Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: Implications for postpartum mood disorder

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Summary
Early life stress is believed to constitute a risk factor for the development of mood disorders later in life. In the present study, we hypothesized that prenatal stress (PS) exerts long-lasting effects in female rat offspring, resulting in impaired adaptations to stress during lactation and, as such, may be a contributory factor to postpartum mood disorders. PS increased anxiety in adult virgin females compared with controls. During lactation, PS dams nursed significantly less and spent less time with pups compared with controls, whereas dams did not differ in pup retrieval or maternal aggression. HPA axis reactivity was elevated in response to a mild stressor in PS dams compared to their controls, but not in virgins, with the delta corticosterone response returning to the higher level seen in virgins. Moreover, corticotropin-releasing hormone (CRH) mRNA expression within the parvocellular region of the paraventricular nucleus (PVN) was increased in both virgins and dams exposed to PS compared with the relative controls, while the attenuation in expression in lactating controls was abolished following PS. In addition, arginine vasopressin (AVP) mRNA was increased in the parvocellular, but not magnocellular part of the PVN, in both PS-exposed virgins and lactating dams compared with their relative controls; although expression was also higher in controls during lactation compared with virgins. Thus, the present study demonstrates that exposure to PS results in long-lasting behavioural and neuroendocrine alterations in the female offspring, which are manifested during the lactation period. Furthermore, it implicates PS as a potential risk factor for the development of postpartum mood disorders, and that alterations in the HPA axis reactivity, at least partially, are involved.

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1. Introduction

Stress during pregnancy and in the peripartum period has been demonstrated to increase the risk of mood disorders in the offspring later in life (O’Hara and Swain, 1996; Kofman, 2002). Importantly, physiological responses to stress exposure in late pregnancy and lactation are significantly attenuated, as seen both in human and animal studies (Stern et al., 1973; Neumann et al., 1998; Russell et al., 1999; Lightman et al., 2001; Kammerer et al., 2002; De Weerth and Buitelaar, 2005). These adaptations are believed to be necessary to protect the fetus from exposure to excessive levels of glucocorticoids (Welberg and Seckl, 2001). Further, gonadal steroids are important regulators of the HPA axis and the corticotropin-releasing hormone (CRH) system (Kirschbaum et al., 1999; Young et al., 2001). Therefore, the attenuation in stress-related brain circuitries may also be important for the well-being of the mother, providing a protective mechanism against the dramatic fluctuations in circulating sex steroids. Correspondingly, general suppression of HPA axis activity during lactation has been hypothesized to prevent the development of depression in vulnerable women (Carter et al., 2001). Accordingly, disruption of such normal adaptations is a likely contributory factor in the development of postpartum affective disorders (Zonana and Gorman, 2005).

The postpartum period is a time of increased vulnerability to mood disorders, with 20–30% of women experiencing mood disorders within the first 6 weeks postpartum (O’Hara and Swain, 1996; Llewellyn et al., 1997; Pedersen, 1999; Mastorakos and Ilias, 2000). A number of animal studies have demonstrated that chronic stress during pregnancy can affect maternal behaviour (Pardon et al., 2000; Meek et al., 2001; Patin et al., 2002; Bosch et al., 2006) and increase anxiety in the dam (Maestripieri et al., 1991; Darnaudery et al., 2004). Furthermore, attenuation of the stress responsiveness of the HPA axis can be partially prevented by pregnancy stress; an effect which was dependent on the genetically determined level of anxiety (Neumann et al., 2005). However, due to the lack of knowledge, both from a clinical and preclinical standpoint, the neurobiological mechanisms underlying postpartum mood disorders, including postpartum depression, remain largely unknown.

Adverse effects of early life stress on adult neuroendocrine parameters and stress coping are well documented both in virgin female and male offspring (Sucheki and Palermo Neto, 1991; Weinstock et al., 1992; Neumann et al., 1998, 2005; Levine, 2001; Welberg and Seckl, 2001; Pedersen and Boccia, 2002; Bosch et al., 2006). For example, CRH and arginine vasopressin (AVP) expression were found to be altered after exposure to prenatal stress (PS; Bosch et al., 2006). These two neuropeptides are well documented to be involved in stress coping and to undergo adaptations during pregnancy and lactation (for review see Neumann, 2003). One study has shown that exposure to early life stress (7d intermittent stress during gestation) in rats characterized as high licking/groomers, reduced not only this behaviour but also brain oxytocin receptor expression to the level of those characterized as low licking/groomers (Champagne and Meaney, 2006). Despite this, a possible link to peripartum mood disorders in adult life of female offspring has not been demonstrated to date.

We hypothesized that PS exerts long-lasting effects in female offspring, resulting in impaired adaptations to stress during lactation and as such, may be a contributory factor to postpartum mood disorders.

In order to study this hypothesis, prenatally stressed, adult female rats were mated and tested for their maternal behaviour early in lactation. Moreover, plasma ACTH and corticosterone levels were monitored in order to assess the responsiveness of the HPA axis to an acute stressor. Finally, the activity of CRH and AVP were monitored by quantification of their respective mRNA expressions in the hypothalamic paraventricular nucleus (PVN).

2. Methods

2.1. Animals

All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Bavaria and the guidelines of the National Institute of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Adult, virgin female Wistar rats purchased from Charles River (Germany; 250–300 g body weight) were mated overnight in groups of three with a sexually experienced male. Pregnancy was confirmed by the presence of semen in vaginal smears the following morning (day 1 of pregnancy). Pregnant rats were exposed daily to a psychosocial stressor or remained unstimulated and housed in groups of two to three rats of the same treatment group under standard laboratory conditions (12:12 light–dark cycle, lights on at 0600 h, 22 °C, 55% humidity and free access to water and standard rat chow) and were housed singly from day 20 of gestation until weaning of PS or control female offspring at the age of 22 days.

Female offspring, after weaning, were housed in groups of 3–4 of the same treatment under standard laboratory conditions as defined above.

2.2. Pregnancy stress

Between days 4 and 10 of pregnancy, half of the pregnant rats (n = 7) were exposed to a psychosocial and restraint stressor daily. The former involved the introduction of the pregnant dams as intruders into a lactating resident dam in its home cage for 45 min (Neumann et al., 2001) whereas the latter comprised of 60 min restraint stress (plexiglas column with ventilation holes; 12 cm diameter; Glavin et al., 1994). The stressors were alternated daily, with one exposure between 0900 h and 1200 h and the other between 1400 h and 1700 h in order to decrease the predictability of the stressor (Anisman and Matheson, 2005). Between days 11 and 18 of pregnancy, dams were only exposed to 60 min daily maternal defeat because of significant weight gain of the pregnant rats. This procedure has been successfully used before (Bosch et al., 2006). Pregnant control rats (n = 8) were left undisturbed (change of bedding once a week). Control and pregnancy-stressed rat dams gave birth to 14.3 ± 0.5 and 12.7 ± 1.3 pups, respectively. The litter sizes were adjusted to 8 pups. The day of birth was considered day 0.
The lactating resident rats used to defeat the experimental rats during pregnancy were purchased as virgins from Charles River (Sulzfeld, Germany). They were kept in a separate room to avoid adaptation to the pregnant intruders and to increase the performance of offensive behaviour towards them (Bosch, unpublished observation). Further, lactating residents were only used between day 2 and 8 of lactation, i.e. the time of highest maternal aggression (Giovanardi et al., 2000; Neumann et al., 2001).

2.3. Elevated plus-maze

In order to confirm the effects of PS on anxiety-related behaviour, virgin female offspring of both the PS group (n = 25) and the control group (n = 29) were tested on the elevated plus-maze at the age of 12 weeks according to a protocol by Pellow et al. (1985) and as described before (Neumann et al., 1998). Briefly, the test is based on the creation of a conflict between the exploratory drive of the rat and its innate fear of open and exposed areas. Thus, reduced open-arm exploration indicates increased anxiety-related behaviour. The plus-maze made of opaque PVC consists of a plus-shaped platform elevated 70 cm from the floor, separated from the rest of the room by a curtain. Two of the opposing arms (50 × 10 cm) are enclosed by 40 cm-high side and end walls (closed arms, 20 lux), whereas the other two arms have no walls (open arms, 80 lux).

Before each animal was tested, the plus-maze was cleaned thoroughly by water with a mild detergent. At the beginning of the test, the rat was placed onto the central area (10 × 10 cm) of the maze facing a closed arm. The following parameters were recorded by means of a video/computer setup during the 5-min exposure between 0800 h and 1200 h: the percentage of time spent on the open arms (ratio of time spent on open arms to total time spent on all arms), the latency until first entry into the open arm and the number of entries into closed arms. All animals were placed into the test room the day before and the scorer was blind to the experimental group.

2.4. Maternal care

Home cage: In order to test the effects of PS on undisturbed maternal behaviour in the home cage, half of the PS and control rats were mated at the age of 12 weeks with an experienced breeder. Pregnancy was confirmed by the presence of semen in vaginal smears. One day after delivery, i.e. on lactation day 2, the undisturbed maternal behaviour of lactating dams was observed and scored in their home cage. The occurrence of arched back nursing as well as the sum of all different nursing postures (arched back, lying on side, blanket position), and the sum of all pup-directed behaviours (i.e. the total time spent in direct pup contact including pup carrying), were recorded by an observer blind to the experimental group. The respective behaviour was monitored every 4 min during a 60-min observation period performed every second hour between 0600 h and 1900 h, i.e. during a total of 7 h with the last observation period in the dark phase under red light conditions.

Pup retrieval: The pup retrieval test was performed as described before (Neumann et al., 2005). Briefly, between 0900 h and 1200 h on day 3 of lactation, PS and control dams were separated from their pups for 1 h. Meanwhile, pups were kept on a heating pad at 32 °C. Eight pups of one litter were then spread into the corners of an arena (32 × 34 × 53 cm, with some saw dust of the original homecage, the arena was cleaned thoroughly by water with a mild detergent before each test), the dam was placed into the centre of the arena and the time of collection of eight pups was monitored during a 15-min observation period.

Maternal defence test: Between 0900 h and 1200 h on day 4 of lactation, maternal aggressive behaviour was monitored during a 10-min maternal defence test as described before (Neumann et al., 2001) with lactating control and PS dams as residents defeating a virgin intruder. Importantly, the outcome of the maternal defence test was found to be independent of prior exposure to the 15-min pup retrieval test the day before (Neumann et al., 2005). The behaviour was videotaped for later analysis by an experienced experimenter blind to the treatment of the dams. Behaviours analysed were the amount of attacks, as well as offensive, defensive and explorative behaviour.

2.5. Jugular vein catheterization and blood sampling

In order to study the effects of PS on neuroendocrine adaptations of the HPA axis in lactating compared with virgin female offspring, PS (virgin n = 8; lactating n = 9) and control (n = 8 both groups) rats were implanted with a chronic jugular vein catheter on lactation day 4. Surgery was performed under isoflurane anaesthesia and semi-sterile conditions as described before (Neumann et al., 1998). The catheter was filled with sterile saline (0.9%) containing gentamicin (30000 IU/ml; Centravet, Germany) and exteriorized dorsally in the cervical region. Following surgery, the rats were housed singly in plexiglas cages and carefully handled each day to familiarize them with the blood sampling procedure and to reduce non-specific stress responses during the experiment.

At 0800 h on lactation day 9, the catheters were connected to a sampling syringe filled with heparinized saline, and the pups were removed. After 2 h, the first blood sample (0.2 ml) was taken under baseline, stress-free conditions in EDTA-coated tubes with trasylol (10 μl) and stored on ice. After 30 min, a second basal sample was taken. Thereafter, the rat was placed on an elevated platform (25 cm diameter, 70 cm above the floor in front of the home cage) for 5 min, which has previously been demonstrated to constitute an acute mild stressor in rats (for details see Neumann et al., 2000). After 5, 15 and 60 min, additional blood samples were taken and replaced by sterile saline. The samples were centrifuged (4000 rpm, 4 °C, 5 min) and the plasma was separated for measuring ACTH (70 μl) and corticosterone (10 μl) and stored at −20 °C. Plasma ACTH and corticosterone were measured radioimmunologically using commercially available kits (ICN Costa Mesa, USA) according to the respective protocols.
2.6. In situ hybridization for CRH and AVP mRNA

In order to study the effects of PS on CRH and AVP mRNA expression within the hypothalamic PVN in lactating (day 12) compared with virgin female offspring, the brains of PS and control virgin and lactating rats were rapidly removed under basal conditions and short (10 s) isoflurane anaesthesia and flash frozen. Cryocut sections (20 μm) were slide mounted. One set of six slide-mounted brain sections per animal was processed, targeting the PVN.

The CRH mRNA in situ hybridization was performed using a highly specific single, 48 base, 35S-labeled oligonucleotide probe (5’ gcg cct gca) directed against the last 16 amino acids of the glycoprotein that AVP does not share with the related peptide oxytocin (Ivell and Richter, 1984; Villar et al., 1994).

In situ hybridization was performed using an established protocol (De Vries et al., 1994; Wang et al., 1994). Following in situ hybridization, the air-dried sections were exposed to BioMax MR film (Eastman Kodak, Rochester, New York, USA) with 14C-labeled autoradiographic standards (Amersham Bioscience Europe GmbH, Freiburg, Germany) for 2 h (AVP mRNA in situ hybridization was performed using a highly specific single, 48 base, 35S-labeled oligonucleotide probe (5’ gcct gca gcc cgg ccc ggc cgg ccc gtc cag) directed against the last 16 amino acids of the glycoprotein that AVP does not share with the related peptide oxytocin (Ivell and Richter, 1984; Villar et al., 1994).

In situ hybridization was performed using an established protocol (De Vries et al., 1994; Wang et al., 1994). Following in situ hybridization, the air-dried sections were exposed to BioMax MR film (Eastman Kodak, Rochester, New York, USA) with 14C-labeled autoradiographic standards (Amersham Bioscience Europe GmbH, Freiburg, Germany) for 2 h (AVP mRNA in the magnocellular PVN), 6 h (AVP mRNA in the parvocellular PVN) or 18 days (CRH mRNA). Slides from all groups were processed simultaneously. The autoradiograms were coded to ensure the identity of the tissue. Brain slices which contained comparable sections of PVN were measured for each subject to provide individual means. Expression of AVP mRNA was measured as optical density and of CRH mRNA as grey density on a Macintosh computer with a computerized image program (ImageJ 1.31, National Institutes of Health, http://rsb.info.nih.gov/ij/). Background activity was automatically subtracted from measured areas to yield values for specific binding.

2.7. Statistical analysis

Data are presented as group means ± SEM. Statistical analysis was performed by means of statistical software (GB-Stat V6.0, Dynamic Microsystems, Silver Spring, MD, USA). One-way (factor reproductive status, or factor treatment) or two-way (factors reproductive status × treatment, treatment × time) analyses of variance (ANOVA) followed by the Newman–Keuls post hoc test were appropriate were utilized. P < 0.05 was considered statistically significant. For comparison of two groups only (plus-maze data, delta values), the non-parametric Mann–Whitney U-test was used.

3. Results

3.1. Effects of PS on anxiety-related behaviour

In confirmation of previous results, exposure to PS significantly increased the anxiety-related behaviour of virgin female offspring, as reflected by a reduction in the percentage of time spent on the open arms of the elevated plus-maze compared with unstressed virgin controls (p = 0.02, Fig. 1). The latency to first open-arm entry and the number of entries performed into the closed arms reflecting locomotor activity, was not altered by PS.

3.2. Effects of PS on maternal behaviour

Exposure to PS significantly altered home cage maternal behaviour of the female offspring during lactation (Fig. 2), whereas maternal behaviour, either in a challenging environment, i.e. during the pup retrieval test or by displaying maternal aggression, i.e. in the maternal defence test, was not affected by PS (Table 1).

In the home cage, on day 2 of lactation, the amount of nursing was dependent on the time of the day (F6,102 14.7, p < 0.0001; Fig. 2A), with a significant decrease at the beginning of the dark phase in both control (p < 0.05) and PS (p < 0.01) groups. Also, PS tended to affect nursing behaviour (treatment × time F6,102 2.0, p = 0.07). Separate analysis of total nursing behaviour in the light and dark phase (inset Fig. 2A) revealed that the amount of nursing is altered by PS (F1,34 5.09, p = 0.03). Specifically, in the dark phase, the reduction in nursing behaviour is more pronounced in PS dams compared with control dams (p < 0.01), whereas no differences were observed within the light phase (Fig. 2A). With respect to different nursing postures and the occurrence of arched back nursing, PS and control dams did not differ at any time point monitored (F6,102 0.67, p = 0.68; Fig. 2B). The occurrence of licking and grooming of the pups was found to depend on both treatment and time (F6,102 2.53, p = 0.03), but no differences were revealed by the post hoc test (data not shown).

The total occurrence of pup-directed behaviour (mother on; Fig. 2C) was found to depend on the early life treatment.
and differed over the day (treatment × time $F_{6,102} = 3.30$, $p = 0.005$) with a decline at the beginning of the dark phase in both control ($p < 0.05$) and PS ($p < 0.01$) dams. The amount of total pup-directed behaviour was significantly reduced in PS dams during the dark phase compared with control dams ($p < 0.01$).

During the pup retrieval test performed on day 3 of lactation, there was no significant difference between the experimental groups, with five pups collected within 5 min (Table 1). Furthermore, PS and control dams did not differ in maternal aggression as reflected by similar amount of attacks as well as offensive, defensive and explorative behaviour (Table 1).

### 3.3. Effects of PS on plasma ACTH and corticosterone

The mean basal levels of ACTH differed between virgin and lactating rats ($F_{1,29} = 9.27$, $p = 0.005$), but the post hoc test revealed no significance. Further, mean basal levels were not affected by PS ($F_{1,29} = 1.06$, $p = 0.31$; data not shown). Exposure to a mild stressor (elevated platform) induced a significant rise in plasma ACTH concentrations both in virgin ($F_{4,70} = 11.7$, $p < 0.0001$) and lactating ($F_{4,60} = 24.8$, $p < 0.0001$) rats (Fig. 3A). In lactating rats, the delta ACTH response was similar compared with virgin female rats. PS did not significantly alter ACTH concentrations in either virgin or lactating rats. However, separate calculation on ACTH delta values revealed that exposure to PS significantly elevated the ACTH response in lactating rats ($p < 0.05$) but not in virgins (n.s.; Fig. 3A inset).

As described for ACTH, the mean basal levels of corticosterone differed between virgin and lactating rats ($F_{1,29} = 9.85$, $p = 0.004$), but the post hoc test revealed no significance. Further, mean basal levels were not affected by PS ($F_{1,29} = 0.17$, $p = 0.68$; data not shown). Exposure to the elevated platform also elevated corticosterone plasma concentrations in virgin ($F_{4,56} = 26.1$, $p < 0.0001$) and lactating ($F_{4,60} = 57.4$, $p < 0.0001$) rats (Fig. 3B) with a significantly blunted response in lactating rats ($p < 0.01$). An effect of PS was seen in lactating, but not in virgin rats, where the corticosterone delta response was more pronounced in lactating PS compared with lactating control dams ($p < 0.05$, Fig. 3B inset).
3.4. Effects of PS on hypothalamic CRH mRNA expression

CRH mRNA expression within the hypothalamic PVN was found to be dependent on the reproductive state ($F_{1,20} 38.2, p<0.0001$) and exposure to PS ($F_{1,20} 12.6, p=0.002$; Fig. 4). In detail, and in confirmation of our own results (Bosch et al., 2006), exposure to PS significantly elevated CRH mRNA expression in virgin female rats ($p<0.05$). Moreover, on day 12 of lactation, CRH mRNA expression within the PVN was significantly lower in unstressed lactating rats compared with unstressed virgin female offspring ($p<0.01$), confirming previous results (Walker et al., 2001). Importantly, exposure to PS prevented the lactation-related reduction in CRH mRNA expression and significantly elevated CRH in PS lactating dams compared with non-stressed lactating rats ($p<0.01$; Fig. 4).

3.5. Effects of PS on hypothalamic AVP mRNA expression

AVP mRNA expression was found to be dependent on reproductive state (parvocellular (pPVN): $F_{1,20} 11.5, p=0.003$; magnocellular (mPVN): $F_{1,20} 14.7, p=0.001$) and PS exposure (only pPVN: $F_{1,20} 27.3, p<0.0001$; Fig. 5).

Fig. 3  (A) ACTH and (B) corticosterone secretory response following exposure to the elevated platform (EPF, arrow) of PS female dams (black symbols and columns) and their unstressed controls (grey symbols and columns) on day 9 of lactation. Hormone delta responses are shown on the insets. Numbers of animals per group (control/PS) were (A) virgin 8/8, lactating 8/9, and (B) virgin 7/8, lactating 8/9. Data represent mean±SEM. ##$p<0.01$, *$p<0.05$ vs. previous basal sample; *$p<0.05$ vs. control; $\dagger p<0.05$ vs. virgin.

In detail, within the pPVN ($p<0.01$), but not the mPVN, exposure to PS elevated AVP mRNA expression in virgin female rats, confirming our recent results (Bosch et al., 2006). Interestingly, AVP mRNA expression was generally elevated in lactating dams both in the pPVN and the mPVN compared with respective virgin female offspring ($p<0.05$). In lactating rats exposed to PS, AVP mRNA levels were found to be further elevated in the pPVN ($p<0.01$), whereas, in the mPVN, AVP expression was not affected by PS.

4. Discussion

The present study demonstrates that exposure to PS results in long-lasting behavioural and neuroendocrine alterations in the female offspring, which are manifested during the lactation period. Thus, PS dams show impaired maternal behaviour and display a more pronounced ACTH and corticosterone response to an acute stressor compared with control dams. Further, PS dams do not display the lactation-related downregulation of CRH mRNA in the PVN. These changes suggest that the attenuation of the HPA axis responsiveness during lactation is impaired in dams, which were subjected to PS.
4.1. Effects of PS in virgin female offspring

There exists substantial evidence, both from human (Meijer, 1985; Susser and Lin, 1996; Brown et al., 2000; Wadhwa et al., 2001; Kofman, 2002; Federenko and Wadhwa, 2004; Owen et al., 2005; van den Bergh et al., 2005; Wadhwa, 2005) and from preclinical studies (for review see Weinstock, 2001) that PS impairs the behavioural and hormonal development of the offspring. Numerous studies in rodents have reported that PS causes anxiety and depression-like symptoms and increase reactivity of the HPA axis to stress in adulthood (Fride et al., 1986; Weinstock et al., 1992; McCormick et al., 1995; Valleé et al., 1997; Bowman et al., 2004). The present study, in agreement with these findings, demonstrates that adult virgin female offspring, which were exposed to PS were more anxious compared with non-stressed controls (Fig. 1). It is likely that alterations of numerous neuronal systems underlie the effects of early life stress in adulthood (Peters, 1986; Insel et al., 1990; Liu et al., 2000). One system implicated in such effects is CRH, as both prenatal (Bosch et al., 2006) and postnatal (Plotsky and Meaney, 1993; Ladd et al., 1996) stress have been reported to increase hypothalamic CRH mRNA expression. The present study replicated these observations in PS-treated virgin female offspring (Fig. 4) but extends these findings to demonstrate for the first time that PS also increases AVP mRNA expression in the parvocellular PVN (Fig. 5). The increase in AVP expression in the pPVN appears highly selective as levels within the magnocellular neurons of PVN (Fig. 5) and SON (data not shown) were not significantly different between control and PS-treated virgin offspring. Exposure to an acute stressor tended to cause a greater increase in ACTH and corticosterone in PS-treated virgin female offspring (Fig. 3), further supporting long-lasting alterations to the HPA axis.

AVP, like CRH (Stenzel-Poore et al., 1994; Landgraf et al., 1995; Liesch et al., 1995; Nemeroff, 1996; Bakshi et al., 2002; van Gaalen et al., 2002; Heinrichs and Koob, 2004) has been shown to induce anxiogenic effects, specifically within the PVN (Wigger et al., 2004) and to regulate neuroendocrine stress responses (Bielsky et al., 2004; Engelmann et al., 2004; Landgraf and Neumann, 2004; Wotjak et al., 1998). Additionally, genetic factors also appear to play a role in these systems, as differences in the vulnerability to PS have been reported between male and female offspring (Weinstock et al., 1992; McCormick et al., 1995; Nischio et al., 2001; Bowman et al., 2004; Gue et al., 2004), between different strains (Stöhr et al., 1998; de Fries et al., 1967) and between rats bred for high or low levels of anxiety (Bosch et al., 2006). Thus, a potential mechanism underlying the increased anxiety and HPA responsiveness found in PS-treated virgin offspring, is the elevated activity of the brain CRH and AVP systems in the PVN. Importantly, and adding further credence to this hypothesis, hyperactivity of both the CRH and AVP neuropeptidergic systems has been linked to neuroendocrine and behavioural aberrations related to...
psychopathologies including depression (Nemeroff, 1996; Purba et al., 1996; Arborelius et al., 1999; Keck et al., 2002; Bakshi and Kalin, 2002; Holmes et al., 2003; Landgraf and Neumann, 2004; Landgraf, 2006).

4.2. Effects of PS on female offspring during lactation

The underlying aetiology of depression, including postpartum mood disorders, remain poorly understood, despite many advances in treatment and diagnostic abilities (Slattery et al., 2004). This in turn hinders the development of animal models based on aetiology (Cryan et al., 2002; Cryan and Slattery, 2007). However, our knowledge regarding behavioural and physiological adaptations during pregnancy and lactation, particularly with respect to stress-coping mechanisms, is rapidly expanding. Although mainly originating from animal studies, such findings and their interpretations, provide an important basis for understanding the aetiologies of postpartum mood disorders. A consistently replicated finding from human studies in breastfeeding women (Nisell et al., 1985; Schulte et al., 1990; Altemus et al., 1995; Kammerer et al., 2002; De Weerth and Buitelaar, 2005) and in lactating rodents (Stern et al., 1973; Walker et al., 1995; Windle et al., 1997a,b; Shanks et al., 1999; Lightman et al., 2001; Neumann, 2003) is an attenuation of HPA axis responses to stress. In confirmation of these findings, a significant blunting of corticosterone secretion following exposure to a mild stressor was observed in lactating control dams in our study (Fig. 3B), likely to be the result of a diminished ACTH secretion (Fig. 3A). Importantly, the lactation-associated attenuation of HPA axis was impaired in PS-treated lactating dams. More specifically, the attenuated corticosterone secretion in response to a mild stressor during lactation was absent in PS dams compared with controls (Fig. 3) despite no difference being observed in basal values. Therefore, it is possible to speculate that the neuroendocrine adaptations of the HPA axis are particular sensitive to chronic stressful experiences, either in the prenatal period (this study) or during pregnancy (Neumann et al., 2005).

HPA axis activity, particularly ACTH secretion, is regulated through a synergistic action of hypothalamic CRH and AVP released from parvocellular neurones of the PVN (Gillies et al., 1982; Aguilera, 1994; Engelmann et al., 2004). Interestingly, CRH mRNA expression within the PVN is reduced during pregnancy (Johnstone et al., 2000) and lactation (Da Costa et al., 2001; Lightman et al., 2001; Walker et al., 2001), a finding which was replicated in the current studies comparing lactating and virgin control rats. Lactation-associated alterations in CRH expression have also been described in the central amygdala (decrease; Walker et al., 2001, but see Da Costa et al., 2001) and the bed nucleus of the stria terminalis (BNST; increase; Walker et al., 2001), two regions important not only for HPA axis regulation, but also for emotionality. Moreover, increased activity of the amygdala is one of the most consistent findings from human neuroimaging studies of depressed patients (Drevets, 2003) and the lesions of the BNST have recently been described to increase behavioural despair in Wistar rats (Pezuk et al., 2006). Thus, alterations in the brain CRH system may be responsible, at least in part, for the behavioural changes observed in lactation, including reduced anxiety (Hard and Hansen, 1985; Toufexis et al., 1999a; Windle et al., 1997b; Neumann, 2003) and display of appropriate maternal behaviour (Pedersen et al., 1991) and aggression (Gammie et al., 2004). Therefore, it follows that the increased anxiety and decreased maternal behaviour in the dark phase in PS-treated dams may be in part the result of the impaired attenuation of CRH mRNA in the PVN, levels of which did not differ from virgin controls (Fig. 3B).

In contrast to the attenuation of the CRH system in the PVN, an elevation of AVP in this region appears to be an essential part of maternal adaptations (Walker et al., 2001). In the present studies, we could recapitulate these findings in lactating control dams compared with control virgins, in both the parvo- and magnocellular regions of the PVN (Fig. 5). The increased vasopressin system should be predominantly viewed in context of regulating osmotic balance in lactation, due to the production and loss of milk. However, this increased vasopressinergic drive is also likely to be involved in maternal behaviour (Pedersen et al., 1994) and maternal aggression (Bosch et al., 2004). Therefore, and in combination with the increased CRH system activity observed following PS, the increased AVP expression, restricted to the pPVN, of PS-treated dams in lactation is likely to play a prominent role in the differences in behaviour. These alterations in the CRH and AVP systems clearly reflect PS-induced abnormalities of the stress circuitries of the brain and are likely to contribute to the elevated HPA axis reactivity in these rats. Interestingly, a recent study has shown that female offspring subjected to PS have decreased receptor expression of the related neuropeptide, oxytocin, during their lactation period (Champagne and Meaney, 2006). This suggests that the adaptations observed in the current study following stress may also be transmitted across generations, causing similar alterations in maternal behaviour in the offspring. Additional experiments are planned to reveal the physiological significance of the high synthetic activity of AVP and CRH neurones in PS-exposed lactating dams. Furthermore, future studies will address whether PS exposure also affects additional systems known to be attenuated in lactation, such as noradrenergic tone within the PVN (Toufexis et al., 1998; Douglas et al., 2005), and the pituitary sensitivity to CRH and enhanced sensitivity to vasopressin (Toufexis et al., 1999b).

As alluded to above, PS exposure resulted in less time the dams spend in direct pup contact (nursing, licking, carrying the pups) during the dark phase, with no differences observed during the light phase (Fig. 2). It is intriguing to speculate that the rise in brain CRH levels underlie the impairment in maternal behaviour observed in PS-treated lactating dams as CRH has previously been reported to influence maternal behaviour (Pedersen et al., 1991). Contrastingly, no difference between prenatal conditions were observed during both the pup retrieval test and the maternal defence test (Table 1). This discrepancy between home cage maternal care and maternal aggression or pup retrieval in a novel environment is possibly explained by previous literature demonstrating that while stressors can affect maternal behaviour, maternal aggression and pup retrieval appear to be a robust behaviour (Neumann et al.,
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2005). More specifically, repeated exposure to stress during pregnancy has been shown to affect maternal behaviour in the home cage, but not pup retrieval in a novel environment (Maestripieri et al., 1991; Pardon et al., 2000; Meek et al., 2001; Neumann et al., 2005). The finding that maternal behaviour is only modestly affected by PS raises the question whether such a model could be established to study postpartum depression, as a significant lack of child attachment and maternal care has been reported in women suffering from postpartum mood disorders (Lyons-Ruth et al., 1986; Bifulco et al., 2004). However, it is unclear to what extent external factors can influence maternal behaviour in rodents and that such a modest decrease may in fact represent a substantial alteration in physiology, which is supported by the findings relating to HPA axis reactivity. Further support is provided by data showing only modest effects on maternal behaviour of mothers exposed to the stress during pregnancy (Neumann et al., 2005).

In summary, the present study reveals that exposure to PS results in long-lasting effects on the female offspring, which persist into the peripartum period. PS results in impairments in lactation-associated adaptations in the CRH and AVP neuropeptidergic systems, which are known to influence emotionality and stress coping, and a corresponding increase in anxiety and decrease in maternal behaviour. Therefore, the present study implicates PS as a potential risk factor for the development of postpartum mood disorders and that alterations in the HPA axis reactivity, at least partially, are involved.

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References


