Hormonal and behavioral effects of prenatal stress: focus on circadian rhythms and sleep

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Abstract
In humans, prenatal stress can induce psychological difficulties, mental retardation and sleep disturbances in the infants. In animals, dams stressed during pregnancy can bear offspring with reduced male sexual activity, enhanced emotional reactivity, modifications of glucocorticoid secretion and increased propensity to self-administer drugs. In adult rats submitted to prenatal stress, we studied the stress-induced corticosterone secretion response and hippocampal corticosterone receptors, their hormonal and behavioral circadian rhythms, and their sleep-wake parameters. We found that prenatal stress prolongs stress-induced corticosterone secretion and decreases hippocampal corticosterone receptors in adult offspring. Under entrainment to a regular light-dark cycle, prenatally-stressed rats of both genders show significant phase advances in the circadian rhythms of locomotor activity (running-wheel behavior) and plasma corticosterone secretion. Compared to controls, PNS rats exhibit higher amounts of paradoxical sleep during both the light and the dark phase (increased number of REM episodes). Light slow-wave sleep is increased, number of stage shifts more frequent and duration of wakefulness episodes reduced. Taken together, those results indicate that prenatal stress in rats is associated with long-term alterations in various hormonal and behavioral parameters, which are comparable to those described in depressed patients, suggesting the usefulness of prenatally-stressed rats as an animal model of human depression.

Influence of perinatal environment changes on individual’s development and HPA axis activity
Changes of prenatal and postnatal environments can exert complex influences on the development of an organism. In particular, life events occurring during those two early periods of life can have different long-term behavioral effects. For example, in humans, prenatal stress (PNS) can induce mental retardation and sleep disturbances in the infant (17). In animals, dams stressed during pregnancy can bear offspring with reduced male sexual activity, enhanced emotional reactivity (22) and an increased propensity to self-administer drugs (2). Conversely, postnatal stimulation has been found to improve the performance of aged offspring in cognitive tasks (10). Although prenatal and postnatal events can have different behavioral consequences, they may also impinge on the same behavioral response, and postnatal manipulations can reverse the behavioral effects of prenatal stress. For example, it has been shown that postnatal handling or adoption (8,9) can reverse the increase in emotional reactivity induced by prenatal stress (21).
Several observations indicate that glucocorticoid secretion could be a substrate of the different long-term behavioral effects of prenatal or postnatal events. Prenatal stress increases stress-induced corticosterone secretion peak in preweaning rats (4) and attenuates its habituation over repeated exposure to stress in the adult (3). In contrast, postnatal handling reduces stress-induced corticosterone secretion in adult and aged rats, probably by strengthening corticosterone feedback (10, 19). Finally, impairment in glucocorticoid feedback, resulting in an increased glucocorticoid secretion, is associated with behavioral disorders in depression (6). We therefore thought to determine whether a modification of fetal hormonal environment by stressing the mother can influence the development of the activity of the hypothalamo-pituitary-adrenal (HPA) axis. It has already be shown that stress during pregnancy sensitizes different neuroendocrine systems, such as gonadal and HPA axis (4). However, it remains unclear which mechanisms are involved in the dysregulation of corticosterone secretion in prenatally-stressed adult rats. Given that hippocampal type I and type II corticosteroid receptors appear to be major regulating factors in corticosterone secretion (15), we assessed stress-induced corticosterone secretion and hippocampal corticosteroid receptors in adult rats that had been submitted to prenatal manipulations (4,8).

**Prenatal stress procedure**

Prenatal stress was performed daily during the last week of pregnancy until delivery. Pregnant females were individually restrained three times a day (at 09:00, 12:00 and 17:00 h) for 45 min in transparent plastic cylinders (7 cm diameter, 19 cm long). Control pregnant females were left undisturbed in their home cages. Offspring were weaned 21 days after birth and housed in same-sex groups of four until the age of two months. Only litters of 8-13 pups with similar numbers of males and females were utilized for the study. Only two male pups per litter were studied as adults to minimize any possible “litter effects” (1) on the measured variables.

**Effects on the HPA axis**

Repeated restraint of the mother during the last week of pregnancy induces prolonged corticosterone secretion in adult offspring (90 days of age), which was indicative of impaired corticosterone feedback. Indeed, corticosterone levels in either basal conditions or 30 min after stress did not differ between the control and prenatally-stressed rats, but two hours after stress, corticosterone secretion was higher in the prenatally stressed than in the control rats (Figure 1a) (2, 8). Prenatal stress also decreased hippocampal type I corticosteroid receptors (Figure 1b) and, in contrast, as described by other authors (23), prenatal stress failed to modify type II corticosteroid receptors (Figure 1c) (8).
Figure 1: Plasma corticosterone secretion after restraint stress (a), type I (b) and type II (c) corticosteroid receptors in control or prenatally-stressed adult rats. a: corticosterone levels 120 min after restraint stress remained elevated in prenatally-stressed rats, whereas they returned to preexposure values in controls. b: Prenatally-stressed rats showed a lower binding capacity of type I corticosteroid receptors compared to controls. c: Prenatal stress did not significantly modify binding capacities of type II corticosteroid receptors. Affinities of type I and type II receptors were not modified by prenatal stress. Mean affinities were: type I = 1.14 ± 0.11 nM, type II = 0.6 ± 0.12 nM. **p<0.01. Vertical line shows S.E.M.

The decrease in hippocampal type I corticosteroid receptors observed in prenatally-stressed rats could account for their prolonged stress-induced corticosterone secretion. It has been shown that a selective reduction in hippocampal corticosteroid receptors is accompanied by a prolonged corticosterone secretion in response to stress (13). In view of their affinities for corticosterone, it is generally thought that type II receptors are involved in stress-induced feedback mechanisms, while type I receptors are involved in the tonic regulation of corticosterone release under basal conditions (11). Thus, the observed decrease in hippocampal type I receptors might not be expected to be involved in stress-modulated feedback control. However, there is also evidence that both receptor types are involved in feedback control mechanisms (14). The prolonged corticosterone secretion observed in prenatally-stressed animals could also account for the behavioral alterations, as for example the increased propensity to amphetamine self-administration observed in prenatally-stressed adult rats (2).
Influence of prenatal stress on circadian rhythms of locomotor activity and corticosterone secretion in adult offspring

The circadian system plays a major physiological role to insure optimal functioning of the organism and its adaptation to the various changes in the environment (18). Alterations in circadian rhythmicity has been associated with aging (18,20), sleep disorders (24) and affective disorders (12). Perinatal events can also influence the functioning of the circadian system. Postnatal maternal environment can influence circadian oscillations in plasma corticosterone: blind rat pups adopted by dams out-of-phase with respect to their original mothers do modify their corticosterone rhythm to reflect that of the foster mother (5). An imposed restricted access to the natural mother can induce a shift in the corticosterone rhythm of rat pups (16). Despite the fact that postnatal events can have complex influences on circadian function, little is known on the long-term effects of prenatal manipulations on circadian rhythms in adult male rats. Therefore, we sought to determine in adult offspring the long-term effects of PNS on circadian rhythms of corticosterone and locomotor activity, two robust markers of the circadian clock.

Locomotor activity rhythm

Control (N = 6) and PNS (N = 6) adult female offspring were kept in individual cages equipped with a running-wheel for continuous recording of locomotor activity via an on-line computer (Chronobiology Kit, Stanford Softwares Systems, CA, USA), and exposed to a 12:12 light-dark (LD) cycle with light dimmed to 20 - 50 lux to reduce possible masking effects of light on actual onset of locomotor activity. Individual 24-hour activity profiles were constructed for each animal over 10 day-intervals. From those activity profiles, individual onsets of locomotor activity were estimated by determining the time of the first 15-min bin when the number of rotations exceeded 10% of the profile’s highest level (peak) and remained at that level for at least 50% of the time in the next 30 minutes. Mean (± s.e.m.) onset of locomotor activity in the group of Control rats occurred 15 ± 12 min after lights-off, while it occurred 39 ± 22 min before lights-off in the group of PNS rats (p < 0.02, unpaired Student’s t test). At the time of lights-off, relative amplitude of locomotor activity (i.e.; % of peak activity value) averaged, respectively, 15 ± 5 % and 35 ± 10 %, in Control and PNS rats (p < 0.05, unpaired Student’s t test).
Figure 2: Mean (± s.e.m.) onset of locomotor activity relative to time of lights-off in control adult female rats (Control) and prenatally-stressed rats (Prenatal Stress). A value above zero line indicates that activity occurred before the time of lights-off, a value below this line indicates that it occurred after the time of lights-off.

**Rhythm of corticosterone secretion**

Control (N = 6) and PNS (N = 6) adult female offspring were housed in individual cages under a dim 12/12 LD cycle (light intensity = 150 lux) and allowed 2 weeks of habituation to this lighting condition. Rats were then implanted with chronic intraveneous cathethers. After at least 12 days of recovery, blood samples were collected at nine different points over the 24-hour cycle. After each withdrawn, blood was immediately replaced with the same volume of saline, and the dead volume of the catheter was filled with heparin. Corticosterone levels were determined by radioimmunoassay using a highly specific corticosterone antiserum (Kit ICN Biomedicals Inc.) with a detection threshold of 0.1 µg/100ml.

PNS rats secreted higher corticosterone levels than Control rats two hours before the onset of the dark period (F(1,10) = 9.12, p<0.01). Corticosterone levels culminated at 45.79 ± 6.9 µg/100 ml in Control females at the beginning of the dark period, and in PNS females at 51.54 ± 4.86 µg/100 ml 2 hours before lights-off, indicating that prenatal stress induced a phase advance of corticosterone secretion in female offspring. Furthermore, the area under the curve was significantly larger in PNS female rats (527 ± 59 µg/100ml/24 h) compared to controls (375 ± 20 (µg/100ml/24 h) (F(1,10)=5.816; p<0.05).
Figure 3: Circadian fluctuations of plasma corticosterone levels measured at 9 points over a 12:12 light/dark cycle in 6 control and 6 prenatally-stressed rats: male on the left panel, female on the right panel. Times are shown at the bottom, with the thick black bar representing the dark phase of the light-dark cycle. Prenatal stress induced a significant phase advance of the corticosterone rhythm relative to the light/dark cycle ($p < 0.01$) and a larger area under the curve ($p < 0.05$).

Prenatal stress can be responsible for major changes in the phase angle of entrainment of circadian rhythms of locomotor activity and corticosterone secretion. Our finding of a similar change (i.e.; a phase advance) in two rhythms driven by the hypothalamic suprachiasmatic nuclei (SCN), the location of the circadian clock in mammals (18), raises the possibility that the circadian clock of those animals has been altered by prenatal stressful events. Various clinical observations in humans suggest a possible pathophysiological link between affective disorders (such as depression) and disturbances in circadian rhythmicity (12). One of the current hypothesis on the neuroendocrinology of depression involves a flattened (and advanced) circadian cortisol rhythm with hypercortisolism, possibly due to an increased sensitivity of the adrenal cortex (6) thought to normalize pituitary ACTH release in spite of an enhanced drive from the hypothalamic CRH neurons (6).
Influence of prenatal environment modifications on sleep-wake parameters in adult offspring

One strong marker of human depression is an alteration in the sleep-wake cycle, including a shortened REM sleep latency, increased amount and frequency of REM sleep during the first part of the night, increased sleep fragmentation, and decreased slow wave sleep amount (7). In the present study we studied sleep in the adult PNS male rat under baseline conditions.

Adult Control and PNS rats were implanted, under deep anaesthesia, with chronic electrodes for polygraphic recordings of fronto-parietal electroencephalogram (EEG), electro-oculogram (EOG) and nuchal electromyogram (EMG). The animals were then habituated to the sleep recording procedure over the next for 14 days. The rats were connected with a cable to a rotating swivel allowing free movements and EEG, EOG and EMG activities were recorded on a polygraph (Nihon~Khoden, EEG-4414 A/K) with an output connected to a computer for on-line spectral analysis of the EEG. Habituation consisted of two sessions of recording for 8 hours and two sessions of 24 hours. At the end of the habituation period, sleep was recorded for a period of 24 hrs beginning at the onset of the light phase.

Figure 4: Distribution per 12-h intervals (light phase, dark phase) of vigilance states in 8 control (CONT) and 8 prenatally-stressed (PNS) rats under baseline conditions. Mean (± s.e.m.) values of wake (W), light slow-wave sleep (SWS1), deep slow-wave sleep (SWS2) and paradoxical sleep (PS) are expressed as percentage of recording time. *p < 0.05, **p < 0.01, ***p < 0.001 (two-tail unpaired Student’s t-test) for between-groups comparisons.

Prenatal stress induced changes in both the structure and the continuity of sleep in adult offspring. Paradoxical sleep (PS) was the most altered state (Fig-
ure 4). Compared to control rats, PNS rats showed increased total PS time, as well as an increase in the percentage of total sleep time in PS, during both the light and the dark phases. The increase in time spent in PS was due to an increase in the number of PS episodes (+31% over the 24-h interval, p = 0.0014) while the mean duration of PS episodes remained the same between the two groups. PNS also induced an increase in total light slow wave sleep (SWS1) time that was restricted to the dark phase. In addition, sleep was more fragmented during the dark phase in PNS animals, as indexed by a larger number of episodes in each vigilance state and a shorter duration of wake (W) episodes. This resulted in less time spent in W during the dark phase in the PNS animals that was particularly pronounced during the last 4 hrs of darkness (Figure 4). Quantitative analysis of EEG activity during specific vigilance states over the 12-h of light phase revealed minor differences in power spectral values (1 - 32 Hz) between the two groups of rats. EEG slow wave activity (SWA; EEG power in the 1-5 Hz range), an indicator of sleep intensity, was evaluated during SWS2 in the light phase. SWA progressively decreased over consecutive 2-h intervals during the light phase and the time course for this decrease was comparable in the two groups. PNS rats showed a non significant tendency for higher SWA levels in each 2-h interval; this tendency was also observed in power intensities within the higher frequency ranges (5-32 hz). During PS, similar tendency for enhanced power spectral values were observed in PNS rats as compared to CONT rats in the entire frequency range studies (1-32 Hz).

Our results demonstrate pronounced effects of PNS on sleep in the adult rat that parallel to some extent changes in sleep architecture found in depressed humans.

**Conclusions**

Added to our previous findings in PNS rats of high anxiety and emotionality, dysfunction of the HPA axis and circadian timing abnormalities, the observation of long-term changes in their sleep homeostasis supports the validity of the “prenatal stress” model as a new animal model of depression. The persistence of all induced abnormalities after removal of the imposed stressors should be seen as advantageous for the design and testing of new therapeutical strategies in mood and sleep disorders.

**References**


Introduction
Recent studies brought us a lot of information about slow wave sleep physiological mechanisms and disclosed several oscillating elements integrated in networks whose functional properties are greatly modified by comparison with waking state. We used topographical quantified EEG analysis in order to study slow waves and spindles activities during sleep in the presence of thalamic lesions compared with control data.

Slow waves
Since RECHTSCHAFFEN and KALES definitions [27], unicellular electrophysiological investigations lead to dissociate delta activities between a slow rhythm (below 1 Hz) and truly intrinsic delta oscillations (between 1 and 4 Hz) generated in cortical neurons or as a clock-like rhythm in thalamo-cortical neurons [5].

A slow oscillation (<1Hz) described in intracellular recordings from cortical and thalamic neurons [35,41,42] is able to synchronise and group other sleep rhythms like spindles and delta into complex sequences [32]. The interplay between such oscillations reflects at the cortical level and yields to patterns that take the shape of polymorphic waves [5]. The slow rhythm is essentially induced by alternative sequences of long-lasting depolarisation and hyperpolarisation phases [11,42]. It was initially observed in cats during anaesthesia and has been more recently demonstrated during natural sleep in animals.