THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

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

Citation for published version:

Brunton, P 2015, 'Programming the brain and behaviour by early life stress: A focus on neuroactive steroids.', *Journal of Neuroendocrinology*, vol. 27, no. 6, pp. 468–480. <https://doi.org/10.1111/jne.12265>

Digital Object Identifier (DOI):

[10.1111/jne.12265](https://doi.org/10.1111/jne.12265)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Journal of Neuroendocrinology

Publisher Rights Statement:

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jne.12265

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Article Type: Review Article

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

Paula J Brunton

Division of Neurobiology, The Roslin Institute & R(D)SVS, University of Edinburgh, Scotland, UK.

Address for correspondence:

Dr. Paula J Brunton, Division of Neurobiology, The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, Scotland, U.K.

Email: p.j.brunton@ed.ac.uk

Telephone: +44 (0) 131 651 9129

Fax: +44 (0) 131 651 9105

Short title: Early life stress and neuroactive steroids

Keywords: 5 α -reductase, allopregnanolone, GABA_A receptor, maternal separation, neurosteroids, prenatal stress.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jne.12265

This article is protected by copyright. All rights reserved.

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 5 α -reduced/3 α -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,

Accepted Article

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, myelination, 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 adrenocorticotrophic 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 interneurons 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 (3 α -hydroxy-5 α -pregnan-20-one, also 3 α ,5 α -tetrahydroprogesterone) and 5 α ,3 α -tetrahydrodeoxycorticosterone (THDOC) a metabolite of deoxycorticosterone (DOC). For allopregnanolone synthesis, progesterone is first converted into dihydroprogesterone (DHP; 20 α -hydroxy-4-pregnen-3-one) by 5 α -reductase (the rate limiting enzyme), which in turn is converted into allopregnanolone by the actions of 3 α -hydroxysteroid dehydrogenase (3 α HSD) (Fig. 2). THDOC is synthesised from the adrenal steroid DOC (via the intermediate 21-hydroxy-5 α -pregnane-3,20-dione; 5 α -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 5 α -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 5 α -reductase and 3 α -HSD (98, 104). 5 α -reductase also converts testosterone into the more potent androgen, dihydrotestosterone (DHT), which in turn can be converted into 3 α -androstadiol (5 α -androstane-3 α ,17 β -diol; hereafter 3 α -diol) by 3 α HSD or 3 β -androstadiol (5 α -androstane-3 β ,17 β -diol; hereafter 3 β -diol) by the actions of

3 α HSD, 3 β HSD and 17 β HSD (105, 106) (Fig. 2), including in the brain (107, 108). In contrast to 3 α -diol, the formation of 3 β -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 3 α -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 3 α -diol, 3 β -diol does not enhance GABA action (113) and instead it can serve as an estrogen receptor- β (ER β) 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

Accepted Article

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 5 α -reduced metabolite of testosterone, DHT and its metabolite, 3 β -diol (114, 119, 120). Moreover, treatment with the 5 α -reductase inhibitor, finasteride results in enhanced/prolonged ACTH and corticosterone responses to acute stress (116, 120).

However, central levels of allopregnanolone and 5 α -reductase activity are reduced following chronic stress exposure (121-124). In mice, chronic social isolation leads to a dramatic reduction in 5 α -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).

5 α -reduced/3 α -hydroxylated neuroactive steroids such as allopregnanolone, THDOC and 3 α -diol have potent anxiolytic properties, in keeping with their ability to act as positive allosteric modulators at the GABA_A receptor. Allopregnanolone, THDOC and 3 α -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, 3 α -diol has also been reported to enhance cognition in rats and mice (136) and 3 β -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 5 α -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 5 α -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 5 α -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 5 α -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 5 α -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 3 α -diol (5 α -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 5 α -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 5 α -reductase. Indeed, we have recently demonstrated that in adult male rats, prenatal stress is associated with reduced 5 α -reductase mRNA expression in the PVN and nucleus tractus solitarius (NTS) (158), whereas in adult females 5 α -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 5 α -reductase activity is programmed during development by testosterone levels: castration increases while testosterone administration reduces hepatic 5 α -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 5 α -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 5 α -reductase expression in the liver and/or brain. Moreover, reduced 5 α -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 5 α -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 5 α -reductase activity may contribute to some of the negative phenotypes observed in individuals exposed to stress in early life. 5 α -reductase type 1 activity is markedly reduced in adult survivors of the World War II Holocaust. Intriguingly the largest reductions in 5 α -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 5 α -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 suppresses 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 5 α -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 5 α -reductase and 3 α -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 3 β -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, 3 α -diol or 3 β -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 suppressing HPA axis activity (44), indicates GABA_A receptors as a likely target. In contrast to allopregnanolone, 3 β -diol evidently exerts its effect on the HPA axis via estrogen receptor- β (114). ER β 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 ER β 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 brain's ability to generate neurosteroids (e.g. by down-regulation of 5 α -reductase gene expression) also requires further study; whether this 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.

ACKNOWLEDGEMENTS

The author would like to thank Prof. John Russell and Ms. Natalia Grundwald for critically reviewing this manuscript. PJB receives Institute Strategic Funding from the Biotechnology and Biological Sciences Research Council (BBSRC).

REFERENCES

1. Maccari S, Krugers HJ, Morley-Fletcher S, Szyf M, Brunton PJ. The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. *J Neuroendocrinol* 2014; **26**: 707-723.
2. O'Donnell K, O'Connor TG, Glover V. Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta. *Dev Neurosci* 2009; **31**: 285-292.
3. Darnaudery M, Maccari S. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. *Brain Res Rev* 2008; **57**: 571-585.
4. Wingenfeld K, Wolf OT. HPA axis alterations in mental disorders: impact on memory and its relevance for therapeutic interventions. *CNS Neurosci Ther* 2011; **17**: 714-722.
5. Bradley AJ, Dinan TG. A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality. *J Psychopharmacol* 2010; **24**: 91-118.
6. Marx CE, Jarskog LF, Lauder JM, Gilmore JH, Lieberman JA, Morrow AL. Neurosteroid modulation of embryonic neuronal survival in vitro following anoxia. *Brain Res* 2000; **871**: 104-112.
7. Guennoun R, Labombarda F, Gonzalez Deniselle MC, Liere P, De Nicola AF, Schumacher M. Progesterone and allopregnanolone in the central nervous system: Response to injury and implication for neuroprotection. *J Steroid Biochem Mol Biol* 2014; **146C**:48-61.
8. Tsutsui K, Ukena K, Sakamoto H, Okuyama S, Haraguchi S. Biosynthesis, mode of action, and functional significance of neurosteroids in the purkinje cell. *Front Endocrinol* 2011; **2**: 61.

- Accepted Article
9. Rasmusson AM, Pinna G, Paliwal P, Weisman D, Gottschalk C, Charney D, Krystal J, Guidotti A. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. *Biol Psychiatry* 2006; **60**: 704-713.
 10. Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. *Psychopharmacology (Berl)* 2006; **186**: 362-372.
 11. Schumacher M, Weill-Engerer S, Liere P, Robert F, Franklin RJ, Garcia-Segura LM, Lambert JJ, Mayo W, Melcangi RC, Parducz A, Suter U, Carelli C, Baulieu EE, Akwa Y. Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog Neurobiol* 2003; **71**: 3-29.
 12. Hellgren C, Akerud H, Skalkidou A, Backstrom T, Sundstrom-Poromaa I. Low serum allopregnanolone is associated with symptoms of depression in late pregnancy. *Neuropsychobiology* 2014; **69**: 147-153.
 13. Schule C, Nothdurfter C, Rupprecht R. The role of allopregnanolone in depression and anxiety. *Prog Neurobiol* 2014; **113**: 79-87.
 14. Strous RD, Golubchik P, Maayan R, Mozes T, Tuati-Werner D, Weizman A, Spivak B. Lowered DHEA-S plasma levels in adult individuals with autistic disorder. *Eur Neuropsychopharmacol* 2005; **15**: 305-309.
 15. Strous RD, Spivak B, Yoran-Hegesh R, Maayan R, Averbuch E, Kotler M, Mester R, Weizman A. Analysis of neurosteroid levels in attention deficit hyperactivity disorder. *Int J Neuropsychopharmacol* 2001; **4**: 259-264.
 16. MacKenzie EM, Odontiadis J, Le Melledo JM, Prior TI, Baker GB. The relevance of neuroactive steroids in schizophrenia, depression, and anxiety disorders. *Cell Mol Neurobiol* 2007; **27**: 541-574.
 17. Vallee M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *J Neurosci* 1997; **17**: 2626-2636.
 18. Koenig JI, Elmer GI, Shepard PD, Lee PR, Mayo C, Joy B, Hercher E, Brady DL. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. *Behav Brain Res* 2005; **156**: 251-261.
 19. Chen J, Evans AN, Liu Y, Honda M, Saavedra JM, Aguilera G. Maternal deprivation in rats is associated with corticotrophin-releasing hormone (CRH) promoter hypomethylation and

- enhances CRH transcriptional responses to stress in adulthood. *J Neuroendocrinol* 2012; **24**: 1055-1064.
20. Aisa B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ. Effects of maternal separation on hypothalamic-pituitary-adrenal responses, cognition and vulnerability to stress in adult female rats. *Neuroscience* 2008; **154**: 1218-1226.
21. Fan JM, Chen XQ, Jin H, Du JZ. Gestational hypoxia alone or combined with restraint sensitizes the hypothalamic-pituitary-adrenal axis and induces anxiety-like behavior in adult male rat offspring. *Neuroscience* 2009; **159**: 1363-1373.
22. Brunton PJ, Russell JA. Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: sex specific effects. *J Neuroendocrinol* 2010; **22**: 258-271.
23. Fride E, Weinstock M. Prenatal stress increases anxiety related behavior and alters cerebral lateralization of dopamine activity. *Life Sci* 1988; **42**: 1059-1065.
24. Takahashi LK, Kalin NH. Early developmental and temporal characteristics of stress-induced secretion of pituitary-adrenal hormones in prenatally stressed rat pups. *Brain Res* 1991; **558**: 75-78.
25. Weinstock M, Matlina E, Maor GI, Rosen H, McEwen BS. Prenatal stress selectivity alters the reactivity of the hypothalamic-pituitary adrenal system in the female rat. *Brain Res* 1992; **595**: 195-200.
26. McCormick CM, Smythe JW, Sharma S, Meaney MJ. Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Dev Brain Res* 1995; **84**: 55-61.
27. Lemaire V, Koehl M, Le Moal M, Abrous DN. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proc Natl Acad Sci USA* 2000; **97**: 11032-11037.
28. Paris JJ, Frye CA. Juvenile offspring of rats exposed to restraint stress in late gestation have impaired cognitive performance and dysregulated progesterone formation. *Stress* 2011; **14**: 23-32.
29. Paris JJ, Brunton PJ, Russell JA, Frye CA. Immune stress in late pregnant rats decreases length of gestation, fecundity, and alters later cognitive and affective behaviour of surviving pre-adolescent offspring. *Stress* 2011; **14**: 652-664.
30. Lee PR, Brady DL, Shapiro RA, Dorsa DM, Koenig JJ. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. *Brain Res* 2007; **1156**: 152-167.

- Accepted Article
31. Patin V, Lordi B, Vincent A, Caston J. Effects of prenatal stress on anxiety and social interactions in adult rats. *Dev Brain Res* 2005; **160**: 265-274.
 32. Frye CA, Orecki ZA. Prenatal stress alters reproductive responses of rats in behavioral estrus and paced mating of hormone-primed rats. *Horm Behav* 2002; **42**: 472-483.
 33. Holson RR, Gough B, Sullivan P, Badger T, Sheehan DM. Prenatal dexamethasone or stress but not ACTH or corticosterone alter sexual behavior in male rats. *Neurotoxicol Teratol* 1995; **17**: 393-401.
 34. Glover V. Prenatal stress and its effects on the fetus and the child: possible underlying biological mechanisms. *Adv Neurobiol* 2015; **10**: 269-283.
 35. Schuurmans C, Kurrasch DM. Neurodevelopmental consequences of maternal distress: what do we really know? *Clin Genet* 2013; **83**: 108-117.
 36. King S, Dancause K, Turcotte-Tremblay AM, Veru F, Laplante DP. Using natural disasters to study the effects of prenatal maternal stress on child health and development. *Birth defects Res C Embryo Today* 2012; **96**: 273-288.
 37. Blair MM, Glynn LM, Sandman CA, Davis EP. Prenatal maternal anxiety and early childhood temperament. *Stress* 2011; **14**: 644-651.
 38. Henry C, Kabbaj M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J Neuroendocrinol* 1994; **6**: 341-345.
 39. Gunn BG, Cunningham L, Cooper MA, Corteen NL, Seifi M, Swinny JD, Lambert JJ, Belelli D. Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response. *J Neurosci* 2013; **33**: 19534-19554.
 40. Zohar I, Weinstock M. Differential effect of prenatal stress on the expression of corticotrophin-releasing hormone and its receptors in the hypothalamus and amygdala in male and female rats. *J Neuroendocrinol* 2011; **23**: 320-328.
 41. Bosch OJ, Musch W, Bredewold R, Slattery DA, Neumann ID. Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: implications for postpartum mood disorder. *Psychoneuroendocrinology* 2007; **32**: 267-278.
 42. Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *J Neurosci* 1995; **15**: 110-116.

- Accepted Article
43. Vazquez DM, Van Oers H, Levine S, Akil H. Regulation of glucocorticoid and mineralocorticoid receptor mRNAs in the hippocampus of the maternally deprived infant rat. *Brain Res* 1996; **731**: 79-90.
 44. Cullinan WE, Ziegler DR, Herman JP. Functional role of local GABAergic influences on the HPA axis. *Brain Struct Funct* 2008; **213**: 63-72.
 45. Uchida T, Furukawa T, Iwata S, Yanagawa Y, Fukuda A. Selective loss of parvalbumin-positive GABAergic interneurons in the cerebral cortex of maternally stressed Gad1-heterozygous mouse offspring. *Transl Psychiatry* 2014; **4**: e371.
 46. Giovanoli S, Weber L, Meyer U. Single and combined effects of prenatal immune activation and peripubertal stress on parvalbumin and reelin expression in the hippocampal formation. *Brain Behav Immun* 2014; **40**: 48-54.
 47. Fride E, Dan Y, Gavish M, Weinstock M. Prenatal stress impairs maternal behavior in a conflict situation and reduces hippocampal benzodiazepine receptors. *Life Sci* 1985; **36**: 2103-2109.
 48. Barros VG, Rodriguez P, Martijena ID, Perez A, Molina VA, Antonelli MC. Prenatal stress and early adoption effects on benzodiazepine receptors and anxiogenic behavior in the adult rat brain. *Synapse* 2006; **60**: 609-618.
 49. Fine R, Zhang J, Stevens HE. Prenatal stress and inhibitory neuron systems: implications for neuropsychiatric disorders. *Mol Psychiatry* 2014; **19**: 641-651.
 50. Laloux C, Mairesse J, Van Camp G, Giovine A, Branchi I, Bouret S, Morley-Fletcher S, Bergonzelli G, Malagodi M, Gradini R, Nicoletti F, Darnaudery M, Maccari S. Anxiety-like behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress. *Psychoneuroendocrinology* 2012; **37**: 1646-1658.
 51. Morley-Fletcher S, Rea M, Maccari S, Laviola G. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *Eur J Neurosci* 2003; **18**: 3367-3374.
 52. Kalinichev M, Easterling KW, Plotsky PM, Holtzman SG. Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. *Pharmacol Biochem Behav* 2002; **73**: 131-140.
 53. Vallee M, MacCari S, Dellu F, Simon H, Le Moal M, Mayo W. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. *Eur J Neurosci* 1999; **11**: 2906-2916.
 54. Abe H, Hidaka N, Kawagoe C, Odagiri K, Watanabe Y, Ikeda T, Ishizuka Y, Hashiguchi H, Takeda R, Nishimori T, Ishida Y. Prenatal psychological stress causes higher emotionality,

- depression-like behavior, and elevated activity in the hypothalamo-pituitary-adrenal axis. *Neurosci Res* 2007; **59**: 145-151.
55. Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress responses? *Brain Res Rev* 1990; **15**: 71-100.
56. Ward HE, Johnson EA, Salm AK, Birkle DL. Effects of prenatal stress on defensive withdrawal behavior and corticotropin releasing factor systems in rat brain. *Physiol Behav* 2000; **70**: 359-366.
57. Cratty MS, Ward HE, Johnson EA, Azzaro AJ, Birkle DL. Prenatal stress increases corticotropin-releasing factor (CRF) content and release in rat amygdala minces. *Brain Res* 1995; **675**: 297-302.
58. Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci* 2008; **28**: 9055-9065.
59. Wang XD, Labermaier C, Holsboer F, Wurst W, Deussing JM, Muller MB, Schmidt MV. Early-life stress-induced anxiety-related behavior in adult mice partially requires forebrain corticotropin-releasing hormone receptor 1. *Eur J Neurosci* 2012; **36**: 2360-2367.
60. Brunton PJ, Donadio MV, Russell JA. Sex differences in prenatally programmed anxiety behaviour in rats: Differential corticotropin-releasing hormone receptor mRNA expression in the amygdaloid complex. *Stress* 2011; **14**: 634-643.
61. Wang X, Meng FS, Liu ZY, Fan JM, Hao K, Chen XQ, Du JZ. Gestational Hypoxia Induces Sex-Differential Methylation of Crhr1 Linked to Anxiety-like Behavior. *Mol Neurobiol* 2013; **48**: 544-555.
62. Rutherford KM, Piastowska-Ciesielska A, Donald RD, Robson SK, Ison SH, Jarvis S, Brunton PJ, Russell JA, Lawrence AB. Prenatal stress produces anxiety prone female offspring and impaired maternal behaviour in the domestic pig. *Physiol Behav* 2014; **129**: 255-264.
63. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol* 2004; **44**: 525-557.
64. Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, Lee KF. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat Genet* 2000; **24**: 410-414.
65. Marrocco J, Mairesse J, Ngomba RT, Silletti V, Van Camp G, Bouwalerh H, Summa M, Pittaluga A, Nicoletti F, Maccari S, Morley-Fletcher S. Anxiety-like behavior of prenatally stressed rats is associated with a selective reduction of glutamate release in the ventral hippocampus. *J Neurosci* 2012; **32**: 17143-17154.

- Accepted Article
66. Marrocco J, Reynaert ML, Gatta E, Gabriel C, Mocaer E, Di Prisco S, Merega E, Pittaluga A, Nicoletti F, Maccari S, Morley-Fletcher S, Mairesse J. The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders. *J Neurosci* 2014; **34**: 2015-2024.
 67. Zuena AR, Mairesse J, Casolini P, Cinque C, Alema GS, Morley-Fletcher S, Chiodi V, Spagnoli LG, Gradini R, Catalani A, Nicoletti F, Maccari S. Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. *PLoS One* 2008; **3**: e2170.
 68. Mueller BR, Bale TL. Early prenatal stress impact on coping strategies and learning performance is sex dependent. *Physiol Behav* 2007; **91**: 55-65.
 69. Ivy AS, Rex CS, Chen Y, Dube C, Maras PM, Grigoriadis DE, Gall CM, Lynch G, Baram TZ. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. *J Neurosci* 2010; **30**: 13005-13015.
 70. Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. *Nat Neurosci* 2004; **7**: 841-846.
 71. Oomen CA, Soeters H, Audureau N, Vermunt L, van Hasselt FN, Manders EM, Joels M, Lucassen PJ, Krugers H. Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. *J Neurosci* 2010; **30**: 6635-6645.
 72. Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Mol Psychiatry* 2002; **7**: 609-616.
 73. Boersma GJ, Lee RS, Cordner ZA, Ewald ER, Purcell RH, Moghaddam AA, Tamashiro KL. Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. *Epigenetics* 2014; **9**: 437-447.
 74. Yang J, Han H, Cao J, Li L, Xu L. Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring. *Hippocampus* 2006; **16**: 431-436.
 75. Yaka R, Salomon S, Matzner H, Weinstock M. Effect of varied gestational stress on acquisition of spatial memory, hippocampal LTP and synaptic proteins in juvenile male rats. *Behav Brain Res* 2007; **179**: 126-132.
 76. Brunson KL, Kramar E, Lin B, Chen Y, Colgin LL, Yanagihara TK, Lynch G, Baram TZ. Mechanisms of late-onset cognitive decline after early-life stress. *J Neurosci* 2005; **25**: 9328-9338.

- Accepted Article
77. Huot RL, Plotsky PM, Lenox RH, McNamara RK. Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Res* 2002; **950**: 52-63.
 78. Oomen CA, Soeters H, Audureau N, Vermunt L, van Hasselt FN, Manders EM, Joels M, Krugers H, Lucassen PJ. Early maternal deprivation affects dentate gyrus structure and emotional learning in adult female rats. *Psychopharmacology* 2011; **214**: 249-260.
 79. Paris JJ, Frye CA. Gestational exposure to variable stressors produces decrements in cognitive and neural development of juvenile male and female rats. *Curr Top Med Chem* 2011; **11**: 1706-1713.
 80. Suenaga T, Yukie M, Gao S, Nakahara D. Sex-specific effects of prenatal stress on neuronal development in the medial prefrontal cortex and the hippocampus. *Neuroreport* 2012; **23**: 430-435.
 81. Mychasiuk R, Gibb R, Kolb B. Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. *Synapse* 2012; **66**: 308-314.
 82. Korosi A, Naninck EF, Oomen CA, Schouten M, Krugers H, Fitzsimons C, Lucassen PJ. Early-life stress mediated modulation of adult neurogenesis and behavior. *Behav Brain Res* 2012; **227**: 400-409.
 83. Oomen CA, Girardi CE, Cahyadi R, Verbeek EC, Krugers H, Joels M, Lucassen PJ. Opposite effects of early maternal deprivation on neurogenesis in male versus female rats. *PloS one* 2009; **4**: e3675.
 84. Bock J, Murmu MS, Biala Y, Weinstock M, Braun K. Prenatal stress and neonatal handling induce sex-specific changes in dendritic complexity and dendritic spine density in hippocampal subregions of prepubertal rats. *Neuroscience* 2011; **193**: 34-43.
 85. Raff H, Jacobson L, Cullinan WE. Augmented hypothalamic corticotrophin-releasing hormone mRNA and corticosterone responses to stress in adult rats exposed to perinatal hypoxia. *J Neuroendocrinol* 2007; **19**: 907-912.
 86. Van Waes V, Darnaudery M, Marrocco J, Gruber SH, Talavera E, Mairesse J, Van Camp G, Casolla B, Nicoletti F, Mathe AA, Maccari S, Morley-Fletcher S. Impact of early life stress on alcohol consumption and on the short- and long-term responses to alcohol in adolescent female rats. *Behav Brain Res* 2011; **221**: 43-49.
 87. Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol* 2000; **428**: 191-212.

- Accepted Article
88. Chen Y, Andres AL, Frotscher M, Baram TZ. Tuning synaptic transmission in the hippocampus by stress: the CRH system. *Front Cell Neurosci* 2012; **6**: 13.
 89. Chen Y, Rex CS, Rice CJ, Dube CM, Gall CM, Lynch G, Baram TZ. Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. *Proc Natl Acad Sci USA* 2010; **107**: 13123-13128.
 90. Chen Y, Bender RA, Brunson KL, Pomper JK, Grigoriadis DE, Wurst W, Baram TZ. Modulation of dendritic differentiation by corticotropin-releasing factor in the developing hippocampus. *Proc Natl Acad Sci USA* 2004; **101**: 15782-15787.
 91. Brunson KL, Eghbal-Ahmadi M, Bender R, Chen Y, Baram TZ. Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. *Proc Natl Acad Sci USA* 2001; **98**: 8856-8861.
 92. Wang XD, Rammes G, Kraev I, Wolf M, Liebl C, Scharf SH, Rice CJ, Wurst W, Holsboer F, Deussing JM, Baram TZ, Stewart MG, Muller MB, Schmidt MV. Forebrain CRF(1) modulates early-life stress-programmed cognitive deficits. *J Neurosci* 2011; **31**: 13625-13634.
 93. de Kloet ER, de Jong IE, Oitzl MS. Neuropharmacology of glucocorticoids: focus on emotion, cognition and cocaine. *Eur J Pharmacol* 2008; **585**: 473-482.
 94. Baulieu EE. Neurosteroids: a new function in the brain. *Biol Cell* 1991; **71**: 3-10.
 95. Paul SM, Purdy RH. Neuroactive steroids. *FASEB J* 1992; **6**: 2311-2322.
 96. Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, Vaudry H. Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. *Pharmacol Rev* 1999; **51**: 63-81.
 97. Hu ZY, Bourreau E, Jung-Testas I, Robel P, Baulieu EE. Neurosteroids: oligodendrocyte mitochondria convert cholesterol to pregnenolone. *Proc Natl Acad Sci USA* 1987; **84**: 8215-8219.
 98. Jung-Testas I, Hu ZY, Baulieu EE, Robel P. Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* 1989; **125**: 2083-2091.
 99. Krieger NR, Scott RG. Nonneuronal localization for steroid converting enzyme: 3 alpha-hydroxysteroid oxidoreductase in olfactory tubercle of rat brain. *J Neurochem* 1989; **52**: 1866-1870.
 100. Melcangi RC, Celotti F, Castano P, Martini L. Differential localization of the 5 alpha-reductase and the 3 alpha-hydroxysteroid dehydrogenase in neuronal and glial cultures. *Endocrinology* 1993; **132**: 1252-1259.

- Accepted Article
101. Purdy RH, Morrow AL, Moore PHJ, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci USA* 1991; **88**: 4553-4557.
 102. Cheney DL, Uzunov D, Costa E, Guidotti A. Gas chromatographic-mass fragmentographic quantitation of 3alpha-hydroxy- 5alpha-pregnan-20-one (allopregnanolone) and its precursors in blood and brain of adrenalectomized and castrated rats. *J Neurosci* 1995; **15**: 4641-4650.
 103. Corpechot C, Young J, Calvel M, Wehrey C, Veltz JN, Touyer G, Mouren M, Prasad VVK, Banner C, Sjoval J, Baulieu EE, Robel P. Neurosteroids: 3alpha-Hydroxy-5alpha-pregnan-20-one and its precursors in the brain, plasma, and steroidogenic glands of male and female rats. *Endocrinology* 1993; **133**: 1003-1009.
 104. Kraulis I, Foldes G, Traikov H, Dubrovsky B, Birmingham. Distribution, metabolism and biological activity of deoxycorticosterone in the central nervous system. *Brain Res* 1975; **88**: 1-14.
 105. Gangloff A, Shi R, Nahoum V, Lin SX. Pseudo-symmetry of C19 steroids, alternative binding orientations, and multispecificity in human estrogenic 17beta-hydroxysteroid dehydrogenase. *FASEB J* 2003; **17**: 274-276.
 106. Steckelbroeck S, Jin Y, Gopishetty S, Oyesanmi B, Penning TM. Human cytosolic 3alpha-hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3beta-hydroxysteroid dehydrogenase activity: implications for steroid hormone metabolism and action. *J Biol Chem* 2004; **279**: 10784-10795.
 107. Selmanoff MK, Brodtkin LD, Weiner RI, Siiteri PK. Aromatization and 5alpha-reduction of androgens in discrete hypothalamic and limbic regions of the male and female rat. *Endocrinology* 1977; **101**: 841-848.
 108. Higashi T, Nagura Y, Shimada K, Toyo'oka T. Studies on neurosteroids XXVI. Fluoxetine-evoked changes in rat brain and serum levels of neuroactive androgen, 5 alpha-androstane-3 alpha,17 beta-diol. *Biol Pharm Bull* 2009; **32**: 1636-1638.
 109. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 1986; **232**: 1004-1007.
 110. Morrow AL, Suzdak PD, Paul SM. Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency. *Eur J Pharmacol* 1987; **142**: 483-485.
 111. Lambert JJ, Cooper MA, Simmons RD, Weir CJ, Belelli D. Neurosteroids: endogenous allosteric modulators of GABA(A) receptors. *Psychoneuroendocrinology* 2009; **34 Suppl 1**: S48-58.

- Accepted Article
112. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci* 2005; **6**: 565-575.
 113. Reddy DS, Jian K. The testosterone-derived neurosteroid androstenediol is a positive allosteric modulator of GABAA receptors. *J Pharmacol Exp Ther* 2010; **334**: 1031-1041.
 114. Lund TD, Hinds LR, Handa RJ. The androgen 5alpha-dihydrotestosterone and its metabolite 5alpha-androstan-3beta, 17beta-diol inhibit the hypothalamo-pituitary-adrenal response to stress by acting through estrogen receptor beta-expressing neurons in the hypothalamus. *J Neurosci* 2006; **26**: 1448-1456.
 115. Patchev VK, Hassan AHS, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. *Neuropsychopharmacology* 1996; **15**: 533-540.
 116. Brunton PJ, McKay AJ, Ochedalski T, Piastowska A, Rebas E, Lachowicz A, Russell JA. Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. *J Neurosci* 2009; **29**: 6449-6460.
 117. Patchev VK, Shoaib M, Holsboer F, Almeida OF. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience* 1994; **62**: 265-271.
 118. Owens MJ, Ritchie JC, Nemeroff CB. 5 alpha-pregnane-3 alpha, 21-diol-20-one (THDOC) attenuates mild stress-induced increases in plasma corticosterone via a non-glucocorticoid mechanism: comparison with alprazolam. *Brain Res* 1992; **573**: 353-355.
 119. Handa RJ, Sharma D, Uht R. A role for the androgen metabolite, 5alpha androstane 3beta, 17beta diol (3beta-diol) in the regulation of the hypothalamo-pituitary-adrenal axis. *Front Endocrinol* 2011; **2**: 65.
 120. Handa RJ, Kudwa AE, Donner NC, McGivern RF, Brown R. Central 5-alpha reduction of testosterone is required for testosterone's inhibition of the hypothalamo-pituitary-adrenal axis response to restraint stress in adult male rats. *Brain Res* 2013; **1529**: 74-82.
 121. Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, Watanabe H, Costa E, Guidotti A. Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. *Proc Natl Acad Sci USA* 2001; **98**: 2849-2854.
 122. Matsumoto K, Pinna G, Puia G, Guidotti A, Costa E. Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. *Stress* 2005; **8**: 85-93.

- Accepted Article
123. Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, Usala L, Purdy RH, Biggio G. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. *J Neurochem* 2000; **75**: 732-740.
 124. Agis-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E, Guidotti A. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *Proc Natl Acad Sci USA* 2007; **104**: 18736-18741.
 125. Agis-Balboa RC, Guidotti A, Pinna G. 5alpha-reductase type I expression is downregulated in the prefrontal cortex/Brodman's area 9 (BA9) of depressed patients. *Psychopharmacology (Berl)* 2014; **231**: 3569-3580.
 126. Evans J, Sun Y, McGregor A, Connor B. Allopregnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress. *Neuropharmacology* 2012; **63**: 1315-1326.
 127. Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR. Effects of gonadal steroids in women with a history of postpartum depression. *Am J Psychiatry* 2000; **157**: 924-930.
 128. Nappi RE, Petraglia F, Luisi S, Polatti F, Farina C, Genazzani AR. Serum allopregnanolone in women with postpartum "blues". *Obstet Gynecol* 2001; **97**: 77-80.
 129. Romeo E, Strohle A, Spalletta G, di Michele F, Hermann B, Holsboer F, Pasini A, Rupprecht R. Effects of antidepressant treatment on neuroactive steroids in major depression. *Am J Psychiatry* 1998; **155**: 910-913.
 130. Rupprecht R, Rammes G, Eser D, Baghai TC, Schule C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, Uzunov V, McAllister KH, Bertaina-Anglade V, La Rochelle CD, Tuerck D, Floesser A, Kiese B, Schumacher M, Landgraf R, Holsboer F, Kucher K. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science* 2009; **325**: 490-493.
 131. Marx CE, Keefe RS, Buchanan RW, Hamer RM, Kilts JD, Bradford DW, Strauss JL, Naylor JC, Payne VM, Lieberman JA, Savitz AJ, Leimone LA, Dunn L, Porcu P, Morrow AL, Shampine LJ. Proof-of-concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia. *Neuropsychopharmacology* 2009; **34**: 1885-1903.
 132. Bitran D, Shiekh M, McLeod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABA(A) receptors. *J Neuroendocrinol* 1995; **7**: 171-177.
 133. Rodgers RJ, Johnson NJ. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. *Pharmacol Biochem Behav* 1998; **59**: 221-232.

134. Wieland S, Belluzzi JD, Stein L, Lan NC. Comparative behavioral characterization of the neuroactive steroids 3 alpha-OH,5 alpha-pregnan-20-one and 3 alpha-OH,5 beta-pregnan-20-one in rodents. *Psychopharmacology (Berl)* 1995; **118**: 65-71.
135. Edinger KL, Frye CA. Testosterone's anti-anxiety and analgesic effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. *Psychoneuroendocrinology* 2005; **30**: 418-430.
136. Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA. Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. *Horm Behav* 2008; **54**: 726-734.
137. Crawley JN, Glowa JR, Majewska MD, Paul SM. Anxiolytic activity of an endogenous adrenal steroid. *Brain Res* 1986; **398**: 382-385.
138. Frye CA, Edinger KL, Lephart ED, Walf AA. 3alpha-androstanediol, but not testosterone, attenuates age-related decrements in cognitive, anxiety, and depressive behavior of male rats. *Front Aging Neurosci* 2010; **2**: 15.
139. Vivian JA, Barros HM, Manitiu A, Miczek KA. Ultrasonic vocalizations in rat pups: modulation at the gamma-aminobutyric acidA receptor complex and the neurosteroid recognition site. *J Pharmacol Exp Ther* 1997; **282**: 318-325.
140. Zimmerberg B, Brunelli SA, Hofer MA. Reduction of rat pup ultrasonic vocalizations by the neuroactive steroid allopregnanolone. *Pharmacol Biochem Behav* 1994; **47**: 735-738.
141. Akwa Y, Purdy RH, Koob GF, Britton KT. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. *Behav Brain Res* 1999; **106**: 119-125.
142. Engin E, Treit D. The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: the amygdala, medial prefrontal cortex, or hippocampus. *Behav Pharmacol* 2007; **18**: 461-470.
143. Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. *Neurobiol Learn Mem* 2006; **86**: 35-46.
144. Frye CA, Sturgis JD. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiol Learn Mem* 1995; **64**: 83-96.
145. Matthews DB, Morrow AL, Tokunaga S, McDaniel JR. Acute ethanol administration and acute allopregnanolone administration impair spatial memory in the Morris water task. *Alcohol Clin Exp Res* 2002; **26**: 1747-1751.
146. Johansson IM, Birzniece V, Lindblad C, Olsson T, Backstrom T. Allopregnanolone inhibits learning in the Morris water maze. *Brain Res* 2002; **934**: 125-131.

147. Osborne DM, Edinger K, Frye CA. Chronic administration of androgens with actions at estrogen receptor beta have anti-anxiety and cognitive-enhancing effects in male rats. *Age (Dordr)* 2009; **31**: 191-198.
148. Holzbauer M, Birmingham MK, De Nicola AF, Oliver JT. In vivo secretion of 3 alpha-hydroxy-5 alpha-pregnan-20-one, a potent anaesthetic steroid, by the adrenal gland of the rat. *J Steroid Biochem* 1985; **22**: 97-102.
149. Akirav I, Sandi C, Richter-Levin G. Differential activation of hippocampus and amygdala following spatial learning under stress. *Eur J Neurosci* 2001; **14**: 719-725.
150. Armario A, Gavalda A, Marti J. Comparison of the behavioural and endocrine response to forced swimming stress in five inbred strains of rats. *Psychoneuroendocrinology* 1995; **20**: 879-890.
151. Melcangi RC, Panzica GC. Allopregnanolone: state of the art. *Prog Neurobiol* 2014; **113**: 1-5.
152. Wang JM, Singh C, Liu L, Irwin RW, Chen S, Chung EJ, Thompson RF, Brinton RD. Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2010; **107**: 6498-6503.
153. Ordyan NE, Pivina SG. Effects of prenatal stress on the activity of an enzyme involved in neurosteroid synthesis during the "critical period" of sexual differentiation of the brain in male rats. *Neurosci Behav Physiol* 2005; **35**: 931-935.
154. McKendry AA, Palliser HK, Yates DM, Walker DW, Hirst JJ. The effect of betamethasone treatment on neuroactive steroid synthesis in a foetal Guinea pig model of growth restriction. *J Neuroendocrinol* 2010; **22**: 166-174.
155. Paris JJ, Brunton PJ, Russell JA, Walf AA, Frye CA. Inhibition of 5alpha-reductase activity in late pregnancy decreases gestational length and fecundity and impairs object memory and central progesterone milieu of juvenile rat offspring. *J Neuroendocrinol* 2011; **23**: 1079-1090.
156. Kehoe P, Mallinson K, McCormick CM, Frye CA. Central allopregnanolone is increased in rat pups in response to repeated, short episodes of neonatal isolation. *Brain Res Dev Brain Res* 2000; **124**: 133-136.
157. Walf AA, Frye CA. Gestational or acute restraint in adulthood reduces levels of 5alpha-reduced testosterone metabolites in the hippocampus and produces behavioral inhibition of adult male rats. *Front Cell Neurosci* 2012; **6**: 40.
158. Brunton PJ, Donadio MV, Yao ST, Greenwood MP, Seckl JR, Murphy D, Russell JA. 5alpha-reduced neurosteroids sex-dependently reverse central prenatal programming of neuroendocrine stress responses in rats *J Neurosci* 2014; **35**: 666-677.

- Accepted Article
159. Purves-Tyson TD, Handelsman DJ, Double KL, Owens SJ, Bustamante S, Weickert CS. Testosterone regulation of sex steroid-related mRNAs and dopamine-related mRNAs in adolescent male rat substantia nigra. *BMC Neurosci* 2012; **13**: 95.
 160. Sanchez P, Torres JM, del Moral RG, de Dios Luna J, Ortega E. Steroid 5alpha-reductase in adult rat brain after neonatal testosterone administration. *IUBMB Life* 2012; **64**: 81-86.
 161. Torres JM, Ortega E. Steroid 5alpha-reductase isozymes in the adult female rat brain: central role of dihydrotestosterone. *J Mol Endocrinol* 2006; **36**: 239-245.
 162. Gustafsson JA, Stenberg A. Irreversible androgenic programming at birth of microsomal and soluble rat liver enzymes active on androstene-3,17-dione and 5alpha-androstane-3alpha,17beta-diol. *J Biol Chem* 1974; **249**: 711-718.
 163. Brunton PJ, Sullivan KM, Kerrigan D, Russell JA, Seckl JR, Drake AJ. Sex-specific effects of prenatal stress on glucose homeostasis and peripheral metabolism in rats. *J Endocrinol* 2013; **217**: 161-173.
 164. Huizink AC, Bartels M, Rose RJ, Pulkkinen L, Eriksson CJ, Kaprio J. Chernobyl exposure as stressor during pregnancy and hormone levels in adolescent offspring. *J Epidemiol Community Health* 2008; **62**: e5.
 165. Bortolato M, Devoto P, Roncada P, Frau R, Flore G, Saba P, Pistritto G, Soggiu A, Pisanu S, Zappala A, Ristaldi MS, Tattoli M, Cuomo V, Marrosu F, Barbaccia ML. Isolation rearing-induced reduction of brain 5alpha-reductase expression: Relevance to dopaminergic impairments. *Neuropharmacology* 2011; **60**: 1301-1308.
 166. Yehuda R, Bierer LM, Andrew R, Schmeidler J, Seckl JR. Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors. *J Psychiatr Res* 2009; **43**: 877-883.
 167. Yehuda R, Bell A, Bierer LM, Schmeidler J. Maternal, not paternal, PTSD is related to increased risk for PTSD in offspring of Holocaust survivors. *J Psychiatr Res* 2008; **42**: 1104-1111.
 168. Yehuda R, Daskalakis NP, Lehrner A, Desarnaud F, Bader HN, Makotkine I, Flory JD, Bierer LM, Meaney MJ. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. *Am J Psychiatry* 2014; **171**: 872-880.
 169. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847-854.

- Accepted Article
170. Grobin AC, Heenan EJ, Lieberman JA, Morrow AL. Perinatal neurosteroid levels influence GABAergic interneuron localization in adult rat prefrontal cortex. *J Neurosci* 2003; **23**: 1832-1839.
 171. Zimmerberg B, Blaskey LG. Prenatal stress effects are partially ameliorated by prenatal administration of the neurosteroid allopregnanolone. *Pharmacol Biochem Behav* 1998; **59**: 819-827.
 172. Patchev VK, Montkowski A, Rouskova D, Koranyi L, Holsboer F, Almeida OFX. Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behaviorial and neuroendocrine consequences of adverse early life events. *J Clin Invest* 1997; **99**: 962-966.
 173. Kapoor A, Matthews SG. Testosterone is involved in mediating the effects of prenatal stress in male guinea pig offspring. *J Physiol* 2011; **589**: 755-766.
 174. Shughrue PJ, Merchenthaler I. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. *J Comp Neurol* 2001; **436**: 64-81.
 175. Miller WJ, Suzuki S, Miller LK, Handa R, Uht RM. Estrogen receptor (ER)beta isoforms rather than ERalpha regulate corticotropin-releasing hormone promoter activity through an alternate pathway. *J Neurosci* 2004; **24**: 10628-10635.
 176. Weiser MJ, Wu TJ, Handa RJ. Estrogen receptor-beta agonist diarylpropionitrile: biological activities of R- and S-enantiomers on behavior and hormonal response to stress. *Endocrinology* 2009; **150**: 1817-1825.
 177. Djebaili M, Hoffman SW, Stein DG. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. *Neuroscience* 2004; **123**: 349-359.
 178. Morali G, Montes P, Hernandez-Morales L, Monfil T, Espinosa-Garcia C, Cervantes M. Neuroprotective effects of progesterone and allopregnanolone on long-term cognitive outcome after global cerebral ischemia. *Restor Neurol Neurosci* 2011; **29**: 1-15.
 179. Stone DJ, Walsh JP, Sebro R, Stevens R, Pantazopolous H, Benes FM. Effects of pre- and postnatal corticosterone exposure on the rat hippocampal GABA system. *Hippocampus* 2001; **11**: 492-507.
 180. Koksma JJ, van Kesteren RE, Rosahl TW, Zwart R, Smit AB, Luddens H, Brussaard AB. Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. *J Neurosci* 2003; **23**: 788-797.

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: $\uparrow$ indicates increased/enhanced and $\downarrow$ indicates decreased/suppressed 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: 5α R, 5α -reductase; HSD, hydroxysteroid dehydrogenase.

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

Rats were pre-treated, 20h and 2h before IL-1 β (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-1 β 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-1 β in PNS male rats (c). Based on data from (158).

Figure 4: Early life stress and neuroactive steroids

This article is protected by copyright. All rights reserved.

Stress in early life results in a persistent reduction in 5 α -reductase (5 α R) activity in the brain and liver, leading to a reduction in central neuroactive steroid levels (e.g. allopregnanolone, AP; dihydroprogesterone, DHP; dihydrotestosterone, DHT; 3 α -androstadiol, 3 α -diol; 3 β -androstadiol, 3 β -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 (ER β) and that AP is less effective in suppressing corticotropin releasing hormones (CRH) neuronal activity. $\uparrow$ indicates increased and $\downarrow$ indicates decreased.

Figure 1

Figure 4