dysfunction may underpin psychiatric disorders and disrupted cognitive processing (4, 5).

Neuroactive steroids (endogenous steroids that exert rapid non-genomic effects on neuronal excitability) play a significant role in neurodevelopment in terms of neuroprotection and neuronal organisation: they promote neuronal survival and differentiation, myelinisation, dendritic growth and synaptogenesis (6-8). Hence, it is perhaps unsurprising that several neurodevelopmental disorders, mood disorders and cognitive decline have been associated with a perturbation in neurosteroid levels (9-16). Thus neurosteroids may provide a link between early life stress and adverse programming of the brain and behaviour.

Here a role for neuroactive steroids in the context of early life stress will be discussed, focussing on 3 adverse consequences of stress exposure during development: HPA axis dysregulation, increased anxiety and impaired cognitive ability. First these detrimental effects of early life stress and what is known about the central mechanisms involved are reviewed. Next the role of neuroactive steroids in modulating HPA axis function, anxiety behaviour and cognitive performance is considered before discussion of the impact of early life stress on neurosteroidogenesis and finally the potential for neuroactive steroids in counteracting the negative effects of early life stress on the brain and behaviour is examined.

EFFECTS OF EARLY LIFE STRESS ON THE BRAIN AND BEHAVIOUR

Much of the work investigating the effects of early life stress on the brain and behaviour has been performed in rodents. Numerous rodent models of prenatal stress exposure are described in the literature, though these typically involve exposing a pregnant animal to the same stressor (e.g. restraint) repeatedly (17) or to a variety of different stressors (e.g. cold, forced swimming, overcrowding, restraint) in an unpredictable fashion (18) during a specific period of gestation. Stress in the early post-natal period is frequently achieved by disrupting dam-pup interaction, for example by repeatedly separating pups from their mothers for a few hours per day during the first 2-3 weeks of life (19, 20). In rats maternal exposure to stress during pregnancy or maternal deprivation in early post-natal life is associated with heightened anxiety-like behaviours (17, 21-23), HPA axis

dysregulation (18, 21, 22, 24-26), impaired neural development (27), cognitive deficits (27-29) and aberrant social behaviours (30-33) in the offspring. In human studies, maternal stress and anxiety during pregnancy is also associated with impaired infant neurodevelopment, including delayed motor development, cognitive impairments, emotional problems, negative temperament and symptoms of attention deficit disorder (34-37).

HPA axis dysregulation

Enhanced or prolonged HPA axis responses to stress is a key feature in animals exposed to stress in early life, either pre- or post-natally (19, 20, 22, 25, 38). The central mechanisms underpinning HPA axis dysregulation appear to involve changes in both excitatory feed-forward and inhibitory feedback mechanisms (Fig. 1).

Excitatory inputs

Exaggerated adrenocorticotropic hormone (ACTH) and corticosterone responses to stress induced by early life stress are associated with marked up-regulation in corticotropin releasing hormone (CRH) mRNA expression in the parvocellular neurones of the paraventricular nucleus (PVN) (22, 39-41), indicative of increased excitatory input to the CRH neurones. Indeed enhanced excitatory glutamatergic drive to the CRH neurones in the PVN has recently been demonstrated in a mouse model of early life stress (39)(Fig. 1).

Inhibitory inputs

Glucocorticoid (GR) and mineralocorticoid receptors (MR) mediate negative feedback control of the HPA axis by glucocorticoids. Enhanced and prolonged HPA axis responses to stress are associated with reduced hippocampal expression of GR, MR or both receptors (22, 25, 38, 42, 43), indicating a possible impairment of glucocorticoid negative feedback (Fig. 1).

Insufficient inhibitory GABA input may also play a role. GABAergic neurones that project to the PVN modulate HPA axis activity, resulting in inhibition of the CRH neurones (via glutamatergic activation of PVN projecting GABAergic neurones) or activation of the CRH neurones (via GABAergic inhibition

of PVN projecting GABAergic neurones, i.e. disinhibition) (44). Prenatal stress results in a reduction in the density of parvalbumin-positive GABAergic interneurons in the medial prefrontal cortex and hippocampus (45, 46) (Fig. 1). Moreover, the number of $GABA_A$ receptors is significantly reduced in the hippocampus and the central amygdala in prenatally stressed offspring when compared with controls (47, 48) (Fig. 1). Whether these changes in GABAergic signaling underlie enhanced HPA axis responses to stress following early life stress remains to be determined, however it is interesting to note that a reduction in inhibitory GABA interneurones and/or GABA receptor expression is reported in several neuropsychiatric disorders such as schizophrenia, autism, anxiety and Tourette's syndrome and that these disorders have also been linked to prenatal stress exposure (49).

Anxiety behaviour

An anxiety-like phenotype is frequently observed in animals exposed to early life stress. This has been demonstrated by increased ultrasonic vocalisations in neonates (50), reduced social play during adolescence (51), reduced open arm entries on the elevated-plus maze test (22, 23, 52) and decreased exploration in an open field (17, 53, 54) (Fig. 1).

Anxious behaviours are organised by the amygdala and CRH is importantly involved in mediating anxiety responses (55, 56). CRH content is increased in the amygdala of prenatally stressed rodents (22, 57, 58), as is CRH release from amygdala homogenates (57) (Fig. 1). Increased anxiety-behaviour in adult prenatally stressed offspring is associated with enhanced CRH receptor binding in the amygdala (56) and can be attenuated by central administration of non-selective CRH receptor antagonists (56), indicating that altered CRH receptor expression is likely to be important in the expression of anxiety-like behaviours induced by early life stress. Indeed, studies using conditional forebrain CRH type 1 receptor (CRH-R1) knockout mice have further highlighted the importance of CRH-R1 in facilitating anxiogenic behavioural responses induced by early-life stress (59). Furthermore, increased anxiety-like behaviour in prenatally stressed rats is associated with increased expression of CRH-R1 mRNA in the central and basolateral nuclei of the amygdala (60), the PVN (21, 61) and more recently this has also been demonstrated in the amygdala of prenatally stressed pigs (62) (Fig. 1). Altered expression of the CRH type 2 receptor (CRH-R2) is also likely to influence anxiety behaviour. In contrast to CRH-R1, activation of CRH-R2 is considered to have anxiolytic actions (63, 64). In line with this, is the finding of reduced CRH-R2 expression in the amygdala of prenatally stressed rats that display an anxious phenotype (40, 60) (Fig. 1).

Altered glutamate neurotransmission has also been implicated in anxious behavioural responses. Heightened anxiety-like behaviour in prenatally stressed rats is correlated with reduced glutamate release in the ventral hippocampus (65), which can be reversed with anti-depressant treatment (66).

Cognitive impairments

Cognitive impairments observed following early life stress have been demonstrated in rodents using learning and memory tasks such as the Morris water maze, Barne's maze and the novel object recognition test (27, 28, 67-69). The hippocampus is highly susceptible to the programming effects of stress during the perinatal period and the resultant cognitive deficits are associated with alterations in hippocampal structure and function, including reduced neurogenesis (27, 70, 71), reduced brain-derived neurotrophic factor (BDNF) expression (72, 73), decreased long term potentiation (LTP) (74-76), altered synaptic plasticity e.g. reduced spine density, reduced dendritic length, dendritic atrophy and altered mossy fibre density in rodents (69, 76-81)(Fig. 1). Importantly the consequences of early life stress on cognitive performance and hippocampal function are long term and evidently persist throughout life (in contrast to the reversible effects of chronic stress on cognitive function in adulthood)(82). Moreover, many of these consequences are sex-specific (67, 68, 80, 81, 83, 84), potentially implicating a role for modulation by sex steroids, and are exacerbated by aging (53, 76).

It is not yet clear what 'factor' mediates these effects of early life stress on hippocampal structure and function and hence impaired cognitive performance, however CRH has been implicated. CRH expression in the PVN is markedly elevated in several models of early life stress (19, 39, 40, 85), but there is also evidence that CRH expression is augmented in the hippocampus in adult prenatally stressed rats (86) and in middle-aged rats exposed to stress (induced by fragmented maternal care) in early postnatal life (69) (Fig. 1). Type 1 CRH receptors are located on hippocampal neurones (87, 88) and CRH is known to mediate the effects of acute stress on hippocampal synaptic plasticity and cognitive performance (89, 90). Indeed, prolonged exposure to CRH reduces dendritic complexity in cultured hippocampal neurones (69) in a similar manner to that induced by early-life stress (76). Moreover central administration of CRH to rats in early life mimics the effects of early life stress in adulthood in terms of impaired memory and hippocampal cell loss and is associated with an up-regulation CRH and CRH-R1 gene expression in hippocampal pyramidal cells (91). Thus increased CRH action in the hippocampus may contribute to the central mechanisms underlying the effects of stress during early life on hippocampal structure and function. In support of this, treatment with a CRH-R1 antagonist shortly after stress exposure in early postnatal life has been demonstrated to

prevent the deficits in learning and memory, dendritic atrophy and suppressed LTP observed in later life (69). Furthermore, impairments in spatial learning and memory and the associated disrupted LTP and reduced hippocampal dendritic spine density, induced by early life stress in wild-type mice are not observed in conditional forebrain CRH-R1 knockout mice raised under the same conditions (92).

Additionally, given the critical role of the hippocampus in performing learning and memory tasks and the well described impact of corticosteroids on hippocampal-dependent learning (93), it is likely that reduced levels of hippocampal GR and/or MR in animals exposed to early life stress (22, 25, 38) may also contribute to alterations in cognition, though this requires further investigation.

NEUROACTIVE STEROIDS

The term 'neuroactive steroid' refers to active metabolites of classical steroid hormones that independent of their origin (i.e. those produced in the brain or in the periphery) have rapid membrane actions on neuronal excitability. The brain can produce these neuroactive steroids and when synthesised centrally they are frequently referred to as 'neurosteroids' (94) (95). Production of neuroactive steroids in the brain (i.e. neurosteroids) is dependent upon the expression of the relevant enzymes, which can show important regional differences (96). Amongst the most extensively studied neuroactive steroids are the progesterone metabolite, allopregnanolone (3α-hydroxy-5α-pregnan-20-one, also 3α,5α-tetrahydroprogesterone) and 5α,3α-tetrahydrodeoxycorticosterone (THDOC) a metabolite of deoxycorticosterone (DOC). For allopregnanolone synthesis, progesterone is first converted into dihydroprogesterone (DHP; 20α-hydroxy-4-pregnen-3-one) by 5α-reductase (the rate limiting enzyme), which in turn is converted into allopregnanolone by the actions of 3α-hydroxysteroid dehydrogenase (3αHSD) (Fig. 2). THDOC is synthesised from the adrenal steroid DOC (via the intermediate 21-hydroxy-5α-pregnane-3,20-dione; 5α-DHDOC) by the action of the same two enzymes (Fig. 2). Both of these enzymes are expressed in the brain by astrocytes and oligodendrocytes (97-99), and 5α-reductase activity is also evident in neurones (100). While the brain is capable of synthesising progesterone from pregnenolone and subsequently reducing progesterone to allopregnanolone (via DHP), DOC is formed from progesterone in the adrenal cortex, but not in the brain (97, 98, 101-103). Nonetheless DOC can be converted to THDOC in the brain via the actions of 5α-reductase and 3α-HSD (98, 104). 5α-reductase also converts testosterone into the more potent androgen, dihydrotestosterone (DHT), which in turn can be converted into 3α-androstandiol (5α-androstane-3α,17β-diol; hereafter 3α-diol) by 3αHSD or 3β-androstandiol (5α-androstane-3β,17β-diol; hereafter 3β-diol) by the actions of

3αHSD, 3βHSD and 17βHSD (105, 106) (Fig. 2), including in the brain (107, 108). In contrast to 3α-diol, the formation of 3β-diol is irreversible (106).

Neuroactive steroids and neuromodulation

Neuroactive steroids can rapidly alter neuronal excitability by binding to membrane bound ion channel-linked receptors (95). The $GABA_A$ receptor is the principal mediator of GABAergic neurotransmission in the central nervous system (CNS). $GABA_A$ receptors are ligand-gated chloride channels comprised of five subunits, with 19 different subunit types having been identified to date. The assembly of 5 subunits to form $GABA_A$ receptors results in a complex heterogeneity in their structure (which determines regional expression in the CNS and their pharmacological profile), however the most common type in the brain is a pentamer consisting of two α subunits, two β subunits and either a γ or δ subunit. When activated by GABA, the channel opens, permitting chloride ion influx and thus hyperpolarisation of the cell membrane.

Allopregnanolone, THDOC and 3α-diol are potent positive modulators of $GABA_A$ receptors; they augment the inhibitory actions of GABA by prolonging the opening time of chloride ion channels within $GABA_A$ receptors (109-111). Subunit composition confers sensitivity of the $GABA_A$ receptor to modulation by neuroactive steroids, with allopregnanolone having greater efficacy at $GABA_A$ receptors containing the δ subunit (112). Thus, neuroactive steroids can modulate neuronal activity by binding to neurotransmitter receptors, hence influencing brain function and behaviour. In contrast to 3α-diol, 3β-diol does not enhance GABA action (113) and instead it can serve as an estrogen receptor-β (ERβ) agonist in the brain (114). Despite different mechanisms of action all of the aforementioned neuroactive steroids are able to modulate neuronal activity. The next section focusses on the role of neuroactive steroids in modulating the HPA axis, anxiety behaviour and cognition.

Role in modulating HPA axis function

Acute stress results in an increase in allopregnanolone and THDOC levels in the blood and brain which negatively modulates HPA axis activity, facilitating termination of the stress response and restoring physiological homeostasis (101). Administration of allopregnanolone has been shown to attenuate stress-induced HPA axis activity in male (115) and female rats (116), to attenuate

stimulated CRH release from hypothalamic explants and to prevent adrenalectomy-induced up-regulation of CRH gene expression in the PVN (117). Similarly THDOC administration attenuates the stress-induced increase in corticosterone secretion (118). In male rats, the suppressive actions of testosterone on stress-induced HPA axis activity are mediated by the 5α-reduced metabolite of testosterone, DHT and its metabolite, 3β-diol (114, 119, 120). Moreover, treatment with the 5α-reductase inhibitor, finasteride results in enhanced/prolonged ACTH and corticosterone responses to acute stress (116, 120).

However, central levels of allopregnanolone and 5α-reductase activity are reduced following chronic stress exposure (121-124). In mice, chronic social isolation leads to a dramatic reduction in 5α-reductase 1 mRNA expression in glutamatergic neurones in the hippocampus, basolateral amygdala and the medial prefrontal cortex (124). Interestingly, in humans a similar reduction is observed in the prefrontal cortex of depressed patients (125). In rats, chronic social isolation also results in reduced circulating and hippocampal levels of allopregnanolone and is associated with a depressive-/anxiety-like phenotype and reduced glucocorticoid feedback sensitivity of the HPA axis (126). Importantly, these effects can be prevented if allopregnanolone is administered from the onset of the stress period or can be reversed when allopregnanolone is administered chronically following cessation of the stress exposure (126).

Role in modulating anxiety behaviour

Down-regulation of neuroactive steroid production has a potential causal role in affective disorders, such as anxiety and depression. Reduced neuroactive steroid levels, particularly of allopregnanolone, have been reported in the blood and cerebrospinal fluid of patients with anxiety disorders, depression (major depression and post-partum depression), post-traumatic stress disorder and schizophrenia (9, 125, 127-131).

5α-reduced/3α-hydroxylated neuroactive steroids such as allopregnanolone, THDOC and 3α-diol have potent anxiolytic properties, in keeping with their ability to act as positive allosteric modulators at the $GABA_A$ receptor. Allopregnanolone, THDOC and 3α-diol have been demonstrated to reduce anxiety-like behaviours in rodent tests of anxiety including the elevated plus maze (132-136), light-dark box (134, 136, 137), defensive freezing task (138) and reduce ultrasonic vocalisations in neonates in response to maternal separation (139, 140). Moreover allopregnanolone blocks CRH-induced anxiogenic behaviour (117). The anxiolytic effects of allopregnanolone can be blocked with

a $GABA_A$ receptor chloride channel blocker (132) indicating that the anxiolytic effects of allopregnanolone are mediated via $GABA_A$ receptors. Furthermore, studies where allopregnanolone has been directly infused into the amygdala or the medial prefrontal cortex, implicate these brain regions as potential sites for anxiolytic actions of allopregnanolone (141, 142).

Role in cognition

Cognitive processing is also influenced by neuroactive steroids, though there are differing reports in the rodent literature on the direction of the effects. For example, allopregnanolone has been demonstrated to improve memory performance in the novel object recognition test (143) and Morris water maze (144) in female rats, whereas other groups have reported allopregnanolone impairs spatial memory in male rats (145, 146). The neuroactive metabolite of testosterone, 3α-diol has also been reported to enhance cognition in rats and mice (136) and 3β-diol improves performance in the Morris water maze (147).

Reasons for the discrepancies may relate to the different dose and drug administration regimes, the age and sex of the animals, the behavioural tests used and the sedative/hypnotic/anaesthetic effects of neuroactive steroids that are positive modulators at $GABA_A$ receptors (148). It is important to note that rodent tests of cognition should be interpreted with caution, especially in the case of neuroactive steroid effects, since many tests rely on an aversive or stress-invoking component. For example, the water maze, commonly used to assess spatial memory involves forced swimming; a robust stressor in rodents (149, 150). The test relies on the animals finding the water 'aversive' and/or being sufficiently 'stressed' in order to motivate them to escape onto the hidden platform. Given the anxiolytic and stress suppressive actions of allopregnanolone (discussed above), one can envisage how findings from this sort of behavioural test could be skewed i.e. an increased latency to find the escape platform may not necessarily represent memory impairment, but rather may result from a reduced motivation to escape if the test is perceived as being less anxiogenic/stressful.

Nevertheless, neuroactive steroids in particular allopregnanolone, have established neuroprotective actions in models of injury or disease (151). For example, in a transgenic mouse model of Alzheimer's disease (where allopregnanolone concentrations in the cerebral cortex are markedly lower than in wildtype mice), allopregnanolone treatment has been shown to promote neurogenesis and to reverse learning and memory deficits (152), implicating allopregnanolone as a potential therapeutic for cognitive deficits.

Effects of early life stress on neurosteroidogenesis and neuroactive steroid actions in the offspring

Given the critical role neuroactive steroids play in neurodevelopment, disruption of neurosteroidogenesis during pregnancy/early post-natal life is potentially damaging to the brain and may lead to altered development of the systems that regulate stress responses, mood, behaviour and cognitive function. In this section the influence of stress exposure in early life on neurosteroidogenesis in the brain of the fetus/neonate will be considered and the evidence for these effects persisting into later life will be discussed.

Neurosteroidogenesis in the placenta and fetal brain

There is evidence that prolonged exposure to elevated glucocorticoid levels or exposure to stress during pregnancy may reduce the capacity of the fetal brain to synthesise neurosteroids. Exposing pregnant rats to immobilisation stress on days 15-18 of pregnancy decreases 5α-reductase activity in the cerebral cortex and hypothalamus of the male fetuses on embryonic day 19 (153) and repeated betamethasone administration during gestation in the guinea pig results in reduced 5α-reductase type 2 expression in the placenta with a concomitant reduction in the fetal hippocampus (154). Moreover, in rats, dams exposed to stress in late pregnancy have lower levels of circulating allopregnanolone at birth and this predicts reduced allopregnanolone production in the brains of the offspring in post-natal life (28). These data suggest that exposure to elevated glucocorticoids as a result of stress, influences the neurosteroidogenic capacity of the placenta and the fetal brain.

Neurosteroidogenesis in the neonate, juvenile, adult

Reduced capacity for neurosteroidogenesis in the fetal brain as a result of prenatal stress exposure appears to persist into post-natal life. In rats, the male and female juvenile offspring of mothers exposed to either repeated restraint or chronic variable stressors during late pregnancy display reduced conversion of progesterone into its 5α-reduced metabolites in the medial prefrontal cortex compared with control offspring; an effect which is associated with impaired cognitive development and reduced dendritic spine density in the dorsal hippocampus (28, 79). Similar cognitive deficits are observed in the offspring of mothers who were treated with finasteride (a 5α-reductase inhibitor which blocks neuroactive steroid generation) in late pregnancy (155). Moreover, progesterone utilisation (the ratio of allopregnanolone and DHP to progesterone) is also markedly reduced in the

hippocampus of female juveniles born to mothers exposed to immune challenge in late pregnancy (29). Reduced DHP production (indicating reduced 5α-reductase activity) is also seen in the brains of neonates exposed to maternal separation and social isolation stress in early postnatal life (156). In adult males prenatal stress is associated with reduced levels of dihydrotestosterone (DHT) and 3α-diol (5α-reduced metabolites of testosterone) in the hippocampus (157) and with increased corticosterone responses to acute stress, anxiogenic behaviour and reduced social interaction (157).

The findings of reduced 5α-reduced metabolites in the brains of animals exposed to early life stress (be it pre- or post-natally) indicate reduced expression and/or activity of 5α-reductase. Indeed, we have recently demonstrated that in adult male rats, prenatal stress is associated with reduced 5α-reductase mRNA expression in the PVN and nucleus tractus solitarii (NTS) (158), whereas in adult females 5α-reductase mRNA expression is down-regulated compared with control females only in the NTS. The reason for the sex difference is not clear, though this may result from differences in circulating androgens (159-161). Indeed, hepatic 5α-reductase activity is programmed during development by testosterone levels: castration increases while testosterone administration reduces hepatic 5α-reductase activity (162). In accordance, circulating testosterone levels are increased in adult male rats whose mothers were exposed to social stress in late pregnancy (158) and there is a concomitant reduction in 5α-reductase mRNA expression in the liver (163). Moreover, testosterone levels are significantly greater in the adolescent children born to women who were exposed to stress (associated with the Chernobyl disaster) from the second trimester of pregnancy onwards, indicating prenatal programming of testosterone levels in humans (164). Thus, elevated circulating testosterone levels in prenatally stressed males may contribute to reduced 5α-reductase expression in the liver and/or brain. Moreover, reduced 5α-reductase activity in the periphery could potentially also contribute to reduced neuroactive steroid levels in the brain.

In juveniles, social isolation rearing post-weaning has also been shown to reduce expression of 5α-reductase isoforms 1 and 2 in the nucleus accumbens and medial prefrontal cortex and is associated with reduced allopregnanolone and THDOC levels in the frontal cortex of male rats (165). Human studies further indicate that reduced 5α-reductase activity may contribute to some of the negative phenotypes observed in individuals exposed to stress in early life. 5α-reductase type 1 activity is markedly reduced in adult survivors of the World War II Holocaust. Intriguingly the largest reductions in 5α-activity are observed in individuals who were youngest at the time of the Holocaust (166), highlighting a potential developmental 'programming' window. Moreover there is evidence for intergenerational transmission of the adverse effects of Holocaust exposure from survivors to their offspring which involves epigenetic mechanisms (167, 168). Whether epigenetic mechanisms

might explain reduced 5α-reductase gene expression following early life stress requires further study, however altered DNA methylation has been demonstrated for other genes (e.g. GR, CRH, CRH-R1) in rodent models of early life stress (19, 58, 61), those exploiting natural variations in maternal care (169), and in humans in the offspring of parents with post-traumatic stress disorder (168).

Early life stress evidently also interferes with neuroactive steroid action in the brain. Gunn and colleagues have elegantly demonstrated that early life stress (using a mouse model of fragmented maternal care) enhances excitatory glutamatergic drive to CRH-expressing neurones in the PVN of neonates and is associated with increased CRH expression in the PVN (39). Moreover, they have shown that while allopregnanolone potently supresses CRH neuronal firing in controls, the same treatment is ineffective on these neurones in hypothalamic slices from neonatal mice exposed to early life stress (39). Importantly, this neuroactive steroid insensitivity is not a result of allopregnanolone becoming less effective in modulating $GABA_A$ receptor function, but rather is a consequence of the increased glutamatergic drive onto the PVN CRH neurones (39). A similar finding is observed in the neonatal offspring of $GABA_A$ receptor δ-subunit knockout mice (which also display abnormal maternal care) (39) indicating that modulation of neuronal activity by neuroactive steroids during the neonatal period may be critical for normal development of the HPA axis (and hence for 'normal' HPA axis responses in later life) as has been demonstrated for the normal development of GABAergic systems in the prefrontal cortex (170).

A role for neuroactive steroids in counter-acting the adverse effects of early life stress

Neuroendocrine stress responses and anxiety-related behaviour

Given the role of neuroactive steroids in modulating neuroendocrine stress responses, anxiety behaviour and cognitive function (discussed above) as well as the findings that stress during early pre- or post-natal life alters neurosteroidogenesis, it can be hypothesised that altered neuroactive steroid production and/or action may underpin some of the adverse effects of early life stress described earlier and thus neuroactive steroid administration may counteract or reverse some of the adverse effects associated with early life stress.

Studies in the 1990's, first demonstrated a role for neuroactive steroids in reversing or preventing some of the neuroendocrine and behavioural consequences of stress exposure in early life.

Allopregnanolone treatment was shown to significantly reduce maternal separation-induced ultrasonic vocalisations (an indicator of anxiety) in neonates (140), with allopregnanolone's effect mediated via $GABA_A$ receptors (139). Moreover, the increased anxiety-like behaviour observed in adult prenatally stressed offspring can be prevented if pregnant dams are administered allopregnanolone in parallel with the stress exposure during the last week of gestation (171). Administration of THDOC, another 5α-reduced metabolite and a potent positive allosteric modulator of the $GABA_A$ receptor, during early postnatal life abolishes the adverse behavioural and neuroendocrine effects induced by repeated maternal separation in early life that are observed in adulthood, such as increased anxiety, enhanced HPA axis responses to stress and impaired glucocorticoid feedback (172). Together, these data indicate that during development, neuroactive steroids that act as positive modulators at the $GABA_A$ receptor may have stress-protective actions in the brain.

More recently it has been demonstrated that peripheral administration of allopregnanolone over a period of 20h is sufficient to normalise ACTH secretory responses to an acute physical stressor in adult female rats born to mothers exposed to repeated social stress during pregnancy (158) (Fig. 3a). Moreover, adenovirus-mediated gene transfer to up-regulate expression of 5α-reductase and 3α-HSD in the NTS also normalises HPA axis responses in female PNS rats (158). Notably, peripheral allopregnanolone treatment is ineffective in the prenatally stressed male siblings which also display hyperactive HPA axis responses to acute stress (Fig. 3b). However, short-term treatment with 3β-diol, a metabolite of testosterone, does reverse the enhanced HPA axis responses to stress in PNS males, measured as significant reductions in ACTH (Fig. 3c) and corticosterone secretion and in CRH mRNA expression in the PVN (158) and reduces anxiety-like behaviour on the elevated plus maze (Donadio, Russell & Brunton, unpubl.). Testosterone replacement also normalises behaviour in the open field and pre-pulse inhibition responses (deficits of which are seen in some neurodevelopmental disorders such as schizophrenia and attention deficit disorder) in adult prenatally stressed guinea pigs, as well as having a tendency for reversing elevated ACTH secretion under basal conditions (173). Whether this is the result of a direct action of testosterone or an indirect action via one of its metabolites, e.g. DHT, 3α-diol or 3β-diol, is not known.

The mechanisms through which neuroactive steroids normalise neuroendocrine responses to stress in animals exposed to early life stress remain to be elucidated. As described above, the potent action of allopregnanolone on $GABA_A$ receptors in supressing HPA axis activity (44), indicates $GABA_A$ receptors as a likely target. In contrast to allopregnanolone, 3β-diol evidently exerts its effect on the HPA axis via estrogen receptor-β (114). ERβ is expressed in the PVN (including in CRH neurones) and

NTS (174, 175), indicating there is the potential for either direct or indirect modulation of HPA axis activity by 3β-diol. Indeed, agonists selective for ERβ have anxiolytic and anti-depressive actions and attenuate swim stress-induced corticosterone secretion in rats (176).

Cognitive deficits

Neuroactive steroids, in particular allopregnanolone, are known to have neuroprotective actions (151). For example, allopregnanolone reduces cell death and cognitive impairments that result from brain injury or cerebral ischaemia (177, 178) and reverse cognitive deficits in a mouse model of Alzheimer's disease (152). Whether neuroactive steroid treatment also reverses cognitive impairments associated with early life stress, as has been shown for HPA axis dysfunction and anxiety behaviour (158, 172), remains to be determined.

SUMMARY AND OUTLOOK

Animal studies clearly demonstrate that exposure to stress during early life programmes the brain and subsequent behaviour. HPA axis dysregulation is a common feature of the 'programmed' phenotype and may contribute to heightened anxiety behaviour and cognitive deficits. Similarly, stress exposure during development in humans seemingly increases the propensity for psychiatric disorders and cognitive impairments.

Neuroactive steroids play a critical role in brain development and can modulate HPA axis activity and influence anxiety behaviour and cognitive performance. There is growing evidence that exposure to stress in early life reduces the capacity of the brain for neurosteroidogenesis and may also alter the ability of neuroactive steroids to exert their actions (Fig. 4). Whether altered neuroactive steroid sensitivity results from variations in the number (47), the subunit composition (179) or the phosphorylation status of the $GABA_A$ receptors (180) remains to be elucidated. Moreover, the mechanisms underlying the reduction in the brains ability to generate neurosteroids (e.g. by down-regulation of 5α-reductase gene expression) also requires further study; whether this is involves epigenetic mechanisms or results from increased exposure to androgens during critical periods of brain development is not yet known. Nonetheless, neuroactive steroids can counteract some of the adverse effects of early life stress exposure, such as HPA axis dysregulation and heightened anxiety behaviour (Fig. 3, 4); however, there are sex differences in the underlying central mechanisms.

The possibility that the adverse effects of early life stress may be reversed by manipulating neuroactive steroids is a promising proposition that warrants further research and may have important implications for the development of new treatments for human stress-related conditions, which could be tailored according to gender.

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FIGURE LEGENDS

Figure 1: Summary of the consequences of early stress and the possible central mechanisms involved

Early life stress (pre- or post-natal) is frequently associated with dysregulation of the HPA axis, increased anxiety-like behaviours and impaired cognitive function. Examples of indicators of these adverse phenotypes are given together with a summary of the neural correlates and potential central mechanisms involved. Abbreviations/symbols: ↑ indicates increased/enhanced and ↓ indicates decreased/supressed compared with control animals; CRH, corticotropin releasing hormone; CRH-R1, CRH receptor type 1; CRH-R2, CRH receptor type 2; EPM, elevated plus maze; GC, glucocorticoid; hippo, hippocampus; HPA, hypothalamo-pituitary-adrenal; LTP, long term potentiation; MWM, Morris water maze.

Figure 2: Neuroactive steroid biosynthetic pathways

The enzymes and intermediates involved in the synthesis of allopregnanolone, 3α-diol, 3β-diol and tetrahydrodeoxycorticosterone (THDOC) from steroid precursors. Production of neuroactive steroids in specific tissues is dependent upon the expression of the relevant enzymes. Common names are in bold with chemical names beneath. Enzymes are in red italics. Dashed arrows indicate a reversible reaction. Steroids in filled black boxes indicate those which act as positive allosteric modulators at $GABA_A$ receptors. 3β-diol exerts its actions via estrogen receptor-β. Abbreviations: 5αR, 5α-reductase; HSD, hydroxysteroid dehydrogenase.

Figure 3: Effect of allopregnanolone or 3β-diol pretreatment on ACTH responses to IL-1β in control and prenatally stressed (PNS) rats

Rats were pre-treated, 20h and 2h before IL-1β (500 ng/kg i.v.), with either vehicle (oil), allopregnanolone (AP: 3 mg/kg and 1 mg/kg s.c.) or 3β-diol (1 mg/kg s.c.). Increase in plasma ACTH concentrations from basal levels in: **a)** control and PNS females treated with and without AP; **b)** control and PNS males treated with and without AP; **c)** control and PNS males treated with and without 3β-diol. #$p<0.05$ versus control/oil group; *$p<0.05$ versus respective oil-treated group (two-way ANOVA). In each case values are group means + SEM. AP significantly reduced the ACTH response to IL-1β in control and PNS females (a), but had no such effect in male rats (b). However, 3β-diol did normalise the ACTH response to IL-1β in PNS male rats (c). Based on data from (158).

Figure 4: Early life stress and neuroactive steroids

Stress in early life results in a persistent reduction in 5α-reductase (5αR) activity in the brain and liver, leading to a reduction in central neuroactive steroid levels (e.g. allopregnanolone, AP; dihydroprogesterone, DHP; dihydrotestosterone, DHT; 3α-androstandiol, 3α-diol; 3β-androstandiol, 3β-diol). This is associated with dysregulation of the HPA axis, heightened anxiety-like behaviour and impaired cognitive ability. Some of these adverse phenotypes can be reversed with neuroactive steroid treatment/replacement. There is also evidence that early life stress leads to a reduction in the central expression of the targets for neuroactive steroid action e.g. $GABA_A$ receptor ($GABA_A$-R) and estrogen receptor-β (ERβ) and that AP is less effective in suppressing corticotropin releasing hormones (CRH) neuronal activity. ↑ indicates increased and ↓ indicates decreased.

Figure 1

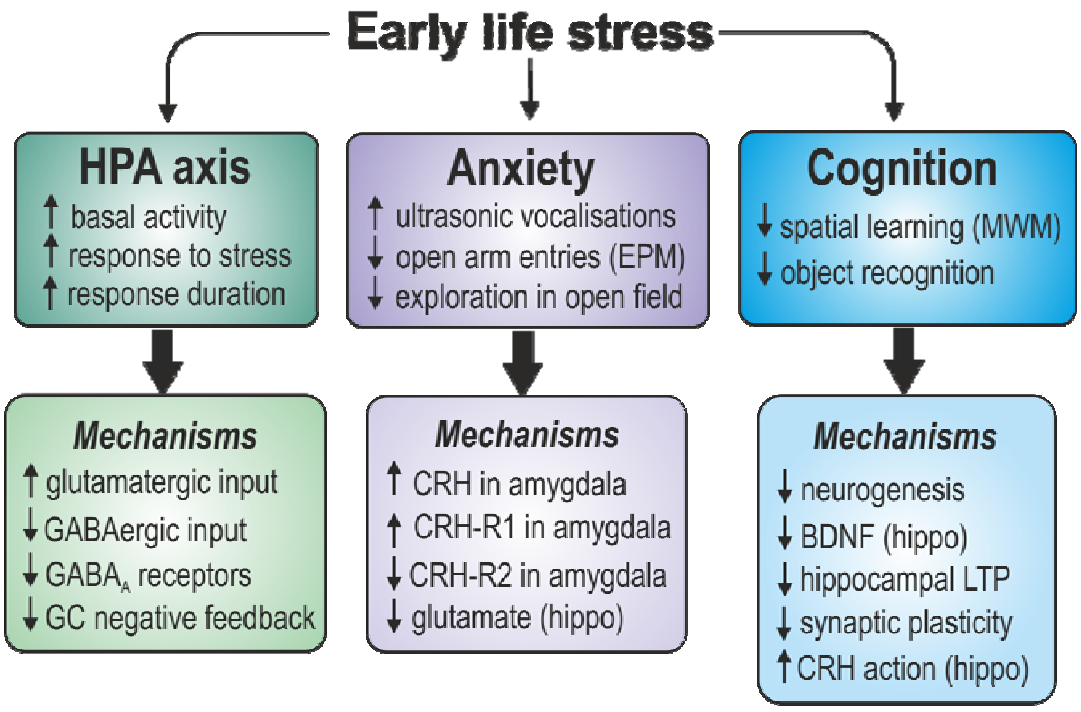

Figure 2

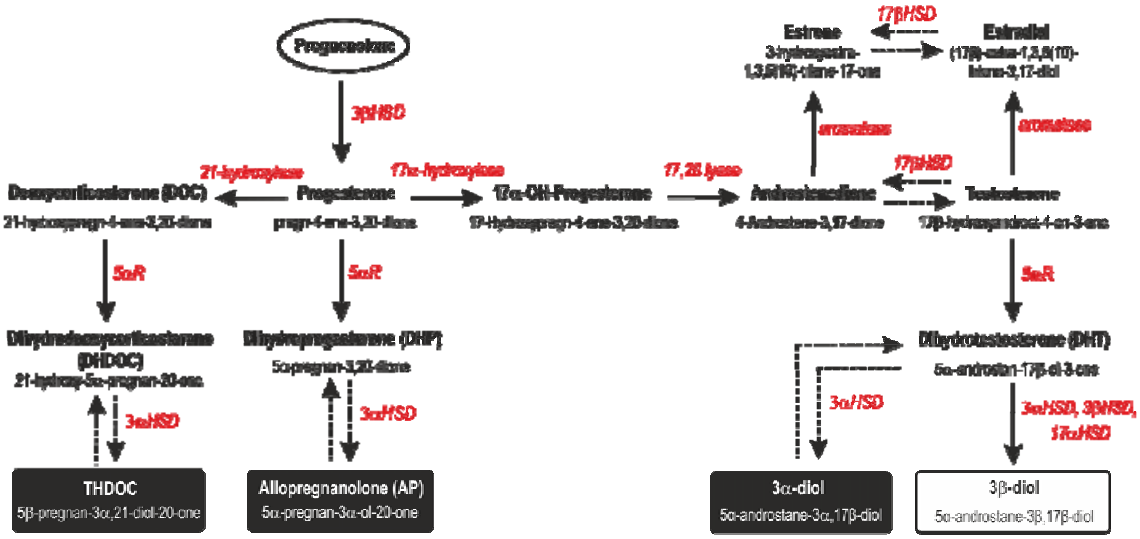

Figure 3

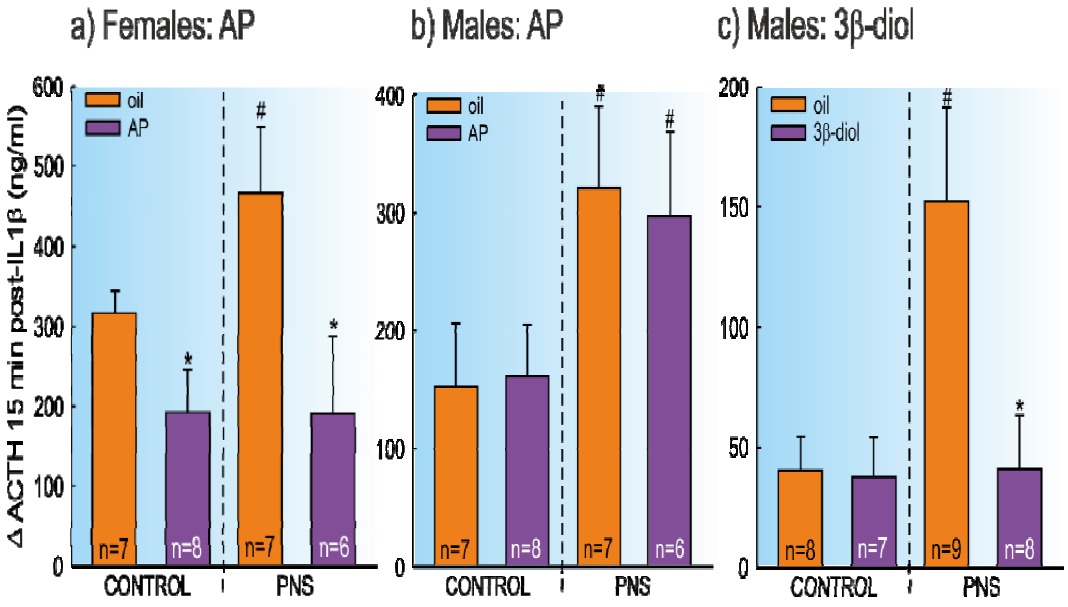

Figure 4