Available online at www.sciencedirect.com
ScienceDirect
Hormones and Behavior 51 (2007) 77–88
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Psychological stressors as a model of maternal adversity: Diurnal modulation of corticosterone responses and changes in maternal behavior

Marion Léonhardt [a], Stephen G. Matthews [b], Michael J. Meaney [a], Claire-Dominique Walker [a,*]

[a] CIHR program in Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) Douglas Hospital Research Center, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3
[b] Department of Physiology, Medicine and Obstetrics and Gynaecology, University of Toronto, Toronto, Canada M5S 1A8

Received 24 May 2006; revised 23 August 2006; accepted 28 August 2006
Available online 10 October 2006

Abstract

Maternal adversity is associated with long-lasting consequences on cognitive development, behavior and physiological responses in rat offspring. Few studies have examined whether repeated maternal stress produces repeated activation of the hypothalamus–pituitary–adrenal (HPA) axis in mothers and whether it modifies maternal behavior. Here, we tested a novel model of perinatal stress using repeated exposure to "purely" psychological stressors throughout the gestation and lactation periods in rats. We first tested the diurnal influences of repeated 1-h strobe light exposure on maternal corticosterone secretion. Despite the hyporesponsiveness to stress documented in late pregnant and lactating mothers, we observed an enhanced response to strobe light in the afternoon compared to the morning in stressed mothers during lactation. Next, dams were exposed to 24-h forced foraging followed by 10-h wet bedding during the diurnal peak of corticosterone secretion. Although no corticosterone responses to forced foraging and wet bedding were observed, the combination of both stressors had a significant effect on maternal behavior. Mother–pup interactions were significantly altered during the first 8 days of lactation. Taken together, these findings suggest that lactating mothers maintain responsiveness to specific and repeated psychological stressors, in particular at the time of the diurnal peak in corticosterone secretion. Depending on the stressor applied, either neuroendocrine activation or changes in maternal behavior might be important determinants of the long-term consequences in the offspring. The combination of forced foraging, wet bedding and strobe light might represent a novel model of mild maternal adversity using "purely" psychological stressors.

Keywords: Maternal stress; Corticosterone; Maternal behavior; Diurnal stress response; Forced foraging; Wet bedding

Introduction

Human epidemiological data as well as animal studies consistently show that early life stress is associated with defects in cognitive and affective development as well as impaired physiological stress responses (Meaney and Szyf, 2005; Pryce et al., 2005; Mirescu et al., 2004; Chapillon et al., 2002; Lemaire et al., 2000; Vallee et al., 1999; Cianfarani et al., 1998; Maccari et al., 1995), which increases the susceptibility to physiological and psychological pathologies later in life (for reviews see Seckl, 2001; Weinstock, 2001; Matthews, 2002). Such programming is thought to be a consequence of complex interactions between differential gene expression or vulnerability factors, stress hormones released by the hypothalamic–pituitary–adrenal (HPA) axis in mothers (Kapoor et al., 2006), and variations in maternal care (for reviews see Fish et al., 2004; De Kloet et al., 2005). In rats, the perinatal period represents a critical window for brain development (Avishai-Eliner et al., 2002; Morgane et al., 1993). A burst of brain growth and a rapid neuroendocrine maturation occur during this period which makes the brain highly vulnerable to environmental programming (Meaney and Szyf, 2005; Ozanne and Hales, 2002; Matthews, 2002). Several paradigms of prenatal and perinatal maternal stress have been used to induce behavioral and biological dysfunctions in rodent offspring, including immobilization or restraint in a plastic cylinder (Maccari et al., 1995; Rojo et al., 1985; Smith et al., 2004), novel environment exposure (Muir et al., 1985; Maestripieri et al., 1991), immersion

* Corresponding author. Douglas Hospital Research Center, 6875 Lasalle Blvd, Verdun, QC, Canada H4H 1R3. Fax: +1 514 762 3034.
E-mail address: waldom@douglas.mcgill.ca (C.-D. Walker).

0018-506X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2006.08.008

in cold water (Guo et al., 1993), hyperthermia (Shiota and Kayamura, 1989) and others. The frequency and duration of exposure to gestational stress as well as the stressor combination have been varied, often to introduce an unpredictable component over the entire gestation (Rojo et al., 1985) or during the last week of gestation (Muir et al., 1985; Maccari et al., 1995). Other experimental protocols have used acute or chronic maternal stress during the lactational period, including maternal separation (Plotsky and Meaney, 1993), reduction of bedding/nesting materials (Brunson et al., 2005), noise stress (Windle et al., 1997) and endotoxin injections (Shanks et al., 1999).

Whereas the effects of repeated perinatal maternal stress exposure have been well documented in the offspring, it is generally assumed that repeated perinatal stress causes a steady release of stress hormones in mothers, despite evidence of a blunted period of stress responsiveness in the mother during late pregnancy, parturition and lactation (Lightman, 1992; Neumann et al., 1998; Toufexis et al., 1999; Douglas et al., 2003) in rodents and possibly also in humans (for review see Tu et al., 2005). This blunted stress response has been suggested as a mechanism to limit the adverse effect of glucocorticoid exposure in fetuses and neonates. In fact, few studies have attempted to determine whether repeated maternal stress does indeed produce a steady release of glucocorticoid and whether habituation occurs under conditions of repeated exposure to psychological stressors. Repeated stressful events can have important consequences on maternal behavior in humans (Llewellyn and Nemeroff, 1993) and in animals (Champagne and Meaney, 2006; Smith et al., 2004; Darnaudery et al., 2004; Patin et al., 2002; Maestripieri et al., 1991). In humans, the early postpartum period is a vulnerable period, often associated with the onset of depression (Mastorakos and Ilias, 2000) and the risk of postpartum depression is increased by exposure to stressful events during pregnancy (Federenko and Wadhwa, 2004). Moreover, parental care may be altered by stressors associated with poverty (McLoyd and Wilson, 1990). In animals, mothers exposed to repeated stress during pregnancy display enhanced anxiety-related behaviors (Darnaudery et al., 2004; Maestripieri et al., 1991), spend more time near their pups and nurse them more (Muir et al., 1985) although others have also reported a reduction of nesting/grouping pups and arched back nursing (Smith et al., 2004). In a recent model of early postnatal adversity and maternal neglect, Brunson et al. (2005) showed that limited availability of nesting material impaired nurturing behavior with reduced and fragmented nursing and grooming of the pups. In addition, in mothers displaying high or low pup-directed licking/grooming (LG) behavior, repeated exposure to restraint stress during the last week of gestation reduces pup licking/grooming in high LG mothers to a level comparable with those of low LG mothers (Champagne and Meaney, 2006). This pregnancy stress effect can be mediated by a reduction in oxytocin receptor levels, an important neuroendocrine parameter of the lactating dam implicated in the regulation of maternal care (Champagne and Meaney, 2006). In addition, prenatally stressed pups might also elicit different maternal care, i.e., maternal licking (Moore and Power, 1986a) and it appeared that the natural trend of foster dams to lick more males than females was eliminated by prenatal stress (Moore and Power, 1986b).

To increase the potential for translational studies from rodents to humans and our understanding of the effects of pre- and perinatal stress in human cohorts, the use of "purely" psychological stressors appears to be more appropriate and powerful. Several paradigms of early life adversity have a mixed composition of psychological and physical stress. For instance, maternal restraint stress in a plastic cylinder under strong light has an obvious psychological component, but an increase in body temperature and reduction in locomotion also constitutes a physical stressor. Thus, the present study was designed to examine a novel model of early perinatal maternal adversity on maternal behavior and maternal glucocorticoid secretion using repeated exposure to ecologically relevant psychological stressors. We first tested whether maternal glucocorticoid secretion following exposure to strobe light was altered during gestation and lactation. Since blunted responses to stress in pregnant and nursing females have been reported in the morning (low basal plasma corticosterone levels), we tested whether maternal responses to this stressor displayed a diurnal component and could be enhanced in the afternoon at the time of the diurnal peak of corticosterone secretion. In a second series of experiments, we used repeated exposure to high foraging demand and wet bedding as psychological stressors applied at the time of high basal glucocorticoid secretion throughout pregnancy and lactation. Forced foraging is known to impair mother–infant interactions and adrenocortical activity in nonhuman primate species (Rosenblum and Andrews, 1994) and in rodents (Hennessy and Sharp, 1990). Similarly, housing on soiled bedding is a powerful stressor that increases heart rate, body temperature and locomotor activity (Harkin et al., 2002). Using this stressor combination, we observed a significant effect of this stressor on maternal behavior and on plasma glucocorticoid concentrations in stressed mothers.

Materials and methods

Animals

Adult Sprague-Dawley virgin female (250 g) and male rats (275 g) were purchased from C. River (St Constant, Quebec) and maintained in the Animal Facility at the Douglas Hospital Research Center in controlled conditions of light (lights on at 0800 h and off at 2000 h), temperature (18–25°C) and humidity (25–40%) and with ad libitum access to food and water. Females were group housed (2–3 per cage) during 2 weeks to synchronize their estrous cycle prior to mating. After mating and the observation of sperm in vaginal smears (gestational day 0), females were singly housed and the conditions of light were changed according to the experiment (experiment 1: lights on between 0700 and 1900 h; experiment 2: lights off between 0800 h and 2000 h). Pregnant females were randomly assigned to the control (*C*) and stress (*S*) groups. The day of birth was considered as postnatal day (PND) 0 and on PND1 all litters were culled to 10 pups per mother. All procedures were approved by the Animal Care Committee of McGill University and followed ethical guidelines from the Canadian Council on Animal Care.

Repeated maternal stress during the gestation and lactation periods

As depicted in Fig. 1, pregnant and lactating females were subjected to repeated stress on GD9, 12, 15, 19 and PND3, 8, 14 and 21. Mothers were left

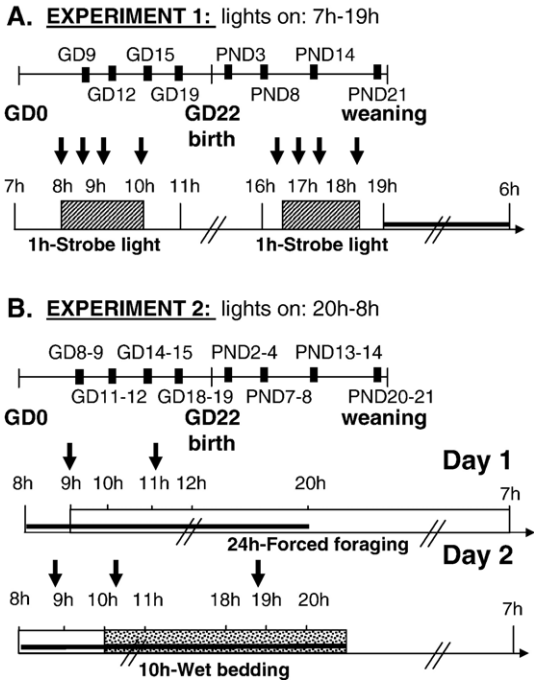

Fig. 1. Experimental design for repeated stress experiments. In experiment 1 (A), stressed females were exposed to 1 h of strobe light twice daily during the light phase on gestation day (GD) 9, 12, 15, 19 and postnatal day (PND) 3, 8, 14, 21. Blood samples (⬇) were taken before, during and after each stress exposure. In experiment 2 (B), stressed females were exposed to 24 h of forced food foraging followed by exposure to wet bedding for 10h during the dark phase on GD8-9, 11–12, 14–15, 18–19 and PND2–4, PND7–8, PND13–14, PND20–21. Blood samples (⬇) were taken 5 times in 34 h; before, during and after each stress. In both experiments, control females were left undisturbed except for blood sampling at the same time as stressed females.

undisturbed between days of stress exposure. Two different stress paradigms were used.

Strobe light stress (experiment 1): On each of the stress days, pregnant and lactating mothers were exposed to high intensity strobe light (white light, 5–7 flash/second; 10 W bulb) for 1 h in their home cage placed in an opaque box lined with black felt. Stress was applied in the morning (between 0800 h and 0900 h) and in the afternoon (1630 h–1730 h). Control mothers were left undisturbed in their home cage. For nursing mothers, pups from stressed mothers were separated from their mother during stress light exposure and maintained on a warming pad. Maternal blood samples were collected from the tail vein by cutting the tip of the tail (approximately 1–2 mm) and collecting blood drops. The tail was only cut once and blood samples were collected prior to stress onset and at 30 min, 60 min and 2 h after the onset of stress. Control mothers were not subjected to stress although blood from these animals was collected at the same time points as for stressed mothers.

Forced foraging and wet bedding stress (experiment 2): Forced food foraging was intermittently applied to pregnant and lactating mothers for 24-h periods (Day 1) by mixing 125% of the food normally eaten by control mothers at the same reproductive stage. This forced the mother to forage for food without creating food deprivation during the critical periods of pup growth. When applied in lactating mothers, food was available ad libitum for the offspring in an area of the cage only accessible to the pups via a small hole in a plexiglass partition placed in the cage. When partitions were placed after parturition, the mother and her litter were housed in a larger cage in order to maintain the original housing space for the mother. This stressor was followed on day 2, by exposure to a wet bedding material for 10 h during the dark period of the light–dark cycle (between 0900 h and 1900 h). The wet bedding procedure was initiated by placing a slowly leaking water bottle over the top of the cage. Mothers were kept on the wet bedding for 10 h, after which they were placed on fresh bedding in clean cages.

Blood samples were collected from control and stressed mothers via the tail vein tip, using a mild restraint procedure at various times prior to and during exposure to these psychological stressors (see Fig. 1). Plasma was separated by centrifugation and stored at −20°C prior to being assayed for corticosterone concentrations.

Plasma corticosterone determination

Plasma corticosterone concentrations were assayed by specific radioimmunoassay (RIA) using a kit from ICN (Medicorp, Montreal, Canada) as previously described (Deschamps et al., 2003). Briefly, 5 µl of plasma was incubated with ^{125}I-corticosterone (8000 cpm) and a specific anti-corticosterone serum (ICN kit). The free fraction was separated from the bound fraction by addition of an excess charcoal buffer and centrifugation. The radioactivity contained in the bound fraction was counted in a gamma counter. The limit of detection of the assay was 0.2 µg/dl and the interassay and intra-assay variability was 12%, and 3%, respectively. All samples were assayed in duplicates.

Maternal behavior

In order to control for the effects of blood sampling on maternal behavior and establish "normal" behavioral conditions as a reference point for both the control and stress groups described above, we introduced an additional control group of mothers which were left undisturbed and were not subjected to blood sampling procedures (Cu). Mothers in the Cu group were only weighed at regular time intervals throughout gestation and lactation.

Maternal behavior was observed on PND3–4 and on PND6–8 during either a "rest" day (PND3 and 6) or a "stress" day (PND4 and 8). Each dam was observed in her home cage for four 72-min observation periods per day including three periods during the dark phase (0930 h, 1330 h and 1730 h) and one period during the light phase (2100 h) of the light–dark cycle. Within each observation period, the behavior of each dam was scored every 3 min (25 observations per period for 4 periods per day, i.e., 100 observations per mother per day). Clusters of behaviors were divided into 3 categories, i.e., actions directed towards the mother, towards the pups and actions aimed at building the nest. Behaviors directed towards mothers consisted of self grooming (GS), eating or drinking (E), wandering (W), and sleeping alone (WP, wander passive). Behaviors directed towards the pups were active nursing (active arched back nursing position, NA), passive nursing (blanket-like position, NP) and pup grooming (GP) and retrieving. For each mother in each experimental group, the overall frequency of behavioral occurrence was computed. Qualitative assessment of behavioral changes was determined using higher occurrence of specific behaviors over the observed days and depicted in a representative analysis grid by color code (cf., legend of Fig. 7).

Statistical analysis

All results are presented as group means ± SEM and the level of significance was set at $P<0.05$. Hormonal results and behavioral data were evaluated by ANOVA with or without repeated measures over time followed by post hoc Newman–Keuls tests for further examination of group differences. One-way ANOVA and Student's two-tailed *t*-tests were performed when appropriate.

Results

Diurnal changes in maternal corticosterone response to strobe light stress

Repeated 1-h strobe light exposure between GD9 and PND21 did not affect maternal body weight gain during gestation ($t=0.76$, $P>0.05$) or lactation ($t=1.10$, $P>0.05$) and did not reduce litter size at birth ($t=-0.61$, $P>0.05$) (Table 1). Moreover, repeated stress did not affect pup's body weight at PND1 (Control: 6.89 ± 0.39 g; Stressed: 6.72 ± 0.27 g, $P>0.05$)

Table 1
Litter size at birth and maternal weight gain during gestation and lactation.

Experiment	Group	Maternal body weight gain (g)		Litter size at birth
		Gestation	Lactation	
Experiment 1 (strobe light)	Control (n=6)	94.00±10.81	33.00±9.24	13.50±0.65
	Stressed (n=5)	86.65±3.70	22.25±5.77	14.30±1.02
Experiment 2 (forced foraging/ wet bedding)	Control (n=6)	99.35±4.66	9.53±5.30	16.33±1.28
	Stressed (n=5)	84.17±8.81	6.26±8.93	18.20±1.02

All values represent mean±SEM. For experiment 1, gestation is from gestational day (GD) 0 to GD19 and lactation from postnatal day (PND) 1 to PND21 while gestation is from GD0 to GD18 and lactation from PND2 to PND22 for experiment 2. Body weight on GD0 was comparable between mothers randomly attributed to control (*C*) and stressed (*S*) groups. Pup weight at PND0–1 at PND20–21 was comparable between *C* and *S* litters. All litters were culled to 10 pups/mother on PND1.

and at PND21 (Control: 49.24±2.91 g, Stressed: 42.14±1.81 g; $P>0.05$). In addition, we determined that the repeated blood sampling protocol did not affect basal corticosterone levels measured within the first hour of lights off in control mothers throughout gestation or lactation (except on PND13, Table 2).

Repeated 1-h strobe light exposure significantly increased maternal corticosterone secretion compared to control mothers in the afternoon, but only on PND3 (Fig. 2). Three-way ANOVA with repeated measures over time and time of the day (AM/PM) showed a significant main effect of time (GD19: $F(3,24)=3.90$ $P<0.05$; PND3: $F(3,24)=6.00$ $P<0.01$; PND8: $F(3,24)=7.86$, $P<0.01$) as well as a significant ($P<0.01$) AM/PM effect ($F(1,8)=17.45$; PND3: $F(1,8)=210.99$, PND8: $F(1,8)=72.89$) at each of these ages. The main effect of group (Control vs. Stress) was only significant on PND8 ($F(1,8=14.99$, $P<0.01$). We observed a significant time×AM/PM interaction at GD19 ($F(3,24)=3.89$ $P<0.05$) and there was a significant increase in corticosterone secretion in the afternoon compared with the morning ($F(3,48)=7.75$ $P<0.001$), at 30 min ($F(3,32)=14.56$, $P<0.01$), 60 min ($F(3,32)=19.24$, $P<0.001$) and 120 min $F(3,24)=19.16$, $P<0.001$). On PND3, the ANOVA showed a three-way interaction (group×time×AM/PM interaction: $F(3,24)=4.68$, $P<0.05$). At this age, the corticosterone response was significantly higher in stressed mothers compared to control mothers during the afternoon at 30 min ($F(1,64)=13.01$ $P<0.001$) and 120 min ($F(1,64)=7.64$ $P<0.01$). Moreover, levels of corticosterone were significantly higher in the afternoon than in the morning at all times in control and stressed mothers (*C*: 0, 30, 60 min, $P<0.001$ and 120 min, $P<0.01$ and *S*: 0 min, $P<0.05$ and 30, 60, 90 min, $P<0.001$). On PND8, we observed a significant group×time interaction ($F(3,24)=3.76$ $P<0.05$), stressed mothers displayed an overall higher corticosterone secretion ($F(3,24)=10.75$ $P<0.001$) at 30 min ($F(1,32)=9.77$, $P>0.01$), 60 min ($F(1,32)=7.53$, $P>0.01$) and 120 min ($F(1,32)=15.67$, $P>0.001$).

Analysis of the area under the curve for the corticosterone secretion following strobe light exposure (Fig. 3) showed a significant effect of group (Control vs. Stressed: $F(1,8)=11.13$, $P<0.05$) and time of day (AM vs. PM: $F(1,8)=26.06$, $P<0.001$), but no effect of age. We observed an age×AM/PM interaction ($F(7,56)=2.38$, $P<0.01$), and corticosterone secretion in the PM was significantly higher than in the AM at GD15 ($F(1,64)=9.02$, $P<0.01$), at GD19 ($F(1,64)=14.65$, $P<0.001$), at PND3 ($F(1,64)=6.69$, $P<0.05$) and PND8 ($F(1,64)=5.62$, $P<0.05$). Although significant effects of stress could not be demonstrated at specific ages using our three-way ANOVA analysis, there was a trend for PM corticosterone secretion to be higher in stressed mothers compared to control mothers on GD9, GD19 as well as all postpartum ages. When the magnitude of the response to strobe light was determined in virgin females, we observed a large corticosterone AUC due to the blood sampling and strobe light exposure (AUC CORT: AM=3954±576 µl/dl×120 min, n=6; PM=2488±754 µl/dl×120 min). In the AM, corticosterone secretion to strobe light in virgin females was higher than that observed in pregnant and lactating mothers. In the PM, the corticosterone response to strobe light in lactating females was comparable or higher than that of virgin females.

Effect of forced foraging and wet bedding stress on maternal plasma corticosterone secretion throughout gestation and lactation

Repeated forced foraging followed by wet bedding starting during the second week of gestation until weaning did not affect maternal body weight gain during gestation ($t=1.98$ $P>0.05$) or lactation ($t=0.36$, $P>0.05$) or the number of pups

Table 2
Basal plasma corticosterone levels in mothers as a function of gestation and lactation age

Group	Maternal basal corticosterone levels (µg/dl)							
	Gestation				Lactation			
	GD8	GD11	GD14	GD18	PND2	PND7	PND13	PND20
Control sampled (n=6)	14.30±3.00	32.56±6.80	34.7 ±4.05	83.27±6.11	51.53±13.50	33.25±5.00	36.77±4.32	31.67±4.05
Control Undisturbed (n=4)	17.98±2.80	ND	32.26±2.40	ND	26.22±5.03	28.15±4.64	23.01±2.41**	28.73±3.83

Blood samples were collected 1 h after lights out at 0900 h in control mothers subjected to the repeated blood sampling paradigm as for stressed mothers (Control sampled) and in control mothers left undisturbed except for blood sampling at the specific ages when corticosterone levels were measured (Control undisturbed). ND: not determined. Values are group means±SEM.
* $P<0.05$ compared to Control sampled group (Student t-test).

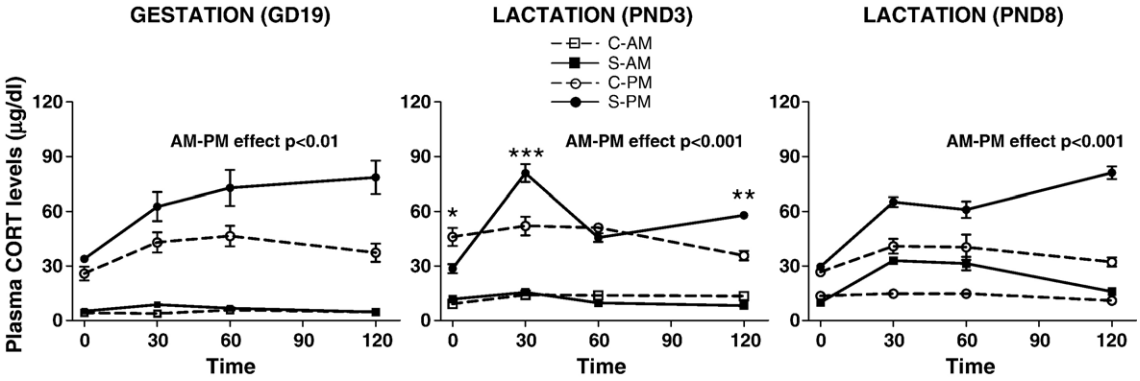

Fig. 2. Diurnal variations in the time course of plasma corticosterone concentrations in response to repeated 1-h strobe light with blood sampling (Stressed mothers, *S*, $n=6$) or blood sampling alone (Control mothers, *C*, $n=4$) on GD19, PND3 and PND8. Values are presented as means±SEM. *$P<0.05$; **$P<0.01$; ***$P<0.001$ S-PM vs. C-PM.

per litter ($t=-1.07$, $P>0.05$) (Table 1). Pup body weight at birth (Control: 6.28±0.14 g, Stress: 5.88±0.14 g; $P>0.05$) or PND20 (50.96±3.98 g, Stress: 48.68±2.66 g, $P>0.05$) was not altered by the psychological stress regimen.

The corticosterone response to the stress of forced foraging (Fig. 4) was expressed as the difference between corticosterone levels at basal time (0 min; 0900 h; dark phase) and 2 h after the onset of foraging (2 h; 1100 h, dark phase). Because forced foraging was initiated at the beginning of the dark phase, we expected that corticosterone levels in control animals would decrease between the 0-min and 2-h time point (thus a negative delta) due to normal diurnal variations in corticosterone secretion. On GD18–19, the delta corticosterone secretion was mostly negative in control mothers, while earlier, on GD11–12 and GD14–15, there was no apparent circadian change in controls. On GD8–9, the positive delta in control mothers might reflect the stress due to the first episode of blood sampling alone. Forced foraging during gestation tended to increase corticosterone secretion in stressed compared to control mothers (positive deltas against the normal circadian decrease) on GD8–9, GD11–12 and GD14–15, although the increase did not reach statistical significance at any of those ages. On GD18–19, there was a large drop in corticosterone secretion in control mothers, due to large circulating value of corticosterone at 0 min ($C=83.27\pm6.11$ µg/dl and $S=75.64\pm12.96$ µg/dl), but no response to forced foraging stress. During lactation, we observed a significant response to this stressor only on PND7–8 ($t=3.58$, $P<0.01$).

Similar to foraging stress, the corticosterone response to wet bedding (Fig. 5) was expressed as the difference between corticosterone levels at time 0 min (0900 h; dark phase, after 24 h of foraging stress) and 1.5 h after the onset of wet bedding (1030 h, dark phase). Because wet bedding was initiated at the beginning of the dark phase, we expected that corticosterone levels in control animals would decrease between the 0-min and 1.5-h time point (thus a negative delta) due to normal diurnal variations in corticosterone secretion. Indeed, this was observed for all ages starting on GD14–15. Although wet bedding was previously reported to cause dramatic cardiovascular responses in male rats (Harkin et al., 2002), this stressor did not significantly influence corticosterone responses in females either during gestation or lactation (Fig. 5). In virgin females, we observed a small, but not significant increase in corticosterone secretion in response to wet bedding (*C*: 5.45±9.67 µg/dl, $n=6$, *S*: 21.39±7.98 µg/dl, $n=6$; $P>0.05$).

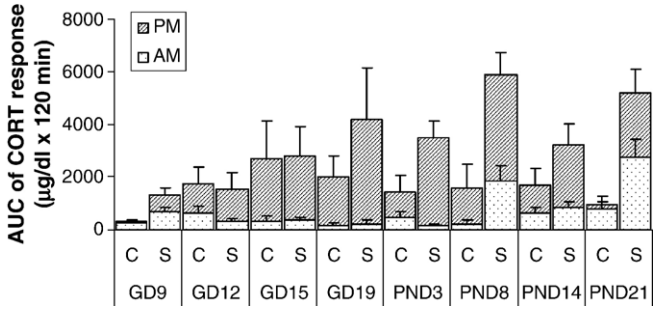

Fig. 3. Total maternal corticosterone secretion (area under the curve over 120 min) in response to repeated 1-h strobe light exposure with blood sampling (Stressed mothers, *S*, $n=6$) or blood samplings alone (Control mothers, *C*, $n=4$) in the morning and in the afternoon. Responses are measured during gestation on GD9, 12, 15, 19 and lactation on PND3, 8, 14, and 21 during the light phase. Values are presented as means±SEM.

Effect of repeated psychological stressors on maternal behavior

For evaluation of maternal behavior, we compared *C* and *S* mothers to an additional group of control unhandled mothers (Cu), which were not disturbed for blood sampling or stress. Behavior was evaluated on both resting days (between stressor exposure, PND3 and 6) and days of stress exposure (PND4 and 8). The repeated combination of forced foraging and wet bedding stress did not significantly affect maternal behavior on days of rest (PND3 and 6) during the dark (0900 h–1012 h) and light (2100 h–2212 h) phases of the cycle (Fig. 6). In contrast to resting days, maternal behavior was disturbed during the days of wet bedding (PND4 and 8) during the dark and light phases of the cycle. At PND4, during the exposure to wet bedding (dark phase), stressed mothers displayed a significant reduction in the frequency of active nursing compared to control mothers (group×behavior effect, $F(4,60)=3.30$, $P<0.05$, post hoc NK, $P<0.01$). No further changes in active nursing were noted

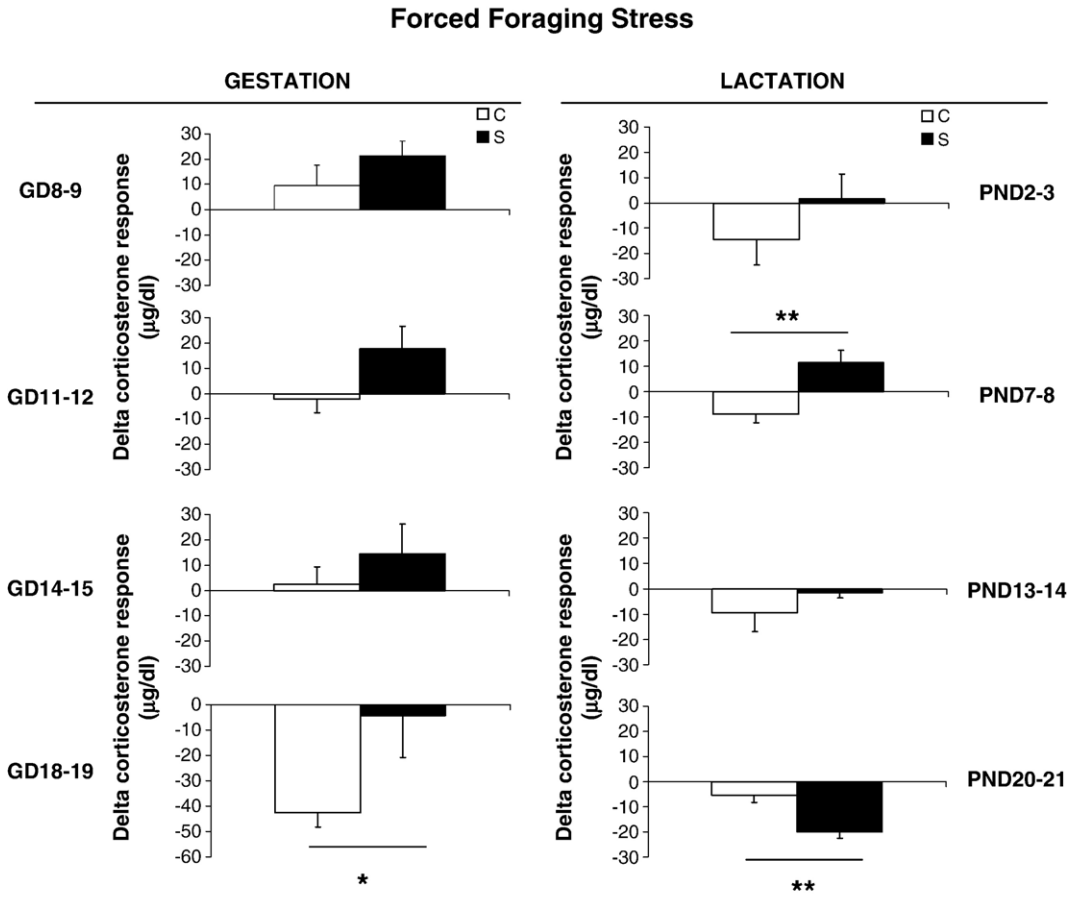

Fig. 4. Changes in plasma corticosterone concentration with forced foraging and blood sampling (Stressed mothers, *S*, $n=5$) or blood samplings alone (Control mothers, *C*, $n=6$). Maternal corticosterone responses are represented at specific times during gestation (GD8–19) and lactation (PND2 through 21) by the delta between plasma CORT levels at 9 h and 11 h during the dark phase. Values are presented as means±SEM. ** $P<0.01$; *$P<0.05$ stressed vs. control mothers.

during the light phase at this age. Exposure to wet bedding during the dark phase on PND8, did not modify the frequency of active nursing in stressed mothers as on PND4, but significantly increased the frequency of pup grooming compared to control mothers (*C* and Cu; group × behavior interaction, $F(4,72)=4.06$, $P<0.01$, post hoc NK, $P<0.05$). In the light phase, however, active nursing was again significantly increased in stressed mothers compared to both control groups (group × behavior effect, $F(4,72)=2.94$, $P<0.05$, post hoc NK, $P<0.05$).

Qualitative analysis of maternal behavior over the 3-min bins of the entire observation period revealed other differences induced by stress (Fig. 7). In this figure, each square represents the dominant behavior observed for all females in one treatment group and color codes discriminate between behaviors directed towards pups and those directed towards mothers. This representation provides a visual and qualitative analysis of the behavioral measures averaged in Fig. 6. On PND3, which is a rest day, stressed mothers tended to exhibit more frequent behaviors directed towards themselves compared to *C* and Cu mothers during the light phase (more grey colors compared to yellow). In the dark phase, all mothers exhibited more behaviors directed towards self than their pups. During the exposure to wet bedding (dark phase), stressed mothers on PND4 spent very little time caring for their pups. Their time was mainly devoted to nest building and retrieving pups into the nest, whether they were burying the pups in the wet bedding or transferring the pups to a part of the cage that was cleaned of bedding. On PND8, however, stressed mothers spent more time grooming their pups during wet bedding exposure and as a consequence, exhibited less frequent behaviors directed towards themselves compared to *C* and Cu mothers. Finally, after termination of exposure to wet bedding (light phase), stressed mothers on PND8 spent almost all their time nursing their pups, contrary to *C* and Cu mothers on PND8 that spent time caring for themselves.

Discussion

The aims of the present study were to determine whether repeated psychological stress throughout gestation and lactation would lead to robust and steady corticosterone responses and be associated with significant behavioral changes in mothers. Since stress hyporesponsiveness is documented in late pregnant (Johnstone et al., 2000; Douglas et al., 2003; Neumann et al., 1998) and lactating (Lightman, 1992; Walker et al., 2001) rats, we tested mothers both outside (GD8–15) and inside the "hyporesponsive" period (GD18-lactation, PND14). The first

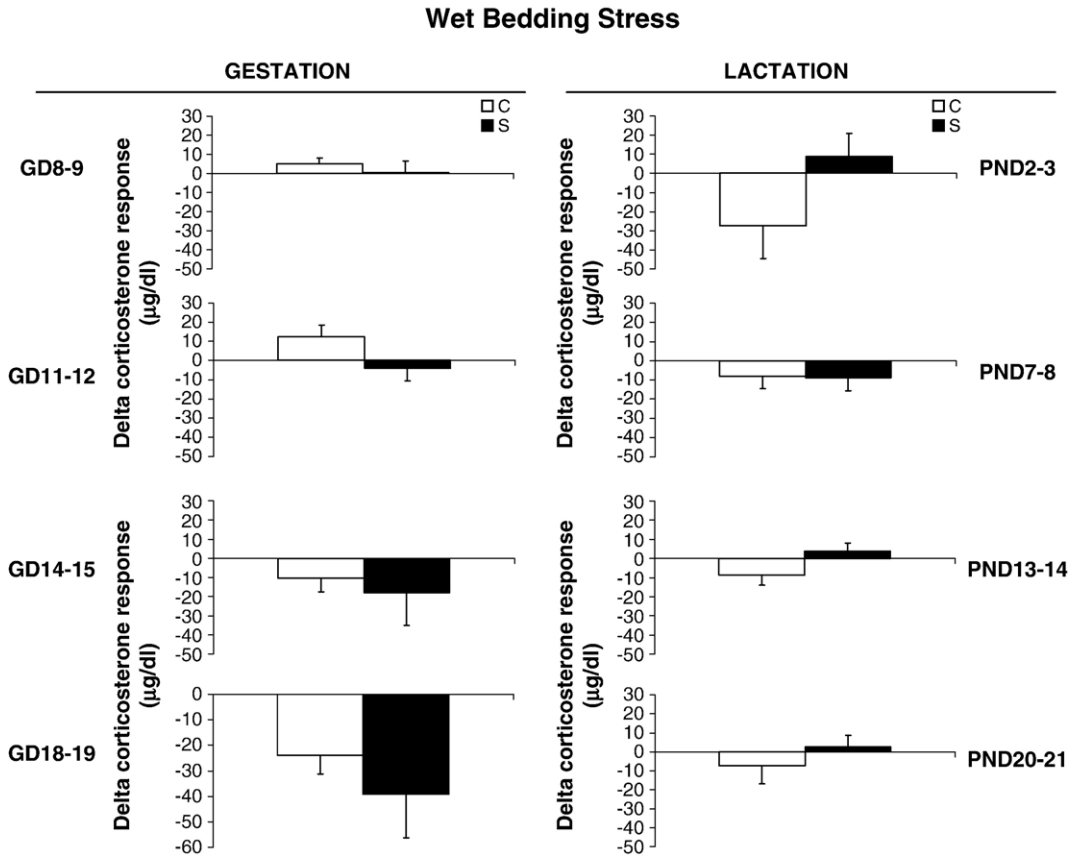

Fig. 5. Lack of acute corticosterone secretion to wet bedding and blood sampling in stressed mothers (*S*, $n=5$) compared to control mothers (*C*, $n=6$) subjected to blood sampling alone. Maternal corticosterone secretion is represented at specific times during gestation (GD8–19) and lactation (PND2 through 21) by the delta between plasma CORT levels at 9 h and 1030 h during the dark phase. Note that wet bedding stress was applied immediately after 24 h of forced food foraging (Stressed mothers) or blood sampling alone (Control mothers).

set of results provided evidence that repeated strobe light exposure in the AM and the PM increased maternal corticosterone responses in the afternoon even when animals were tested during the hyporesponsive period of lactation. During gestation, there was a trend for stressed mothers to display increased corticosterone secretion compared to control mothers in the PM, although this increase did not reach statistical significance. Our second experiment showed that the combination of repeated forced foraging and wet bedding did not alter corticosterone secretion in pregnant rats and caused a small response only on days 7–8 of lactation. However, this stressor induced significant changes in maternal behavior during the first 8 days of lactation.

In contrast to several models of maternal adversity using a variety and/or combination of physical and psychological stressors that often lead to reduced birth weight in the offspring, we chose to determine maternal corticosterone responses to exclusively psychological stressors applied during the prenatal and postnatal periods. These included exposure to strobe light or to a combination of forced foraging and wet bedding stress. Repeated exposure to these stressors did not cause any significant changes in maternal weight gain, litter size or offspring's weight at birth. In our first experiment, repeated 1-h strobe light exposure, a purely psychological stressor induced significant corticosterone responses in lactating rats (PND3) in the afternoon without affecting maternal corticosterone secretion when the stressor was presented in the morning. During, gestation, stressed mothers tended to display higher corticosterone secretion than control mothers in the PM although the difference in secretion was not significant. This is an interesting observation given that the late gestation and lactation periods in the rat have been associated with blunted HPA axis responses to a variety of stressors (Lightman, 1992; Neumann et al., 1998; Toufexis et al., 1999; Douglas et al., 2005, for review see Douglas, 2005 and Tu et al., 2005). However, most of the data reported in the literature have been obtained from pregnant or lactating animals exposed to stress in the morning as this time of day is associated with higher stress responsiveness when the HPA axis activity is at its lowest (Bradbury et al., 1991). Significant corticosterone responsiveness to stress at the time of the diurnal peak of corticosterone might be related to a greater CNS drive to the hypothalamic PVN that overrides blunted neuronal responses observed in lactating females in the morning (Da Costa et al., 1996, 1997; Abbud et al., 1994). Unfortunately, as our blood collection protocol did not allow for the determination of plasma ACTH responses, we cannot at present be certain that the hypothalamus–pituitary unit remains fully responsive to stress in lactation. Alternatively, increased corticosterone secretion after stress in the PM might

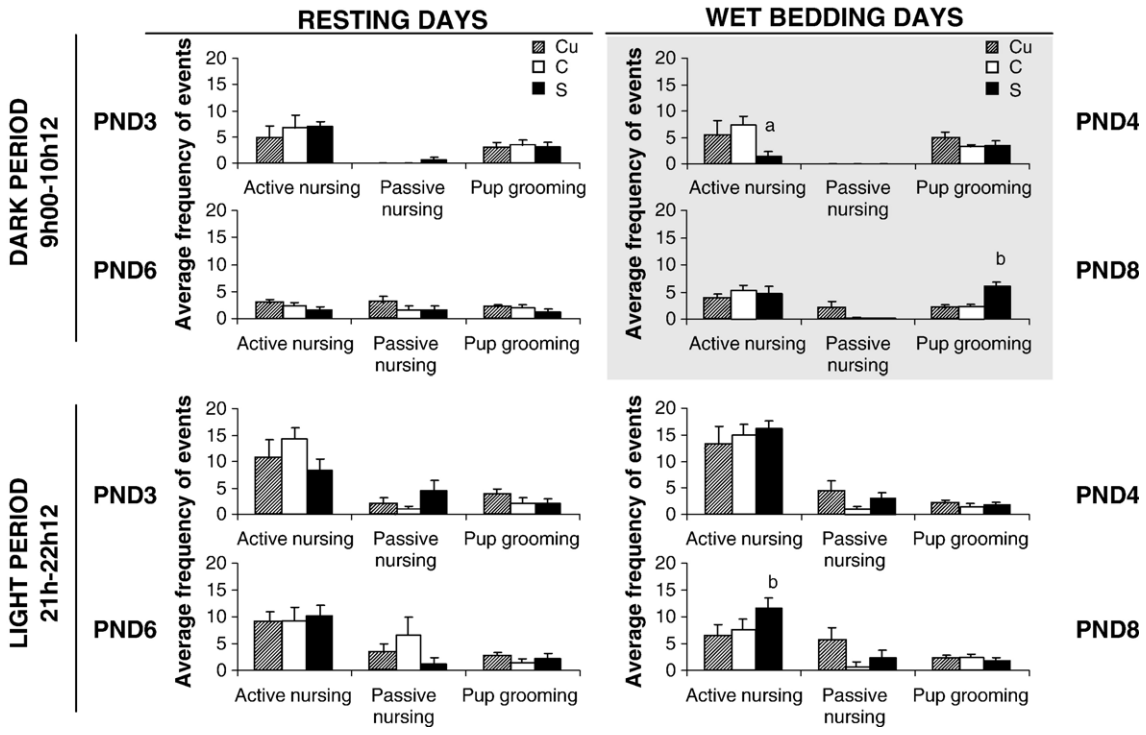

Fig. 6. Effect of repeated stress (forced foraging + wet bedding) and blood sampling during the gestation and lactation periods (see Fig. 1 for experimental design of experiment 2) on maternal behavior on PND3–4 and 6–8. Frequency of active nursing, passive nursing and grooming of the pups was calculated over a period of 72 min in observation bins of 3 min during the dark (top) or light (bottom) phase of the cycle. Maternal behavior was observed either during rest periods (in between days of exposure to stress, left panels) or during periods of wet bedding stress (right panels). Note that wet bedding occurred during the dark phase (top, right panel). An additional control undisturbed group (Cu) was added to determine whether blood sampling alone (*C*) modified maternal behavior. Values are presented as means ±SEM of 5–9 mothers per group. [a]$P<0.01$ vs. control (*C*) mothers, [b]$P<0.05$ vs. control undisturbed (Cu) and control (*C*) mothers.

be due to increased adrenal sensitivity to ACTH in lactating females compared to earlier in gestation. This might be possible for females during late pregnancy where the increase in corticosterone secretion under basal conditions was shown to be dissociated from significant increases in plasma ACTH secretion (Atkinson and Waddell, 1995). However, in an earlier report (Walker et al., 1992), we showed that the adrenal sensitivity of mothers on day 10 of lactation was not significantly different from that of virgin females in the AM or in the PM, suggesting that important changes in adrenal sensitivity might not be a primary factor mediating increased stress responses, at least during the lactation period. A third mechanism that could explain the increased PM response to strobe light in animals that had been exposed in the AM to the same stressor might be a facilitation of PM responses by the previous AM stress. Such a facilitatory trace has been demonstrated to occur in male rats for at least 12 h and cause elevated ACTH responses to the diurnal input at lights off (Akana et al., 1992). It is therefore interesting to speculate that facilitation of corticosterone responses to a second exposure to stress by a prior stress remains present at least during the lactation period. Further experiments will need to address this issue.

In our second experiment, we used two psychological stressors with ecological relevance such as forced foraging for food and exposure to wet bedding. Based on our previous results with the strobe light, and because these stressors might be more disruptive during the active phase of the diurnal cycle, we exposed mothers to the combination of stressors during the dark period of the cycle. We hypothesized that exposure to 24-h high foraging demand followed by 10-h wet bedding at the time of high basal glucocorticoid secretion would induce a significant corticosterone response in mothers as for strobe light exposure. Indeed, strobe lighting is known to be a milder stress than soiled bedding that directly influences the home cage environment (Harkin et al., 2002). Moreover, forced foraging is also a powerful stressor that affects the rearing environment by increasing the number of maternal separation episodes due to the effort required to obtain food (Lyons et al., 1998; Champoux et al., 2001). Surprisingly, the impact of these two stressors on maternal corticosterone secretion was mild and varied as a function of the stressor. Whereas forced foraging only increased corticosterone secretion on PND7–8, wet bedding failed to elicit a corticosterone response at any age studied. Maternal and pup weight were not affected indicating that the amount of food scattering was sufficient to avoid caloric restriction that could in itself activate the HPA axis. Stressful foraging demand was used in earlier studies in guinea pigs and squirrel monkeys (Hennessy and Sharp, 1990; Lyons et al., 1998; Coplan et al., 2005; Champoux et al., 2001) and for nonhuman primates, a robust rise in glucocorticoid secretion and an elevation of CSF CRF were observed. In rodents, few studies have used this paradigm and none has done so during lactation.

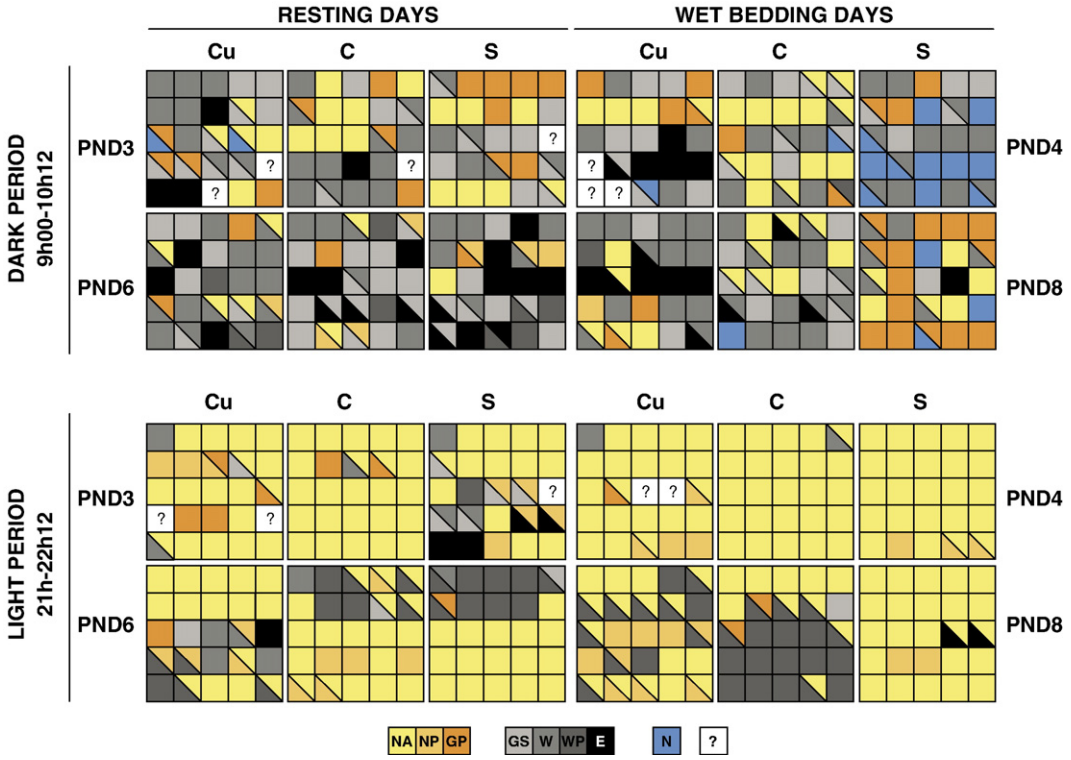

Fig. 7. Qualitative analysis grids of representative maternal behavior in nursing mothers subjected to stress (forced foraging + wet bedding and blood sampling), or in control mothers subjected to blood sampling alone (*C*) or left undisturbed (Cu) during days of rest (left columns) or wet bedding (right columns). Maternal behavior was scored on PND3–4 and 6–8 in the dark (upper panel) or light (lower panel) phase of the cycle. Each grid depicts 25 consecutive 3-min periods of observation during which the most frequent characteristic of maternal behavior among mothers from a same group is illustrated ($n = 5–9$ mothers per group). Behaviors directed towards the mother are indicated in grey shades as follows: light grey = self-grooming (GS), medium grey = wander (W), dark grey = sleep alone (WP, wander passive), black = eat or drink. Behaviors directed towards the pups are indicated in shades of yellow as follows: yellow = active nursing (NA), light orange = passive nursing (NP), orange = licking/grooming of pups (GP). Blue squares indicate nest building and white squares with a question mark indicate no main behavior observed.

Although increased maternal separation episodes induced by this stressor are clearly stressful for the rat offspring (Francis and Meaney, 1999; Meaney, 2001), our data demonstrate that forced foraging does not constitute a stressor for the mother (except at one time point). It is possible that the lack of significant responses to this manipulation might be related to habituation or to a relative tolerance to the disruption of contact with the litter.

Wet bedding exposure did not induce a significant corticosterone response at any of the ages tested. Soiled or wet bedding is currently used in models of chronic mild stress in association with other stressors to induce depression-like symptoms in rats (Willner et al., 1987, for review see Willner, 2005; Moreau et al., 1992). However, few studies have used wet or soiled bedding as an individual stressor. In one of these studies, Harkin et al. (2002) have shown that wet bedding is a powerful stressor that elicits large changes in heart rate, body temperature and activity in male rats during almost the entire duration of the stressor. We also observed that wet bedding induced a small corticosterone response in virgin females. In our study, wet bedding was applied after 24-h forced foraging and therefore, the HPA axis might not have returned to a "basal" state at the onset of wet bedding, masking a potential adrenocortical response to wet bedding. If this hypothesis were correct, then blood levels of corticosterone at the onset of wet bedding (0900 h) should be higher than those measured 24 h earlier at the onset of forced foraging. When this comparison was performed, no significant increases in "basal" corticosterone concentrations were found at any age studied (data not shown). Alternatively, we suggest that the ability of the mother to cope behaviorally with the stressor might be greater than that of males, at least during the lactation period. As shown with our behavioral observations, wet bedding exposed the pups to cold temperature and triggered specific behaviors in the mother that aimed at regulating the temperature of her offspring. Indeed, the changes in maternal behavior that we observed in our experiments were a consequence of trying to overcome the wet bedding situation and included two main strategies: mothers either tried to bury their pups in the wet sawdust or retrieved them all and attempted to nurse them in a place cleared of any soiled bedding material. During the first exposure of the nursing mother to wet bedding on PND4, mothers spent significantly less time nursing their pups, but seemed to increase the huddling behavior by spending more time to build the nest (Fig. 7). On PND8, mothers coped with the decreased temperature by spending more time in the nest licking and grooming their pups, in order to remove the wet bedding from their offspring. Maternal behavior remained affected by wet bedding after termination of the stressor

during the light phase of the cycle on PND8. At this time, stressed mothers continued to spend more time with their pups, but in active nursing whereas control mothers were off the nest more often. It is well established that natural variations in maternal care can program the HPA axis of the rat offspring (Ladd et al., 2000; Liu et al., 1997; Fish et al., 2004), thus suggesting that alterations of maternal behavior such as those induced by wet bedding might have consequences on the HPA axis of the offspring later in life. The extent of changes in maternal behavior during forced foraging was not determined in our experiments. However, we observed that mothers spent less time with their pups during the light phase at PND3, the day after forced foraging exposure. This alteration in maternal behavior could be a consequence of the 24 h of previous foraging stress. Finally, it might be important to note that even if blood sampling is considered as a stressful procedure, there are no differences in maternal behavior between control and control undisturbed mothers, suggesting that alterations in maternal behavior are induced mainly by exposure to the combination of forced foraging and wet bedding.

In conclusion, our studies demonstrate that lactating females can mount a significant corticosterone response to the repeated application of strobe light. The response is observed specifically in the PM, at the time of the diurnal peak in endogenous glucocorticoid production but not consistently in the AM. A similar trend is observed during late gestation although significant level of responses was not reached at that stage. This suggests that the known stress hyporesponsiveness of lactation might be dependent upon the diurnal state of HPA axis activation and/or be modified by stress-induced facilitation in cases of repeated exposure to stressors within a short time period. Other psychological stressors such as forced foraging and wet bedding induced no significant corticosterone responses in the dark phase of the diurnal cycle, but had a major impact on maternal behavior towards the pups. Our results demonstrate that while repeated strobe light exposure might trigger neuroendocrine changes in mothers, the combination of forced foraging and wet bedding throughout gestation and lactation might provide a model of adversity that disturbs maternal behavior without significantly activating corticosterone secretion. This model has the advantage of including primarily psychological stressors, thus mimicking somewhat situations of maternal adversity in humans. Contrary to several other models of maternal adversity, our combination of stressors does not lead to significant metabolic consequences in the mother or offspring at birth, thus allowing to dissociate the neuroendocrine and behavioral effects from those usually associated with restricted intra-uterine growth. The long-term consequences of this regimen of maternal adversity on cognitive, behavioral and neuroendocrine development in offspring remain to be determined.

Acknowledgments

We would like to thank Hong Long and Zhifang Xu for their excellent technical help with these experiments. This work was supported by a group grant from the Canadian Institutes for Health Research (CHIR, HPG #64850) to M.J.M., S.G.M. and C.D.W. (MAVAN: Maternal Adversity Vulnerability and Neurodevelopment). M.M.L. was a recipient of a fellowship from the Genes–Environment training program.

References

Abbud, R., Hoffman, G.E., Smith, M.S., 1994. Lactation-induced deficits in NMDA receptor-mediated cortical and hippocampal activation: changes in NMDA receptor gene expression and brainstem activation. Brain Res. Mol. Brain Res. 25, 323–332.

Akana, S.F., Scribner, K.A., Bradbury, M.J., Strack, A.M., Walker, C.D., Dallman, M.F., 1992. Feedback sensitivity of the rat hypothalamo-pituitary–adrenal axis and its capacity to adjust to exogenous corticosterone. Endocrinology 131, 585–594.

Atkinson, H.C., Waddell, B.J., 1995. The hypothalamic–pituitary–adrenal axis in rat pregnancy and lactation: circadian variation and interrelationship of plasma adrenocorticotropin and corticosterone. Endocrinology 136 (2), 512–520.

Avishai-Eliner, S., Brunson, K.L., Sandman, C.A., Baram, T.Z., 2002. Stressed-out, or in (utero)? Trends Neurosci. 25, 518–524.

Bradbury, M.J., Cascio, C.S., Scribner, K.A., Dallman, M.F., 1991. Stress-induced adrenocorticotropin secretion: diurnal responses and decreases during stress in the evening are not dependent on corticosterone. Endocrinology 128, 680–688.

Brunson, K.L., Kramar, E., Lin, B., Chen, Y., Colgin, L.L., Yanagihara, T.K., Lynch, G., Baram, T.Z, 2005. Mechanisms of late-onset cognitive decline after early-life stress. J. Neurosci. 25, o9328–o9338.

Champagne, F.A., Meaney, M.J., 2006. Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. Biology 31.

Champoux, M., Hwang, L., Lang, O., Levine, S., 2001. Feeding demand conditions and plasma cortisol in socially-housed squirrel monkey mother–infant dyads. Psychoneuroendocrinology 26, 461–477.

Chapillon, P., Patin, V., Roy, V., Vincent, A., Caston, J., 2002. Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. Dev. Psychobiol. 41, 373–387.

Cianfarani, S., Germani, D., Rossi, L., Argiro, G., Boemi, S., Lemon, M., Holly, J.M., Branca, F., 1998. IGF-I and IGF-binding protein-1 are related to cortisol in human cord blood. Eur. J. Endocrinol. 138, 524–529.

Coplan, J.D., Altemus, M., Mathew, S.J, Smith, E.L., Sharf, B., Coplan, P.M., Kral, J.G., Gorman, J.M., Owens, M.J., Nemeroff, C.B., Rosenblum, L.A., 2005. Synchronized maternal–infant elevations of primate CSF CRF concentrations in response to variable foraging demand. CNS Spectr. 10, 530–536.

Da Costa, A.P., Wood, S., Ingram, C.D., Lightman, S.L., 1996. Region-specific reduction in stress-induced c-fos mRNA expression during pregnancy and lactation. Brain Res. 742, 177–184.

Da Costa, A.P., Kampa, R.J., Windle, R.J., Ingram, C.D., Lightman, S.L., 1997. Region-specific immediate-early gene expression following the administration of corticotropin-releasing hormone in virgin and lactating rats. Brain Res. 770, 151–162.

Darnaudery, M., Dutriez, I., Viltart, O., Morley-Fletcher, S., Maccari, S., 2004. Stress during gestation induces lasting effects on emotional reactivity of the dam rat. Behav. Brain Res. 153, 211–216.

De Kloet, E.R., Sibug, R.M., Helmerhorst, F.M., Schmidt, M., 2005. Stress, genes and the mechanism of programming the brain for later life. Neurosci. Biobehav. Rev. 29, 271–281.

Deschamps, S., Woodside, B., Walker, C.-D., 2003. Pups presence eliminates the stress hyporesponsiveness of early lactating females to a psychological stress representing a threat to the pups. J. Neuroendocrinol. 15 (5), 486–497.

Douglas, A.J., 2005. Central noradrenergic mechanisms underlying acute stress responses of the hypothalamo-pituitary–adrenal axis: adaptations through pregnancy and lactation. Stress 8, 5–18.

Douglas, A.J., Brunton, P.J., Bosch, O.J., Russell, J.A., Neumann, I.D., 2003.

Neuroendocrine responses to stress in mice: hyporesponsiveness in pregnancy and parturition. Endocrinology 144, 5268–5276.
Douglas, A.J., Meddle, S.L., Toschi, N., Bosch, O.J., Neumann, I.D., 2005. Reduced activity of the noradrenergic system in the paraventricular nucleus at the end of pregnancy: implications for stress hyporesponsiveness. J. Neuroendocrinol. 17, 40–48.
Federenko, I.S., Wadhwa, P.D., 2004. Women's mental health during pregnancy influences fetal and infant developmental and health outcomes. CNS Spectr. 9, 198–206.
Fish, E.W., Shahrokh, D., Bagot, R., Caldji, C., Bredy, T., Szyf, M., Meaney, M.J., 2004. Epigenetic programming of stress responses through variations in maternal care. Ann. N. Y. Acad. 1036, 167–180.
Francis, D.D., Meaney, M.J., 1999. Maternal care and the development of stress responses. Curr. Opin. Neurobiol. 9, 128–134.
Guo, A., Nappi, R.E., Criscuolo, M., Ficarra, G., Amram, A., Trentini, G.P., Petraglia, F., Genazzani, A.R., 1993. Effect of chronic intermittent stress on rat pregnancy and postnatal development. Eur. J. Obstet. Gynecol. Reprod. Biol. 51, 41–45.
Harkin, A., Connor, T.J., O'Donnell, J.M., Kelly, J.P., 2002. Physiological and behavioral responses to stress: what does a rat find stressful? Lab. Anim. 31, 42–50.
Hennessy, M.B., Sharp, K., 1990. Voluntary and involuntary maternal separation in guinea pig pups with mothers required to forage. Dev. Psychobiol. 23, 783–796.
Johnstone, H.A., Wigger, A., Douglas, A.J., Neumann, I.D., Landgraf, R., Seckl, J.R., Russell, J.A., 2000. Attenuation of hypothalamic–pituitary–adrenal axis stress responses in late pregnancy: changes in feedforward and feedback mechanisms. J. Neuroendocrinol. 12, 811–822.
Kapoor, A., Dunn, E.A., Kostaki, A., Andrews, M.H., Matthews, S.G., 2006. Fetal programming of hypothalamo-pituitary–adrenal (hpa) function: prenatal stress and glucocorticoids. J. Physiol. 572, 31–44.
Ladd, C.O., Huot, R.L., Thrivikraman, K.V., Nemeroff, C.B., Meaney, M.J., Plotsky, P.M., 2000. Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog. Brain Res. 122, 81–103.
Lemaire, V., Koehl, M., Le Moal, M., Abrous, D.N., 2000. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 97, 11032–11037.
Lightman, S.L., 1992. Alterations in hypothalamic–pituitary responsiveness during lactation. Ann. N. Y. Acad. Sci. 652, 340–346.
Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., Meaney, M.J., 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. Science 277, 1659–1662.
Llewellyn, A.M., Stowe, Z.N., Nemeroff, C.B., 1993. Depression during pregnancy and the puerperium. J. Clin. Psychiatry Suppl. 15, 26–32.
Lyons, D.M., Kim, S., Schatzberg, A.F., Levine, S., 1998. Postnatal foraging demands alter adrenocortical activity and psychosocial development. Dev. Psychobiol. 32, 285–291.
Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H., Le Moal, M., 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J. Neurosci. 15, 110–116.
Maestripieri, D., Badiani, A., Puglisi-Allegra, S., 1991. Prepartal chronic stress increases anxiety and decreases aggression in lactating female mice. Behav. Neurosci. 105, 663–668.
Mastorakos, G., Ilias, I., 2000. Maternal hypothalamic–pituitary–adrenal axis in pregnancy and the postpartum period. postpartum-related disorders. Ann. N. Y. Acad. Sci. 900, 95–106.
Matthews, S.G., 2002. Early programming of the hypothalamo-pituitary–adrenal axis. Trends Endocrinol. Metab. 13, 373–380.
McLoyd, V.C., Wilson, L., 1990. Maternal behavior, social support, and economic conditions as predictors of distress in children. New Dir. Child Dev. 4, 49–69.
Meaney, M.J., 2001. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu. Rev. Neurosci. 24, 1161–1192.
Meaney, M.J., Szyf, M., 2005. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. Dialogues Clin. Neurosci. 7, 103–123.
Mirescu, C., Peters, J.D., Gould, E., 2004. Early life experience alters response of adult neurogenesis to stress. Nat. Neurosci. 7, 841–846.
Moore, C.L., Power, K.L., 1986a. Prenatal stress affects mother–infant interaction in Norway rats. Dev. Psychobiol. 19, 235–245.
Moore, C.L., Power, K.L., 1986b. Prenatal stress eliminates differential maternal attention to male offspring in Norway rats. Physiol. Behav. 38, 667–671.
Moreau, J.L., Jenck, F., Martin, J.R., Mortas, P., Haefely, W.E., 1992. Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. Eur. Neuropsychopharmacol. 2, 43–49.
Morgane, P.J., Austin-LaFrance, R., Bronzino, J., Tonkiss, J., Diaz-Cintra, S., Cintra, L., Kemper, T., Galler, J.R., 1993. Prenatal malnutrition and development of the brain. Neurosci. Biobehav. Rev. 17, 91–128.
Muir, J.L., Pfister, H.P., Ivinskis, A., 1985. Effects of prepartum and postpartum enrichment on mother–infant interaction and offspring problem-solving ability in *Rattus norvegicus*. J. Comp. Psychol. 99, 468–478.
Neumann, I.D., Johnstone, H.A., Hatzinger, M., Liebsch, G., Shipston, M., Russell, J.A., Landgraf, R., Douglas, A.J., 1998. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. J. Physiol. 50, 289–300.
Ozanne, S.E., Hales, C.N., 2002. Early programming of glucose-insulin metabolism. Trends Endocrinol. Metab. 13, 368–373.
Patin, V., Lordi, B., Vincent, A., Thoumas, J.L., Vaudry, H., Caston, J., 2002. Effects of prenatal stress on maternal behavior in the rat. Brain Res. Dev. Brain Res. 139, 1–8.
Plotsky, P.M., Meaney, M.J., 1993. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res. Mol. Brain Res. 18, 195–200.
Pryce, C.R., Ruedi-Bettschen, D., Dettling, A.C., Weston, A., Russig, H., Ferger, B., Feldon, J., 2005. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. Neurosci. Biobehav. Rev. 29, 649–674.
Rojo, M., Marin, B., Menendez-Patterson, A., 1985. Effects of low stress during pregnancy on certain parameters of the offspring. Physiol. Behav. 34, 895–899.
Rosenblum, L.A., Andrews, M.W., 1994. Influences of environmental demand on maternal behavior and infant development. Acta Paediatr. Suppl. 397, 57–63.
Seckl, J.R., 2001. Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. Mol. Cell. Endocrinol. 185, 61–71.
Shanks, N., Windle, R.J., Perks, P., Wood, S., Ingram, C.D., Lightman, S.L., 1999. The hypothalamic–pituitary–adrenal axis response to endotoxin is attenuated during lactation. J. Neuroendocrinol. 11, 857–865.
Shiota, K., Kayamura, T., 1989. Effects of prenatal heat stress on postnatal growth, behavior and learning capacity in mice. Biol. Neonate 56, 6–14.
Smith, J.W., Seckl, J.R., Evans, A.T., Costall, B., Smythe, J.W., 2004. Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. Psychoneuroendocrinology 29, 227–244.
Toufexis, D.J., Tesolin, S., Huang, N., Walker, C.D., 1999. Altered pituitary sensitivity to corticotropin-releasing factor and arginine vasopressin participates in the stress hyporesponsiveness of lactation in the rat. J. Neuroendocrinol. 11, 757–764.
Tu, M.T., Lupien, S.J., Walker, C.D, 2005. Measuring stress responses in postpartum mothers: perspectives from studies in human and animal populations. Stress 8, 19–34.
Vallee, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M., Mayo, W., 1999. 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. 11, 2906–2916.
Walker, C.D., Lightman, S.L., Steele, M.K., Dallman, M.F., 1992. Suckling is a persistent stimulus to the adrenocortical system of the rat. Endocrinology 130, 115–125.
Walker, C.D., Toufexis, D.J., Burlet, A., 2001. Hypothalamic and limbic expression of CRF and vasopressin during lactation: implications for the

control of ACTH secretion and stress hyporesponsiveness. Prog. Brain Res. 133, 99–110.

Weinstock, M., 2001. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. Prog. Neurobiol. 65, 427–451.

Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural–neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90–110.

Willner, P., Towell, A., Sampson, D., Sophokleous, S., Muscat, R., 1987. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93, 358–364.

Windle, R.J., Wood, S., Shanks, N., Perks, P., Conde, G.L., da Costa, A.P., Ingram, C.D., Lightman, S.L., 1997. Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity. J. Neuroendocrinol. 9, 407–414.