Prenatal stress and long-term consequences: implications of glucocorticoid hormones

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Abstract

We have shown that prenatal restraint stress (PNRS) induces higher levels of anxiety, greater vulnerability to drugs, a phase advance in the circadian rhythm of locomotor activity and an increase in the paradoxical sleep in adult rats. These behavioral effects result from permanent modifications to the functioning of the brain, particularly in the feedback mechanisms of the hypothalamic-pituitary–adrenal (HPA) axis: the secretion of corticosterone is prolonged after stress and the number of the central glucocorticoid receptors is reduced. These abnormalities are associated with modifications in the synthesis and/or release of certain neurotransmitters. Dysfunction of the HPA axis is due, in part, to stress-induced maternal increase of glucocorticoids, which influences fetal brain development. Some biological abnormalities in depression can be related to those found in PNRS rats reinforcing the idea of the usefulness of PNRS rats as an appropriate animal model to study new pharmacological approaches.

1. Perinatal events in humans

There is increasing evidence that variations in prenatal environment can influence the responses of the new-born. Barker [1] has emphasized how adult vulnerability to cardiovascular disease may be programmed during the fetal period. Indeed, non-genetic factors that could act early in life to organize or imprint permanently physiological systems are known as perinatal programming [2,3]. It can be speculated that prenatal plasticity of physiological systems allows environmental factors, acting on the mother and/or the fetus, to alter the differentiate functions of an organ or tissue system to prepare the unborn animal optimally for the environmental conditions ex utero. However, in extreme conditions like stress and/or undernutrition, offspring of stressed mothers during pregnancy displayed short and long-term physiological and behavioral abnormalities such as reduced birth weight, increased infant morbidity, locomotion and cognition retardation, increased anxiety or sleep disturbances [4–6].

The fetus is sensitive to maternal environment and it has been shown that anxious pregnant women, who present an altered blood flow in the uterine arteries [7], can influence the development of the fetus she carries [1,8,9]. Similar results are also observed later, indeed there is an evidence showing that adverse environmental experiences early in life predispose individuals to the development of affective and anxiety disorders in adulthood [10].

Glucocorticoids may underlie the association between low birth weight and adult stress-related cardiovascular, metabolic and neuroendocrine disorders such as hypertension, type 2 diabetes, ischaemic heart disease and affective disorders [11]. These intriguing findings have spawned the fetal origins hypothesis of adult disease [1]. The brain is very sensitive to prenatal programming and glucocorticoids in particular have powerful brain-programming properties [11]. One of the most intensively systems studied is the hypothalamic-pituitary–adrenal (HPA) axis. Substantial evidence suggests that prenatal stress programs the HPA
axis, and that plasticity of developing brain monoamine systems underlies, in part, these changes. Because an important feature of the stress response is the secretion of high levels of glucocorticoids, this steroid has become an obvious candidate for the role of programming factor in the prenatal stress paradigm. Thus, in human cohorts, it has been shown associations between low birth weight and adult hyperactivity of the HPA axis [12–14].

2. An animal model characterization

In order to better understand mechanisms involved in the long-term effects of such early experiences and considering the obvious difficulties inherent to human research in this particular field, different kind of prenatal stress animal models have been developed. Pregnant rats have been subjected to various types of stressors: conditioned avoidance training [15], tail suspension [16], crowding [17], repeated electric shocks [18], noise [19] or saline injections [20]. During the last years we have studied the influences of prenatal restraint stress (PNRS) in a rat animal model according to a revised model of Ward and Weisz [21].

The prenatal stress procedure we have used consisted in restraining the mothers. Adult virgin Sprague-Dawley female rats (Iffa Credo, France) were group-housed (10 per cage size 60 × 80 cm²) for at least 10 days after arrival, to eliminate stress resulting from shipping and to coordinate their estrous cycle. Animals were then individually housed in the presence of a sexually experienced male Sprague-Dawley rat. Pregnant females were then randomly assigned to prenatal stress or control groups, and individually housed in plastic breeding cages. For all experiments, animals were allowed ad libitum access to food and water, and maintained on a regular light–dark cycle (lights-on 07:00–19:00 h) with constant temperature (23 °C) and humidity (60%).

Prenatal stress was started between the days 11–15 of pregnancy until delivery at 21 days according to a revised model of Ward and Weisz: pregnant females were individually placed in plastic transparent cylinders (7 cm diameter, 19 cm long) and exposed to bright light for 45 min. Animals were daily submitted to three stress sessions starting at 09:00, 12:00 and 17:00 h, whereas control pregnant females were left undisturbed in their home cages. Male and female offspring were weaned 21 days after birth, and only offspring from litters containing 10–14 pups with similar numbers of males and females were used in the experiments. A maximum of two male pups were taken from each litter to remove any ‘litter effects’ [22,23].

2.1. Behavioral long-term consequences

It is clear from animal studies that the behavior of the adult offspring can be altered by PNRS. In rats, PNRS can exert profound influences on offspring’s development, inducing abnormalities which extend from early [24–26] to later life [27]. Adult PNRS rats (4–7 months) exhibit increased ‘anxiety’ (Fig. 1) [28,29], drug addiction [30] ‘emotionality’ [15,31,32] or depressive-like behaviors [16,33–35]. We also reported in PNRS rats enhanced age-related (16–22 months) recognition memory impairment in the Y-maze compared to controls, and altered working memory in the radial-maze [27]. Furthermore, our data provide evidence of a long-term effect of a prenatal stressful procedure on the circadian system. We have shown significant phase advances in the circadian rhythms of locomotor activity relative to the entraining light–dark cycle in both male and female stressed rats [36]. When subjected to an abrupt shift in the light–dark cycle, male and female PNRS rats resynchronized their activity rhythm to the new light–dark cycle slower than control rats [37,38]. Those results raise the possibility that the circadian clock in the hypothalamic suprachiasmatic nuclei (SCN) [39,40] of those animals has been altered by prenatal stressful events. The altered phase-relationship between the circadian clock and lights-off could be due to a change in the underlying period of the circadian clock during entrained conditions. In order to test this hypothesis, we analyzed the free-running period of locomotor activity in temporal isolation in constant darkness. The free-running period was significantly shorter in PNRS rats compared to control rats.

2.2. Neurobiological long-term consequences

The HPA axis has been shown to be affected by PNRS (Fig. 2), showing increased responsiveness to a novel stimulus [41,42]. Levels of both glucocorticoid type I and type II receptors were reduced in
the hippocampus at 90 days, showing a possible mechanism for the long lasting effects on the HPA axis [24,41]. Associated with HPA axis hyperactivity, PNRS rats also displayed hyperglycemia [43]. Moreover, prenatal dexamethasone exposure, a synthetic glucocorticoid, has recently been implicated in the development of adult hyperglycaemia and hypertension as well as behavioral changes and HPA activation [44–46]. PNRS accelerated the age-related HPA axis dysfunction. Indeed, the HPA axis period of hyporesponsiveness was abolished in new-born PNRS rats [24] and circulating glucocorticoid levels of PNRS middle-aged animals were similar to those found in old control ones [27].

We have shown that PNRS altered circadian rhythms. More precisely, PNRS induced higher levels of total and free corticosterone secretion at the end of the light period in both males and females, and hypercorticism over the entire diurnal cycle in females (Fig. 3) [47]. Those effects could be mediated, at least in part, by a reduction in hippocampal glucocorticoid receptors at specific times of day [42]. PNRS induces a reduction of type I glucocorticoid receptors both at the beginning of the light period, which is in agreement with previous data [41] and at the end of the light period at a time when total corticosterone levels are increased in PNRS rats.

Furthermore, prenatal noise stress rats also showed a decrease in benzodiazepine receptors in the hippocampus [48], which could explain the profile of anxiety observed in those rats. Prenatal stress is likely to influence not only the HPA axis but also other endocrinological factors such as sexual hormones in the mother and in the fetus [21]. Recently, the effect of prenatal stress was investigated on the sympathoadrenal response to novelty and footshock by measuring the time course of the changes in circulating catecholamines and their metabolites. Plasma NA levels were significantly higher in prenatal stress than in control rats immediately after footshock, indicating a greater activation of the sympathetic nervous system in prenatal stress rats. The findings demonstrate for the first time that prenatal stress can induce long-term changes in the sensitivity of the sympathoadrenal system to stress [49].

PNRS has been reported to affect neurotransmission. Adult PNRS offspring shows increased 5-hydroxytryptamine (5-HT) contents in several brain regions, e.g. the hypothalamus and the cortex [50–53]. Prenatal dexamethasone exposure mimics prenatal stress procedure, and induces a reduction of 5-HT turnover in the hypothalamus, the hippocampus and neocortex in the offspring moreover at 3 weeks of age [54]. This change in 5-HT function could be involved in alterations of behavioral and hormonal
responses to environmental stimuli, including the HPA axis given that serotonin is a major modulator of the HPA axis [55]. Furthermore, corticosterone regulates the activity of serotonergic system, including tryptophan hydroxylase in the raphe nuclei [56]. Prenatal stressed rats also show reduced noradrenaline contents, and increased noradrenaline turnover in the hippocampus and neocortex [54,57,58] and reduced dopamine turnover in the hypothalamus [54, 59]. Finally, PNRS has long-term effects on the forebrain cholinergic systems inducing an increased hippocampal acetylcholine release after a mild stress and CRH injection (Fig. 4) [60]. A higher release of acetylcholine in the hippocampal area in PNRS rats could be responsible for the decreased corticosterone feedback mechanisms, normally observed in those animals, by reducing glucocorticoid receptors.

This early life experience, the prenatal stress, seems to significantly influence the development of the brain. In this regard, prenatal stress induces structural abnormalities in the hippocampal formation and recently, it has been shown that PNRS produces learning deficits associated with an inhibition of neurogenesis in the hippocampus [61]. Given that glucocorticoids inhibit hippocampal cell proliferation [62] the increased HPA axis activity of prenatally stressed animals [41] could explain their reduced neurogenesis.

3. Parallel between prenatal stress effects and biological abnormalities found in depression

Taken together, our results indicate that prenatal stress induces an increased stress response and abnormal circadian and sleep function in adult rats, suggesting an underlying dysfunction of their circadian clock and a global bad adaptation to challenges. These biological alterations indicate that PNRS adult rats have similar biological correlates of depressed patients [63,64]. Thus, like depressed patients, PNRS rats do escape from the feedback inhibition responsible for returning corticosterone secretion to basal levels after stress [41]. Like in depressed patients in which cholinergic hyperactivity is described [65], PNS rats exhibit cholinergic hypersensitivity after a CRH challenge [60].

Fig. 3. Circadian fluctuations of plasma corticosterone levels at various points over the light–dark cycle in CONT and PNRS male (left panel) and CONT and PNRS female (right panel) adult rats. Prenatal stress induces in both males and females higher levels of the corticosterone during the light-phase, and only in females an hypercorticism over the entire light–dark cycle. *p < 0.05. Error bars show SEM.

Fig. 4. Effects of prenatal stress on saline-stimulated hippocampal ACh output (Delta fmol/min) in males. Prenatal manipulation enhanced the transient increase of ACh release in males (*p = 0.004).
Various clinical observations in humans suggest a possible pathophysiological link between depression and disturbances in circadian rhythmicity, including rhythms of body temperature, various peripheral hormone concentrations and urinary levels of neurotransmitter metabolites [66]. Circadian abnormalities include changes in free-running period, amplitude, cohesion, and entrainment to photic and/or social ‘zeitgebers’ [67]. One of the current hypotheses 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 [68] thought to normalize pituitary ACTH release in spite of an enhanced drive from the hypothalamic CRH neurons [68,69]. Those hormonal features of depression can be related to those found in PNRS rats. Previous reports on the long-term effects of prenatal stress have already suggested its usefulness as an animal model to develop new pharmacological approaches of depression.

One of the hallmarks of human depression is an alteration in the sleep–wake cycle including shortened rapid eye movement (REM) sleep latency, an increase in the amount and frequency of REM sleep during the first part of the night, an increased sleep fragmentation, and a decrease in the amount of slow wave sleep [70]. Significant correlations between sleep abnormalities and dysfunctions of the HPA axis have been shown in depressed patients [71,72], and may result from a stress component [67,73]. In view of those data, we investigated the effects of PNRS on the sleep–wake cycle in adult rats. PNRS rats exhibited a significant increase in the amount of paradoxical sleep (PS) over the 24 h recording session that is positively correlated to plasma corticosterone levels (Fig. 5). Other changes include increased sleep fragmentation, total light slow wave sleep time, and a slight decrease in the percentage of deep slow wave sleep relative to total sleep time [74]. Although there are reports of abnormal ‘sleep-like behaviors’ in PNS monkeys [75] and PNS humans [5], our data provide a polygraphic demonstration of long-term effects of PNRS on the sleep–wake cycle when the animals reach adulthood. These results are also in agreement with the work of Poland group’s [76].

Another important point is that the persistence of REM alterations in PNRS rats dramatically contrasts with the reversibility of these sleep effects in other chronic stress animal models. For example, in both the learned helplessness [77] and the intermittent foot shock paradigms [78], REM was increased only during the first day of recovery. In rats exposed to chronic mild stress, an increase in REM sleep was only observed during the first day of stress recovery [79]. Interestingly, we have noted that even at 6 months of age sleep–wake differences between PNRS and control rats were still present (unpublished data).

Added to our previous findings in PNRS rats of high anxiety and emotionality, dysfunction of the HPA axis and circadian timing abnormalities, the observation of long-term changes in their sleep organization supports the validity of the ‘prenatal stress’ model to develop new pharmacological approaches of depression.

### 4. Predictive validity

The PNRS seems to be an interesting animal model because of the permanent disturbances it produces in the long-term. We determined whether PNRS rats are sensitive to a chronic treatment with antidepressants. There is one single report showing efficacy of tricyclic antidepressants in prenatally stressed female rats [80].

We investigated the behavioral response of PNRS rats in the forced swimming test (Porsolt test), a behavioral test classically used to study antidepressant efficacy. The procedure of the forced swimming test consisted in plunging rats into a glass cylinder (height 59.5 cm; diameter 24.6 cm) containing 36 cm of water maintained at 24 °C for 15 min. A second trial was performed 24 h later for 5 min. PNRS rats show an increased PS over 24 h. (*p < 0.05, **p < 0.01). Positive correlations between individual stress-induced plasma corticosterone AUC values and amounts of PS expressed as percentage of total recording time in CONT and PNRS rats. $r$, coefficient of Pearson’s correlation analysis.

Fig. 5. Distribution per 4 h intervals of vigilance states in CONT and PNRS rats under baseline conditions. PS are expressed as percentage of recording time. PNRS rats show an increased PS over 24 h. (*p < 0.05, **p < 0.01). Positive correlations between individual stress-induced plasma corticosterone AUC values and amounts of PS expressed as percentage of total recording time in CONT and PNRS rats. $r$, coefficient of Pearson’s correlation analysis.
rats showed increased immobility and decreased swimming in the Porsolt test. In another study we assessed, by means of various behavioral tests, the effects of a chronic treatment (once daily in the morning for 21 days) with imipramine, a classical tricyclic antidepressant (10 mg/kg i.p.). Chronic imipramine reversed the immobility behavior, increased hippocampal corticosteroid receptors and reduced cortical 5-HT1A receptor mRNA in PNRS rats. Levels of 5-HT1A receptor mRNA in the cortex were also measured and imipramine decreased levels of these cortical 5-HT1A mRNA in PNRS rats. In order to extend this study we examined the effects of the antidepressant on anxiety related parameters such as the elevated plus maze test and the social test. Interestingly, although showing as expected an increased profile of anxiety such as reduced exploration of the open arm and marked self-grooming behavior, PNRS rats were not affected by the antidepressant treatment in these behavioral parameters [81]. Overall, these results indicate PNRS animals as more sensitive than controls to the effects of antidepressant treatment, and reinforce the idea of the usefulness of this animal’s model as an interesting tool for the design and testing of new pharmacological drugs.

5. Role of maternal factors

In order to understand the pathophysiological mechanisms by which stress in the mother reaches the fetus and influences its development, we studied the effects of blocking maternal corticosterone secretion during PNRS on stress-induced corticosterone secretion and hippocampal corticosteroid receptors in adult offspring [82]. Dams were adrenalectomized, at 13 days of pregnancy to block the increased in corticosterone secretion induced by restraint stress. Adrenalectomized dams were implanted with a corticosterone substitutive treatment (100 mg corticosterone pellet containing 50% corticosterone 21-hemisuccinate and 50% cholesterol). These adrenalectomized dams were submitted to a repeated restraint stress during the last 10 days of pregnancy. In a second experiment, we also studied the effects of a corticosterone injection (3 mg/kg) given concomitantly with the restraint stress to the adrenalectomized mothers implanted with a corticosterone pellet. The corticosterone injections in adrenalectomized pregnant rats elicited plasma corticosterone levels approximating those found in intact mothers in response to stress. The results show that hyperactivity of HPA axis in adult offspring induced by prenatal stress is related to the high levels of maternal corticosterone secretion during restraint stress. In fact, blocking stress-induced corticosterone secretion by adrenalectomy with corticosterone substitutive treatment suppresses the prolonged stress-induced corticosterone response and the reduction in hippocampal corticosteroid receptors observed in PNRS rats at 3 months of age (Fig. 6). Furthermore, administration of bolus injections of corticosterone reinstated the effects of prenatal stress [82].

Results also show that restraint stress increased the basal corticosterone secretion only in the evening in pregnant females [42]. The setting of the circadian rhythmicity of the HPA axis has been shown to depend on maternal factors both during the pre and the postnatal periods [83,84]. The abnormal corticosterone secretion in pregnant females submitted to prenatal stress may then perturb the development of the fetal circadian clock through an action on glucocorticoid receptors, which are notably present in the SCN during early development [85]. It has also been clearly demonstrated that the prenatal dexamethasone exposure was a potent factor that could directly influence the development of the central monoaminergic systems, e.g. noradrenergic, dopaminergic, and serotonergic systems [54].

Taken together, those results suggest that disruption of the normal hormonal response to stress observed in PNRS individuals and the developmental alterations in brain monoamine metabolism depend on stress-induced increase in maternal glucocorticoids during pregnancy. Those findings are in agreement with data showing that exposure of pregnant rats to alcohol (i.e. a procedure that stimulates maternal glucocorticoid secretion) results in a hyperactive HPA axis in the offspring [86,87].

Similarly, non-abortive maternal infections, which increase maternal glucocorticoids [88,89], compromise the development of the fetal brain and alter HPA axis
functioning in the adult [90]. Thus, maternal hormones seem to be good candidates for communication between the dam and developing fetus [91,92]. Interestingly, those data in rats have been confirmed by recent human studies showing that a correlation exists between maternal and fetal plasma cortisol [93]. It should be noted that neuroactive substances other than glucocorticoids could be involved in these long-term prenatal stress effects, including catecholamines, ACTH or beta-endorphin [94,95].

Maternal factors other than hormones may contribute to the long-term changes in HPA activity in the offspring. For example, we have shown that chronic restraint stress during pregnancy can persistently affect mother’s behavior. After several days of restraint stress the reactivity of the pregnant mothers was altered with an increased of their anxiety measured in the white dark box test. One month after the end of stress at weaning time, stressed mothers seemed to be more anxious as shown by their reduced time spent in the open arms in the elevated plus maze test [96]. Such behavioral disturbances may affect maternal care during the lactating period and finally contribute to the long-term effects of PNRS on offspring. Indeed, we have also previously shown that early adoption, which increases maternal behavior, prevents PNRS-induced impairments in glucocorticoid feedback [41] (Fig. 7). Furthermore, handling, a postnatal manipulation, results in changes in maternal behavior [97,98] and contributes to improve behavioral performances in handled offspring.

In conclusion, we have shown that PNRS can cause long-term deficits of different biological systems, reproducing alterations of biological parameters that occur in human depression. So the PNRS rats, especially for what concern neuroendocrinological aspects, may represent an appropriate animal model to evaluate pharmacological interventions to the depression.

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