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Review

Stress, genes and the mechanism of programming the brain for later life

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Abstract

de Kloet ER, Sibug RM, Helmerhost FM, Schmidt M. Stress, genes and the mechanism of programming the brain for later life. Neurosci Biobehav Rev XXX–XXX, 2005 adverse conditions during early life are a risk factor for stress-related diseases such as depression and post-traumatic stress disorder (PTSD). How this long-term effect of early adversity occurs is not known, although evidence accumulates that the action of stress hormones is an important determinant. In rodents after a variety of experiences, even minor ones, during postnatal life permanent changes in emotional and neuroendocrine reactivity have been observed. Also stressful events occurring prenatally and even the pre-implantation hormonal conditions can have permanent consequences. Here we will focus on evidence obtained from (i) the blastocyst implantation during conditions of ovarian hyperstimulation, which is commonly used in the generation of transgenic mice; (ii) the stress system activity in the newborn under various conditions of maternal care; (iii) the long-term consequences of maternal separation procedures. The results clearly demonstrate that early experiences trigger immediate changes in the stress system that may permanently alter brain and behaviour.

Keywords: Brain; Stress; Glucocorticoids; Development; Ovarian hyperstimulation; Blastocyst implantation; Maternal deprivation; Aging; Cognition; HPA response

Contents

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1. Introduction

For more than 50 years, the stress response is monitored by measuring the activity of the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis. The exposure to a brief stressor such as a novel environment causes through HPA axis activation a rise in circulating corticosteroid concentration that reaches maximal levels after 15–30 min, while returning to baseline 60 min later. The elevated corticosteroid concentrations attenuate these initial stress reactions in the very same pathways that have led to HPA activation and mobilize for this purpose the necessary energy substrates. Brain and body functions are coordinated by these effects of the hormone on information processing with the goal to facilitate adaptation and to be prepared if the stressor is encountered another time [1–3].

Much has been learned over the past 50 years about the way the corticosteroids operate in the stress system. It was found that corticosterone interacts with numerous factors in afferent pathways to the paraventricular nucleus (PVN) that either activate or attenuate the HPA response [4]. These afferents include pathways that signal inflammatory and immune reactions or that monitor imbalances in volume regulation and energy metabolism. Signalling pathways in higher 'limbic' brain regions that mediate the cognitive and/or emotional manifestations of the stress response are also targets of corticosterone. In the 'limbic' brain, e.g. hippocampus, amygdala, frontal cortex, the corticosterone action appears to be bi-modal [5–7]. One (fast) mode, possibly involving glutamatergic signalling [8,9], determines the threshold or sensitivity of the stress system. Once a stressor has activated the stress system through corticotrophin-releasing hormone (CRH) and its CRH-1 receptors, the circulating corticosterone concentrations rise and progressively operate the other slower mode as part of a late sustained coping system that synergizes with the activity of the urocortin-CRH-2 receptor system. The actions exerted by corticosterone in the fast and slow mode are mediated by mineralocorticoid (MR) and glucocorticoid receptors (GR), respectively [2]. The MR helps to control the threshold or sensitivity of the stress response system, while via the GR the stress response is terminated and recovery from stress is promoted. Very recent evidence points to the implication of membrane-mediated corticosteroid effects perhaps involving these receptors also [10].

Numerous studies have identified changes in HPA axis activity and corticosteroid action that are associated with disease states [11,12]. The cause of these changes can be inability to cope with a chronic stressor [12], an exposure to a single acute life event or a genetic risk factor such as a single nucleotide polymorphism (SNP) disabling efficient MR and/or GR functioning [13,14]. The altered HPA activity is reflected in a change in frequency and/or amplitude of the pulsatile secretion of the steroid [15] suggesting it is rather the pattern than the actual level of circulating corticosteroid that matters. In susceptible individuals an aberrant corticosteroid patterns may precipitate disease as is the case in, e.g. psychotic depression [16]. In the latter disease the brain is overexposed to cortisol resulting in relatively more activation of GR than MR. As predicted, psychotic depression responds positively to treatment with anti-glucocorticoids that attenuate the overactive GR function favouring health through MR. The sluggish response pattern of the corticosteroids to daily variation and stress becomes upon recovery 'reactive' again [17,18].

In the newborn rodent, mild common stressors are unable to trigger an ACTH or cortisol response, but the brain is in many ways still very responsive to stressors [19]. It is well known that more severe stressors such as infection are capable to break through the apparent quiescence of the early postnatal HPA axis. What came as a surprise, however, is that the most powerful effect is achieved when the pup is deprived from the dam's feeding, licking and grooming. Separation of mother and pup not only activates and sensitises the HPA axis to subsequent stressors, but also can produce permanent changes in HPA reactivity.

In this contribution evidence is presented suggesting that conditions as early as ovarian hyperstimulation applied to stimulate egg production and induce ovulation, may affect postnatal life. Next, the progress in understanding the mechanism underlying the stable quiescent HPA activity during the stress hypo-responsive period (SHRP) is examined. Finally, the experiments showing a lasting outcome of early manipulations for stress regulation and cognitive performance are evaluated. The 'leitmotiv' is that stress-related events mediated by corticosteroids occurring from the blastocyst stage to the postnatal stage can impact on the offspring.

2. Peri-implantation effects

Epidemiological data have shown an association between children with a low birth weight and an increased risk to develop several metabolic, cardiovascular and behavioural pathologies in later life [20]. This programming phenomenon known as the 'fetal origins of adult diseases' (FOAD) proposes that these disorders are derived from fetal adaptations due to intra-uterine perturbations. The FOAD hypothesis has been tested in animal models using maternal undernutrition, stress and glucocorticoids during early-, mid-, late-trimester or throughout pregnancy. These in utero 'stressors' altered birth weight, neuroendocrine responses (i.e. hypothalamo–hypophyseal–adrenal axis, serotonergic system and release of neurotrophic factors), immune functions, cognitive and nociceptive behaviour. Furthermore, cardiovascular and metabolic disorders such as hypertension, hyperglycemia, hyperinsulinemia occur and persist throughout life.

The exact period of the initiation of programming is not known. However, data show that programming may initiate already at the pre-implantation period. The pre-implantation embryo is sensitive to epigenetic modifications that may have programming consequences [21,22]. In a maternal low protein diet paradigm using rats from embryonic day 0 (ED0) to ED4.5, the blastocysts have a reduced number of inner cell mass. Postnatally, female offspring showed low birth weight, increased systolic blood pressure and abnormal organ/body weight ratios in comparison with males [23]. Very recent data show that in mice in vitro culture of pre-implantation embryos until the blastocyst stage showed significant alterations in anxiety, locomotor activity and spatial memory in adult life [24,25]. Moreover, large organs and an increase in body weight and pathologies have been observed [25]. Similar observations after in vitro culture of embryos of large domestic animals have been characterized as the so-called 'large offspring syndrome' [26].

There are data in the literature indicating that the onset of programming may take place even earlier than the pre-implantation period. Fetal growth and weight in humans [27] and sheep [28] can be influenced by poor nutrition around the time of conception. In mouse, maternal protein undernutrition for 15 or 30 days before mating reduced the number of cleaving embryos and caused delayed and asynchronous cleavage leading to retarded differentiation of morulae to blastocysts [29]. In humans, low preconceptional (24 months before conception) intake of minerals and vitamins is associated with spina bifida offspring [30] and a high preconceptional intake of fruits and vegetables reduces the risk for orofacial clefts [31].

Glucocorticoids appear to exert also programming effects around the period of preconception. Mice treated with dexamethasone consecutively for 3 days before mating produced offspring with significantly greater body weight in adult life in comparison with those treated with saline (Sibug et al., unpublished data).

2.1. Effects of ovarian hyperstimulation on embryonic development

Ovarian hyperstimulation (OHS) with gonadotropins is routinely used for ovulation induction in human in vitro fertilization programs and in the generation of transgenic animals. In humans [32–34] and rodents [35,36], assisted conception preceded by OHS has been associated with a low fetal/birth weight. In a matched twin study the in vitro fertilization effects were found to override genetic factors [33]. The mechanism whereby OHS leads to a low birth weight remains to be elucidated.

Blastocyst implantation is intimately associated with vascular permeability and angiogenesis. These two processes are potently stimulated by the vascular endothelial growth factor (VEGF), which binds to the two tyrosine-kinase receptors, flt-1 and KDR/flk-1. Messenger RNA expression of VEGF and its receptors correlates spatially and temporarily with changes in angiogenesis and vascular permeability at implantation sites [37,38].

The role of gonadotropins in processes underlying blastocyst implantation is still poorly understood. Since glucocorticoids are known to exert detrimental effects during pregnancy we hypothesized that treatment with gonadotropins to induce OHS evokes a (stress) response of which the effects persist after conception and cause impairment of angiogenesis during the peri-implantation period. We investigated the effects of gonadotropin treatment on mRNA expression of VEGF120 and its receptors during the implantation process using in situ hybridisation. Adult female CD1 mice were either injected with urinary human follicle-stimulating hormone and urinary human chorionic gonadotropin to stimulate folliculogenesis and ovulation, respectively. Spontaneously ovulating mice served as controls and received saline injections. Treatment with urinary gonadotropins led to a delayed blastocyst implantation, smaller size of the implantation site and prolonged gestational period. These were accompanied by reduced expression of VEGF, flt-1 and flk-1 and increased levels of resorption and progesterone (Fig. 1).

Although the murine model has become indispensable in elucidating the mechanisms involved during the

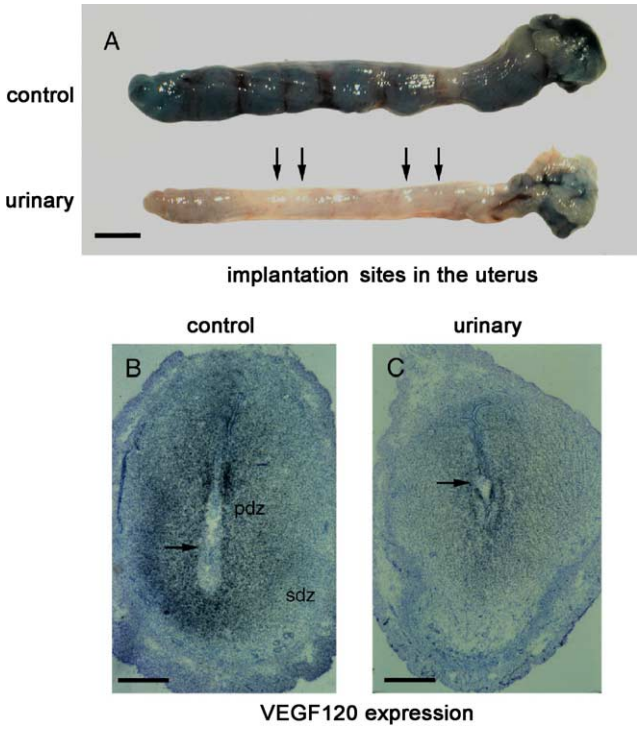

Fig. 1. Effects of urinary and recombinant gonadotropins on vascular permeability and expression of VEGF120 in the embryonic implantation site on embryonic day 5. (A) Chicago blue dye penetration along the length of the uterus in the different groups. Arrows indicate implantation sites without dye staining. Lower panels: autoradiograms showing the expression of VEGF120 in saline (control, B) and urinary (C)—treated implantation sites. Arrows indicate the site where the blastocyst implants. Bars: (A) = 2.5 mm, (B–C) = 0.6 mm; exposure period: (B–C) = 17 days. pdz, primary decidual zone; sdz, secondary decidual zone.

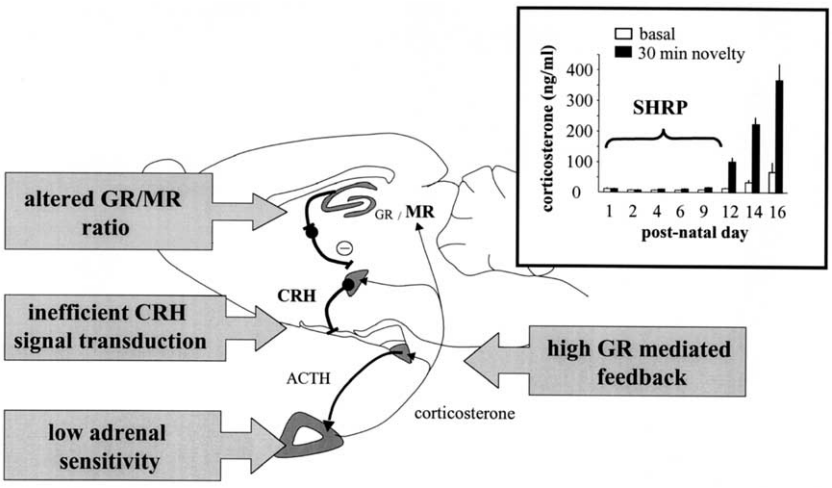

Fig. 2. Schematic overview of the activity of the neonatal stress system. Peripheral HPA axis activity is low due to a high inhibitory tone of corticosterone on the POMC gene via the GR and the low adrenal sensitivity to ACTH. In contrast, the activity of central HPA components as CRH is high due to the lack of efficient negative feedback.

implantation process, there are species-specific differences and caution should be applied in extrapolating the present results in the human situation. Nonetheless, the implications of these data in human assisted reproduction programs deserve careful attention since a study showed that children procreated via assisted reproduction are often preterm, have low APGAR scores and showed poorer health than control siblings [39]. (The APGAR score is a practical method for assessing a newborn infant and is done routinely 60 s after the birth of the infant and then it is commonly repeated 5 min after birth. The APGAR score is a number arrived at by scoring the heart rate, respiratory effort, muscle tone, skin colour, in response to a catheter in the nostril.) In addition, in vitro produced sheep blastocysts showed an alarming tenfold increase in perinatal mortality [40] Hence, there is a clear need to study the possible long-term consequences of the programming effects of ovarian hyperstimulation with urinary gonadotropins before conception.

3. Immediate effects of early life manipulations

When talking about environmental alterations, which permanently affect the adult phenotype and the lifetime vulnerability to disease, the timing of these early disruptions of the stress system development is an essential factor. In rats and mice there seem to exist certain time windows, where a quiescence of the stress system is crucial for the normal development of the brain circuitry, and where a prolonged activation of the HPA axis is only resorted to under severe physiological or psychological stress. In rodents, this period of low stress system activity is known as stress hypo-responsive period (SHRP), and lasts depending on the species (rat or mouse) for about the first two weeks following birth [41–44]. While the historical definition of the SHRP is mainly based on the low peripheral concentration of the stress hormone corticosterone and the inability of mild stressors to induce a marked increase of this hormone, we and others could demonstrate that the SHRP is also characterized by a number of specific features, which underlie the function of the neonatal HPA axis (Fig. 2). One of the first hypotheses regarding the cause of the stress hypo-responsiveness of the HPA axis during postnatal development was the postulation of immature neural projections to the PVN [45]. A number of recent studies could indeed show, that some of the neural connections to the PVN only develop postnatally, and are not fully functional during the SHRP [46–48]. Thus, it seems feasible that the neonatal brain is unable to relay some specific stressors to the PVN in an appropriate manner, making the adequate activation of the HPA axis impossible.

On the other hand numerous studies could show that young rat pups are quite capable of a robust HPA axis activation, dependent on the type of the stressor [49–51]. Furthermore, studies by the group of Levine could demonstrate that the HPA axis of a neonatal rat can be activated by the lack of maternal care (maternal deprivation), responding to mild stressors (e.g. novelty exposure) in a more or less adult-like fashion [52]. Hence, even though an immaturity of the neural connections may in part contribute to the hypo-responsiveness of the HPA axis during postnatal development, it is unlikely that this is the main, underlying mechanism.

In addition to the structural differences of the neonatal brain the expression patterns of a number of central HPA factors are very characteristic during the SHRP. Corticotropin releasing hormone (CRH) is already expressed as early as fetal day 18 [53], but there is some discrepancy in the literature about the postnatal ontogeny of this neuropeptide. While some early papers reported a low [54] or steadily rising [55] CRH expression in the hypothalamus,

more recent studies demonstrated CRH expression in the PVN at adult-like levels during the SHRP [56,57]. In an extensive study in the mouse we could recently demonstrate, that indeed the CRH expression of the neonates during the SHRP is quite high, and decreases abruptly at the end of the SHRP [58]. This high CRH expression correlates with the low corticosterone release from the adrenal and a different MR/GR ratio in the developing brain. Apparently CRH is not under a strict negative feedback control during the SHRP, or this control is disabled due to the low corticosterone concentrations. However, even though the central aspects of the neonatal HPA axis are under a different regulation than in the adult, this does not seem to be the primary cause for the lack of peripheral HPA activity during the SHRP.

Paradoxically, the high CRH production in the PVN does not enable the HPA axis of the neonate to induce an ACTH response from the pituitary. The answer to the question of why the pituitary of the neonate is not responding to mild stressors with an adult-like ACTH response seems to lie in the negative feedback control of ACTH production. Already shortly after the first description of the SHRP it was hypothesized that an enhanced GR-mediated negative feedback at the level of the anterior pituitary might present the most proximal cause of the HPA hyporesponsiveness [43,59–61]. This hypothesis was supported by a number of findings. First, Sakly and Koch [62] measured adult-like levels of GRs in the pituitary throughout postnatal ontogeny. Secondly, circulating levels of the plasma corticosterone-binding globulin (CBG) were found to be very low during the SHRP, implying that most of the circulating corticosterone would be in its unbound, biologically active form [63]. Further support came from experiments using adrenalectomy (ADX) or treatment with metyrapone during the SHRP. Under both conditions, where corticosterone levels are minimal, basal and stress-induced ACTH secretion was found to be largely elevated [64,65]. Also, elimination of circulating corticosterone levels by adrenalectomy greatly enhanced the expression of the ACTH precursor gene POMC in the pituitary, but had no or only little effect on CRH expression in the PVN [66]. Yi and colleagues [67] could further demonstrate, that chronic blockade of GRs in the PVN in neonatal rats only slightly enhanced CRH expression and corticosterone secretion.

In a recent series of experiment we could now support this hypothesis. When treated intra peripherally (i.p.) with the GR antagonist RU486, mouse pups during the SHRP respond with a largely increased ACTH and corticosterone secretion. In contrast to this enormous peripheral effect of the antagonist, CRH expression in the PVN remained unchanged. No or only a very small effect was seen when instead of the GR antagonist the MR antagonist 28318 was applied. Thus, it can be concluded that a high inhibitory tone of the pituitary GR is likely to be the main cause for the low responsiveness of the pituitary during the SHRP. With this elegant biological regulation the neonatal brain manages to create a situation, where the activity of the peripheral and the central part of the HPA axis is separated. That way a high activity of the central, i.e. CRH stress circuitry during development is possible without the damaging effect of high corticosterone concentrations.

Although less apparent, a similar period as the SHRP has recently also been postulated in human children [68]. This analogous phenomenon in humans appears to develop gradually during the first year of life; it is unclear, however, how long it extends. While healthy newborn infants exhibit a highly reactive adrenocortical response to stressors [69], the HPA axis of the average 12–18-month-old child does not respond to mild challenges [70,71]. Even though these children exhibit a clear behavioural response to for example approaching strangers, or novel events or environments, this apparent distress does not result in elevated circulating cortisol levels. Thus, humans and rodents seem to share certain similarities in their patterns of stress system development, which probably underlie a similar biological phenomenon and rationale.

Another similarity between humans and mice (or rats) is that both species profoundly rely on a strong mother–infant interaction during development. Maternal (or in the human situation in general parental) care seems to be a crucial factor for the normal development of the infants. In humans, the quality of childcare has been described to influence the cortisol response to mild stressors, especially during the period where an increase of cortisol is difficult (human stress hypo-responsive period). For instance, Nachmias and colleagues [72] showed that 18-month-olds, withdrawing from strange events and seeking comfort with their mothers, only showed a cortisol response, if the attachment to the mother was insecure. In addition, prolonged or severe childhood trauma appears associated with a permanent alteration in the function of the HPA system, thereby being a risk factor for the development of psychiatric diseases during adulthood [73,74]. In spite of these similarities there are obvious differences as well between mice and rats on the one hand, and humans on the other with respect to, e.g. litter size and rate of development.

In rodents, alterations in maternal care have a strong influence on the HPA activity of the pups. Historically, two different models of altered maternal care have been used, handling and maternal separation. In handling, the pups are separated from the dam for 15 min per day [75]. A number of studies could demonstrate, that this short disruption results in an average increase of maternal care, like licking, grooming and arched back nursing as compared to non-handled animals [76,77]. In contrast, prolonged maternal separation results in an overall lack of maternal care and can be regarded as a laboratory model for neglect. It can be argued, that because a separation of the dam from the litter for a prolonged period of time creates a dangerous and potentially life threatening situation for the infants, the stress system of these animals adapts to this situation regardless of the adverse consequences for later

development. For the correct interpretation of the long-term consequences of decreased (and also increased) maternal care it is therefore crucial to understand the immediate effects of this treatment. For that reason, we and others have studied the model of 24 h of maternal deprivation, especially with the focus on the immediate effects of a mother–pup separation.

Even when applied during the SHRP, where mild stressors do not elicit a corticosterone response, 24 h of maternal deprivation result in a marked activation of the HPA axis. In the rat as well as in the mouse, corticosterone levels are largely increased following maternal deprivation [58,78]. In that respect it was astonishing, that ACTH levels are not (in the rat) or only slightly (in the mouse) elevated in deprived pups compared to controls. We therefore recently conducted a study, where we examined the activity of the HPA axis during the 24 h deprivation period [79]. The results show, that both ACTH and corticosterone largely increase during the first 8 h of maternal absence. However, during the second part of the 24 h deprivation period circulating ACTH levels are restrained and ultimately suppressed by negative feedback, while corticosterone levels continue to be elevated. This seemingly paradoxical effect is likely to be related to a higher adrenal sensitivity to ACTH at the end of the deprivation period [80,81]. Also in the brain the activation of negative feedback mechanisms could be shown. The relatively high expression levels of CRH in the PVN decreased dramatically following the increase in circulating corticosterone. Expression of MR and GR in the hippocampus as well as GR in the PVN is also decreased following maternal deprivation. Thus, the prolonged absence of the dam triggers the activation of the HPA axis in the pups, which consequently initiates negative feedback mechanisms in the brain. Therefore, the developing brain is suddenly exposed to a variety of neurotransmitters, neuromodulators and transcription factors, which are normally not present, or at least not in that quantity. There is no doubt that activated corticosteroid receptors play an important part in this process, which may ultimately alter the developmental trajectory of specific brain circuits, for example the ones involved in stress system regulation.

A variety of studies indicate that these changes are actually due to the lack of maternal care. In the rat, Suchecki and colleagues demonstrated, that if the maternal licking behaviour is replaced during the deprivation period by mild stroking with a wet brush, some of the deprivation effects can be prevented [82,83]. Stroking combined with artificial feeding was even able to reverse all the measured effects of maternal deprivation. Indeed it seems that the initial activation of the HPA axis during the first 8 h of maternal absence occurs via metabolic signals. After all, a lack of nutrition is one of the most life threatening events that can occur to a mouse or rat pup. Studies in adult animals have shown very clearly that the metabolic system is in close interaction with the activity of the HPA axis. Much attention has been given to the recently discovered metabolic hormones leptin and ghrelin, which both have been shown to bind to specific receptors in the brain and to be able to modulate HPA activity in the adult [84,85] and in the case of leptin also in the neonate [86,87]. Especially ghrelin is an interesting candidate for the regulation of HPA activity. A recent study by Hayashida et al. [88] demonstrated, that circulating ghrelin plasma levels are increased following 8 h of milk deprivation. In addition, ghrelin has been shown to increase neuropeptide Y (NPY) expression in the arcuate nucleus, which in turn could increase CRH production and release in the PVN [89]. Together with the recently demonstrated trophic function of leptin during development [48], it is feasible that a similar pathway is employed in the neonate to initiate the activation of the HPA axis during maternal deprivation. In contrast to feeding, maternal care (e.g. licking) seems to modulate the central response to this peripheral activation.

In light of the strong corticosterone response to maternal deprivation it seems logical, that in the literature this hormone is held responsible for most effects of mother–pup separations. Nevertheless, caution should be taken in the interpretation of these data, as firm proof for the causality of corticosterone is lacking. For instance, pretreatment of pups with dexamethasone completely abolished the corticosterone response to maternal deprivation, but did not affect the central effects of mother–pup separation [83]. Our group could recently demonstrate, that CRH1 receptor knockout mice respond to maternal deprivation with a marked decrease of GR expression in the hippocampus and the PVN, even though they are completely unable to elicit a corticosterone response to this treatment [90]. Yet unpublished results from our group further indicate a strong regulatory influence of limbic CRH1 receptors on HPA function during development. Thus, even though high corticosterone levels acting via GR and MR are undoubtedly fundamental for the direct effects of maternal deprivation, other signals may as well contribute to the described effects.

4. Long-term consequences

Of all the different models on early life effects the handling paradigm is probably the historically most used and best documented paradigm. It has convincingly been demonstrated that handling of rat pups during their postnatal development permanently alters the function of their HPA axis. Adult rats handled during infancy show a reduced ACTH and corticosterone response to stress in the hippocampus as well as lower levels of CRH and AVP mRNA expression and immunoreactivity when compared to non-handled animals [91–93]. Furthermore, handling has been shown to reduce anxiety-like behaviour [94]. However, as Pryce and colleagues [95] recently pointed out, some care has to be taken in the interpretation of these data,

as the non-handled animals, which are commonly used as control group, also present an experimental extreme.

The control group problem has been elegantly avoided by Meaney's group, who introduced the model of low vs. high grooming mothers. In this model, naturally occurring differences in maternal care are scored in a population of Long-Evans rat mothers, and used as criterion for sub grouping the females according to the intensity of their maternal care. They could show, that the offspring of high grooming mothers had significantly lower ACTH and corticosterone responses to stress, a lower CRH mRNA expression in the PVN and a higher GR mRNA expression in the hippocampus [76]. In addition, these rats from 'high grooming' mothers also displayed a higher spatial learning and memory ability compared to the offspring of low grooming mothers [96]. Taken together, these data underscore that maternal care matters, and that alterations of maternal care during development affect the function of the individual during adulthood. It should be pointed, however, that such differences in outcome, if pups are exposed to low- and high-grooming mothers, represent correlates rather than causality. For causality cross-fostering studies are essential [97].

Long-term effects on the receptors for corticosteroids also have been measured. In the handled animals Meaney and colleagues always report elevated expression levels of GR solely for the hippocampus. In studies using maternal deprivation for 24 h Sutanto et al. [98] reported in adult male rats down-regulation of hippocampal GR, which was further enhanced if the adrenals were at the time of maternal deprivation stimulated with ACTH. Down-regulation of MR was also observed in the deprived males. In contrast, in females deprived as pups GR was increased at adulthood and this increase was further enhanced upon neonatal ACTH injection. MR was not affected in females by any of the neonatal manipulations. It seems logical that if increased maternal care is beneficial for the development of the infants, then a prolonged maternal absence or neglect is unfavourable or even harmful. Most of the procedures aiming for long term effects on HPA function and behaviour have in common that it is attempted to induce a HPA axis activation during the SHRP. As previously mentioned this can be achieved by disruption of maternal care, but also by other means, e.g. exposure to cold or ether fumes [51]. Shanks and colleagues treated 3-day old rat pups with lipopolysaccaride (LPS), mimicking a mild bacterial infection of the infants. Similar to maternal separation this treatment induced a temporarily enhanced ACTH and corticosterone secretion in the neonates, an effect mediated by hypothalamic CRH [99]. As adults, these animals exhibited a greater ACTH and corticosterone response to stress, an effect possibly related to a decreased glucocorticoid feedback ability of these animals [100]. In subsequent studies it was shown that the elevated circulating levels of corticosterone were reflected in an increased amplitude and frequency of the pulsatile corticosterone secretory bursts [100].

It has to be pointed out that the timing of these treatments is crucial for the long term effects. Some of the applied paradigms aiming for a disruption of HPA function circumvent this problem by simply extending the treatment throughout postnatal development [92,95]. This strategy is based on the assumption, that the induced effects are cumulative and unidirectional. However, this may not be the case at all. In other words, maternal separation at the beginning of the SHRP may have very different long-term consequences as the same treatment towards the end of the SHRP. Studies with a single 24 h separation period indicated, that especially at the beginning of the SHRP the stress system of the pups is vulnerable to external disturbances [101,102]. Thus, the stage of development is crucial for the effect of maternal neglect.

One question that has not yet been addressed directly, but is nevertheless of great importance, is the role of the genetic background on the possible long-term consequences of a disrupted stress system development. While virtually all mice (and rats) subjected to maternal deprivation of more than 4 h will react with an activation of the HPA axis, the long term consequences of maternal deprivation are much more subtle. They depend besides the duration of the separation, also on the time point of the separation during the SHRP, the gender as well as the genetic background. Oitzl and co-workers [103] could demonstrate that some Brown Norway rats subjected during their development to maternal deprivation aged very successfully in regard to their learning ability, while others did not. Controls progressively aged to a large group (45% of the animals) of partially impaired animals, but the maternally deprived animals were mostly either good or bad learners, with only a few (12% of the animals) partially impaired. This implied that after deprivation the number of good performers increased at senescence twofold and bad performers with a factor 1.5. This dissociation in good and bad performers correlated with the expression of Brain Derived Neurotrophic Factor (BDNF) in the hippocampus [104]. The better the animals learned, the higher was the expression of BDNF.

What is the role of the stress system and of genetic background in this dichotomization of cognitive performance at senescence as a result of maternal deprivation? In the same study parameters of HPA activity were measured. If exposed to novelty the response of corticosterone slowly attenuated during the aging process and was lowest at senescence. In deprived rats peak levels of stress-induced corticosterone levels were at midlife far higher than in the controls, but much lower at young age. At senescence particularly after exposure to more severe stressors the corticosterone response was attenuated [105]. It would be of interest, therefore, to examine whether the extent of 'mid-life stress' is a determinant in selecting a trajectory towards either successful aging or senility, and if so which gene patterns are being activated under such conditions.

5. Perspectives

This contribution was focused on the phenomenon that events early in life, even the seemingly minor ones, can program the brain for a pattern of neuroendocrine and behavioural responses in later life. These events may occur as early as during the conditions of ovarian hyper-stimulation used for facilitation of embryo implantation in the uterus. The exposure of the pregnant dam to stress and drugs like dexamethasone has received a lot of attention and that will be the topic of other contributions. However, one intriguing paradox is that the poor nutritional state during pre-natal life may present a risk factor for metabolic and cardiovascular disease. This is a paradox because a caloric restriction regime during adulthood has on the contrary a life extending potential. Furthermore, in postnatal life the permanent effects of variations in mother–infant interaction are well documented. The outcome in the adult rodent deprived as infant varies depending on the time and the duration of the separation as well as the gender and genetic background of the animals.

In the mechanism underlying these programming effects of early life events we have taken the position that the stress hormones are key mediators. It is now known that glucocorticoid feedback in the anterior pituitary is a prominent mechanism in maintenance of the SHRP with adrenal hypo-responsiveness to ACTH as one of its predictable consequences. During the SHRP the brain's stress system is, however, fully capable to respond. In the rodent variations in mother–infant interaction only disrupt the quiescent HPA axis if the infant is deprived once for several hours from maternal care. It is reasonable to assume that the activated CRH and corticosteroid systems then are the primary signals engaged in programming the brain. This would then be reminiscent to the sex steroids which act during postnatal days 2–4 on the brain to program sexual behaviour at adulthood. However, solid evidence confirming a similar 'conductor's' role for the stress hormones, as observed for sex hormones, is still lacking.

In a very recent study Meaney's group demonstrated that the level of maternal licking and grooming correlated with DNA methylation, histone acetylation and binding of transcription factor NGF1A binding to the GR and that the processes were reversed with cross-fostering. Along with these chromatin changes hippocampal GR expression was found increased and the stress-induced HPA activation was suppressed. The GR related changes induced by maternal care were all abolished upon administration to the pups of a histone deacetylase inhibitor [106]. This evidence supports the reasoning by Meaney and colleagues that the programming effects of maternal behaviour may proceed in part through one single gene, e.g. GR, through epigenetic processes. It is reasonable to assume that GR mediated actions affect the wiring and synaptic organization of the brain's stress circuitry [48]. Understanding this circuitry requires the identification of neuronal pathways using tracing techniques and knowledge of 'patterning' genes under peri-natal conditions, for instance by analysing large scale genomic responses [107].

The importance seems to be that early experiences are capable to enhance or to suppress the expression of certain genetic traits and by doing so may change the outcome for behavioural performance in later life. An intriguing question is whether all individuals suffer uniformly from such early life experiences. The outcome of the 'early handling' studies seem to support the thesis that all individuals are affected in the same mode and direction [76,91,108]. An alternative view on the emergence of individual differences is that depending on genetic background some individuals after early trauma actually 'gain' while others 'loose' the ability to cope with challenging conditions. At least, our studies clearly demonstrate that the maternal deprivation paradigm amplifies genetically determined individual differences, particularly during the aging process rather that all individuals are affected in the same way as the éarly handling studies suggest [7]. In favour also of a role for 'gene X environment' interaction for individual variation in coping with stress is the recent report demonstrating that individuals carrying the long allele of the 5HT transporter are resistant to depression [109].

In conclusion, current evidence demonstrates that in particular stress-related events that occur between conception and the postnatal period can impact on the offspring and permanently change brain and behaviour. How the stress factors exert these long-lasting changes will be an important avenue of future research.

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