Effects of prenatal restraint stress on the hypothalamus–pituitary–adrenal axis and related behavioural and neurobiological alterations

Stefania Maccari\textsuperscript{a,b,\ast,1}, Sara Morley-Fletcher\textsuperscript{a}

\textsuperscript{a}Perinatal Stress Team, University of Lille 1, Bât SN4.1 59655 Villeneuve d’Ascq, France
\textsuperscript{b}University of Rome La Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

Received 1 April 2007; received in revised form 5 June 2007; accepted 5 June 2007

KEYWORDS
Gestational stress; Limbic structures; Anxiety; Locomotor activity; Fos protein; Animal model; HPA axis

Summary
Chronic hyper-activation of the hypothalamus–pituitary axis is associated with the suppression of reproductive, growth, thyroid and immune functions that may lead to various pathological states. Although many individuals experiencing stressful events do not develop pathologies, stress seems to be a provoking factor in those individuals with particular vulnerability, determined by genetic factors or earlier experience. Exposure of the developing brain to severe and/or prolonged stress may result in hyper-activity of the stress system, defective glucocorticoids-negative feedback, altered cognition, novelty seeking, increased vulnerability to addictive behaviour, and mood-related disorders. Therefore, stress-related events that occur in the perinatal period can permanently change brain and behaviour of the developing individual. Prenatal restraint stress (PRS) in rats is a well-documented model of early stress known to induce long-lasting neurobiological and behavioural alterations including impaired feedback mechanisms of the HPA axis, disruption of circadian rhythms and altered neuroplasticity. Chronic treatments with antidepressants at adulthood have proven high predictive validity of the PRS rat as animal model of depression and, reinforce the idea of the usefulness of the PRS rat as an interesting animal model for the design and testing of new pharmacologic strategies in the treatment of stress-related disorders.

1. The hypothalamus–pituitary–adrenal (HPA) axis

The adaptation of an organism to environmental challenges involves mechanisms of response to stress activating central and peripheral circuitries: the HPA axis, the sympathetic
system and the central limbic stress loop. Such response is under the control of stimulating and inhibiting inputs to the hypothalamic paraventricular nuclei (PVN), which control the secretion of corticotropin-releasing hormone (CRH), vasopressin (VP) into the pituitary portal circulation and other neuropeptides (De Kloet et al., 1998). CRH and VP secretion leads to pituitary release of adrenocorticotropic (ACTH) and adrenal glands activation, with release of glucocorticoids. Corticosteroids that bind preferentially to hippocampal mineralocorticoid receptors appear to be involved in maintaining basal activity of the HPA axis, while glucocorticoid receptors mediate the effects of corticoids aimed at restoring the homeostasis in the reactive mode. The activity of the HPA axis is involved in the regulation of important physiologic functions of individuals for life, as well as, growth and reproduction. The effects of chronic hyper-activation of the HPA axis associated with the suppression of reproductive, growth, thyroid and immune functions may lead to disease vulnerability, like central obesity (metabolic syndrome X), hyperthyroidism and diabetes mellitus (Pasquali et al., 2006). Recently, a link between other HPA axis/glucocorticoids dysfunctions and increased susceptibility to depressive/anxiety disorders (Holsboer, 2001) as well as to drug abuse (Huizink et al., 2006; Prendergast and Little, 2007) has also been proposed although direct evidence is still lacking.

2. Early environmental influences on HPA axis development

Although many individuals experiencing stressful events do not develop pathologies, stress seems to be a provoking factor in those individuals with particular vulnerability, determined by genetic factors or earlier experience (McEwen and Sapolsky, 1995). The chronic hyper-activation of HPA axis can be determined by multiple factors including genetic and environmental factors. The perinatal life, infancy, childhood and adolescence are periods of increased plasticity for the stress system and are, therefore, particularly sensitive to stressors. Adverse stressors during these critical periods of life, may affect behaviours and physiologic functions, such as growth, metabolism, reproduction and the inflammatory/immune response (Seckl, 2001). These environmental triggers or stressors may not have a transient, but rather a permanent effect on the organism. Barker (1995) has emphasised how adult vulnerability to disease may be programmed during the foetal stage. Indeed, non-genetic factors that could act early in life to organise or imprint permanently physiological systems are known as perinatal ‘programming’ (McEwen and Sapolsky, 1995). It can be speculated that prenatally plasticity of physiological systems allows environmental factors, acting on the mother and/or the foetus, to alter the set-point or ‘hard-wire’ the differentiated functions of an organ or tissue system to prepare the unborn animal optimally for the environmental conditions ex utero.

The intrauterine under-growth and low birth weight are considered as an index of prenatal stress in humans. 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. These intriguing findings have spawned the ‘fetal origins’ hypothesis of adult disease. The brain is very sensitive to prenatal programming and glucocorticoids in particular have powerful brain-programming properties. In rats, substantial evidence suggests that prenatal stress programs the HPA axis as well as behaviour, and that plasticity of developing brain monoamine system underlies, in part, these changes. Because an important feature of the stress response is the secretion of high levels of glucocorticoids, these steroids have become an obvious candidate for the role of ‘programming factor’ in the prenatal stress paradigm. A large number of animal studies have described the effects of prenatal exposure to the synthetic glucocorticoid dexamethasone, which relatively readily passes the placenta. Moreover, prenatal dexamethasone exposure has recently been implicated in the development of adult hyperglycaemia and hypertension as well as behavioural changes and HPA activation (Welberg et al., 2001).

3. The prenatal restraint stress model in the rat

Numerous animal models of early stress are currently being developed because early stress results in long-term disruptions of neuronal functions and the development of long-term behavioural disorders. During the last 15 years, we have studied the influences of a prenatal restraint stress (PRS) in a rat animal model. The prenatal stress procedure we have used consisted in restraining the pregnant rat in a transparent Plexiglas cylinder, 3 times/day for 45 min under bright light at the day 11 of pregnancy until delivery at 21–22 days (Maccari et al., 1995, 2003; Morley-Fletcher et al., 2003). The HPA axis functioning of the PRS offspring is long-term impaired with a prolonged corticosterone stress response (Maccari et al., 1995, 2003; Koehl et al., 1999) and reduced levels of both mineralocorticoid and glucocorticoid receptors in the hippocampus at the adolescent and adult stage (Henry et al., 1994; Maccari et al., 1995; Van Waes et al., 2006). The age-related HPA axis dysfunctions are enhanced by PRS. Indeed, the HPA axis period of hyper-responsiveness was abolished in new-born PRS rats (Henry et al., 1994) and circulating glucocorticoid levels of PRS middle-aged animals were similar to those found in old non-stressed animals (Vallée et al., 1999). Recently, we have showed pro-inflammatory consequences on the immune system of PRS adult animals (Vanbesien-Maillot et al., 2007).

The impact of PRS is already detectable at the foetal stage, giving further support to prenatal stress programming in adult pathophysiology. In the placenta of PRS rats, the expression of glucose transporters type 1 (GLUT1) was decreased, whereas GLUT3 and GLUT4 were slightly increased. Moreover, placental expression and activity of the glucocorticoid barrier enzyme 11beta-hydroxysteroid dehydrogenase type 2 was strongly reduced. At E21, PRS foetuses exhibited reduced body weight and decreased weight of the adrenals, pancreas and testis. These alterations were associated in the offspring with reduced pancreatic beta-cells mass, plasma levels of glucose, growth hormone and ACTH, whereas corticosterone, insulin, IGF-1 and CBG levels were unaffected (Mairesse et al., 2007a).
The hyper-activity of the HPA axis observed in PRS rats is behaviourally accompanied in adult rats (4–7 months) by enhanced sensitivity to drug abuse (Deminiere et al., 1992; Henry et al., 1995; Koehl et al., 2000; Morley-Fletcher et al., 2004a), learning impairments in aged animals (Vallée et al., 1999; Darnaudery et al., 2006) and an increase in anxiety- and depression-related behaviour (Vallée et al., 1997; Morley-Fletcher et al., 2003, Fig. 1A). Moreover, PRS increases the levels of 5-HT_2 receptors (Peters, 1988), induces a higher expression of 5HT_1A mRNA in the prefrontal cortex (Morley-Fletcher et al., 2004b), and increases acetylcholine release in the hippocampus after mild stress (Day et al., 1998). High maternal corticosterone levels may contribute to the long-term effect described in the offspring after PRS (in addition to possible internal vaconstrictions that would affect blood supply to the placenta). The reduction or the increase of maternal glucocorticoids by adrenalectomy of stressed mothers results in suppressed or reinstated PRS effects on HPA axis offspring (Barbazanges et al., 1996). The immediate postnatal environment also plays an important role on PRS-related outcome on HPA axis that can be reversed by an early postnatal manipulation such as early adoption (Maccari et al., 1995). Indeed, adoption modifies maternal behaviour, increasing pup-directed behaviour in foster mothers, and decreases the stress-induced corticosterone secretion in the adult PRS offspring.

PRS alters not only reactive adaptation, but also predictive adaptation by changing circadian rhythms. Significant phase advances are observed in the circadian rhythms of locomotor activity relative to the entraining light–dark cycle in both male and female PRS rats that

---

**Figure 1** Long-term effects of prenatal restraint stress (PRS). Results are expressed as mean±SEM and have been obtained from male adult Sprague–Dawley control and PRS rats. (A) Positive correlation between individual stress-induced (60 min after stress extinction) time spent in immobility in the forced swim test in controls and PRS rats. *P*<0.05 and **P**<0.01 vs. controls. (B) Positive correlations between individual stress-induced plasma corticosterone (AUC values) and amounts of REM sleep expressed as percentage of total recording time in controls and PRS rats. *r* = coefficient of Pearson’s correlation analysis. (C) Effect of chronic imipramine treatment (10 mg/kg, IP, daily, 21 days) on binding capacities of total corticosteroid (MR+GR) hippocampal receptors in control and PRS adult rats. *P*<0.05 vehicle-treated PRS animals vs. vehicle-treated control group; **P**<0.05 imipramine-treated PRS group vs. vehicle-treated PRS group. (D) Neural activity in the hippocampus, as measured by Fos immunoreactivity at the basal level and in response to a mild stress (open arm of the elevated-plus maze) in controls and PRS rats. Original data are reported in Morley-Fletcher et al. (2003b, 2004b) (A, C), Dugovic et al. (1999) (B) and Viltart et al. (2006) (D).
resynchronised their activity rhythm to the new light–dark cycle slower than control rats when subjected to an abrupt shift in the light–dark cycle (Van Reeth et al., 1998; Maccari and Van Reeth, 2007). Also, PRS induced higher levels of corticosterone secretion at the end of the light period in both males and females, and hypercorticism over the entire diurnal cycle only in females (Koehl et al., 1997). The sleep–wake cycle is dramatically modified by PRS with a significant increase in the amount of REM sleep over the 24-h recording session, positively correlated to plasma corticosterone levels (Fig. 1C). 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 (Dugovic et al., 1999), thus providing a polygraphic demonstration of long-term effects of PRS on the sleep–wake cycle when the animal reach adulthood.

The neurobiological mechanism underlying dysfunctions induced by PRS are still unknown. In addition to glucocorticoids, other factors may be involved in the long-term effects of PRS on sleep–wake cycle. The 5-HT system could be a good alternative, in view of the developmental alterations in brain 5-HT metabolism induced by prenatal restraint stress. Finally, experiments recently conducted by our group have proven a long-term effect of PRS on group-I metabotropic glutamate (mGlu-I) receptors with an opposite outcome in male and female rats (Maccari et al., 2004), by reducing activity of mGlu receptors in males and increasing it in females. Giving that glutamate systems may participate in sleep alterations (Feinberg et al., 2002; Ngomba et al., 2005), their role in PRS-induced disruptions of circadian rhythms need to be explored.

4. Prenatal restraint stress in the rat as animal model of altered neuroplasticity

We have identified some of anatomical substrates and neural mechanisms sustaining the HPA axis hyper-activity classically described in PS rats after stress exposure. Fos protein expression after exposure to a mild stressor (open arm of the elevated-plus maze) was evaluated in hippocampus and locus coeruleus, brain areas involved in the feedback control of the HPA axis and in the PVN, that reflect the magnitude of the hormonal response to stress (Viltart et al., 2006; Fig. 1D). At basal level, PRS rats exhibited higher number of Fos-immunoreactive neurons than controls in the hippocampus and locus coeruleus, whereas they presented a higher basal expression of hypothalamic vGAT a marker of GABAergic synapses. After exposure to the open arm, number of Fos-immunoreactive neurons increased in the PVN, whereas no changes were observed in the hippocampus and locus coeruleus of PRS rats compared to basal condition. Moreover, only PRS rats presented an elevation of the number of activated catecholaminergic neurons in the locus coeruleus. Mairesse et al. (2007b) also examined whether behavioural reactivity was correlated with neuronal activation, by assessing Fos expression in limbic regions of rats exposed to a low or high anxiogenic environment (the closed and open arms of the elevated-plus maze, respectively). A negative correlation was found between behavioural and neuronal activation, with a lower behavioural reactivity and a higher neuronal response observed in rats exposed to the more anxiogenic environment (the open arm) with respect to the less anxiogenic environment (the closed arm). Interestingly, the variation in the neurobiological response between the two arms of the maze was less pronounced in rats that had been subjected to PRS. These studies provide evidence of long-lasting changes in brain plasticity induced by PRS that affect the ability of limbic neurons to cope with anxiogenic stimuli of different strength.

5. Prenatal stress in the rat as an animal model of depression

PRS rats display biobehavioural alterations that can parallel to some extent indices in human depression research, thus becoming a useful tool for the design and testing of new pharmacologic strategies in mood and sleep disorders. The criteria proposed by Willner (1997) require that animal models of depression exhibit face, predictive and construct validity. Face validity refers to the phenomenological similarity, whereas predictive validity refers to the accuracy of a model in forecasting the course and outcome of a human syndrome. Finally, construct validity represents the degree to which both the human syndrome and the animal model are unambiguously defined such that a rational theory can be constructed to explain the pathophysiology of disorder. However, because mental disorder is a human pathology, the perfect homology of an animal model to a human psychiatric condition cannot be absolutely demonstrated. In contrast, it is possible to use animal models to highlight some similar symptoms and develop new pharmacological strategies. The stress-driven theory of mood disorders (Sapolsky, 1996; Kessler, 1997) suggests that a stress-induced model of depression such as the PRS model has good construct validity (Rosenwasser and Wirtz-Justice, 1997). In this regard, early-life stress at moments when critical developmental processes are taking place in parts of the nervous system or neuronal circuits involved in (later) HPA-axis functioning may induce in some individuals distinct and stable patterns of dysregulations that are associated with altered emotional processing and heightened responsiveness to stress. Various clinical observations in humans suggest a possible pathophysiological link between depression and disturbances in hypothalamus–pituitary axis, circadian rhythmicity, body temperature fluctuations, various peripheral hormone concentrations and urinary levels of neurotransmitter metabolites (Holboer, 2001). Added to our previous findings in PRS rats of high anxiety and emotionality, dysfunction of the HPA axis and circadian timing abnormalities, the observation of long-term changes in their sleep structure supports the validity of the PRS model as a valid animal model of anxiety/depression. Our group has provided increasing evidence for the predictive validity of PRS model by means of chronic treatment with different classes of antidepressants in adult rats (Fig. 1C). Indeed, imipramine (tricyclic), tianeptine (a selective serotonin reuptake enhancer, structurally similar to the tricyclic antidepressants) or agomelatine (a dual antidepressant with melatonergic agonist and 5-HT2C antagonist properties) reverse several PRS-induced alterations at the behavioural, neurochemical and neuroanatomical level.
Thus, following antidepressant treatment, PRS rats displayed reduced immobility behaviour in the forced swim test, increased exploration of the open arm in the elevated-plus maze, enhanced mineralocorticoid and glucocorticoid receptors densities in the hippocampus and modified 5-HT1A mRNA expression (Morley-Fletcher et al., 2003, 2004b). Also, since preclinical and clinical research has increasingly focused on the interaction between stress and depression and their effect on hippocampus (Duman et al., 2001), we have recently tested the effects of antidepressant treatment on hippocampal neurogenesis. Interestingly, PRS induces a lifespan reduction of hippocampal neurogenesis in male rats (Lemaire et al., 2000, 2006) but not in females (Darnaudery et al., 2006), and a chronic agomelatine treatment increases hippocampal neurogenesis especially in the ventral part of the hippocampus of male rats (Maccari et al., 2005). This latter finding gives further support to the validity of the PRS model.

6. Conclusions

In an animal model of early stress, it has been showed that stress-related events that occur during the foetal and early postnatal period may have lifelong programming effects on HPA axis functioning and different body functions with a considerable impact on disease susceptibility. Stress-related disease can be interpreted broadly, including cardiovascular disease, components of the metabolic syndrome and emotional alterations, for which the evidence of foetal origins is most abundant. Preclinical and clinical studies have shown that lifelong programming of the HPA axis function by foetal life conditions is likely to be a key factor in mediating associations with these disorders. It is therefore highly plausible that susceptibility to different stress-related disorders originates in a similar manner during early life, although direct evidence is still lacking. The development of animal models involving early-life environmental manipulations, should allow to study the concept of vulnerability applied to stress-related disorders and help to improve the prevention by developing new therapeutic strategies.

Role of the funding source

University of Lille 1, USTL (France) contributed with editorial assistance, reviewed drafts of the manuscript and contributed to the decision to submit the manuscript for publication. The authors retained full editorial control and responsibilities throughout the preparation of the manuscripts.

Conflict of interest

None declared.

Acknowledgements

This supplement is based upon the proceedings from Lille Summer School in Neurosciences-Brain Plasticity in Life Span held in France in 2006. The supplement is supported financially by University of Lille 1, USTL (France), Servier (France) and Nestlé (Switzerland).

References


Please cite this article as: Maccari, S., Morley-Fletcher, S., Effects of prenatal restraint stress on the hypothalamus–pituitary–adrenal axis and related behavioural and neurobiological.... Psychoneuroendocrinology (2007), doi: 10.1016/j.psyneuen.2007.06.005


