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REVIEW ARTICLE

Stress and the Reproductive Axis

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There exists a reciprocal relationship between the hypothalamic-pituitary-adrenal (HPA) and the hypothalamic-pituitary-gonadal (HPG) axes, wherein the activation of one affects the function of the other and vice versa. For example, both testosterone and oestrogen modulate the response of the HPA axis, whereas activation of the stress axis, especially activation that is repeating or chronic, has an inhibitory effect upon oestrogen and testosterone secretion. Alterations in maternal care can produce significant effects on both HPG and HPA physiology, as well as behaviour in the offspring at adulthood. For example, changes in reproductive behaviour induced by altered maternal care may alter the expression of sex hormone receptors such as oestrogen receptor (ER)α that govern sexual behaviour, and may be particularly important in determining the sexual strategies utilised by females. Stress in adulthood continues to mediate HPG activity in females through activation of a sympathetic neural pathway originating in the hypothalamus and releasing norepinephrine into the ovary, which produces a noncyclic anovulatory ovary that develops cysts. In the opposite direction, sex differences and sex steroid hormones regulate the HPA axis. For example, although serotonin (5-HT) has a stimulatory effect on the HPA axis in humans and rodents that is mediated by the 5-HT_{1A} receptor, only male rodents respond to 5-HT_{1A} antagonism to show increased corticosterone responses to stress. Furthermore, oestrogen appears to decrease 5-HT_{1A} receptor function at presynaptic sites, yet increases 5-HT_{1A} receptor expression at postsynaptic sites. These mechanisms could explain the heightened stress HPA axis responses in females compared to males. Studies on female rhesus macaques show that chronic stress in socially subordinate female monkeys produces a distinct behavioural phenotype that is largely unaffected by oestrogen, a hyporesponsive HPA axis that is hypersensitive to the modulating effects of oestrogen, and changes in 5-HT_{1A} receptor binding in the hippocampus and hypothalamus of social subordinate female monkeys that are restored or inverted by oestrogen replacement. This review summarises all of these studies, emphasising the profound effect that the interaction of the reproductive and stress axes may have on human reproductive health and emotional wellbeing.

Key words: reproduction, stress, maternal care, oestrogen, serotonin, ovary

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Introduction

The present review examines the results from four laboratories in both North and South America that are studying the interaction of the stress and reproductive axes at several levels.

It has been shown that the adult hypothalamic-pituitary-adrenal (HPA) axis reactivity can be altered early in life by differences in maternal care. In laboratory rats, the neuroendocrine and behavioural effects of postnatal environmental manipulations of the infant–mother relationship have been studied experimentally for more than 50 years. Among these, the most frequently applied postnatal manipulations are neonatal handling (NH), which consists

of brief periods of daily separation of mothers and offspring (usually < 15 min) taking place any time before weaning, and maternal separation (MS), which includes repeated removal of either pups or mother from the nest for periods ranging from 3 to 8 h per day during the first two postnatal weeks (1–3). Although the effects of early-life manipulations on the HPA axis have been extensively characterised, few investigators have examined reproductive markers in rats following the MS stress paradigm, and there is evidence that NH can alter reproductive behaviour in various ways. Thus, in many instances, early maternal care can set the stage for the interaction of the hypothalamic-pituitary-gonadal (HPG) and HPA axes in adult life.

There is ample evidence that gonadal steroids, the end product of the HPG axis, actively modulate the function of the HPA axis in adults. Studies on female rats have found higher adrenocorticotrophin hormone (ACTH) levels subsequent to acute stress at pro-oestrus or following treatment with pro-oestrus levels of oestrogen, and longer lasting post-stress elevations of corticosterone in female rats treated with oestradiol or oestradiol and progesterone (4). 17β-Oestradiol (E_2) has been shown to increase ACTH secretion in female baboons (5) and increase ACTH and cortisol by decreasing glucocorticoid negative-feedback in female monkeys (6) and, in women, exercise stress enhances ACTH and arginine vasopressin (AVP) only in the mid-luteal stage when ovarian hormones are rising (7). In male rats, testosterone decreases glucocorticoid and adrenocorticotrophin responses to stress (8,9). Furthermore, gonadectomy increases both corticosterone and ACTH in male rats and this can be normalised by replacement with testosterone or dihydrotestosterone (10). These studies suggest that gonadal steroids modulate the HPA axis in both sexes.

By contrast, activation of the stress axis, especially activation that is repeating or chronic, has an inhibitory effect upon gonadal hormone secretion. For example, stress and stress hormones inhibit the release of gonadotrophin-releasing hormone from the hypothalamus, and glucocorticoids inhibit the release of luteinising hormone from the pituitary and E_2 and progesterone secretion by the ovary (11,12), as well as testosterone from the testes (12,13). One way that stress acts to mediate HPG activity in females is through activation of a sympathetic neural pathway originating in the hypothalamus and releasing norepinephrine (NE) into the ovary (14,15). The deleterious effect that this sympathetic pathway can have on the ovary is likely a main contributor to the effect of stress on the HPG axis.

Data garnered from these substantially different experimental paradigms emphasise that the interaction of the reproductive and stress axes has far-reaching implications for human health.

Maternal separation stress and reproductive function: effects on male and female rats

Given that a substantial amount of brain development occurs after birth, it is consequently subject to environmental influences, which may negatively or positively affect brain maturation. Even natural variations in the quality or quantity of maternal care can have a long-term impact on offspring brain and behaviour. Human epidemiological and animal experimental studies show that early social experiences influence the functioning of physiological processes even into adulthood (3,16–22).

In both sexes, rat sexual behaviour can be divided into two components: appetitive and consummatory (23). In females, appetitive behaviours, also named proceptive behaviours, consist of anogenital investigation, solicitations, hops and darts, and ear wiggling, whereas males display anogenital investigation, chase the females and attempt to mount them. The consummatory/receptive phase in females consists of the expression of the lordotic posture, which allows the male to mount, perform several intromissions and ejaculate, the three main copulatory behaviours shown by males (24). Although results are not consistent across the literature, MS induces sexually dimorphic outcomes. Although reproductive physiology is not significantly affected in females, an MS protocol has been described as producing significant effects on male reproductive physiology, such as longer mount latencies, longer intromission latencies and a reduction in the percentage of animal ejaculating but it does not affect female reproductive function (25). On the other hand, Greisen *et al.* (26) found that MS led to a male phenotype with heightened sexual performance, reflected in decreased mount latency, decreased intromission latency and decreased post-ejaculatory interval, whereas mating behaviour was not affected in females. The discrepancies observed between these two studies may be explained because they employed different MS protocols and different control groups. However, although the results may differ depending upon the experimental conditions, MS is a good animal model of early-life stress that has been extensively used over the past decades. Further studies are still needed to determine the impact of early-life stress on later life.

Interestingly, studies employing NH protocols, on the other hand, have found reduced sexual behaviour in males and females, reduced sexual receptivity, reduced lordosis quotient (LQ), an increased frequency of anovulatory oestrous cycles, and an altered hormonal profile of several hormones related to ovulation and sexual behaviour (27–29). This effect relates neatly with the effects of natural variations in maternal care because the effects of early handling have been ascribed, at least in part, to the enhanced maternal care the pups receive upon their return to the dam. Upon the return of the mother, NH increases maternal licking and grooming (LG) of the pups.

Findings suggest that the quality of parental care received during the early postnatal period programmes the HPG axis in rats, subsequently influencing adult sexual behaviour, especially in female rats, in which offspring of high LG showed reduced LQ, higher percentages of mounts without intromission (reflecting a decreased quality of lordosis), received fewer ejaculations and were less likely to achieve pregnancy (30,31). Also in the brain areas involved in the control of the HPG axis and sexual behaviour (the ventromedial hypothalamus and anterior ventral periventricular nucleus), high LG female offspring show lower ERα expression, which correlates with the reproductive strategy displayed by these animals (32).

It is proposed that maternal care induces internal modifications that can 'programme' reproductive strategies in the female rat. Such neuroendocrine programming biases towards increased fecundity (i.e.

the offspring of low LG mothers) or increased investment in the offspring (the offspring of high LG mothers), adapting female offspring to respond to subtle variations in parental care to adapt to the everyday environmental conditions that they will face. Under high-risk environmental conditions, when the probability of survival is low, the optimal strategy is to maximise the number of offspring through accelerated mating. By contrast, a more propitious environmental favours greater investment in individual offspring at the cost of mating (31,33).

In conclusion, early-life experience affects adult sexual behaviour. Unfortunately, parental influences on progeny remain not entirely understood. However, as researchers steadily gather more information about this system, it is becoming clear that, as in the rat, human parental programming of the reproductive system is likely to involve gene–environment interactions.

Sympathetic stress and ovarian function

Sympathetic nerves affect ovarian function

Sympathetic nerves arrive at the ovary originate from two sources (34,35): (i) the ovarian plexus nerve, which travels along the ovarian artery, and (ii) the superior ovarian nerve, which is associated with the suspensory ligament. Superior ovarian nerve fibres innervate the secretory components of the ovary (i.e. interstitial glands and follicles) (36). A detailed tracing study by Gerendai *et al.* (37) demonstrated that the sympathetic pathway to the ovary originates in the paraventricular region of the hypothalamus, results that have been confirmed by functional studies (37–39), leading us to propose the neuroanatomical organisation shown in Fig. 1.

We propose that stimulation originating from the paraventricular area of the hypothalamus travels by a multisynaptic pathway arriving at the celiac ganglion that then projects to the ovary by post-ganglionic sympathetic fibres where it regulates steroidogenesis and early follicular development (15). It has also been demonstrated that NE facilitates follicular development, as seen by the inhibition of follicular growth following the ovarian denervation (40). Chronic changes (either decreases or increases) in the sympathetic input to the ovary can cause profound changes in ovarian function.

The sympathetic nerve participates in the development of the polycystic ovary (PCO)

PCO syndrome (PCOS), the most common cause of infertility in women during their reproductive years, is a complex disease characterised by anovulatory failure and the presence of ovarian cysts, amenorrhoea, hyperandrogenaemia, and variable levels of circulating gonadotrophins (41). Because sympathetic nerves stimulate androgen secretion from the ovary, the possibility exists that a hyperactivation of sympathetic nerves could participate in the development and maintenance of ovarian cysts in the rat. In accordance with this hypothesis, sympathetic nerve activation induced by oestradiol valerate administration to rats is causally related to both the development and maintenance of PCO and surgical ablation of the sympathetic nerves at the level of the supra optic

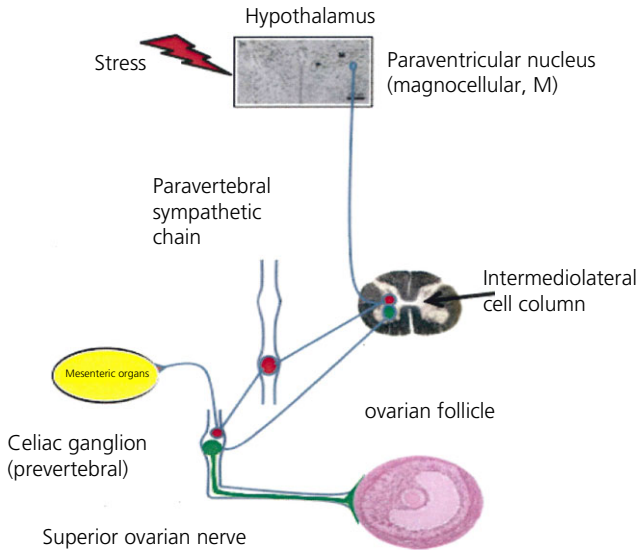

Fig. 1. Sympathetic nerve control of the ovary. Retrovirus tracing mapped the anatomical nerve connection between the brain and the ovary (37). Functional studies either changing the activity of neurones of the paraventricular nucleus or pharmacological blocking of the stress-activated sympathetic nerve pathway (38,39,44) has enabled verification of the relevance of sympathetic innervation with respect to the function of the ovary.

nucleus of the hypothalamus results in the reversal of the anovulatory PCO and diminishes ovarian androgen secretion (42,43). In addition, it has been shown that the hyperandrogenic condition is causally related with enhanced ovarian steroidal responsiveness to β-adrenoceptor stimulation, a condition also prevented surgical elimination of supraoptic nucleus projections to the ovary (42,43). This recovery of the ovulation was confirmed by the presence of corpus luteum in the denervated ovary and by the recovery of the oestrous cycling in rats.

PCOS is also characterised by metabolic abnormalities that are consistent with the metabolic syndrome. Enhanced sympathetic and adrenal medullar activities are important links between defects in insulin action and the development of hypertension. Despite extensive research seeking the pathogenesis of PCOS, there is still disagreement on the underlying mechanisms. The potential contribution of the sympathetic nervous system to the syndrome has been suggested in several studies, especially because of the role of NE to enhance androgens and progesterone secretion from the mammalian ovary (44,45). It has been suggested that androgen excess early in life may provide a hormonal 'insult' that results in manifestation of PCOS in adulthood (46), especially because PCOS is highly associated with conditions in which the foetus was exposed to high amounts of sex steroids during pregnancy. We have data demonstrating that mothers with PCOS maintain their hyperandrogenic condition during pregnancy, although their HPA axis has been suppressed (47). Hence, if chronically increased androgens reach the placental tissue in which the foetus is developing, the internal milieu can 'programme' its reproductive axis to be disturbed at the onset of puberty and adulthood. Therefore, one possibility to consider is that increased

superior ovarian nerve input may contribute toward the aetiology of PCOS through a stimulatory action on androgen secretion. This would explain the effectiveness of ovarian wedge resection or laparoscopic laser cauterisation to increase ovulatory response in women with PCOS because procedures are likely to disrupt superior ovarian innervation.

Sympathetic stress and β-adrenergic system spur the development of the PCO

The fact that the ovary communicates with the hypothalamus through a multisynaptic pathway implies that a centrally-originated stimulus could affect the function of the ovary independent of the well-known ovarian control mediated by gonadotrophins. It has been demonstrated that cold stress, either acutely or chronically, selectively activates the sympathetic nerves without altering the ACTH response. Cold stress has been described as stressor that activates the sympathetic nervous system and alters ovarian function (44). When the cold stress procedure is sufficiently chronic to affect a group of ovarian follicles (more than 4 weeks), it modifies follicular development by accelerating the transition from antral follicles to a group of preovulatory follicles that are not able to be released at ovulation, and therefore moves follicles towards a precystic appearance in which there was a hypertrophied theca cells compartment in parallel with an increase in ovarian NE concentration (44).

The stress response is a multifactorial event that involves orchestrated neuroendocrine responses required to maintain homeostasis but, when stress becomes chronic, it may induce pathology. To focus on the sympathetic nerve activity as one of the multiple factors involved in the chronic stress response, we recently applied a method to directly stimulate β-adrenoceptors by the *in vivo* administration of the β-adrenoceptor agonist isoproterenol (48). We administered isoproterenol (125 μg/kg/day) for 10 days to study the changes induced by β-adrenoceptor overstimulation in ovarian follicular development. Thirty days after isoproterenol withdrawal, there was a clear increase in the number of follicular cysts. The direct relationship between the β-adrenergic receptor activation and follicular cyst development was demonstrated by the capacity of propranolol (a β-adrenergic antagonist) to reverse both the isoproterenol-induced hyperandrogenic condition and the ovarian cyst formation (48).

We can conclude that the neural axis originating at the hypothalamic paraventricular nucleus controls the function of the ovary and that changes in the activity of this neural network regulate ovulation. Therefore stress, if chronic, could be harmful to reproduction. Experimental procedures aiming to attenuate the sympathetic activity could be a method for treating women with PCOS.

Afferent mediators of gonadal status on the paraventricular nucleus of the hypothalamus

There are sex differences in HPA axis function

The HPA axis involves the sequential release of a chain of hormones from the brain to the periphery, ultimately regulating the release of glucocorticoid steroids from the adrenal gland. Acute elevations in circulating glucocorticoids are adaptive because they provide sources of energy to meet the metabolic demands of homeostatic threat. On the other hand, chronic elevations in glucocorticoids are pathological and linked to several types of disorders, including anxiety and depression. Thus, the HPA axis must be both tightly regulated and equally responsive to the demands of stress (49). Our research focuses on sex differences and sex steroid hormone regulation of the paraventricular nucleus of the hypothalamus (PVH), the final common pathway regulating adaptive neuroendocrine responses. The hypophysiotropic zone of the PVH houses corticotrophin-releasing hormone (CRH) and AVP expressing neurones that synergise on the synthesis and release of ACTH from the anterior pituitary, which then stimulates the release of glucocorticoids from the adrenals [cortisol in humans, corticosterone (CORT) in rodents].

Rodent studies have shown that females secrete higher levels of CRH than males and higher levels of CORT in response to various challenges (50–52). The gonadal hormones are at least partly responsible for these sex differences in the rat because androgen administration decreases ACTH and CORT secretion, whereas oestrogens increase these measures (4,53). In humans without psychiatric illness, the sex difference in stress HPA axis function is not so apparent on the surface. Thus, men often show similar, if not higher, levels of cortisol than women in response to various acute challenges (54,55). However, this does not discount an underlying influence for androgens and oestrogens in regulating the HPA axis in humans because manipulations of gonadal status in women and men often provoke changes in CRH and cortisol release similar to the results in rodents (56–60). Several disorders associated with chronic stress are more prevalent in women than in men, including depression and such anxiety-related disorders as post-traumatic stress disorder (61–63). Depression is frequently associated with abnormalities of the HPA axis, including hypercortisolaemia (64), and cortisol levels have been reported to be higher in depressed women compared to men (65). Large variations in individual cortisol release patterns feature prominently in humans exposed to acute and repeated challenges (66) and the biological determinants for this variation are not understood. Thus, the neurobiological basis for the sex differences in stress-related disorders remains unresolved and, as argued elsewhere, extensive phenotyping of HPA axis function remains essential (58,67).

Serotonin modulates the HPA axis

Several lines of evidence support a stimulatory influence of 5-HT on the HPA axis in humans and rodents (68,69), mediated, in part, by the 5-HT_{1A} receptor subtype (70–72). Sexual dimorphisms in HPA axis function and in the 5-HT system provide evidence to suggest that the brain 5-HT system has a higher potential for stimulating the HPA axis in females. Thus, females express higher levels of 5-HT and/or metabolites than males in brainstem, limbic forebrain and cortex under basal conditions (73,74) and in response to various challenges (75–77).

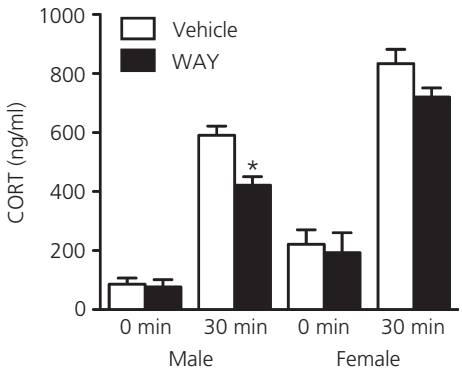

Fig. 2. Mean ± SEM plasma corticosterone responses in males and females given vehicle or 5-HT 1A receptor antagonist (WAY) injections prior to 30 min of restraint exposure. *P < 0.05 vs. vehicle counterpart (n = 7–8 per group). Adapted with permission from Goel et al (83).

Reported sex differences in 5-HT_{1A} receptor binding and/or expression have not been consistent (78,79). However, oestrogen has been shown to desensitise 5-HT_{1A} receptor coupling at both pre- and postsynaptic sites in unstressed animals. Presynaptic 5-HT_{1A} (somatodendritic) receptors diminish neuronal excitability of raphe neurones to reduce serotonin synthesis and release, whereas postsynaptic 5-HT_{1A} (heteroreceptors) receptors mediate the signal transfer to nonserotonergic forebrain neurones (80,81). Taken together, the stimulatory effect of the 5-HT system on the HPA axis could reflect the net of inhibitory and stimulatory influences of the 5-HT_{1A} receptor on the PVH, as well as its extended circuitries.

Sex differences modulate stress and 5-HT_{1A} receptor interactions

In humans and rodents, females show higher neuroendocrine responses to a systemic injection of the 5-HT_{1A} receptor agonist, 8-OH DPAT. We suspect that the endogenous requirements for 5-HT_{1A} receptors to regulate the HPA axis may also be sexually dimorphic under stressful conditions. Previous studies in the male rodent have shown that 8-OH-DPAT decreases the number of raphe neurones recruited to express Fos protein in responses to immobilisation, whereas the 5-HT_{1A} receptor antagonist, WAY 100635, counteracts this effect (82). Building on the utility of this antagonist to unmask how 5-HT_{1A} receptors participate in HPA axis control circuitry, we recently examined neuroendocrine and Fos responses in male and female rats bearing systemic injections of vehicle or WAY 30 min in advance of restraint exposure (83). In line with a stimulatory role for the 5-HT_{1A} receptor on the HPA axis, WAY administration decreased the CORT response to restraint in males but not in females (Fig. 2). This sex difference in HPA output was not recapitulated at the level of the PVH, where males and females showed similar decrements in Fos protein induction in response to WAY. This result warrants further exploration on connectional and phenotypic grounds, given the heterogeneity of cell types localised to the hypophysiotropic zone of the PVH.

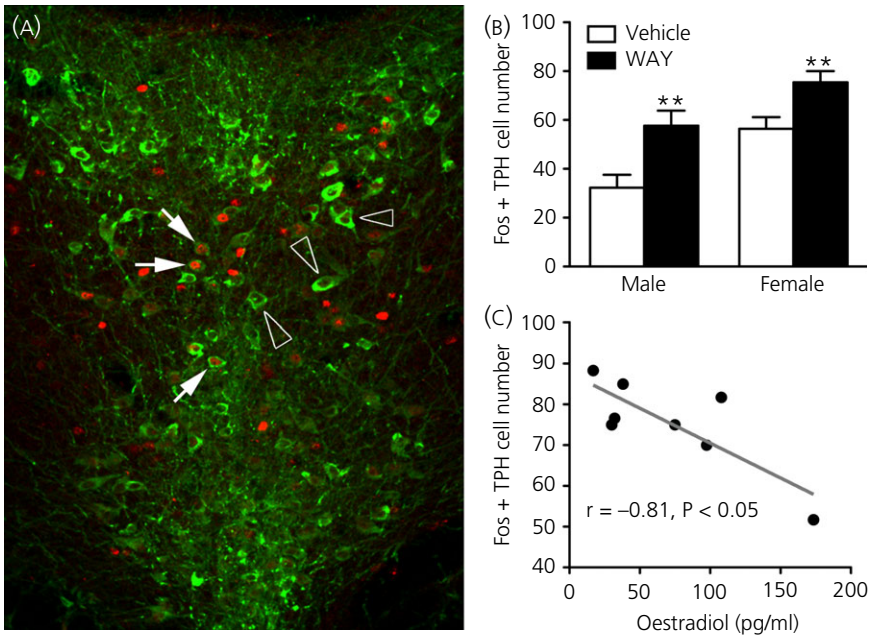

Fig. 3. Dual fluorescence confocal image showing overlap in nuclear Fos-immunoreactive (red) and cytoplasmic tryptophan hydroxylase (TPH; green) staining within the dorsal raphe nucleus (DRD) (A). Solid arrows show doubly-labelled neurones and open arrowheads mark Fos-positive, TPH-negative cells. Mean ± SEM relative numbers of double-labelled (Fos + TPH) neurones within the dorsal subdivision of the dorsal raphe nucleus in males and females (B). Scatterplot showing a significant negative correlation between plasma oestradiol concentrations and Fos + TPH-labelled cells in the DRD of WAY females (C). **P < 0.01 versus vehicle counterpart (n = 7–8 per group). Adapted with permission from Goel *et al.* (83).

By contrast to the PVH, WAY administration had the opposite effect to potentiate the stress-induced activation of dorsal raphe nuclei identified as serotonergic (tryptophan hydroxylase expressing), in both males and females (Fig. 3). However, a negative correlation between oestrogen and Fos responses was identified in WAY-treated females, to emphasise a role for oestrogen to decrease 5-HT_{1A} autoreceptor function. This could provide mechanisms for increasing 5-HT release in projecting structures and the heightened HPA axis responses in females. Analysis of the relative levels of 5-HT_{1A} mRNA revealed no sex differences in the size or distribution of the transcript within various forebrain nuclei or the dorsal raphe nucleus. However, a positive relationship was found between oestrogen and 5-HT_{1A} mRNA expression in females that was unique to the area of the zona incerta (Fig. 4). Based on previous connectivity experiments, the zona incerta represents a key relay for 5-HT raphe projections to the hypophysiotropic zone of the PVH, as well as for several limbic related structures (84–86). Thus, the organisation of zona incerta projections implies that this region may be in a position to integrate neocortical and emotionally relevant information to changes in oestrogen, as well as to coordinate central 5-HT and neuroendocrine responses.

The results emphasise the important sex differences in 5-HT_{1A} receptor regulation of the acute HPA axis response at both pre- and postsynaptic sites. The nature by which functional changes in 5-HT_{1A} receptors underlie a sex difference in HPA axis responses to chronic or repeated forms of stress remains to be seen. The 5-HT_{1A} receptor not only drives the stimulatory effect of serotonin on the HPA axis, but also is a critical determinant of the antidepressant response (87). Thus, our current findings provide several new starting points for understanding the connectivity of 5-HT_{1A} sensitive projections to the HPA axis and how these may contribute to the sex disparity in affective disease.

Social subordination disrupts the effects of oestrogen on behaviour and physiology in female rhesus monkeys

Social stress modulates the effects of oestrogen in female rhesus macaques

As emphasised above, rodents represent an appropriate model for studying interactions between stress and short-term changes in reproductive function. Of note, the human reproductive cycle is radically different to that of the female rat (88). Indeed, the magnitude and duration of endogenous oestrogen exposure and, consequently, the reactivity of brain systems responding to oestrogen, may not be entirely the same between female humans and rats. Similar to women, however, female rhesus monkeys display changes in ovarian hormones over a comparable 28-day cycle during the breeding season (89–92). Thus, the female rhesus monkey is perhaps more suitable for modelling psychopathologies in women attributed to major changes in ovarian hormone secretion (93–102).

Although there is utility in studying the effects of chronic psychogenic stress in the rodent, this can never approach the inherent complexities of psychosocial stress experienced by humans. By comparison, female macaques naturally form social hierarchies, in which subordinate (SUB) females are constantly harassed both physically and psychologically by their dominant (DOM) counterparts (103). This social organisation provides an advantageous and translatable model for characterising the effects of psychosocial stress on a multitude of physiological and psychological endpoints. Thus, chronic psychogenic stress exposure in SUB female macaques (104,105) induces a number of phenotypes (106–112) that are similar to patients suffering from mood, metabolic and immune disorders (113–121). Moreover, female macaques also display remarkable similarities with women in other physiological domains, including central nervous system mediators of neuroendocrine and emotional responses to stress (122–127).

In a series of experiments completed over the last several years, we have utilised this animal model to examine the effects of chronic psychosocial stress on the physiology and behaviour of ovariectomised (OVX) SUB female monkeys, and to determine how these are modulated by the replacement of the major ovarian hormone E_2. To control for previous life-experiences and any possible genetic propensity that may predispose a female towards a particular social rank, middle-ranking, unrelated adult females were selected from large social groups to form 10 new groups of five females and one male. Females were randomly selected and sequentially added to the new group following which the domi-

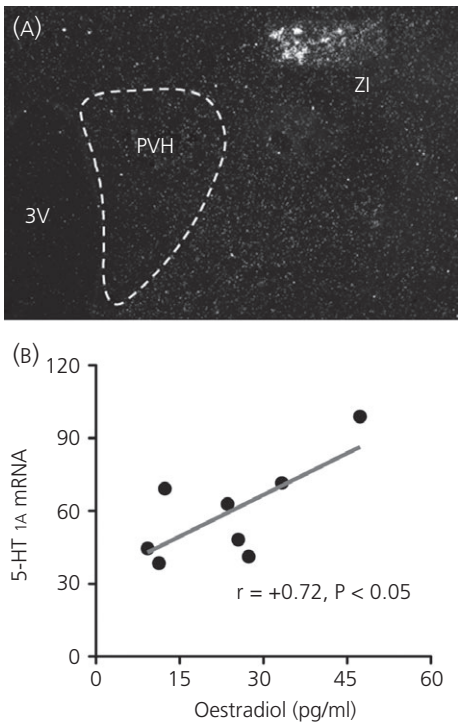

Fig. 4. Dark-field photomicrograph to show the distribution of 5-HT_{1A} receptor mRNA expression in the vicinity of the paraventricular nucleus of the hypothalamus (PVH) (A). Dashed line defines the nuclear border of the PVH to emphasise the absence of the transcript relative to the distinct cluster of 5-HT_{1A} receptor expressing cells within the zona incerta (ZI). Scatterplot (B) showing a significant positive correlation between plasma oestradiol concentrations and 5-HT_{1A} mRNA levels in the ZI of females. 3V, third ventricle. Adapted with permission from Goel *et al.* (83).

nance hierarchy quickly emerged (128). These small social groups functioned to exacerbate the social subordination stress that is usually dispersed throughout the normally large social groups favoured by this species. In addition, because it has been shown that short promoter polymorphism of the serotonin transporter gene (SERT) interacts with stress to increase the occurrence of affective disorders in individuals (129–132) and also increases both behavioural and HPA reactivity in rhesus monkeys (128,133–136), we evaluated the effect of the SERT polymorphism in our female monkey studies and reported these findings in experiments in which there was a statistically significant effect.

The results from such studies demonstrate that social subordination has profound effects on many aspects of behaviour and physiology, of which some are enhanced, blunted or unaffected by E_2 replacement (107,108,111,112,137–145). Here, we elaborate on three of these findings.

Social subordination results in increased anxiety behaviour and a disruption of socio-sexual behaviour, which are not consistently modulated by E_2

It had previously been shown that social subordination in female macaque monkeys increases depressive- (110,146) and anxiety-like behaviours (116). To evaluate whether the well-established anxiolytic effects of E_2 in rodents (147–155) were significantly affected by social status as well as by SERT polymorphism in female monkeys, a study by Michopoulos *et al.* (106) evaluated the effects of E_2 on behaviour in females prior to the addition of

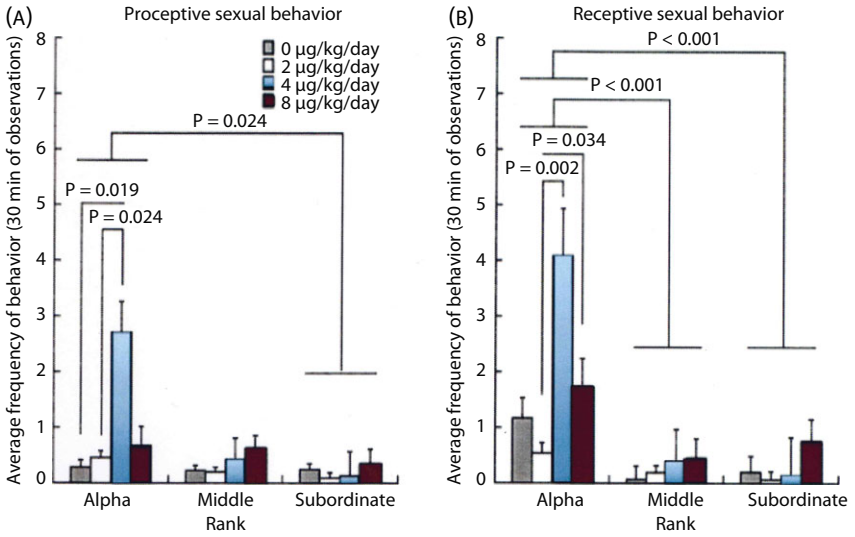

Fig. 5. The interaction between social rank and 17β-oestradiol (E_2) dose on sexual behaviour toward males. Both (A) proceptive and (B) receptive behaviour showed a main effect of social rank, a main effect of E_2 dose, as well as an interaction effect between the two. Post-hoc analysis showed activational effects only in dominant (DOM) alpha females. Data are presented as the mean ± SE of frequencies (140).

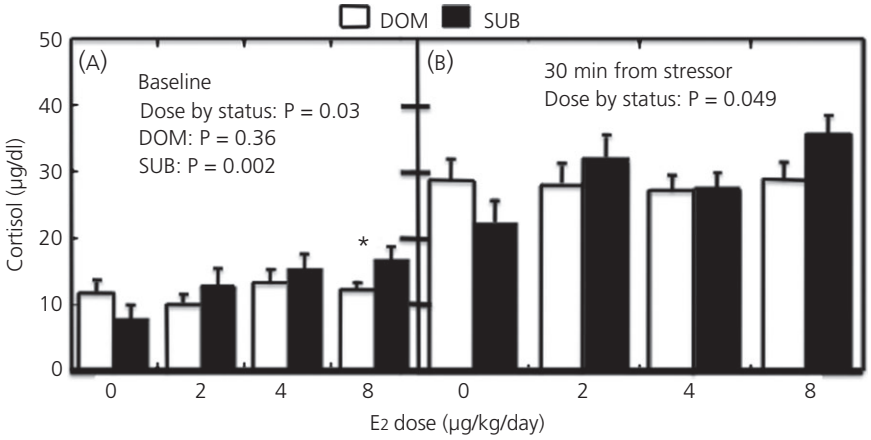

Fig. 6. Repeated measures analysis of variance was used to determine the effects of 17β-oestradiol (E_2) dose and social status [dominant (DOM) versus subordinate (SUB)] on cortisol levels at baseline and following stressor exposure. (A) Basal cortisol is dose-dependently increased by E_2 in SUB females only. (B) E_2-replacement increases plasma cortisol 30 min following an acute stressor in SUB females alone. Asterisk denotes a status difference in basal cortisol levels.

males to the group. The data showed that E_2 reduced rates of anxiety in DOM females with the short promoter length SERT variant and SUB females with the long SERT variant. DOM females with the long SERT genotype already showed the lowest levels of anxiety behaviour. By contrast, SUB females with the short SERT variant showing high levels of anxiety like behaviour were unaffected by E_2. Thus, the ability of E2 to attenuate anxiety is affected by both social subordination and SERT genotype in female macaques because E_2 is ineffective in modulating the high anxiety rates in SUB monkeys with the short SERT genotype. To determine the interaction between psychosocial stress and E_2 on socio-emotional behaviours when males were present, a study by Reding *et al.* (140) evaluated the effect of social status on reproduction, affiliation, aggression, submission and anxiety-like behaviours in these small groups. The data obtained (Fig. 5) showed that E_2 dose-dependently increased sexual motivation in DOM females but was without an effect in SUB females at any dose. E_2 replacement also increased male affiliation behaviour in DOM but not SUB females. Contact and noncontact aggression were also attenuated in DOM females. Overall, these results suggest that chronic social subordination stress attenuates the anxiolytic effects of E_2 and reduces the activational effects of E_2 on sexual behaviour and affiliation with males, and these latter effects cannot be overcome in SUB monkeys even with higher doses of E_2. Thus, the behavioural effects of E_2 are significantly blunted by social subordination in female macaque monkeys.

Social subordination results in altered HPA axis reactivity that is significantly modulated by E_2

Although SUB female monkeys appear to suffer from many conditions that are related to chronic stress (146,156–159), it has been difficult to establish differences in HPA axis activity as a result of social status. Previously, the only consistent findings indicating HPA dysregulation in SUB female monkeys comprised an increased adrenal size (127,160) and decreased glucocorticoid negative-feedback following dexamethasone injection (127,128,146,161). It has been shown that sex steroids modulate adrenal morphology and function (162–164) and that E_2 alters the diurnal release of cortisol (165) and glucocorticoid-induced negative-feedback on the HPA axis (6), although the use of naturally cycling female macaques in many previous studies (146,156,166) may have confounded some of these outcomes. Therefore, as with the studies described above, we first examined several features of HPA activity in OVX females and then determined the effect of E_2-replacement on some of these endpoints. Our results showed that, compared to OVX DOM females, OVX SUB females had flattened morning cortisol secretion, reduced dexamethasone-induced glucocorticoid negative-feedback, and a decreased adrenal cortisol response to an ACTH challenge (167). These results indicate that the ability to initiate and curtail glucocorticoid release is significantly reduced in OVX SUB female monkeys. Interestingly, this suggests that SUB females have a hyporesponsive HPA phenotype resembling that observed in several human psychopathologies, including post-traumatic stress disorder. Because previous work by our group had shown that SUB females were hypersensitive to the effect of E_2 on HPA activation (6), we next examined both basal and stress-induced cortisol levels in the same females during three different E_2 replacement regimens. The results depicted in Fig. 6 showed that pre-stressor cortisol was dose-dependently increased by E_2 in SUB but not DOM females. Furthermore, the increase in cortisol 30 min after the start of the stressor also showed a significant dose by status interaction, with nonreplaced SUB females having a blunted increase compared to nonreplaced DOM females and a greater increase than DOM females at the highest E_2 dose. These data show that DOM females exhibit a robust cortisol response irrespective of E_2 dose, whereas the CORT response of the SUB females is E_2 dose-dependent. This suggests a reduced response to stress in SUB females lacking E_2 and, as with the previous study, a hypersensitivity in E_2-replaced SUB females. This hypersensitivity to E_2 caused by chronic social stress may be very important when evaluating the stress response in

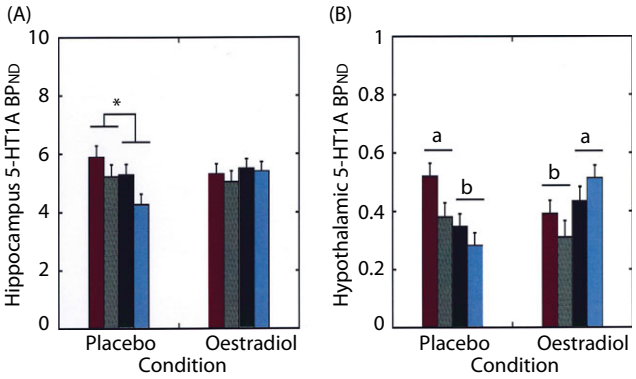

Fig. 7. Mean ± SEM 5-HT_{1A} receptor binding potential (5-HT1A BP_{ND}) during the placebo and 17β-oestradiol (E_2)-replacement for dominant (DOM), long serotonin transporter gene (SERT) genotype (red bars), DOM, short SERT genotype (s-variant) (grey bars), subordinate (SUB) long SERT genotype (black bars) and SUB short SERT genotype (s-variant) females (blue bars). (A) Hippocampal 5HT1A BP_{ND} is attenuated in subordinate females during the placebo condition compared to dominant females (denoted by asterisk), an effect that is normalised upon E_2 replacement. (B) Letters denote that hypothalamic $5HT_{1A}$ BP_{ND} is attenuated in subordinate females during the placebo condition compared to dominant females, an effect that is reversed upon E_2 replacement (144).

women under chronic social stress who have experienced trauma or other adverse emotional events.

Social subordination results in differences in 5-HT_{1A} receptor binding potential in brain regions implicated in emotional regulation and stress reactivity that is modified by E_2 only in the hippocampus and hypothalamus

Because central reduction of the serotonin 5-HT_{1A} receptor is associated with psychopathology in humans (168,169) and has been related to behavioural depression in monkeys (110), we conducted a study to determine the effect of social status and SERT genotype on serotonin 5-HT_{1A} receptor binding potential (5-HT1A BP_{ND}) in brain regions associated with emotional control and HPA activity in OVX female monkeys, and then assessed how these effects were modulated by E_2 replacement. Positron emission tomography using a 5-HT_{1A} receptor-specific ligand was performed to determine the levels of 1A receptor binding under a non-E_2 condition and a 3-week E_2 replacement condition in several brain regions, including anterior cingulate, medial prefrontal cortex, dorsolateral prefrontal cortex, orbitofrontal prefrontal cortex, amygdala, hippocampus, hypothalamus and raphe nucleus. The results show that female monkeys with the short SERT genotype have reduced 5-HT_{1A} binding potential in the medial prefrontal cortex irrespective of social status, and that SUB females with the short SERT variant show a reduction in 5-HT_{1A} binding potential within the anterior cingulate cortex (144). Moreover, the 5-HT_{1A} binding potential in these two regions was unaffected by E_2 replacement. By contrast, as shown in Fig. 7, hippocampal and hypothalamic 5-HT_{1A} BP_{ND} was attenuated in subordinate females regardless of SERT genotype during the non-E_2 condition, and this difference was normalised in the hippocampus and inverted in the hypothalamus with E_2 (144). These data suggest that E_2 can only alter central 5-HT_{1A} BP_{ND} in brain regions that show no SERT genotype-linked control of 5-HT_{1A} binding.

Overall, these experiments show that social stress in OVX female macaque monkeys produces a distinct behavioural phenotype that is largely unaffected by E_2, a hypo-responsive HPA axis that is hypersensitive to the modulating effects of E_2, and changes in serotonin 1A receptor binding in the hippocampus and hypothalamus that are restored or inverted by E_2 replacement. The results reported here elaborate the interaction between psychosocial stress and oestrogen in the modulation of a range of emotional and social behaviour, and begin to characterise the neurophysiology underlying these changes. This may be particularly relevant to women marginalised by low socio-economic status, who experience prolonged psychosocial stress and are disproportionately affected by psychopathology.

Concluding remarks

The HPA and HPG endocrine axes function in a tandem, flexible and bi-directional manner to ensure both reproductive viability and survival. The development of stress responsivity, as well as reproductive function, is influenced by early environmental factors that alter maternal care. This, in turn, creates a framework onto which the imperative to reproduce is balanced against the need to maintain homeostasis. This balance is tested (or challenged) when environmental contingencies (stressors) acutely upset homeostasis, which may result in the sex-specific modulation of neurotransmitter systems, as with 5-HT and stress HPA axis interactions. Intermittent or repeated stress exposure may place a greater load on the HPA–HPG equilibrium, as indicated by reduced ovarian function and pathologies associated with decrements in oestrogen release. Finally, the actions of gonadal hormones to mediate adaptive neuroendocrine and behavioural responses may be completely impaired in the face of chronic stress exposure. As emphasised here, where and how this breakpoint occurs to explain individual- and sex-based differences in stress related disease remains worthy of pursuit.

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