Hormones, monoamines and peripartum affective symptoms

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Chapter 2

Abrupt rather than gradual hormonal changes induce postpartum blues-like behavior in rats

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Abstract

Aims: Postpartum blues is thought to be related to hormonal events accompanying delivery. We investigated whether blues-like symptoms depend on the rate of the decline of hormones, by comparing the behavioral consequences of an abrupt versus a gradual decline of gonadal hormones in an animal model

Methods: Female rats were treated with estrogen and progesterone for 23 days, administered either by injections or by subcutaneously implanted tubes filled with hormones. A gradual hormone decline was achieved by discontinuation of the injections; and rapid decline by removal of the tubes. Control groups received either a continued treatment or no hormones. In the period following the decline the stress-reactivity was tested with an acoustic startle test on 3 consecutive days, and anxiety behavior with an open-field test on the 2nd day. The Hypothalamus-, Pituitary-, Adrenal-axis (HPA-axis) response to stress was measured by assessing the corticosterone levels and hypothalamic c-fos expression stress-response at the 4th day.

Key findings: The rapid decline of hormones induced an increased startle response lasting for two days, and increased anxiety-like behavior in the open field. This was not found in the gradual-decline and control groups. The HPA-axis response to stress was decreased in all hormone-treated animals.

Significance: This animal study suggests that: 1) abrupt rather than gradual hormonal changes induce increased stress-reactivity and anxiety-like behavior; 2) postpartum blues may result from differences the capacity to adapt to the changes of gonadal hormones; 3) Recovery of pregnancy-induced diminished HPA-axis response is independent of the postpartum hormone kinetics.

Key words: postpartum blues, animal model, estrogen, progesterone, silastic implants, acoustic startle, anxiety, stress, HPA-axis.

Introduction

Postpartum blues (PPB) is a benign and transient syndrome of affect lability that occurs in 50% of the women in the first week after delivery (Henshaw 2003). Estrogen (E) and progesterone (P) are proposed to be involved in the precipitation of PPB, as withdrawal of an eight week treatment of E and P induced depressive symptoms and disturbances of the Hypothalamus-, Pituitary-, Adrenal axis (HPA-axis) in women with a history of postpartum depression (Bloch et al. 2000; Bloch et al. 2005).

Investigations on E and P withdrawal in animal models elucidated that a decrease of the P metabolite 3α-OH-5α-pregnan-20-one (allopregnanolone) is associated with anxiety behavior (Gallo and Smith 1993; Smith et al. 1998b). Alloprenalone modulates the $GABA_A$ (γ-aminobutyric-acid-A) receptor thereby enhancing the inhibitory effects of the neurotransmitter GABA on the brain (Smith 2002). Withdrawal of allopregnalone induced an increase of $GABA_A$ binding sites, containing particularly α-4 subunits, this correlated with both a decrease in $GABA_A$ gated Cl^- current as well as anxiety behavior (Bitran and Smith 2005a; Smith et al. 1998a). Blockage or suppression of the expression of the α-4 subunit prevented these changes, emphasizing their role in withdrawal behavior (Hsu and Smith 2003; Smith et al. 1998a).

These observations elucidated the physiology of withdrawal behavior, but do not explain why only a subgroup of women develops postpartum blues. We suggest that differences in the capacity to adapt to the postpartum hormonal changes might be involved in the etiology of postpartum blues. As the adaptational capacity will be less challenged during a gradual than during a rapid hormonal-decline, this might serve as a model to test this hypothesis. Here we investigate the consequences of a rapid and a gradual decline following a three week E and P treatment on behavior and HPA-axis functioning in an animal model. HPA-axis functioning was investigated as Bloch also observed HPA-axis changes related to post-withdrawal psychopathology.

The behavioral effects were determined with the acoustic startle response (ASR) and the pre-pulse inhibition (PPI) of this response and an Open Field Test (OFT). The startle elicits defensive whole body responses to a sudden, intense acoustic stimulus (Faraday 2002; Koch 1999). The involved neuronal circuitry of this response in animals and humans is comparable (Koch 1999). PPI shows whether a non-startling pre-pulse reduces the startle response amplitude, and is a measure of sensorimotor-gating. An increased ASR was found following P withdrawal (Gulinello et al. 2003) and in lactating rats (Byrnes et al. 2007). In pregnant women a decreased PPI was observed (Kask et al. 2008). We expected an increased ASR only following a rapid-hormonal decline. The OFT

measures the behavioral response to a novel environment, and is widely used as a measure of anxiety in rats (Ramos and Mormede 1998). P withdrawal is found to increase anxiety behavior in the OFT (Lofgren et al. 2006), which we expect to occur after a rapid hormonal decline. The HPA-axis response after exposure to a stressful environment was assessed at the 4th day after withdrawal, both on a peripheral (plasma corticosterone) as a central level (c-fos expression in the hypothalamus).

Animals, materials and methods

Animals

Forty-four female Wistar rats (Harlan, The Netherlands) weighting 185-210 gram at the start of the experiment were individually housed in cages (42 x 26 x 17 cm) with a grid roof and a layer of wood shavings, with a 12 h/12 h light/dark cycle (lights on at 7.00 h) in a temperature-controlled room hold at 21^{o} C. Each rat had a small piece of wood (0.5 x 0.5 x 3 cm) as cage enrichment material. Food and water were available ad libitum (standard rat chow, Hopefarms). Protocols were approved by The Animal Ethics Committee of the University of Groningen.

Experimental design

Evaluation of the hormone profile

In this experiment the hormone profile of the two treatments used the behavioral experiment (injections and silistic filled tubes) were evaluated. Eight animals were used. The first group (n=4), (gradual-decline group) was treated with hormone injections for 23 days, as described below. The second group (n=4) (rapid-decline group) received hormone-filled implants for 23 days, as described below. Blood samples (approximately 0.5-0.75 ml) were collected from a small tail wound (Fluttert et al. 2000) using short (<2 min) general anesthesia with isoflurane on day -3, 2 and 4 of the experiment. In the collected samples plasma progesterone was measured.

Evaluation of hormone withdrawal induced behavior

The second experiment was performed to evaluate the behavioral changes induced by a gradual-and rapid hormone withdrawal. The general design of the experiment is depicted in figure 1 and the hormone treatments are described below. Four groups were used. The gradual-decline group (n=10) received hormone injections for 23 days. The no-decline group (n=10) received hormone injections for 27 days and the control group (n=10) received vehicle injections for 27 days. The rapid-decline group (n=6) was treated

with hormone filled silastic tubes for 23 days. The first three days after hormone withdrawal, the startle response was assessed. On day 2 after hormone withdrawal an open field test was performed for eight minutes. Four days after withdrawal the HPA-axis stress response was evaluated in all animals, as described below in the section 'Stress procedure'. The cerebral response to the stressor was established by measuring the c-fos response in the Periventrucular Nuclues of the Hypothalamus (PVN), which is the part of the brain regulating pituitary activity. For this reason animals were perfused 2 hours after exposure to the stressor, when the c-fos response is maximal, and the brains were taken out. The peripheral response to the stressor was evaluated in all animals, by measuring plasma corticosterone in blood collected from a cardiac puncture during the perfusion procedure. After the perfusion the thymus and adrenals were extracted and weighted as a measure of corticosterone production in the preceding days.

Hormone injections

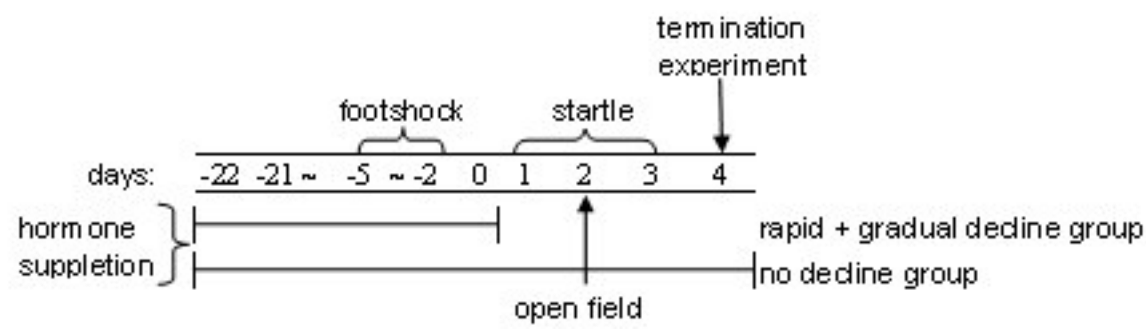


Fig. 1: Design of the behavioral experiment. the experiment started at day -22 with either the injection of progesterone and estrogen in oil or the implantation of hormone filled silastic tubes. Control groups consisted of rats that were either treated and handled as the experimental groups (received oil) or were daily injected for 26 days.

Silastic implant preparation and implantation

Hormone-filled implants were made a day prior to surgery by filling silastic tubes, I.D. 1.96 mm and O.D. 3.18 mm (Dow Coring Corp, Midland MI) with crystallized progesterone and 17-β-estradiol benzoate, closed with 3 mm silicon glue on both sides and stored in NaCl 9% solution for 24 h. The implants placed on day -22 were 5 mm for the estrogen and 3 x 30 mm for the progesterone implants. On day -13 implants of 10 mm estrogen and 5 x 30 mm progesterone were placed. These additional implants were placed to mimic the increase in the levels of estrogen and progesterone found in the normal pregnancy of the rat (Bridges 1984). Procedure of implant placing: Animals were anesthetized with 3% isoflurane. Incisions were made in the skin of the neck and the left flank in which the estrogen- and progesterone-filled silastic tubes were placed subcutaneously. On day -13 the implants were replaced by the larger ones as described

above, in the same position with the same surgical techniques. Implants were removed on day 0 of the experiment.

Hormone analysis

Blood was collected in an tube filled with 100 µl EDTA (30mM) per 5 ml of blood, centrifuged (500 g), and the plasma was stored at -20°C until assay.

Corticosterone was extracted from the plasma (10 µl) with Chromosorb (Alttech) and 30% dichloromethane (Rathburn RH1001). Before the extraction a trace amount of ^{3}H labeled (200 Bq) corticosterone (TRK-406 Amersham) was added to the samples to determine the recovery. A standard curve was determined by using non-labeled corticosterone (0 pg-1000 pg), which was treated in the same way as the extracted plasma samples. For the quantification, samples were mixed with ^{3}H (500 Bq) labeled corticosterone and polyclonal antibody that were raised against corticosterone. The tubes were incubated at 60 °C for 30 min and the equilibrium reaction was established in a water/ice bath for 1 h. The reaction was stopped by adding charcoal suspension and incubated for 20 min in the water/ice bath. The tubes were centrifuged for 15 min at 3000 g and 4 °C to spin down the charcoal bound with free corticosterone. The supernatant was poured into 1 ml of scintillation fluid (Ultimagold XR, Canberra Packard) and samples were counted in β-counter for 4 min or 4000 preset counts. The amount of corticosterone was calculated from the standard curve. All measurements were measured in duplicate and averaged. The intra- and inter-assay variation for corticosterone is 6% and 9.6% respectively and the minimal detection is 3 nMol.

Progesterone was extracted from the plasma (200 µl) with Chromosorb (Alttech) and n-Pentaan (Rathburn RH1001). Before the extraction a trace amount of ^{3}H labeled (185 Bq) progesterone (TRK-641 Amersham) was added to the samples to determine recovery. A standard curve (0-500 pg) was determined by using non-labeled progesterone (Sigma P-0130), which was treated in the same way as the extracted plasma samples. For the quantification, samples were mixed with ^{3}H (350 Bq) labeled progesterone and polyclonal antibody raised against progesterone. The tubes were incubated at 60 °C for 30 min and the equilibrium reaction was established in a water/ice bath for 1 h. The reaction was stopped by adding charcoal suspension and incubated for 20 min in the water/ice bath. The tubes were centrifuged for 15 min at 3000 g and 4 °C to spin down the charcoal bound with free progesterone. The supernatant was poured into 1 ml of scintillation fluid (Ultimagold XR, Canberra Packard) and samples were counted in β-counter for 4 min or 4000 preset counts. The amount of progesterone was calculated from the standard curve. All measurements were measured in duplicate and averaged. Intra and inter assay variation for progesterone are 8,1% and 13% respectively.

Stress procedure

Stress perception was measured at the last day of the experiment (day 4). Previously all rats were exposed to a foot-shock protocol from day -5 to -2, during the light period. The rats were placed in a foot-shock box with a grid-floor and received 5 inescapable foot-shocks with an intensity of 0.8 mA during 8 sec. To achieve maximal response to the stress, the shock interval and duration of the session were varied daily (30-120 min.). Each foot-shock was preceded by a 5 s light signal as a conditioning stimulus. This created an aversive environment and added a 'psychological' component. At day 4 all animals were placed in the shock box and were exposed 5 times to the light signal only. Accordingly, the endocrine and the cerebral responses reflect the consequence of exposure to 'psychological' stress rather than to pain (Gerrits et al. 2005).

Termination of the experiment

At the last day of the experiment (day 4), two hours after the exposure to the stress box, rats were anesthetized with isoflurane and transcardially perfused for 2 minutes with heparinized saline and for 18 minutes with a 4% paraformaldehyde solution (in 0.1 M sodium phosphate buffer pH 7.4) The brains were removed, post fixed in 4% paraformaldehyde solution and stored in 0.02M TBS + 0.1% Na-azide (pH 7.4) at 4°C. The adrenal glands and thymes were removed and weighted.

Immunohistochemistry

Following an overnight cryoprotection in 30% sucrose solution, serial 30 μM coronal sections were made with a cryostat microtome and collected in Tris 0.01M + 0.9 % NaCl (TBS) + 0.01% Na-azide. c-Fos immunostaining was performed as described (Gerrits et al. 2005). C-Fos positive cells in the PVN were quantified using a computerized imaging analysis system. The selected area was digitized, using a Sony charge coupled device camera mounted on a LEICA Leitz DMRB microscope (Leica, Wetzlar, Germany) at 100 x magnification. Regions of interest were outlined and the Fos-positive nuclei were counted in a single focus plane, by an observer blind to the experimental groups, using a computer-based image analysis system (Leica Imaging System Ltd., Cambridge, England) .

Startle test

The acoustic startle response was measured, using a Startle Response System (TSE GmbH, Bad Homburg, Germany) in a dark, sound-attenuating chamber (320 x 320 x 320 mm). The rat was placed in a small plexi-glass cage (270 x 100 x 125 mm) with a grid floor restricting major movement and exploratory behavior. The cage was placed on a transducer platform; which allows accurate measurements of the animals' motor

reactions (g). Acoustic stimuli were generated by means of high-quality high-linearity speakers mounted in the stimulus base unit. Both speakers and startle cages were connected to a PC, which detected and analyzed response with costume software. The startle program consisted of 5 min adapting to the startle box followed by 35 trials; 8 times trial 'no stimulus', 8 times trial 'prepulse alone' (80 dB), 8 times trial 'prepulse + startle pulse' (120 dB) and 11 trials of 'startle pulse alone' (120 dB). The first 3 trials always consisted of 3 'startle pulse alone' stimuli; from trial 4 on the trials were randomized. During the adapting period and testing a background noise of 70 dB was present. The pre-pulse inhibition was defined as the percentage reduction in mean startle response magnitude (SRM) in the presence of the prepulse compared to the mean SRM in the absence of the prepulse: (100-[100 x (mean SRM PP+SP trials / mean SRM SP alone trials)]. Habituation was measured as percent reduction in motor response to the first conditioned stimulus of the day.

Open Field Test

The open field (OF) consisted of a circular black arena with a diameter of 1 m. On day 2 of the experiment rats were placed for eight minutes in the open field and loco motor behavior was recorded with a video tracking system (EthoVision, Nodulus information Technology, Wageningen, the Netherlands) positioned above the open field. The total distance moved in the open field, en the time spent in the inner zone (the inner circle of the OF with a diameter of 50 cm) were calculated.

Statistical analysis

Statistical analyses were done with SPSS (version 12.0), and $p<0.05$ was considered significant. Blood samples were analyzed with repeated-measures ANOVA, with Huynh-Feldt correction, with group (injections and implants) as between subject factor and days as within subject factor. Weight gain was analyzed with repeated-measures ANOVA, with days as within subject factors and groups (control, no-decline, gradual-decline and rapid-decline) as between subject variable. Adrenal weights and loco motor activity in the open field were analyzed with an ANOVA, with group (control, no-decline, gradual-decline and rapid-decline) as between subject factor. The startle data were analyzed with repeated-measures ANOVA, with days as within subject factors and group (control, no-decline, gradual-decline and rapid-decline) as between subject factor. A Tukey's HSD post hoc test was used for pair wise comparison.

Results

Hormone profiles

During hormone administration with implants and injections the blood levels of progesterone nmol/l (± SEM) were 257.8 ± 27.9 and 587.0 ± 19.7 respectively, which is in the range of the 300 -500 nmol/l found during the second half of pregnancy in rats (Bridges 1984). The relative decreases of progesterone levels on day 2 and 4 are depicted in figure 2, showing a faster decrease in the implant-removal group (75%) as compared to the oil-injection rats, (25%) on day 2. Repeated measures ANOVA of the plasma progesterone showed a significant within subject effect ($F_{(2, 10)} = 18.97$, p= 0.001) and a significant between subject effect ($F_{(1, 5)} = 230, 55$ p<0.001). Post hoc ANOVA showed that plasma progesterone levels were significantly higher in the gradual-decline group than in the rapid-decline group during all three days (day -3 (p<0.001), day 2 (p<0.001) and day 4 (p=0.042)).

Post hoc analyses further revealed a significant decrease in progesterone levels between day -3 and 2 in both the gradual-decline and rapid-decline group (p= 0.029 and 0.013 respectively) the difference between day 2 and 4 was only significant in the gradual-decline group (p=0.013).

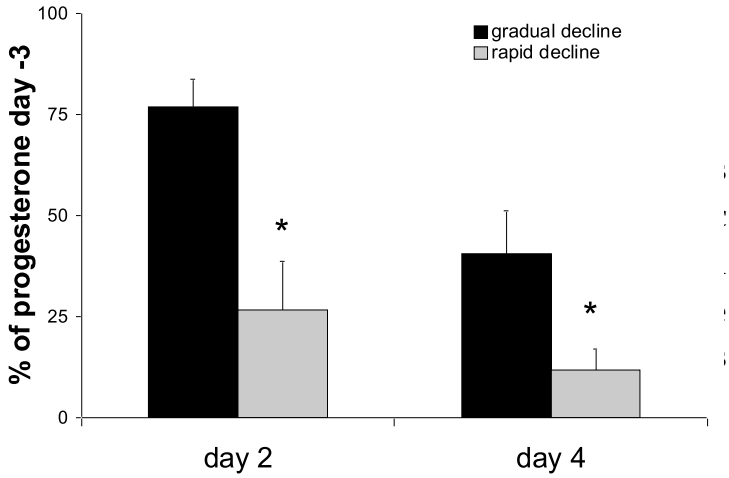


Fig. 2. *Plasma progresterone levels of experiment 1 on days 2 and 4 as a percentage of day -3. Data are expressed with SE. *Significant effect, p< 0,05.*

Open field

Data are shown in figure 3. ANOVA
in the open field showed a significa
testing showed that the rapid-declin
when compared with the control (p=
time spent in the centre of the
significantly lower in the rapid-decli

Startle response

The mean reactions to the startle pulse alone are depicted in figure 4. Repeated measured ANOVA of these data showed a significant between subject effect ($F_{(3, 32)}$ =10.81 p<0.001). Post hoc analyses revealed a significantly stronger startle reaction in the rapid-decline group on day 1 and 2 when compared with the other groups (p<0.01). On day 3 this difference existed only in the comparison with the no-decline group (p<0.01). No significant differences were found in prepulse inhibition (PPI), and the habituation within a session (data not shown).

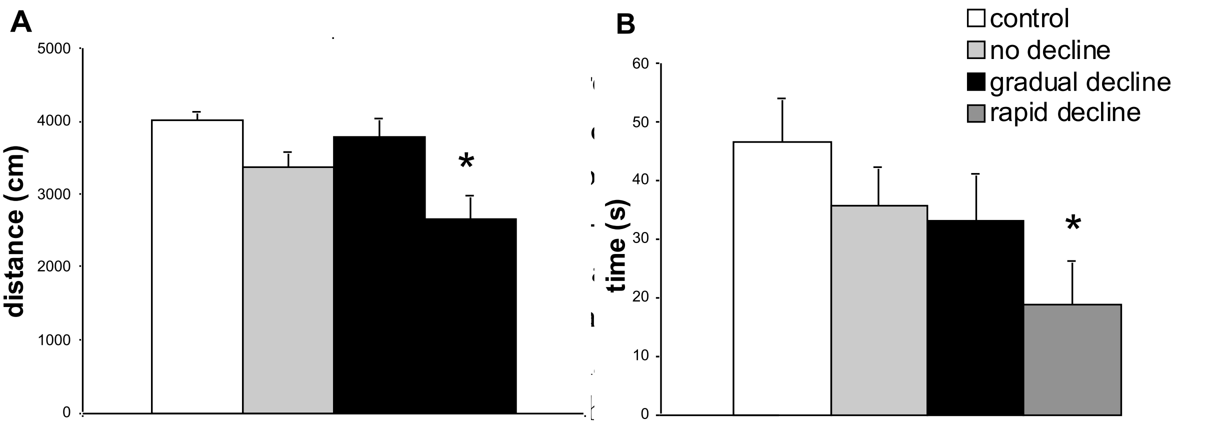
HPA-axis parameters

The groups did not differ in weight gain. The corticosterone levels at day 4 did not differ between the different groups. No significant differences were found in the adrenal weight, however the difference between the rapid-decline and no-decline group reached borderline significance (p=0.07). C-fos in the PVN was significantly higher in the control group ($F_{(3, 32)}$ =6.706; p<0.03) when compared with all other groups. Thymus weight in the control groups was significantly higher than in the other, hormone treated groups ($F_{(3, 32)}$ = 27.643, p<0.001). Results are summarized in table 1.

Discussion

The present data support the hypothesis that a rapid, but nor a gradual decline of E and P, evokes –transient- behavioral changes, as we observed that a rapid but not a gradual decline of progesterone levels induced changes in open field activity and acoustic startle response. Long term hormone exposure resulted in lower stress-induced c-fos response in the PVN, lower weights of thymus but not in differences in cortication levels at 4 days after discontinuation of injections or implants. The latter results indicate that normalization of the endocrine response to stress and reactivity to external stimuli are essentially independent.

This study has several specific features and strong points. 1) The experimental paradigm allows the evaluation of temporal changes of hormones and related behavioral effects. 2) Reliable kinetics of progesterone was obtained. Most of the pub 3) witl day: surg moc grou use estr



(Hiroi and Neumaier 2006). The estrous cycle stage was not evaluated in this study. In addition, we did not measure the hormone kinetics of implants and injections during pseudo-pregnancy, and differences in P levels were found at the end of pregnancy; both

*Fig. 3. Open field data from experiment 2. A) Distance moved (cm) in the open field during the 8 min. registration period. *Differs significant from control (p=0.007) and no decline group (p=0.033). B) Time (s) spend in the inner zone of the open field during de 8 min. registration period. *Differs significant from the control group (p= 0.05). Data are depicted with SE.*

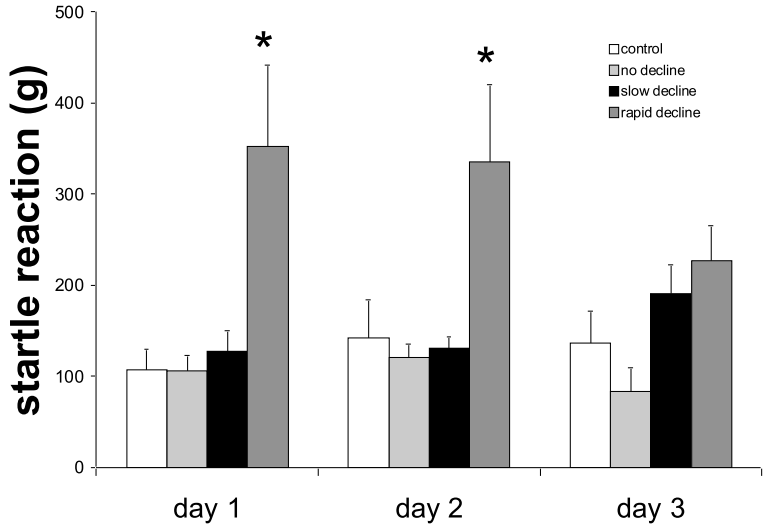


Fig. 4. *Reaction to the startle pulse (magnitude in g) on days 1-3 from experiment2. * Differs significant from all other groups at the same day, p<0.05. Data are depicted with SE.*

y. A last limitation of the current obtained.

ity and decreased activity in the inner-zone activity is generally and Mormede 1998), suggesting her tested animals. The rapid-ed startle reaction, which we Both, the transiently increased nxiety behavior in the open field humans (a few days). Likewise, creased anxiety (Henshaw 2003; Kennerley and Gath 1989), reactivity / hypersensitivity (Kennerley and Gath 1989) and irritability (Pitt 1968; Pitt 1973).

Galea et al performed an experiment with the same injection model as described here and reported that discontinuation of injections induced significantly changes in open field and forced swim behavior, compared with ovariectomized (OVX) controls (Galea et al. 2001). We did not replicate these findings. We suggest that the use of OVX animals both as a treatment and as a as a control group might explain the discrepant results. Ovariectomizing of rats induces large changes in physiology and behavior, like increased weight gain, a higher vulnerability for the aversive effects of chronic stress (Gerrits et al. 2005) and more depressive-like behavior which could be reversed by both hormones (Hiroi and Neumaier 2006) and antidepressants.

Several studies investigated anxiety behavior following E or P withdrawal, but due to differences in the employed hormone regimes the results cannot be compared directly to the here reported findings. Stoffel et al performed a study in which P injections were stopped after 16 days and the E injections after 23 days (Stoffel and Craft 2004). This induced a prolonged time of immobility in the forced swim test but no changes in elevated plus maze behavior. As the combined decrease in P and E was not measured, these results cannot be compared with the present study. Two studies reported that termination of pseudo-pregnancy, induced by injections with pregnant mare serum gonadotropin and human chorionic gonadotropin, caused anxiety-like behavior in the elevated plus which could be resolved by progesterone injections (Bitran and Smith 2005b; Bitran and Solano 2005). In contrast to the above mentioned studies open field behavior in lactating rats did not differ from diestrous females at 2 days and 14 days postpartum. (Byrnes et al. 2007). As the rats were lactating, the hormonal changes accompanying lactation might have opposed the anxiogenic effects of progesterone

withdrawal, since anxiolytic effects of the lactation hormone oxytocin have been reported (Uvnas-Moberg 1998).

In female rats withdrawal of a three week P treatment with implants induced an increased startle response without differences in PPI and habituation (Gulinello et al. 2003). However, PPI calculated as a percentage of the integral response was found to be increased after P withdrawal. P withdrawal induced an increase in $GABA_A$ receptor α4 subunit expression in the amygdala. We replicated these findings only in our rapid-decline group. Although α4 subunit expression was not measured; it is tempting to speculate that the gradual-decline hormonal facilitates a diminished increase of the α4 subunit, thus preventing the increase in startle response. In lactating rats a decrease in PPI has been reported. Furthermore, the startle amplitude was increased at the 14th but not on the 2nd day postpartum (Byrnes et al. 2007). In humans the startle response magnitude and PPI do not change during the first 3 postpartum days when compared to pregnancy. However, when compared to the late postpartum, pregnant women exhibit lower levels of PPI (Kask et al. 2008). Both studies contrast with our and the previous mentioned results found in pregnancy models. Accordingly the endocrine events of 'simplified' pregnancy models are not comparable with the complexity of normal pregnancy. For instance, progesterone decreases a few days before parturition in rats and other hormones like oxytocin and prolactin are increased postpartum both in humans an rats, all these endocrine changes influence startle and PPI (Feifel and Reza 1999; Gogos and Van den Buuse 2004).

Others reported that a rapid but not a gradual progesterone decline induces anxiety behavior in rats (Saavedra et al. 2006). The latter studies together with the present data emphasize that the fast but not a gradual decline of progesterone induces anxiety-like behavior, indicating the differences in the capacity to adapt to the endocrine changes following parturition might underlie the vulnerability for postpartum blues in a subgroup of women. Very recently this hypothesis is confirmed in a study in which mice with an genetically altered capacity to regulate $GABA_A$ receptors – who were consequently unable to modulate the expression of $GABA_A$ receptors according to the changing levels of neurosteroids in the course of pregnancy - exhibited depressive like behavior in the postpartum period (Maguire and Mody 2008).

In addition to these rapid behavioral effects, we observed a persistently diminished PVN c-fos response to stress in hormone treated animals irrespective of the wash-out rate of the gonadic hormones. Despite this decreased hypothalamic stress-response the corticosterone response to stress did not differ between the groups. This might be explained with an effect described by Magiakou et al. (Magiakou et al. 1996) who found

in humans that during the puerperium the Corticotropin-releasing hormone CRH induced adrenocorticotrope hormone ACTH response is blunted but that corticosterone levels are normal. The differences in the time course of the behavioral and endocrine postpartum adaptation processes may diverge, emphasizing that reactivity to external stimuli and a normalization of the endocrine response to stress are essentially independent. In humans the emergence of mood disorders postpartum also differs; the blues appears early, whereas depression is a later reaction. The rapid drop of hormones may therefore be related to the development of blues, whereas the delayed recovery of the endocrine response might be associated with depressive symptoms (Magiakou et al. 1996). Indeed, deviating stress hormone physiology has often been associated with depressive disorders unrelated to the partus (Gillespie and Nemeroff 2005; Saavedra et al. 2006).

Conclusion

Results of this study have three major implications:

1.) The relation between a parturition-like hormone drop and the increased reactivity and anxiety indicates that differences in adaptational capacity of the GABA-nergic system might underlie the vulnerability for post partum blues in humans.

2.) We and others found experimental evidence supporting the idea (also described by (Halbreich 2005)), that rapid changes in levels of the gonadic hormones rather than persistently high or low levels 'destabilize' the organism (and brain) and thus rendering the subject vulnerable to develop mood switches.

3.) The divergence of the HPA-axis response and behavioral reactivity during the postpartum period suggests that the endocrine and behavioral responses to stress are essentially independent.

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