Early life experience : neuroendocrine adaptations to maternal absence

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Chapter 1

Introduction

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1.1 Stress

The term stress, as used in this thesis, was originally introduced by Hans Selye as "the biological phenomenon of a disrupted homeostasis" [174]. Since the 1950s this field of research has evolved substantially and McEwen recently presented this definition of stress as follows [125]: "Stress may be defined as a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and/or behavioural responses. In biomedical terms, stress often refers to situations in which adrenal glucocorticoids and catecholamines are elevated because of an experience".

In this chapter I will describe the role of the hypothalamic-pituitary-adrenal axis (HPA axis) in response to stress and focus on factors controlling this axis' responsivity in early life. In humans, adverse events occurring early in life can permanently alter the set point of HPA axis activity and affect the onset and termination of the HPA axis response to stress with consequences for resilience and coping. To draw a parallel to the human situation animal models have been developed to study this so-called programming of the HPA axis. I will first describe the normal HPA axis response to stress (***section 1.1***) and will then focus on the effects of adverse early life experiences in humans (***section 1.2***). Finally, this chapter will give an overview of normal rodent HPA axis development (***section 1.3***), the consequences of disrupting the axis in rodents (***section 1.4.***) and present a scope and outline of this thesis (***section 1.5***).

1.1.1 The HPA axis response to stress

When an organism experiences a challenging situation threatening to disrupt homeostasis, which is stress, the HPA axis is activated (see ***Figure 1.1***). The central response to stress is a highly integrated process in which diverse neuronal systems are involved [38, 77]. Both physical and psychological stressors can activate the central component of the HPA axis, although through different neuronal pathways. Physical stressors, such as for example infections, temperature changes and dehydration, mainly activate catecholaminergic systems located in the brainstem area, like the locus coeruleus and the nucleus tractus solitarii. Psychological stressors, on the other hand, mainly depend on activation of limbic structures in the brain, such as the prefrontal cortex, amygdala and hippocampus. These limbic circuits harbour structures involved in emotion (amygdala) and planning (prefrontal cortex). In the hippocampus these emotional and cognitive events are placed in a context of place and time. Behavioural processes involve attention and appraisal, while also autonomic and neuroendocrine processes are orchestrated through the paraventricular nucleus of the hypothalamus (PVN) [42, 78]. The PVN is the central assembly point of the neural afferents mediating the stress response. Here all the "threatening" information converging from different brain areas, either directly or indirectly, ultimately activates the HPA axis [77, 95, 123].

The PVN consists of a magnocellular and parvocellular part. Magnocellular neurons produce vasopressin (AVP), which is released at the posterior pituitary into the blood circulation [141, 143]

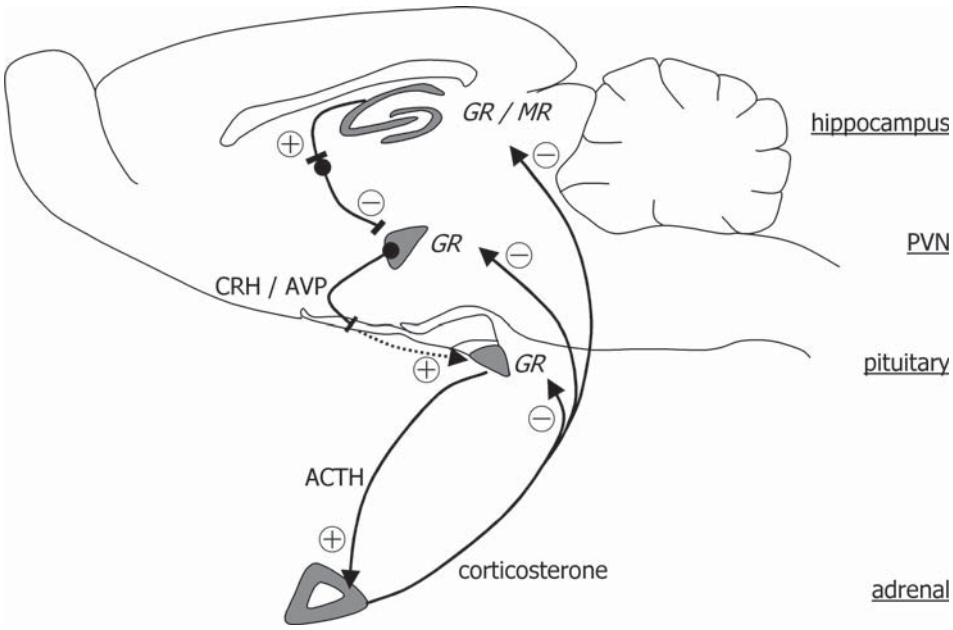

Figure 1.1
Schematic overview of the involvement of the hippocampus and hypothalamic-pituitary-adrenal axis (HPA axis) in the neuroendocrine stress response in rodents. Activation of the paraventriculuar nucleus (PVN) of the hypothalamus from brain stam and higher brain areas initiates the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the median eminence. At the pituitary CRH and AVP act in synergy to release adrenocorticotropic hormone (ACTH) into the blood stream. ACHT stimulates the adrenal cortex to secret corticosterone, which, in turn binds to the brain glucocorticoid (GR) and mineralocorticoid (MR) receptors to control the activity of the HPA axis via negative feedback mechanisms and thereby restores homeostasis. For a more detailed description of the cascade of events see ***Sections 1.1*** and ***1.2***.

to regulate water reabsorption by concentrating urine in the kidneys [18, 142, 201]. Parvocellular neurons produce besides AVP also corticotropin-releasing hormone (CRH). Both hormones are, upon stimulation, released from vesicles into the portal blood system at the median eminence. The portal blood system forms a direct link between the median eminence and the anterior pituitary. Upon reaching the pituitary CRH and AVP bind to their respective receptors, stimulating the production and release of adrenocorticotropic hormone (ACTH) into the blood stream. Although CRH is considered to be the main activator of ACTH, its effects are amplified by AVP [140, 153]. ACTH then travels via the blood stream to the adrenals, where it binds to its melanocortin (MC2) receptor located in the adrenal cortex [22]. Here, ACTH stimulates the synthesis and secretion of glucocorticoids in the circulation, *i.e.* corticosterone in rodents and cortisol as main product in man. The main effects of glucocorticoids on the brain are modulation of their own secretion (negative feedback) [36, 95], modification of neuronal integrity and function [62, 86, 122] and modulation of memory and learning processes [40, 42, 119]. All these processes ultimately serve to restore homeostasis at the level of behaviour and physiology. A more detailed description on glucocorticoid functions and effects is presented in the next section (***section 1.1.2***).

1.1.2 Glucocorticoid functions and effects

This section describes glucocorticoid functions and effects as observed in adult rodents. During early development not every component is fully developed yet, which can influence these

glucocorticoid-mediated effects. This aspect of glucocorticoid function will be discussed in more detail in ***section 1.3***.

Since almost every cell type in an organism is sensitive to glucocorticoids, these molecules have a wide range of actions. When plasma levels of glucocorticoids rise in response to stress they stimulate catabolism, mobilise lipid and glucose reserves, suppress immune responses and increase cardiovascular tone [132, 133]. Glucocorticoids also affect emotional responses and cognitive processes. Hence, they are necessary for behavioural adaptation to any stressful situation. For example, increased corticosterone directly following a learning task promotes consolidation of memory in rats and mice. These animals then "remember" the task and are better able to cope with the same situation a second time they encouter it. On the other hand, when this rise in corticosterone takes place "out-of-context", indicating that no direct relation to the task can be made, memory storage and retrieval of the task might even be impaired [40, 87, 137].

In rodents, the active glucocorticoid is corticosterone. In humans two glucocorticoids are secreted, cortisol and corticosterone. Cortisol is believed to be the main glucocorticoid based on its 10 to 20 times higher concentrations present in blood [89, 186]. However, whether the central effects of glucocorticoids in humans are also dominated by cortisol remains to be investigated. Karssen *et al.* [92] showed that cortisol is selectively hampered from passing the blood-brain barrier by a multi-drug resistance protein called P-glycoprotein, which is likely to result in (only) a 6-fold higher concentration of cortisol compared to corticosterone in the brain. The lack of P-glycoprotein-mediated transport of corticosterone in contrast to cortisol suggests a more important role for corticosterone in modulating human brain function than previously recognised [92] and increases the validity of investigating corticosterone effects in regulation of the stress response in rodent models.

Although increased glucocorticoid levels in response to stress are beneficial to an organism, exposure to high levels for a longer time can also have deleterious effects and lead, for example, to atrophy of apical dendrites in the hippocampus [56, 62, 122]. To prevent the production of excessive amounts of corticosterone by the HPA axis, glucocorticoids produce a negative feedback control on various levels in the cascade of a stress response, thereby reducing their own secretion.

In order for glucocorticoids to exert their effect they bind to two receptors: the mineralocorticoid and glucocorticoid receptor (MR and GR, respectively) [38, 126]. Both MR and GR are cytosolic receptors, which are transported to the nucleus upon binding to their ligand [42]. In the nucleus they act as transcription factors and modify gene expression in various ways. In the form of homodimers MR and GR can bind to glucocorticoid responsive elements located in the genome and hence cause transactivation or repression of genes [11, 46, 185]. MR and GR can also interact, as monomers, with other transcription factors in a complex of protein-protein interactions and thus affect gene expression in a process that is called transrepression [60].

Both MR and GR are expressed throughout the body and the brain, but differ in their

distribution and affinities for corticosterone [38, 151]. Though MR is expressed throughout the body [100], its expression in the brain is predominantly located in limbic regions, like the hippocampus [177, 189]. In epithelial cells, such as in the kidney, the ligand for MR is aldosterone, which becomes corticosterone if inactivated by 11ß-hydroxysteroid dehydrogenase (11ß-HSD). Non-epithelial cells in the heart and brain contain a non-selective MR predominantly occupied by circulating corticosterone. The GR, on the other hand, is bound by glucocorticoids both in the body and in the brain [42]. Central GR has a more widespread expression pattern in the brain than MR. Particularly high expression is found in the hippocampus, PVN, hypothalamic nuclei, the cortex, amygdala and brainstem, all regions involved in regulation of the behavioural and endocrine stress response [177, 189].

Even though both central MR and GR can bind glucocorticoids [38, 126, 197], their pharmacological properties are quite different. MR has a 10-fold higher affinity for corticosterone than GR [151]. As a consequence, the MR is already occupied at basal circulating corticosterone levels regulating the tonic activity of the HPA axis (proactive mode). Additional GR occupation at the circadian peak or after a stress response is essential for the HPA axis to return to homeostasis (reactive mode) [41, 42]. For an appropriate function of MR and GR in, for example, the hippocampus the balance in actions mediated by these two receptors is critical and important for neuronal excitability, stress responsiveness and behavioural adaptation. A deregulation of this balance thus increases vulnerability for psychiatric disorders [42].

1.2 Adverse early life events in humans

1.2.1 Association between glucocorticoids and depression

Many studies nowadays point to the involvement of the stress system in the aetiology of affective psychopathology [12, 13, 27, 58, 69, 71, 72, 82, 152]. For example, an estimated 50% of patients suffering from major depressive disorder displays a dysfunctional HPA axis [152]. The main characteristics are increased cortisol levels (blood plasma, urine and cerebrospinal fluid), an increased cortisol response to ACTH, blunted circadian rhythm and impairment of cortisol suppression after dexamethasone treatment [82]. One of the most compelling pieces of evidence describing a relation between depressive symptoms and stress is the observation that deregulation of the HPA axis even precedes the onset of depression. Furthermore, the relieve of these depressive symptoms are preceded by a normalisation of the HPA axis [82].

Belanoff and colleagues, who successfully treated patients suffering from psychotic depressive disorder with GR antagonists, have recently provided further proof of the close relation between depressive mood disorders and the HPA axis. They showed that short-term use of a high dose of GR antagonist reduced psychotic and depressive symptoms by 50% [13].

One of the clearest functional relations between psychotic depression and HPA axis functioning is revealed by research on Cushing's syndrome. Cushing's syndrome is caused by an adrenal or pituitary adenoma and is besides high blood pressure, hyperglycaemia, hypokalaemia

and obesity, in a significant number of patients also characterised by an impaired memory function, insomnia, anxiety and psychotic depression. Since a pituitary or adrenal adenoma causes excessive levels of cortisol in the body (hypercortisolemia), GR antagonists were used to normalise the physical parameters mentioned above, influenced by these high cortisol levels. Strikingly, this treatment also revealed that the observed neuropsychological symptoms were directly correlated with circulating cortisol levels that were resolved quickly when the hypercortisolemia was treated [152, 166].

1.2.2 Epidemiology of depression

Depression is a widespread mental disorder occurring regardless of gender, age or ethnical background. It is characterised by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep and/or appetite, low energy and a poor concentration [1] and leads to substantial impairments in an individual's ability to take care of his or her everyday responsibilities. Major depressive disorder is characterised by a severely depressed mood that persists for at least two weeks and may be specified as either "a single episode" or "recurrent". At its worst depression can lead to suicide, associated with the loss of about 850 000 lives every year worldwide [205] and affects about 16% of the population on at least one occasion in their lives [15]. Almost without exception, epidemiological studies have documented higher rates of depression in women than in men (mean prevalence of 7.3% versus 4.0%) [15].

The World Health Organisation ranked depression in 2000 as the leading cause of disability in the US as well as in other countries and as the fourth leading contributor to the global burden of disease, which is classified as "Disability Adjusted Life Years" (DALYs; the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability). Nowadays, depression is already the second cause of DALYs in the age category 15 to 44 years and by the year 2020 depression is expected to reach the second place after heart disease of the ranking of DALYs calculated for all ages and both sexes [134].

1.2.3 Genotype - environment interactions in the onset of depression

The onset and persistence of depressive episodes can be influenced by genetic, biological and psychosocial factors (see ***Figure 1.2***). To examplify the importance of psychosocial factors, such as adverse living conditions, 18% of preadolescents and 40% of adolescents with a history of child maltreatment meets the current diagnostic criteria for major depressive disorder [51, 127]. The consistent finding that many individuals with a depressive disorder exhibit an abnormal functioning of the stress system has led to the development of animal models that emphasise the role of development, environment and genotype in shaping pathways susceptible to affective illness [28]. In particular exposure to prenatal or early postnatal stress, like under-nutrition during pregnancy, postnatal separation of the infant from the parents and sexual or physical abuse during childhood may interact with an individual's genetic predisposition and thus increase the risk of developing depression or any other mood or anxiety disorder [48, 69, 73, 131].

A relatively common observation in psychiatry is the association of major depressive disorder with elevated levels of plasma cortisol in these patients [59, 67]. Since activity of the HPA axis can be affected by exposure to repeated and/or chronic stressors in ways hypothesised to contribute to mood disorders [157], early adverse experiences are believed to modify programming of the brain [74]. Early life stress has been associated with (juvenile) onset of major depressive disorder [85, 96] with a linear dose response relation between the severity of abuse and risk of depressive episodes [204]. Early life stress is furthermore associated with persistent hyperactivity of the HPA axis and the autonomous nervous system as well as with increased sensitivity of these systems in adult patients [27, 71, 72]. The observation that neglect in childhood can suppress growth of the long bones is another example of how an early experience can have a tremendous impact on development [4, 57, 175]. This may in part reflect the effect of chronic stress operating via the HPA axis, as CRH and glucocorticoids are known to influence the growth hormone system and

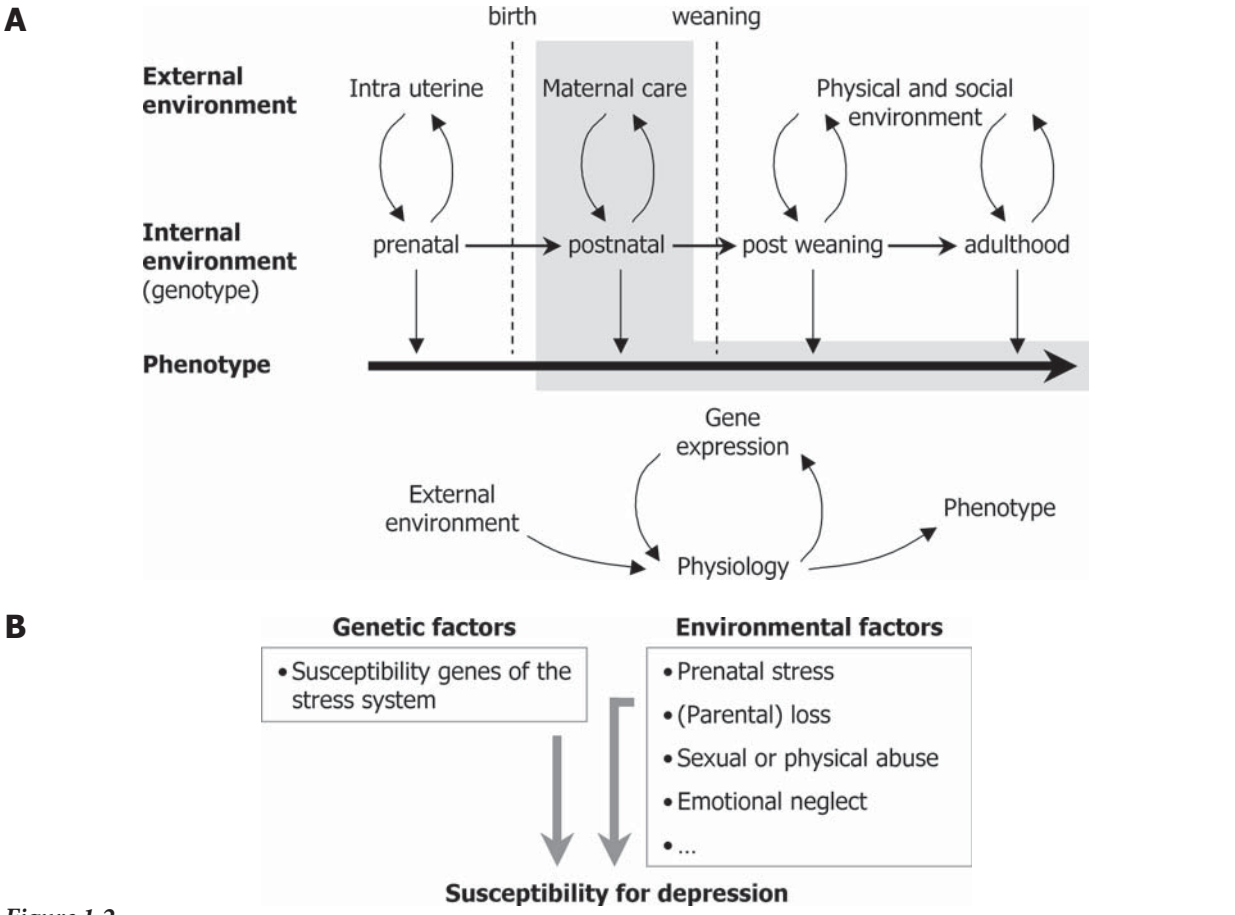

Figure 1.2
(***A***) A schematic overview of the interactions between the genome and environment shaping the phenotype of an organism throughout its life. The focus of this thesis is on early postnatal period between birth and weaning in mice (gray area). Variations in maternal care or physiological changes in the pup, whether experimentally induced or naturally occurring, influence long-term development of structure and function of both physiological and behavioural characteristics. Disruption of infant's homeostasis by atypical maternal care represents a critical period of sensitivity of these interactions and is used in this thesis as an animal model to study the consequences of traumatic early life events. The design of the figure is based on a scheme kindly provided by Christopher R. Price, with minor modifications [150].
(***B***) Some examples of environmental factors that increase the susceptibility for developing depressive disorders as acquired from human epidemiological studies.

the production of growth factors in ways that suppress growth [67, 88].

Results from animal studies also indicated that stress early in life can promote long-term changes in multiple neurotransmitter systems and brain structures involved in the aetiology of major depressive disorders in adults [8, 68, 74, 94]. It is therefore hypothesised that neurobiological changes associated with adverse early experiences can confer vulnerability for the development of depression [93].

1.3 Characteristics of normal HPA axis development

In psychobiology it has long been recognised that postnatal parental care is an important environmental regulatory factor for an individual's development [80]. Human epidemiological and animal experimental studies show that early social experiences influence the functioning of physiological processes even into adulthood [70, 80, 145]. As noted in ***section 1.2.3*** and illustrated in ***Figure 1.2.B***, early exposure to adverse experiences increases the risk for development of, among others, posttraumatic stress, depression and anxiety disorders [70]. However, to understand the molecular mechanisms underlying the effects of adverse early life events and the effects on HPA axis functioning it is eminent to have knowledge on the normal development of the stress system and factors that regulate this development. In the following sections the main issues related to development of the stress system in rodents, important for the understanding of the research described in this thesis, will be discussed.

1.3.1 Stress hypo-responsive period

The stress hypo-responsive period (SHRP) lasts from postnatal day (pnd) 4 to 14 in rats [112, 113, 167, 200, 207] and from pnd 1 to 12 in mice [169]. Very low circulating basal levels of ACTH and corticosterone characterise this period. In response to most mild stimuli, like isolation, novelty or saline injection, pups do not show an activation of the pituitary-adrenal axis like their older conspecifics and are therefore called hypo-responsive. Additionally, these pups have no circadian rhythmicity in ACTH and corticosterone secretion. For rat pups it was shown that they do not display the characteristic early evening peak, corresponding to the start of the active period and daytime trough of these hormones until they are 21 to 25 days old [2].

In a recent study it was furthermore demonstrated that in early postnatal development of the HPA axis mice show a high expression of CRH mRNA in the PVN [169] (see ***Figure 1.3***). This corresponds to earlier data published for both mice and rats [44, 45, 172]. Expression levels of GR mRNA in the hippocampus were only measurable for the CA1 area, since other areas remained below the detection limit. Expression in this area was low at birth, but increased significantly during the SHRP reaching highest expression at pnd 12. Earlier reports on the ontogeny of GR mRNA expression in the rat also showed a gradual increase with age [17, 130, 161, 165], though most of these studies examined the regional ontogeny in detail. Interestingly, one study reported that GR immuno-reactivity in the CA3 region of the hippocampus was only clearly present

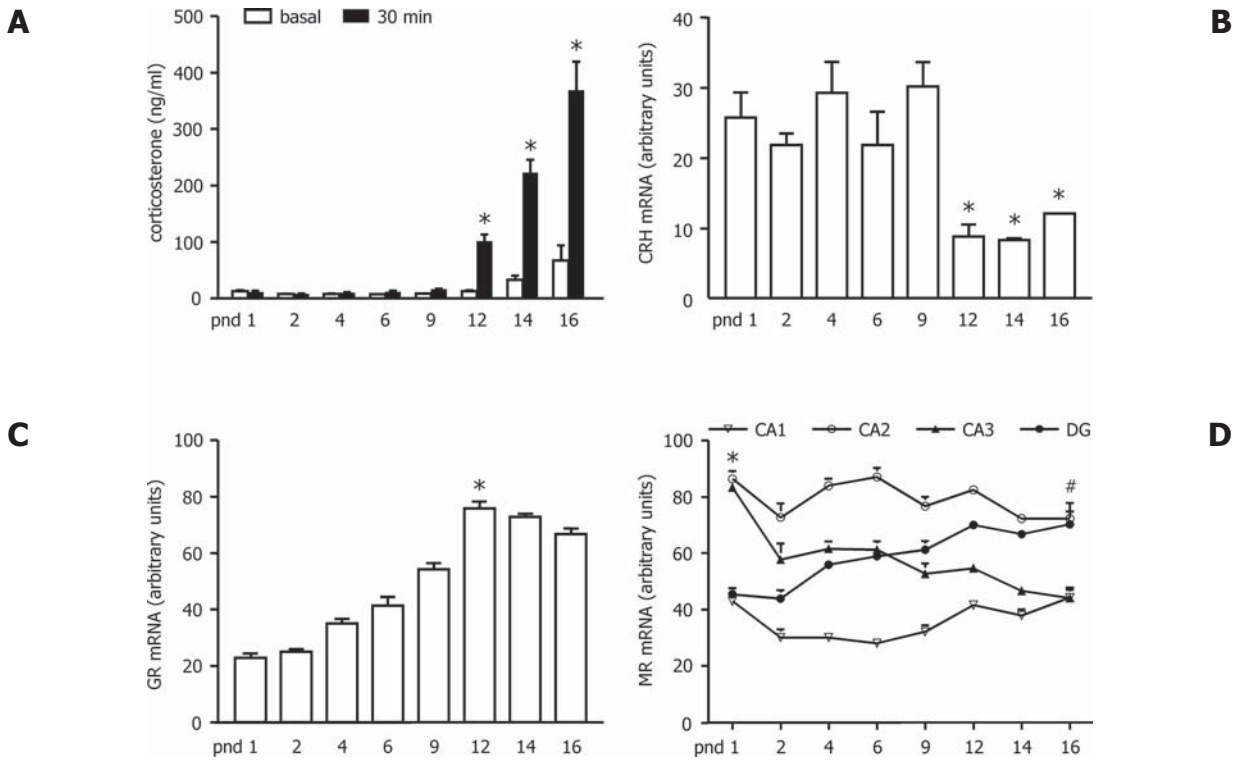

Figure 1.3
Four sample figures illustrating the dynamic developmental changes occurring in various HPA axis markers in CD1 mouse pups at different postnatal ages. (***A***) Basal plasma corticosterone concentrations are low from pnd 1 to 12 and increase from pnd 12 onwards. Between pnd 1 and 9 there is no increase in corticosterone secretion following novelty stress. In contrast, pups at pnds 12, 14 and 16 do respond to 30 minutes of novelty with increasing elevations of corticosterone. * $P<0.05$ versus basal. (***B***) Basal expression of CRH mRNA in the PVN is high at birth (pnd 1) and shows a sudden drop in expression at pnd 12. * $P<0.05$ versus pnd 1-9. (***C***) Basal expression of GR mRNA in the CA1 area of the hippocampus is low at birth (pnd 1), starts to increase from pnd 4 onwards and reaches highest expression at pnd 12. * $P<0.05$ versus pnd 1-9. (***D***) Basal expression of MR mRNA in the CA1, CA2, CA3 and dentate gyrus (DG) area of the hippocampus. MR mRNA expression is higher in the CA1 and CA3 area compared to CA2 and DG at birth. During further development expression remains constant for CA1 and CA2, while expression levels for CA3 and DG reverse. * $P<0.05$ versus CA1 or DG at pnd 1, $^{\#}P<0.05$ versus CA1 or CA3 at pnd 16. Adapted from Schmidt *et al.* [159].

during the first week of life in rats and then disappeared [161]. When mice had developed past the SHRP they exhibited enhanced corticosterone basal levels and a response of both ACTH and corticosterone to mild novelty stress. CRH mRNA expression decreased significantly, while expression of GR in the CA1 area of the hippocampus remained high with a small decrease at pnd 16. In mice the expression of MR in the hippocampus was as high as in adults throughout the postnatal development of the HPA axis and changed very dynamically in a time- and subregion-specific manner [169].

1.3.2 Maintaining HPA axis hypo-responsiveness in early development

During the SHRP the HPA axis differs in a number of functions relative to the adult rodent, resulting in an inhibition of peripheral stress responses. Several intrinsic factors have been identified that are (partly) involved in maintaining the pituitary-adrenal hypo-responsivity. The adrenal cortex is relatively insensitive to ACTH stimulation, releasing only a minimal amount of corticosterone in response to exogenous ACTH stimulation [159, 178]. Furthermore, while a

mild stressor is able to rapidly activate CRH mRNA expression during the SHRP [45], this does not result in increased circulating ACTH. This indicates an inefficient CRH release or signal transduction to the pituitary. Possibly corticosterone itself plays a role in the maintenance of the SHRP as well. During the SHRP the concentration of corticosteroid-binding protein, which binds approximately 75% of the glucocorticoids in the circulation of adult rats, is very low and results in relatively high levels of biologically active corticosterone in the blood [75]. Though the total corticosterone concentration is low, the levels of free corticosterone could be sufficient to exert enhanced feedback at several levels of the HPA axis during the SHRP (see also ***Figure 1.4***). The pituitary has been identified as the main site of glucocorticoid feedback action in rat pups, partly due to its adult-like levels of GR mRNA expression [163]. The PVN as well as an altered balance between MR and GR in the hippocampus which projects to the PVN, might also contribute to the suppression of the HPA axis during this hypo-responsive period [169, 209].

Maintaining minimal corticosterone in plasma is hypothesised to prevent harmful effects of either too high or too low hormone concentrations on the developing nervous system. High levels of glucocorticoids are known to cause structural changes in pyramidal neurons of the hippocampus, *i.e.* dendritic atrophy and an impairment of neurogenesis in dentate gyrus neurons [55, 56, 61]. These effects, though, were shown to be reversible when normal glucocorticoid levels were reinstated. A total absence of corticosteroids, on the other hand, leads to apoptosis [42]. Neonatal treatment of rat pups with glucocorticoids affected the pattern and reduced the rate of postnatal granule cell genesis in the hippocampus [16]. Furthermore, neonatal exposure to hydrocortisone decreased HPA axis responsivity to stress at weaning (approximately pnd 21) and an impairment of the adrenocortical response to stress at 45 to 48 days of age [49]. Furthermore, after a single 24 hours maternal deprivation increased cell death of neurons and glia in several

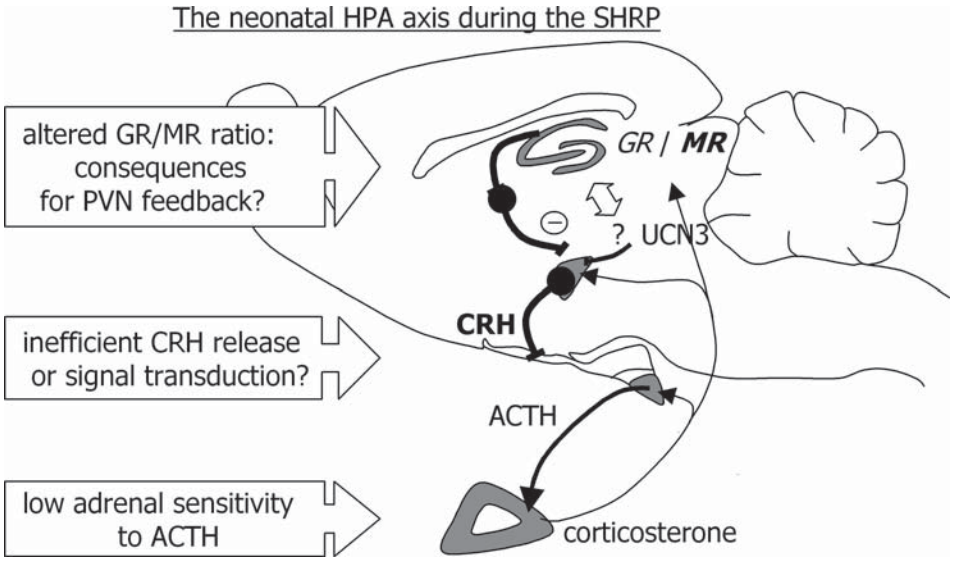

Figure 1.4
Schematic illustration of the stress system during the SHRP indicating the significantly altered state and function of the HPA axis at different organisational levels as compared to the adult. See ***Figure 1.1*** for abbreviations and an explanation of the adult stress response. Adapted from Schmidt *et al.* [159].

cortex regions was observed when the deprivation was performed within the SHRP, but not when performed outside the SHRP [210]. Consequently, disturbances of normal HPA axis development during the SHRP are expected to result in long-lasting alterations in neuroendocrine functioning and behaviour.

1.3.3 Maternal influence on rodent development

Apart from intrinsic factors (see ***section 1.3.2***) maternal factors also contribute to the pituitary-adrenal hypo-responsivity during early development [39, 113]. The mother is the only providing source for many features essential for pup survival. She provides food (milk), warmth (temperature regulation) and active interaction (licking and grooming) [80, 103, 158] and rodent mothers rarely leave their offspring for more than 15 to 20 minutes [3]. Experimental manipulation of the mother-pup interaction (*i.e.* maternal separation) provides clues on the role of maternal influences on pup development. Already after several hours of maternal absence, basal and stress-induced ACTH release are enhanced, followed by an increased corticosterone secretion from sensitised adrenals [102, 160, 170, 178]. Furthermore, CRH and GR mRNA expression in the PVN and MR and GR mRNA expression in the hippocampus decreased [170, 172]. The expression of *c-fos*, an immediate early gene used as marker for neuronal activation, showed a 100-fold increase in the PVN [176]. Interestingly, when mimicking part of maternal behaviour by stroking the anogenital region of rat pups for 30 to 45 seconds every 8 hours, the rise in ACTH and decrease in CRH and MR mRNA expression could be prevented [180]. Strikingly, even the *c-fos* activation was then abolished [192, 194]. Feeding and stroking restored corticosterone and GR mRNA levels as well, indicating that starvation increases the adrenal sensitivity for ACTH [192, 194].

Recently, Schmidt *et al.* [171] provided further evidence that the activation of the HPA axis due to prolonged maternal absence during the SHRP may be closely related to metabolic responses. As in adults, food deprivation in pups leads to a decrease in blood plasma glucose and leptin and an increase in plasma ghrelin levels [19, 25, 90, 91, 97, 99, 208]. High levels of glucose suppress ghrelin release from the stomach [91, 99] and therefore increasing ghrelin levels are a direct reflection of decreasing glucose levels. These responses are further substantiated by the observed ACTH response to 24 hours of maternal absence [170], which is also observed for food deprivation of adult rats [35]. By blocking the metabolic signals of glucose (by applying a high dose of glucose) and ghrelin (by applying a ghrelin antagonist) during 8 hours of maternal separation, Schmidt *et al.* were able to (partially) prevent the pituitary-adrenal activation normally associated with prolonged maternal separation [171]. Concomitantly, this treatment prevented changes in expression of neuropeptide Y (NPY) mRNA in the arcuate nucleus and CRH mRNA in the PVN indicating the importance of these signals in the activation of the HPA axis during the SHRP. Leptin treatment, however, did not affect separation induced effects on the HPA axis [171] (see also ***Figure 1.5***).

Further evidence for maternal influence on rodent development comes from studies using inter-individual differences in licking and grooming (LG) and arched back nursing (ABN)

displayed by rat mothers of the same strain during the first two weeks after delivery. Mothers exhibiting high levels of LG-ABN have offspring that is less fearful and shows a modest HPA axis response to stress compared to offspring from low LG-ABN mothers [52, 118, 203]. When pups from low LG-ABN mothers are then cross-fostered to high LG-ABN mothers, the adult offspring will display the phenotype of the high LG-ABN mothers and *vice versa*, when pups from high LG-ABN mothers are cross-fostered to low LG-ABN mothers, the adult rats will display the phenotype of the low LG-ABN mothers, both on molecular characteristics as in behaviour [52, 203]. These studies clearly show that maternal behaviour plays a very important role in HPA axis development in rodents and that this maternal behaviour (within a strain) is learned.

Apart from inter-individual variation of maternal behaviour within strains, effects as a consequence of the genetic background are also known. BALB/c mice, which are characterised to be very fearful, as illustrated by their elevated endocrine responses to stress, become less fearful when raised by C57BL/6J mothers [7]. The higher licking and grooming frequency of

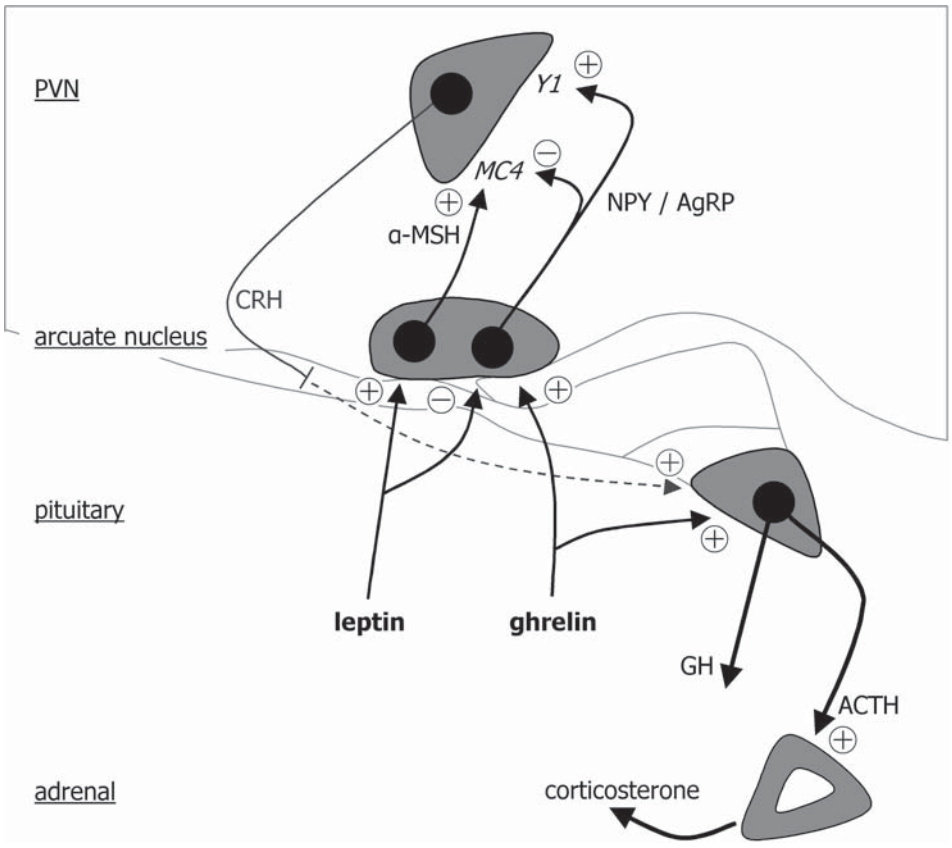

Figure 1.5
The dynamics in the influence of metabolic signals leptin and ghrelin on HPA axis functioning. Ghrelin is synthesised in the stomach and reaches the arcuate nucleus of the hypothalamus via the blood stream. Here ghrelin stimulates GABA-ergic neurons to produce and release neuropeptide Y (NPY) and agouti-related protein (AgRP). Via the NPY receptor (*Y1*) in the paraventricular nucleus (PVN) of the hypothalamus NPY and AgRP stimulate CRH release. However, NPY and AgRP have inhibitory effects via the melanocortin 4 receptor (*MC4*) receptor. In the pituitary ghrelin functions as a releasing agent for, *e.g.* growth hormone (GH) and adrenocorticotropic hormone (ACTH).
Leptin inhibits the GABA-ergic neurons of the arcuate nucleus, but stimulates melanocortin neurons. Upon stimulation, these pro-opiomelanocortin (POMC) neurons produce and release α-MSH, which in its turn via MC4 stimulates CRH release from the PVN.

C57BL/6J mother compared to BALB/c mothers explained this reduced fearfulness. On the other hand, C57BL/6J pups raised by BALB/c mothers did not "acquire" the BALB/c phenotype, but remained indistinguishable from C57BL/6J pups raised by their own mothers. To make things more complex, C57BL/6J pups that were cross-fostered as embryos to BALB/c mothers and raised by BALB/c mothers did show the BALB/c phenotype [53]. These studies indicated that for gene-environment interactions both the pre- and postnatal environment interact and determine the adult behaviour of inbred mouse strains [30].

1.3.4 Analogy between rodents and humans

The validity of animal models used to investigate the consequences of adverse early life events on HPA axis functioning clearly depends on the parallels that can be drawn between rodent and human early development of the HPA axis. Unlike rodents, humans (and also Rhesus macaques) exhibit an early morning peak and an evening trough in corticosterone within a few weeks of birth [147]. Similar to the rodent, the human HPA axis functioning is not adult-like at birth and the circadian regulation continues to mature into the third year of life in humans. However, at this age the daytime production of cortisol is still not fully consistent with the mature pattern [202].

The human HPA axis is furthermore quite responsive to stressful situations roughly up to 3 months of age, but then becomes increasingly difficult to activate over the course of the first year [63, 66]. This shows close similarity with the rat development, which displays a responsive HPA axis during the first few days of life, but becomes hypo-responsive to stress from pnd 4 onwards [112, 167, 207]. Tentatively, the period between 6 and 12 months of age marks the transition into a human functional equivalent of the rodent SHRP [64, 66]. However, what appears to be a period of hypo-responsiveness may more correctly be characterised as a period of intense psychosocial buffering of the HPA axis. This buffering is "caused" by the presence of an adult with whom the child has formed a secure attachment relationship or with an unfamiliar substitute caregiver who shows sensitive and responsive behaviour towards the child [66, 135].

During early development in rodents and primates, contact with responsive, nurturing caregivers appears to be critical for the development of the neuroendocrine system. As noted before, researchers have speculated that adverse early rearing environments in humans will enhance vulnerability to behavioural disorders in part through disturbing the development of stress-sensitive neurobiological systems, including the HPA axis [37, 65, 74]. For infants and children neglect has already been shown to produce an apparent loss of daytime rhythm in corticosterone. Similar effects were demonstrated in nursery-reared Rhesus infants [20].

1.4 Adverse early life event modelling in rodents

Investigating the fundamental biological mechanisms that underlie the enhanced vulnerability to develop behavioural disorders in humans is troublesome. Apart from an ethical perspective,

both genetic and environmental factors that may induce the disorder cannot be controlled and are difficult to quantify. Furthermore, subjects cannot be randomised for treatment and studies may take decades to complete. Since an infant has numerous, changing neuroendocrine interactions during development, it is also impossible to model this accurate in *in vitro* conditions. Much of the current knowledge on human biology originates largely from research in experimental animals. Therefore, animal studies in which it is possible to control in a laboratory, at least to a certain extent, for environmental influences are essential to study behaviour in combination with a neurochemical analysis of brain function and hormone balance [31, 199].

1.4.1 Pre- and postnatal manipulations

The objective of this thesis deals with the influence of early life experiences on the development of the stress system (***section 1.5***). For this purpose, we chose to intervene in the normal development of a mouse by applying an experimentally-induced stress factor. Experimental intervention can be performed either pre- or postnatally.

Using prenatal stress, pregnant rodents are subjected to, for instance, crowding [34], noise [54], saline injections [144] or restraint stress [120]. This prenatal restraint stress does indeed affect adult offspring's behaviour, resulting in increased anxiety [188] and enhanced age-related recognition memory impairment [187]. On the other hand, also HPA axis responsiveness is altered, showing increased responsiveness [98, 121], reduced hippocampal MR and GR density [76, 121], impaired glucocorticoid feedback [121], an abolished SHRP [76] and accelerated the age-related HPA axis dysfunction [120, 187]. Furthermore, the hyperactivity of the offspring's HPA axis induced by prenatal stress is related to high levels of maternal corticosterone secretion during restraint stress [10, 120]. Interestingly, also maternal behaviour towards the pups after birth has been shown to be affected by prenatal stress. Adoption of prenatally stressed pups reared by undisturbed dams prevented the impairments in glucocorticoid feedback [121].

Since adverse experiences early in human life are shown to affect normal development (see ***section 1.2.3***), also postnatal animal models have emerged to investigate causes and consequences using the separation of mother and pups in rodents and primates to experimentally introduce such an adverse life event. This separation is a stressful situation as maternal care plays a major role in the postnatal development. Besides food, the mother also provides thermal, somatosensory, kinaesthetic, olfactory, visual and auditory stimulation. Already after short periods of separation rat pups show a strong ultrasonic vocalisation [81]. When the separation is prolonged their heart rate declines, sleep/wake cycles get disturbed and growth is affected [79, 101]. All these observations indicate that the maternal environment is capable of influencing the postnatal development (see also ***section 1.3.3***). Indeed, these effects on offspring's behaviour and neuroendocrinology have been reported and are described in detail in ***sections 1.4.3*** to ***1.4.6***.

The most often applied postnatal manipulation models are "(early) handling", involving daily separations of mother and infants for up to 15 minutes [32, 33, 111, 115, 117, 198] and the "repeated separations" paradigm that comprises of repeated separations of the pups from the

mothers for a period of 3 to 6 hours per day [104, 105, 107, 117, 145, 146, 155, 156] (see also ***Figure 1.6***). Both paradigms are applied during the SHRP or during the period from birth until weaning. Generally, the consequences of handling are considered to be beneficial for the development of the HPA axis. In contrast, repeated separations are considered to be detrimental and result in increased pituitary-adrenal responses to stress in adult animals and in a decreased negative feedback [117, 145]. A third model to simulate an adverse early life event in rodents is "maternal deprivation", which consists of the separation of mother and pups for a single period of 24 hours at some stage during the SHRP [39, 179, 181, 184, 192, 194, 206, 207] (see also ***Figure 1.6***). In this thesis, both the "repeated separations" and the single 24 hours "maternal deprivation" model are used as an experimentally-induced adverse early life event to investigate the consequences for HPA axis development in mice.

Pre- or postnatally, any experimental paradigm applied in rodents is a model to gain knowledge about stress system development to eventually draw parallels for the understanding of human brain development. The corresponding critical time period between humans and mice, in which to (experimentally) intervene in normal development can be addressed from different view points. Judging the mouse pup by its state of brain development, *i.e.* neuronal growth and neuron connectivity, this period corresponds to the last trimester of human pregnancy [6]. However, comparing the functional state of the HPA axis, resemblance is shown to a 6-12 months old human infant [64, 66]. This clearly shows that, though there are similarities, there also remain differences between rodent and humans. For the studies described in this thesis the choice was made to use a postnatal manipulation paradigm. Not only because there is already a large amount of data on both short- and long-term effects of a traumatic early life events, but the prospect of possible pharmacological interventions when the functional state of the HPA axis is altered due to an early life experience also provides a wealth of experimental options not yet possible with prenatal manipulations.

As indicated in ***section 1.1.1***, several brain structures are involved in or affected by an HPA axis response to stress. Many systems are affected by changes in the early environment of rodent pups, but also of primate infants, which are more closely related to humans. These effects are described in reviews by, for example, Pryce [149, 150], Sanchez [164], Ladd [104], Suomi [182, 183] and Roman [154]. Since this thesis mainly focuses on the interaction of HPA axis development and the early life environment, the effects of adverse early life events on the HPA axis will be highlighted in the description of the short and long-term effects of the two applied models, *i.e.* repeated separations and maternal deprivation.

1.4.2. Methodological considerations

The choice of the control group in studies investigating the role of environmental factors on development proved to be very complex. In many studies manipulated rats are compared to rats that do not experience any direct human environmental disturbance during infancy ("non-

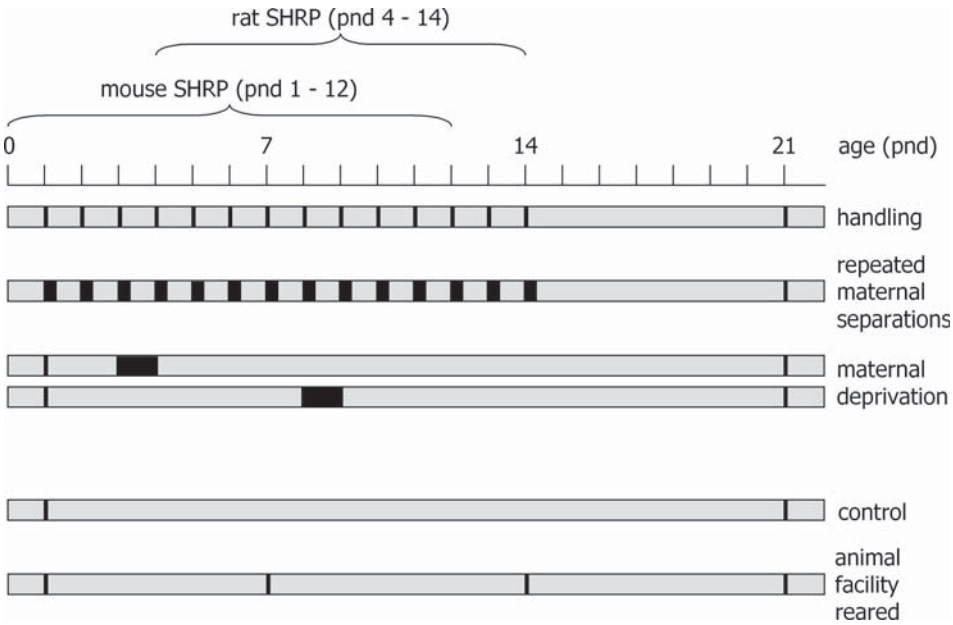

Figure 1.6
Illustration of the various types of maternal separation paradigms that are used. The top line indicates the age of the rodent in postnatal days (pnd). The day of birth is indicated as pnd 0. Above, the stress hypo-responsive period of the mouse (pnd 1 to 12) and the rat (pnd 4 to 14) is marked by large brackets. Usually, at pnd 1 the number of male and female pups is determined and reduced to a fixed litter size with an equal number of male and female pups (culling). Gray bars show different maternal separation/deprivation paradigms. The first bar represents a typical "handling" paradigm: rodent pups are separated from their mother daily for short period of time, usually 3 to 15 minutes. The second bar represents a typical "repeated maternal separations" paradigm: pups are separated from their mother daily for longer periods of time, usually 3 to 6 hours. The third and fourth bar indicate a single 24 hours "maternal deprivation", which can be performed at any stage during early development (as an example pnd 3 and pnd 8 are indicated). The developmental stage at which maternal separation is performed determines the short- and long-term consequences.
The two lowest bars present the most frequently used control groups in literature. "control": pups experience the standard procedures for culling and determining sex ratio's in the nest, but then remain undisturbed until weaning. "animal facility reared" is treated like "control", but the pups also experience the human interventions inherent to standard cage cleaning in animal facility husbandry.

handling") [108, 118, 124, 145, 148], while in other studies control litters experience the lab's routine husbandry regimen, including occasional brief handling for cage cleaning ("animal facility reared") [108, 148] (see also ***Figure 1.6***). Depending on the control group used repeated separations result in, for example, a hyper-reactive, hypo-reactive or non-affected HPA axis (for a detailed analysis see review [149]). In addition, any (experimental) manipulation will also affect maternal behaviour, so the relative effects observed in the treatment group might be diluted or exaggerated due to an altered maternal behaviour in the control group [150]. The effects of these manipulations might lead to increased maternal care following reunion, disturbance of pup homeostasis related to prolonged absence of the mother or a combination of these factors. To complicate this issue even further, the adult phenotype might also be affected by the maternal physiology during lactation (*i.e.* a stress-induced corticosterone increase in the dam, which is transmitted via the milk), resulting in lifelong changes in neuroendocrine functioning and behaviour [26, 150]. From the above it is clear that a careful selection of the control groups is therefore of eminent importance and should always be taken into consideration when comparing results from varies studies.

The best way to control for effects on neonate development induced by maternal behaviour would be the observation of maternal care for 24 hours from birth until weaning. This, however, is barely feasible. Since also nest size [158] and the ratio of male and female pups [5, 158] affects maternal behaviour, keeping these two factors constant for all the nests within and between experiments is workable. Furthermore, the pups used in each experimental treatment group should come from a number of different nests to control for inter-litter variation. Of course, also a clear description of the materials and methods is required.

1.4.3 Repeated maternal separations – 'short-term' effects

Very little data is published on acute (short-term) effects of repeated maternal separations on neuroendocrine development in rodents. The studies that do present observations on the acute results show mainly data on ACTH and corticosterone. Repeated maternal separations of rat pups for 1 hour per day on pnds 2 to 9 resulted in increased ACTH and corticosterone releases in response to the 1 hour maternal separation period at pnd 9 [124].

Also when pups were separated daily for a longer period of 3 hours, pups displayed elevated ACTH and corticosterone 5 minutes after the reunion with their mothers [83, 164]. Furthermore, when these pups were exposed to stress directly following the maternal separation period by pnd 14, like restraint or rat odour, they displayed an enhanced ACTH and corticosterone response when compared to pups that are either animal facility reared or handled [164].

The short-term changes induced by manipulation of the early environment of the pups are of major importance for the interpretation of data observed at later stages in life. It is hypothesised that these short-term changes modulate ongoing programming effects during development with long-term consequences. It is therefore crucial to take these short-term effects into account when the HPA axis development is studied in more detail.

1.4.4 Repeated maternal separations – 'long-term' effects

In contrast to 'short-term' effects, there is much more data published on the long-term effects of repeated maternal separations, although the results are rather inconsistent. The following paragraphs will describe the available data (including (some) conflicting findings) and a 'consensus' on the main findings.

Daily 3 hours maternal separation from pnd 2 to 14 is one of the best-characterised models with respect to long-term neuroendocrine changes in rats (age approximately 3 to 4 months). These separations lead to increased CRH mRNA expression in the PVN with a consequent increase in CRH peptide content in the median eminence. Surprisingly, basal and stress-induced plasma corticosterone levels are similar to non-handled rats, but are higher compared to handled rats [52, 145]. A 33% downregulation of CRH receptor binding in the anterior pituitary and concomitantly an increased noradrenergic response to stress might provide explanations for the increased CRH reactivity [52, 117]. Furthermore, these repeatedly separated animals show an upregulation of CRH gene expression in the bed nucleus of the stria terminalis (BNST) and central amygdala

(CeA), with a consequent increase in CRH peptide content in terminal fields of these neurons when compared to non-handled animals [129, 145].

The neuroendocrine responses to stress in adulthood seem to display varying results. Relative to animal facility reared controls, daily 3 hours maternally separated animals exhibit greater (2- to 3-fold) ACTH and corticosterone peak responses and more prolonged responses to air puff [104], restraint or novelty stress [128]. However, a prolonged, but not enhanced peak response of ACTH was observed by others of the same research group [104, 117]. Interestingly, rats that were repeatedly separated showed a 40 to 50% downregulation of hippocampal GR binding and mRNA levels as well as an upregulation of hippocampal MR binding and mRNA levels as adults. Also a decreased GR mRNA expression was observed in the medial prefrontal cortex [129]. Furthermore, these animals exhibited an altered GR functioning in the CA1 area of the hippocampus, discussed as a molecular basis for defective feedback regulation [14]. These data support the hypothesis of glucocorticoid feedback resistance at the hippocampus, suggesting an enhancement of HPA axis responsiveness to stress with enhanced and prolonged ACTH and corticosterone responses.

Apart from increased neuroendocrine responsiveness, repeated separations in both rats and mice also resulted in increased behavioural responses to stress [14, 52, 145]. Compared to handled controls these animals showed an increased anxiety-like behaviour in the elevated plus maze [84] and a higher emotional response in a light-dark box [14]. Interestingly, repeated maternal separations do not result in altered behavioural responses in, for example, exploration in open field, novelty-induced suppression of feeding, acoustic startle or a two-compartment exploratory task when these animals are compared to non-handled control animals [14, 24].

Despite conflicting data, which are most likely due to the various control groups used (see also ***section 1.4.2***), the overall consensus is that animals experiencing repeated maternal separations (3 to 6 hours) from pnds 2 to 14 display a fundamental reorganisation of neurocircuits involved in neuroendocrine regulation (PVN, hippocampus, frontal cortex), regulation of arousal and vigilance behaviour and autonomic nervous system tone as expressed in altered fear- or contextual-conditioning [164]. Therefore, repeated maternal separations is forwarded as a model to study the consequences of adverse early life events in humans [27].

1.4.5 Maternal deprivation – 'short-term' effects

Maternal deprivation procedures, most often for 24 hours, are used when the pups are still in the SHRP, which lasts from pnd 4 to 14 in rats [112, 113, 167, 200, 207] and from pnd 1 to 12 in mice [169] (***section 1.3.1***). Irrespective of the age within the stress hypo-responsive period, one of the most renowned effects of maternal deprivation is that it induces an increased ACTH and corticosterone secretion. Additionally, an endocrine stress response to a variety of mild stressors, like exposure to cold, novelty or saline injection, is observed when applied directly sequential to the maternal deprivation period [116]. Thus, the pituitary-adrenal axis of young animals is

activated and responsive to mild stressors, even though they are in the SHRP [178]. However, a single separation of mother and infant must last at least 8 to 24 hours to sensitise the adrenal cortex for a response to ACTH or novelty stress [116] to increase corticosterone secretion and to activate the sympatho-adrenal system [139].

Furthermore, the activity of the peripheral and central HPA axis is enhanced following maternal deprivation, as judged from the activation of *c-fos* and tyrosine hydroxylase mRNA expression in the adrenal and of *c-fos* and NGFI-B in the brain [139, 176]. These changes are accompanied by a downregulation of CRH and GR mRNA in the PVN and of GR and MR mRNA in the hippocampus [9, 170, 172, 196]. Also AVP mRNA expression in the PVN and $5HT_{1A}$ mRNA expression in the hippocampus are upregulated [21, 44, 47].

As mentioned in ***section 1.3.3***, maternal factors play an important role in HPA axis development. Mimicking properties of maternal behaviour has been shown to prevent some of the deprivation-induced effects [180, 192, 194]. There are, however, also intrinsic factors regulating the response to maternal deprivation. Recently it was shown that mice lacking the CRH receptor (CRHr1) in the pituitary did not show the characteristic increase in circulating plasma corticosterone and decrease in CRH and MR mRNA expression as is seen in response to maternal deprivation. This indicated that CRHr1 in the pituitary was essential in the initiation of maternal deprivation effects [173]. On the other hand, the absence of CRHr1 in the limbic system and forebrain resulted in a hyperactive corticosterone and ACTH response to maternal deprivation, whereas maternal deprivation was not able to downregulate MR mRNA expression in the hippocampus when CRHr1 in the limbic system was absent. This indicates a role for this receptor in mediating the suppression of the HPA axis [168].

The direct effects of maternal deprivation are relatively consistent across species and age at deprivation, provided the deprivation procedure is applied within the SHRP. When pups are deprived at a later age during the SHRP, the direction of the induced effects remains, but the amplitude increases due to maturing of the HPA axis [29, 116]. The longer-lasting, persistent effects on HPA axis activity, however, depend on the age of deprivation. Rats deprived at either pnd 3 or 11 are hyper- or hypo-responsive to stress at pnd 20 [190, 191]. Also maternal behaviour towards the pups varies with age and the absence of specific time-dependent maternal factors alters the HPA axis functioning both acutely and persistently [192]. Combined with a more mature state of the HPA axis of the pup, reactive negative feedback could restrain the HPA axis more effectively in older pups [169, 193]. All studies thus indicate that the age at deprivation might be a determining factor for the long-lasting consequences of maternal deprivation.

1.4.6 Maternal deprivation – 'long-term' effects

The long-term effects of maternal deprivation at pnd 3 resulted in an atypical pattern of corticosterone secretion during life. Where resting secretion of corticosterone in control animals hardly changed, deprived rats showed elevated levels of basal corticosterone secretion at young

age, dropped to bottom levels in the adult animal and slowly increased to reach levels comparable to control animals at senescence [206]. The responsiveness to novelty stress shows another age-dependent pattern. As adults (12 months old) maternally deprived rats were hyper-responsive compared to control rats, whereas both young (3 months old) and senescent rats (30-32 months old) were hypo-responsive [206].

When focussing on approximately pnd 50, pnd 3 deprived male rats then exhibit elevated basal ACTH and corticosterone and reduced hypothalamic and hippocampal MR and GR binding capacity, suggesting glucocorticoid feedback resistance [42, 184]. However, mRNA expression of both MR and GR were unaltered, although GR mRNA expression in the PVN and pituitary were reduced and hypothalamic CRH mRNA expression was reduced in the face of elevated ACTH [162]. Consequently, the actual pathway(s) leading to the adult phenotype thus remain poorly understood.

In a test on spatial learning and memory with these maternally deprived rats, the impairment in acquisition of information was observed at 3 and 12 months of age extending to disturbances in reversal learning compared to non-deprived litter mates. In addition, cognitive performance decreased with age. Surprisingly, at senescence there was no difference between the average performance of deprived and control animals, but the individual variation in cognitive performance increased dramatically. Most of the control animals were partially impaired, while the number of partially impaired animals of the deprived group was strongly diminished: they performed either excellent or were severely impaired. These findings suggest that maternal deprivation drives spatial learning abilities of senescent rats to the extremes at the expense of average performance [138].

The observation that not all rats behaved similarly underlines data from human studies, indicating that the consequences of adverse early life events vary per individual (see ***section 1.2.3***). Besides effects on learning abilities, this was also demonstrated for HPA axis regulation. Maternal deprivation at pnd 3 resulted in approximately 20% of adult rats in an HPA axis hyper-responsivity as compared to non-handled or animal facility reared control animals, when tested with a mild psychological stressor at postnatal day 90 [164]. Though very few studies investigated the long-term effects, the majority of the effects of this model seem to be confined to a several weeks period immediately following the date of 24 hours maternal deprivation [164].

1.4.7 Concluding remarks

On the basis of pre-clinical research with early adverse experience, maternally separated infant rats provide a model of depressive-like syndrome exhibited by *e.g.* a dysregulation of the HPA axis [24, 104, 106, 136]. This is, among others, shown by dexamethasone resistance [50, 104], enhanced anxiety-like behaviour and anhedonia [23, 104]. Data from the studies presented in ***section 1.2.3*** and ***sections 1.4.2*** to ***1.4.6*** indicate that the postnatal environment may modulate the neurobiological development of the infant, which thus has consequences for behaviour,

emotional responses and cognitive performance. The infant rodent is protected by the mother for most environmental influences, particularly if these concern relatively mild stimuli. For instance, exposure to a novel environment, which is a potent stressor in the adult animal, causes only minor activation of the HPA axis in the first two weeks of life [39, 112, 113, 167, 173, 207]. However, the infants HPA axis emerges from this hypo-responsive period when the mother is removed for a prolonged period of time. A pup deprived of maternal care for 24 hours shows enhanced basal activity and a profound HPA response when exposed to a novel environment [39, 113]. The impact of such an adverse experience in the developing infant furthermore depends on the age of the pup, duration of the separation procedure, gender and rodent strain of investigation [29, 116, 138, 190, 191]. These differential effects are related to the development of the brain, which shows profound changes during the first two weeks of life [169]. Additionally, maternal behaviour plays a very important role in the pup's development. In fact, it is the quality of maternal behaviour encountered by the pup upon reunion that plays a significant role in determining individual differences in stress-responsiveness of the progeny [43].

1.5 Scope and outline of the thesis

1.5.1 Rationale and objectives

As described in the various sections above, adverse early life events can program the susceptibility of the stress system for stressful experiences later in life. Early life adversity therefore may form a risk factor in subjects who already have a genetic predisposition to develop stress-related psychiatric disorders. The separation of infants from their mother is used as a laboratory model to study the immediate and long-term consequences of early adversity. For this purpose in rodents the most frequently used paradigms are daily handling, repeated maternal separations for 3-6 hours and a single 24 hours maternal deprivation. The outcome of each of these treatments is different depending on age, gender and strain of the pup and also on the duration and frequency of the separation.

With respect to the outcome of daily handling and repeated maternal separations most data refer to long-term effects (***section 1.4.4***) and only a limited database is available on the immediate effects of these procedures (***section 1.4.3***). In contrast, in numerous studies only the short-term effects have been examined of a single 24 hours maternal deprivation (***section 1.4.5***) and long-term effects are sparse, especially in mice (***section 1.4.6***). The studies described in this thesis were done to fill these gaps and are presented in two parts. The objective of the studies in the first part of this thesis ("Repeated maternal separations"; ***Chapters 2*** and ***3***), is to determine the short-term cumulative effects of repeated maternal separations on the HPA axis (re)activity. In the second part ("Maternal deprivation"; ***Chapters 4*** to ***6***) the aim is to determine the long-term effects of a single 24 hours maternal deprivation.

1.5.2 Hypotheses and approaches

Part I "Repeated maternal separations": The data described in ***sections 1.4.3*** and ***1.4.4*** have led to the hypothesis that repeated maternal separations produce a sustained activation of the HPA axis. Hence, excessive circulating levels of corticosterone are expected. For this purpose an 8 hours maternal separation procedure was applied that was repeated for three consecutive days. CD1 mice were used in the experiments and the separation procedures were applied between postnatal days 3-5. As read out parameters of the neonatal HPA axis activity circulating ACTH and corticosterone levels were measured. The levels of glucose and ghrelin were measured as indices of the effect of food deprivation associated with the absence of maternal care and feeding. In addition, mRNA expression was measured of markers for neuronal activation (*c-fos*), HPA axis activity (POMC, CRH, MR, GR) and metabolism (NPY). The effects exerted by maternal separation were measured under basal conditions and after exposure to novel environment. Moreover, the response to separation and novelty was also measured under conditions of selective blockade of the either one of the two corticosteroid receptor types. For this purpose MR and GR antagonists were administered either at the beginning of the separation period or at the start of the novelty procedure. The results may shed light on the role of corticosteroid feedback control in early life HPA activity under the various maternal care conditions.

Part II "Maternal deprivation": Long-term effects of a single 24 hours maternal deprivation on cognition and endocrine responsiveness have been observed in rats (see ***section 1.4.6***). In this second part the hypothesis is tested that this paradigm produces a similar outcome in mice and that it is therefore preceded by an altered development trajectory of the HPA axis. To determine these effects the consequences of a single 24 hours maternal deprivation were measured on cognitive performance and endocrine responsiveness in CD1 mice at 6 months of age. A single 24 hours maternal deprivation was also applied at different ages during the SHRP to determine the effects on HPA axis reactivity at weaning. Finally, the effect of maternal deprivation early in the SHRP was investigated on the further development of the HPA axis.

1.5.3 Outline of this thesis

In mice repeated maternal separations have fairly consistent and robust long-term effects on both the cognitive performance and the endocrine stress response, at least in a subgroup of animals (reviewed in [104, 154, 164]). Literature on short-term consequences measured in this model on HPA axis (re)activity during the SHRP is sparse. To better understand and appreciate the long-term consequences using this paradigm, the cumulative immediate effects of repeated maternal separations in CD1 mice were measured. First, the effects of repeated 8 hours maternal separations at postnatal days 3, 4 and 5 were determined on (1) the HPA axis responses to maternal separation in the absence or presence of an additional novelty stressor and (2) the mRNA expressions of selected HPA axis and stress markers in brain and pituitary (***Chapter 2***). Both groups were compared with results observed for experimentally-undisturbed controls and

with the outcome obtained by a single 24 hours maternal deprivation (as described in ***Chapter 7***). *C-Fos* and CRH mRNA expression were measured in the PVN in addition to plasma ghrelin levels and NPY mRNA expression in the arcuate nucleus of the hypothalamus as markers for the metabolic pathway known to be activated by prolonged maternal absence [171]. In the subsequent chapter (***Chapter 3***) the effects of glucocorticoid feedback were explored as a possible explanation for the immediate effects observed in ***Chapter 2***. The HPA axis feedback via the MR and GR was examined using selective antagonists administered in such a way that dissociation between their involvement in response to maternal absence and to novelty became possible.

In contrast to the well documented long-term effects of repeated maternal separations, the single 24 hours maternal deprivation model is best known for its immediate effects on the HPA axis (reviewed in [39, 114, 149, 150, 195]). In rats, but not in mice, only in a few studies long-term effects have been reported [39, 109, 110, 138]. Therefore, it was first investigated if a single 24 hours maternal deprivation in CD1 mice can affect cognitive performance in a spatial learning task and the endocrine response to a stressful event later in life (***Chapter 4***). To gain more insight in the pattern of effects observed in adult mice, experiments were designed (1) to compare the effects at weaning of a single 24 hours maternal deprivation applied at different ages during the SHRP on endocrine responsiveness and on basal mRNA expression of selected HPA axis markers (***Chapter 5***) and (2) to study the consequences of a single 24 hours maternal deprivation at postnatal day 3 on the further development of the HPA axis and the duration of the SHRP (***Chapter 6***).

In ***Chapter 7*** the results of the studies described in ***Chapters 2*** to ***6*** will be summarised and discussed in a broader perspective and conclusions are drawn. Finally, it will be shown in ***Appendix I*** that in male and female CD1 mice the degree of novelty is reflected by the corticosterone response to a stressor but at the same time contains a methodological consideration for the application of tail sampling in mice.

1.6 References

1. American Psychiatric Association (1994) Diagnostic criteria from DSM-IV. Washington, DC. APA .
2. Ader R (1969) Early experiences accelerate maturation of the 24-hour adrenocortical rhythm. Science 163(872): 1225-1226.
3. Ader R and Grota LJ (1970) Rhythmicity in the maternal behaviour of Rattus norvegicus. Anim. Behav. 18(1): 144-150.
4. Albanese A, Hamill G, Jones J, Skuse D, Matthews DR and Stanhope R (1994) Reversibility of physiological growth hormone secretion in children with psychosocial dwarfism. Clin. Endocrinol. 40(5): 687-692.
5. Alleva E, Caprioli A and Laviola G (1989) Litter gender composition affects maternal behavior of the primiparous mouse dam (Mus musculus). J. Comp. Psychol. 103(1): 83-87.
6. Andersen SL (2003) Trajectories of brain development: point of vulnerability or window

of opportunity? Neurosci. Biobehav. Rev. 27(1-2): 3-18.
7. Anisman H, Zaharia MD, Meaney MJ and Merali Z (1998) Do early-life events permanently alter behavioral and hormonal responses to stressors? Int. J. Dev. Neurosci. 16(3-4): 149-164.
8. Arborelius L, Owens MJ, Plotsky PM and Nemeroff CB (1999) The role of corticotropin-releasing factor in depression and anxiety disorders. J. Endocrinol. 160(1): 1-12.
9. Avishai-Eliner S, Hatalski CG, Tabachnik E, Eghbal-Ahmadi M and Baram TZ (1999) Differential regulation of glucocorticoid receptor messenger RNA (GR mRNA) by maternal deprivation in immature rat hypothalamus and limbic regions. Brain Res. Dev. Brain Res. 114(2): 265-268.
10. Barbazanges A, Piazza PV, Le Moal M and Maccari S (1996) Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. J. Neurosci. 16(12): 3943-3949.
11. Beato M, Chavez S and Truss M (1996) Transcriptional regulation by steroid hormones. Steroids 61(4): 240-251.
12. Belanoff JK, Kalehzan M, Sund B, Fleming Ficek SK and Schatzberg AF (2001) Cortisol activity and cognitive changes in psychotic major depression. Am. J. Psychiatry 158(10): 1612-1616.
13. Belanoff JK, Rothschild AJ, Cassidy F, DeBattista C, Baulieu EE, Schold C and Schatzberg AF (2002) An open label trial of C-1073 (mifepristone) for psychotic major depression. Biol. Psychiatry 52(5): 386-392.
14. Biagini G, Pich EM, Carani C, Marrama P and Agnati LF (1998) Postnatal maternal separation during the stress hyporesponsive period enhances the adrenocortical response to novelty in adult rats by affecting feedback regulation in the CA1 hippocampal field. Int. J. Dev. Neurosci. 16(3-4): 187-197.
15. Bland RC (1997) Epidemiology of affective disorders: a review. Can. J. Psychiatry 42(4): 367-377.
16. Bohn MC (1980) Granule cell genesis in the hippocampus of rats treated neonatally with hydrocortisone. Neuroscience 5(11): 2003-2012.
17. Bohn MC, Dean D, Hussain S and Giuliano R (1994) Development of mRNAs for glucocorticoid and mineralocorticoid receptors in rat hippocampus. Brain Res. Dev. Brain Res. 77(2): 157-162.
18. Bonilla-Felix M (2004) Development of water transport in the collecting duct. Am. J. Physiol. Renal. Physiol. 287(6): F1093-F1101.
19. Bouret SG, Draper SJ and Simerly RB (2004) Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304(5667): 108-110.
20. Boyce WT, Champoux M, Suomi SJ and Gunnar MR (1995) Salivary cortisol in nursery-reared rhesus monkeys: reactivity to peer interactions and altered circadian activity. Dev. Psychobiol. 28(5): 257-267.
21. Brabham T, Phelka A, Zimmer C, Nash A, Lopez JF and Vazquez DM (2000) Effects of prenatal dexamethasone on spatial learning and response to stress is influenced by maternal factors. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279(5): R1899-R1909.
22. Buckley DI and Ramachandran J (1981) Characterization of corticotropin receptors on adrenocortical cells. Proc. Natl. Acad. Sci. U. S. A. 78(12): 7431-7435.
23. Caldji C, Diorio J and Meaney MJ (2000) Variations in maternal care in infancy regulate the development of stress reactivity. Biol. Psychiatry 48(12): 1164-1174.
24. Caldji C, Francis DD, Sharma S, Plotsky PM and Meaney MJ (2000) The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22(3): 219-229.

25. Casanueva FF and Dieguez C (2005) Leptin and ghrelin: what is the impact on pituitary function? Rev. Endocr. Metab. Disord. 6(1): 39-45.
26. Catalani A, Casolini P, Scaccianoce S, Patacchioli FR, Spinozzi P and Angelucci L (2000) Maternal corticosterone during lactation permanently affects brain corticosteroid receptors, stress response and behaviour in rat progeny. Neuroscience 100(2): 319-325.
27. Checkley S (1996) The neuroendocrinology of depression and chronic stress. Br. Med. Bull. 52(3): 597-617.
28. Cicchetti D (1984) The emergence of developmental psychopathology. Child Dev. 55(1): 1-7.
29. Cirulli F, Santucci D, Laviola G, Alleva E and Levine S (1994) Behavioral and hormonal responses to stress in the newborn mouse: effects of maternal deprivation and chlordiazepoxide. Dev. Psychobiol. 27(5): 301-316.
30. Crabbe JC and Phillips TJ (2003) Mother nature meets mother nurture. Nat. Neurosci. 6(5): 440-442.
31. Crabbe JC, Wahlsten D and Dudek BC (1999) Genetics of mouse behavior: interactions with laboratory environment. Science 284(5420): 1670-1672.
32. D'Amato FR and Cabib S (1987) Chronic exposure to a novel odor increases pups' vocalizations, maternal care and alters dopaminergic functioning in developing mice. Behav. Neural Biol. 48(2): 197-205.
33. D'Amato FR, Cabib S, Ventura R and Orsini C (1998) Long-term effects of postnatal manipulation on emotionality are prevented by maternal anxiolytic treatment in mice. Dev. Psychobiol. 32(3): 225-234.
34. Dahlof LG, Hard E and Larsson K (1978) Influence of maternal stress on the development of the fetal genital system. Physiol. Behav. 20(2): 193-195.
35. Dallman MF, Akana SF, Bhatnagar S, Bell ME, Choi S, Chu A, Horsley C, Levin N, Meijer OC, Soriano LR, Strack AM and Viau V (1999) Starvation: early signals, sensors and sequelae. Endocrinology 140(9): 4015-4023.
36. Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L and Levin N (1987) Regulation of ACTH secretion: variations on a theme of B. Recent Prog. Horm. Res. 43: 113-173.
37. Dawson G, Ashman SB and Carver LJ (2000) The role of early experience in shaping behavioral and brain development and its implications for social policy. Dev. Psychopathol. 12(4): 695-712.
38. de Kloet ER, Joels M, Oitzl MS and Sutanto W (1991) Implication of brain corticosteroid receptor diversity for the adaptation syndrome concept. Methods Achiev. Exp. Pathol. 14: 104-132.
39. de Kloet ER and Oitzl MS (2003) Who cares for a stressed brain? The mother, the kid or both? Neurobiol. Aging 24 Suppl. 1: S61-S65.
40. de Kloet ER, Oitzl MS and Joels M (1999) Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22(10): 422-426.
41. de Kloet ER and Reul JM (1987) Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. Psychoneuroendocrinology 12(2): 83-105.
42. de Kloet ER, Vreugdenhil E, Oitzl MS and Joels M (1998) Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 19(3): 269-301.
43. Denenberg VH (1964) Critical periods, stimulus input and emotional reactivity: a theory of infantile stimulation. Psychol. Rev. 71: 335-351.
44. Dent GW, Okimoto DK, Smith MA and Levine S (2000) Stress-induced alterations in corticotropin-releasing hormone and vasopressin gene expression in the paraventricular

nucleus during ontogeny. Neuroendocrinology 71(6): 333-342.
45. Dent GW, Smith MA and Levine S (2000) Rapid induction of corticotropin-releasing hormone gene transcription in the paraventricular nucleus of the developing rat. Endocrinology 141(5): 1593-1598.
46. Drouin J, Sun YL, Chamberland M, Gauthier Y, de Lean A, Nemer M and Schmidt TJ (1993) Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. EMBO J. 12(1): 145-156.
47. Eghbal-Ahmadi M, Avishai-Eliner S, Hatalski CG and Baram TZ (1999) Differential regulation of the expression of corticotropin-releasing factor receptor type 2 (CRF2) in hypothalamus and amygdala of the immature rat by sensory input and food intake. J. Neurosci. 19(10): 3982-3991.
48. Ehlert U, Gaab J and Heinrichs M (2001) Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder and stress-related bodily disorders: the role of the hypothalamus-pituitary-adrenal axis. Biol. Psychol. 57(1-3): 141-152.
49. Erskine MS, Geller E and Yuwiler A (1979) Effects of neonatal hydrocortisone treatment of pituitary and adrenocortical response to stress in young rats. Neuroendocrinology 29(3): 191-199.
50. Evans DL and Nemeroff CB (1983) The dexamethasone suppression test in mixed bipolar disorder. Am. J. Psychiatry 140(5): 615-617.
51. Famularo R, Kinscherff R and Fenton T (1991) Posttraumatic stress disorder among children clinically diagnosed as borderline personality disorder. J. Nerv. Ment. Dis. 179(7): 428-431.
52. Francis DD and Meaney MJ (1999) Maternal care and the development of stress responses. Curr. Opin. Neurobiol. 9(1): 128-134.
53. Francis DD, Szegda K, Campbell G, Martin WD and Insel TR (2003) Epigenetic sources of behavioral differences in mice. Nat. Neurosci. 6(5): 445-446.
54. Fride E and Weinstock M (1984) The effects of prenatal exposure to predictable or unpredictable stress on early development in the rat. Dev. Psychobiol. 17(6): 651-660.
55. Fuchs E and Flugge G (1998) Stress, glucocorticoids and structural plasticity of the hippocampus. Neurosci. Biobehav. Rev. 23(2): 295-300.
56. Fuchs E, Uno H and Flugge G (1995) Chronic psychosocial stress induces morphological alterations in hippocampal pyramidal neurons of the tree shrew. Brain Res. 673(2): 275-282.
57. Gohlke BC, Khadilkar VV, Skuse D and Stanhope R (1998) Recognition of children with psychosocial short stature: a spectrum of presentation. J. Pediatr. Endocrinol. Metab. 11(4): 509-517.
58. Gold PW and Chrousos GP (2002) Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol. Psychiatry 7(3): 254-275.
59. Gold PW, Kling MA, Whitfield HJ, Rabin D, Margioris A, Kalogeras K, Demitrack M, Loriaux DL and Chrousos GP (1988) The clinical implications of corticotropin-releasing hormone. Adv. Exp. Med. Biol. 245: 507-519.
60. Gottlicher M, Heck S and Herrlich P (1998) Transcriptional cross-talk, the second mode of steroid hormone receptor action. J. Mol. Med. 76(7): 480-489.
61. Gould E, McEwen BS, Tanapat P, Galea LA and Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. 17(7): 2492-2498.
62. Gould E and Tanapat P (1999) Stress and hippocampal neurogenesis. Biol. Psychiatry

46(11): 1472-1479.
63. Gunnar MR (2003) Integrating neuroscience and psychological approaches in the study of early experiences. Ann. N. Y. Acad. Sci. 1008: 238-247.
64. Gunnar MR (2005) Early experiences, stress and humand development. (personal communication)
65. Gunnar MR, Bruce J and Grotevant HD (2000) International adoption of institutionally reared children: research and policy. Dev. Psychopathol. 12(4): 677-693.
66. Gunnar MR and Donzella B (2002) Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology 27(1-2): 199-220.
67. Gunnar MR and Vazquez DM (2001) Low cortisol and a flattening of expected daytime rhythm: potential indices of risk in human development. Dev. Psychopathol. 13(3): 515-538.
68. Heffelfinger AK and Newcomer JW (2001) Glucocorticoid effects on memory function over the human life span. Dev. Psychopathol. 13(3): 491-513.
69. Heim C, Ehlert U and Hellhammer DH (2000) The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25(1): 1-35.
70. Heim C and Nemeroff CB (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol. Psychiatry 49(12): 1023-1039.
71. Heim C, Newport DJ, Bonsall R, Miller AH and Nemeroff CB (2001) Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. Am. J. Psychiatry 158(4): 575-581.
72. Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH and Nemeroff CB (2000) Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA 284(5): 592-597.
73. Heim C, Newport DJ, Miller AH and Nemeroff CB (2000) Long-term neuroendocrine effects of childhood maltreatment. JAMA 284(18): 2321.
74. Heim C, Owens MJ, Plotsky PM and Nemeroff CB (1997) Persistent changes in corticotropin-releasing factor systems due to early life stress: relationship to the pathophysiology of major depression and post-traumatic stress disorder. Psychopharmacol. Bull. 33(2): 185-192.
75. Henning SJ (1978) Plasma concentrations of total and free corticosterone during development in the rat. Am. J. Physiol. 235(5): E451-E456.
76. Henry C, Kabbaj M, Simon H, Le Moal M and Maccari S (1994) Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. J. Neuroendocrinol. 6(3): 341-345.
77. Herman JP and Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. 20(2): 78-84.
78. Herman JP, Prewitt CM and Cullinan WE (1996) Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. Crit. Rev. Neurobiol. 10(3-4): 371-394.
79. Hofer MA (1981) Toward a developmental basis for disease predisposition: the effects of early maternal separation on brain, behavior and cardiovascular system. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 59: 209-228.
80. Hofer MA (1994) Early relationships as regulators of infant physiology and behavior. Acta Paediatr. Suppl. 397: 9-18.
81. Hofer MA (1996) Multiple regulators of ultrasonic vocalization in the infant rat. Psychoneuroendocrinology 21(2): 203-217.
82. Holsboer F (2000) The corticosteroid receptor hypothesis of depression. Neuropsycho-

pharmacology 23(5): 477-501.
83. Huot RL, Smith MA and Plotsky PM (1997) Alteration of maternal-infant interaction as a result of maternal separation in Long-Evans rats: behavioural and neuroendocrine consequences. (personal communication)
84. Huot RL, Thrivikraman KV, Meaney MJ and Plotsky PM (2001) Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology 158(4): 366-373.
85. Jaffee SR, Moffitt TE, Caspi A, Fombonne E, Poulton R and Martin J (2002) Differences in early childhood risk factors for juvenile-onset and adult-onset depression. Arch. Gen. Psychiatry 59(3): 215-222.
86. Joels M and de Kloet ER (1992) Control of neuronal excitability by corticosteroid hormones. Trends Neurosci. 15(1): 25-30.
87. Joels M, Pu Z, Wiegert O, Oitzl MS and Krugers HJ (2006) Learning under stress: how does it work? Trends Cogn. Sci. 10(4): 152-158.
88. Johnson EO, Kamilaris TC, Chrousos GP and Gold PW (1992) Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. Neurosci. Biobehav. Rev. 16(2): 115-130.
89. Kage A, Fenner A, Weber B and Schoneshofer M (1982) Diurnal and ultradian variations of plasma concentrations of eleven adrenal steroid hormones in human males. Klin. Wochenschr. 60(13): 659-666.
90. Kalra SP and Kalra PS (2004) NPY and cohorts in regulating appetite, obesity and metabolic syndrome: beneficial effects of gene therapy. Neuropeptides 38(4): 201-211.
91. Kalra SP, Ueno N and Kalra PS (2005) Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action. J. Nutr. 135(5): 1331-1335.
92. Karssen AM, Meijer OC, van der Sandt I, Lucassen PJ, de Lange EC, de Boer AG and de Kloet ER (2001) Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. Endocrinology 142(6): 2686-2694.
93. Kaufman J and Charney DS (2001) Effects of early stress on brain structure and function: implications for understanding the relationship between child maltreatment and depression. Dev. Psychopathol. 13(3): 451-471.
94. Kaufman J, Plotsky PM, Nemeroff CB and Charney DS (2000) Effects of early adverse experiences on brain structure and function: clinical implications. Biol. Psychiatry 48(8): 778-790.
95. Keller-Wood ME and Dallman MF (1984) Corticosteroid inhibition of ACTH secretion. Endocr. Rev. 5(1): 1-24.
96. Kendler KS, Karkowski LM and Prescott CA (1999) Causal relationship between stressful life events and the onset of major depression. Am. J. Psychiatry 156(6): 837-841.
97. Kishi T and Elmquist JK (2005) Body weight is regulated by the brain: a link between feeding and emotion. Mol. Psychiatry 10(2): 132-146.
98. Koehl M, Darnaudery M, Dulluc J, Van Reeth O, Le Moal M and Maccari S (1999) Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. J. Neurobiol. 40(3): 302-315.
99. Korbonits M and Grossman AB (2004) Ghrelin: update on a novel hormonal system. Eur. J. Endocrinol. 151 Suppl. 1: S67-S70.
100. Krozowski ZS and Funder JW (1983) Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. Proc. Natl. Acad. Sci. U. S. A. 80(19): 6056-6060.
101. Kuhn CM, Butler SR and Schanberg SM (1978) Selective depression of serum growth

hormone during maternal deprivation in rat pups. Science 201(4360): 1034-1036.
102. Kuhn CM, Pauk J and Schanberg SM (1990) Endocrine responses to mother-infant separation in developing rats. Dev. Psychobiol. 23(5): 395-410.
103. Kuhn CM and Schanberg SM (1998) Responses to maternal separation: mechanisms and mediators. Int. J. Dev. Neurosci. 16(3-4): 261-270.
104. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ and Plotsky PM (2000) Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog. Brain Res. 122: 81-103.
105. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB and Plotsky PM (2004) Long-term adaptations in glucocorticoid receptor and mineralocorticoid receptor mRNA and negative feedback on the hypothalamo-pituitary-adrenal axis following neonatal maternal separation. Biol. Psychiatry 55(4): 367-375.
106. Ladd CO, Owens MJ and Nemeroff CB (1996) Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. Endocrinology 137(4): 1212-1218.
107. Ladd CO, Thrivikraman KV, Huot RL and Plotsky PM (2005) Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates. Psychoneuroendocrinology 30(6): 520-533.
108. Lehmann J and Feldon J (2000) Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Rev. Neurosci. 11(4): 383-408.
109. Lehmann J, Pryce CR, Bettschen D and Feldon J (1999) The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. Pharmacol. Biochem. Behav. 64(4): 705-715.
110. Lehmann J, Russig H, Feldon J and Pryce CR (2002) Effect of a single maternal separation at different pup ages on the corticosterone stress response in adult and aged rats. Pharmacol. Biochem. Behav. 73(1): 141-145.
111. Levine S (1956) A further study of infantile handling and adult avoidance learning. J. Pers. 25(1): 70-80.
112. Levine S (1994) The ontogeny of the hypothalamic-pituitary-adrenal axis. The influence of maternal factors. Ann. N. Y. Acad. Sci. 746: 275-288.
113. Levine S (2001) Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. Physiol. Behav. 73(3): 255-260.
114. Levine S (2005) Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology 30(10): 939-946.
115. Levine S, Chevalier JA and Korchin JS (1956) The effects of early shock and handling on later avoidance learning. J. Pers. 24(4): 475-493.
116. Levine S, Huchton DM, Wiener SG and Rosenfeld P (1991) Time course of the effect of maternal deprivation on the hypothalamic-pituitary-adrenal axis in the infant rat. Dev. Psychobiol. 24(8): 547-558.
117. Liu D, Diorio J, Day JC, Francis DD and Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat. Neurosci. 3(8): 799-806.
118. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis DD, Freedman A, Sharma S, Pearson D, Plotsky PM and Meaney MJ (1997) Maternal care, hippocampal glucocorticoid receptors and hypothalamic-pituitary-adrenal responses to stress. Science 277(5332): 1659-1662.
119. Lupien SJ and McEwen BS (1997) The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain Res. Brain Res. Rev. 24(1): 1-27.
120. Maccari S, Darnaudery M, Morley-Fletcher S, Zuena AR, Cinque C and Van Reeth O (2003) Prenatal stress and long-term consequences: implications of glucocorticoid hormones. Neurosci. Biobehav. Rev. 27(1-2): 119-127.

121. Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H and Le Moal M (1995) Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J. Neurosci. 15(1 Pt 1): 110-116.
122. Magarinos AM, Verdugo JM and McEwen BS (1997) Chronic stress alters synaptic terminal structure in hippocampus. Proc. Natl. Acad. Sci. U. S. A. 94(25): 14002-14008.
123. Makara GB (1985) Mechanisms by which stressful stimuli activate the pituitary-adrenal system. Fed. Proc. 44(1 Pt 2): 149-153.
124. McCormick CM, Kehoe P and Kovacs S (1998) Corticosterone release in response to repeated, short episodes of neonatal isolation: evidence of sensitization. Int. J. Dev. Neurosci. 16(3-4): 175-185.
125. McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. Brain Res. 886(1-2): 172-189.
126. McEwen BS, de Kloet ER and Rostene W (1986) Adrenal steroid receptors and actions in the nervous system. Physiol. Rev. 66(4): 1121-1188.
127. McLeer SV, Callaghan M, Henry D and Wallen J (1994) Psychiatric disorders in sexually abused children. J. Am. Acad. Child Adolesc. Psychiatry 33(3): 313-319.
128. Meaney MJ, Diorio J, Francis DD, LaRocque S, O'Donnell D, Smythe JW, Sharma S and Tannenbaum B (1994) Environmental regulation of the development of glucocorticoid receptor systems in the rat forebrain. The role of serotonin. Ann. N. Y. Acad. Sci. 746: 260-273.
129. Meaney MJ, Diorio J, Francis DD, Widdowson J, LaPlante P, Caldji C, Sharma S, Seckl JR and Plotsky PM (1996) Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. Dev. Neurosci. 18(1-2): 49-72.
130. Meaney MJ, Sapolsky RM and McEwen BS (1985) The development of the glucocorticoid receptor system in the rat limbic brain. II. An autoradiographic study. Brain Res. 350(1-2): 165-168.
131. Meyer SE, Chrousos GP and Gold PW (2001) Major depression and the stress system: a life span perspective. Dev. Psychopathol. 13(3): 565-580.
132. Munck AU, Guyre PM and Holbrook NJ (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5(1): 25-44.
133. Murphy BE (2000) Glucocorticoids, overview. (2): 244-260. Encyclopedia of stress. editor Fink G., Academic Press, San Diego, CA.
134. Murray CJ and Lopez AD (1997) Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 349(9064): 1498-1504.
135. Nachmias M, Gunnar MR, Mangelsdorf S, Parritz RH and Buss K (1996) Behavioral inhibition and stress reactivity: the moderating role of attachment security. Child Dev. 67(2): 508-522.
136. Nemeroff CB (1998) The neurobiology of depression. Sci. Am. 278(6): 42-49.
137. Oitzl MS and de Kloet ER (1992) Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. Behav. Neurosci. 106(1): 62-71.
138. Oitzl MS, Workel JO, Fluttert M, Frosch F and de Kloet ER (2000) Maternal deprivation affects behaviour from youth to senescence: amplification of individual differences in spatial learning and memory in senescent Brown Norway rats. Eur. J. Neurosci. 12(10): 3771-3780.
139. Okimoto DK, Blaus A, Schmidt MV, Gordon MK, Dent GW and Levine S (2002) Differential expression of c-fos and tyrosine hydroxylase mRNA in the adrenal gland of the infant rat: evidence for an adrenal hyporesponsive period. Endocrinology 143(5): 1717-1725.

140. Owens MJ and Nemeroff CB (1991) Physiology and pharmacology of corticotropin-releasing factor. Pharmacol. Rev. 43(4): 425-473.
141. Palkovits M (1982) Neuropeptides in the median eminence: their sources and destinations. Peptides 3(3): 299-303.
142. Palkovits M (1984) Neuropeptides in the hypothalamo-hypophyseal system: lateral retrochiasmatic area as a common gate for neuronal fibers towards the median eminence. Peptides 5 Suppl. 1: 35-39.
143. Palkovits M (1992) Peptidergic neurotransmitters in the endocrine hypothalamus. Ciba Found. Symp. 168: 3-10.
144. Peters DA (1982) Prenatal stress: effects on brain biogenic amine and plasma corticosterone levels. Pharmacol. Biochem. Behav. 17(4): 721-725.
145. Plotsky PM and Meaney MJ (1993) Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res. Mol. Brain Res. 18(3): 195-200.
146. Plotsky PM, Thrivikraman KV, Nemeroff CB, Caldji C, Sharma S and Meaney MJ (2005) Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. Neuropsychopharmacology 30(12): 2192-2204.
147. Price DA, Close GC and Fielding BA (1983) Age of appearance of circadian rhythm in salivary cortisol values in infancy. Arch. Dis. Child. 58: 454-456.
148. Pryce CR, Bettschen D and Feldon J (2001) Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev. Psychobiol. 38(4): 239-251.
149. Pryce CR and Feldon J (2003) Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. Neurosci. Biobehav. Rev. 27(1-2): 57-71.
150. Pryce CR, Ruedi-Bettschen D, Dettling AC and Feldon J (2002) Early life stress: long-term physiological impact in rodents and primates. News Physiol. Sci. 17: 150-155.
151. Reul JM and de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117(6): 2505-2511.
152. Reus VI and Wolkowitz OM (2001) Antiglucocorticoid drugs in the treatment of depression. Expert. Opin. Investig. Drugs 10(10): 1789-1796.
153. Rivier C, Brownstein M, Spiess J, Rivier J and Vale W (1982) In vivo corticotropin-releasing factor-induced secretion of adrenocorticotropin, beta-endorphin and corticosterone. Endocrinology 110(1): 272-278.
154. Roman E and Nylander I (2005) The impact of emotional stress early in life on adult voluntary ethanol intake-results of maternal separation in rats. Stress. 8(3): 157-174.
155. Romeo RD, Fossella JA, Bateup HS, Sisti HM, Brake WG and McEwen BS (2004) Maternal separation suppresses TGF alpha mRNA expression in the prefrontal cortex of male and female neonatal C57BL/6 mice. Brain Res. Dev. Brain Res. 152(1): 73-77.
156. Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS and Brake WG (2003) Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. Horm. Behav. 43(5): 561-567.
157. Rosen JB and Schulkin J (1998) From normal fear to pathological anxiety. Psychol. Rev. 105(2): 325-350.
158. Rosenblatt JS (1975) Prepartum and postpartum regulation of maternal behaviour in the rat. Ciba Found. Symp.(33): 17-37.
159. Rosenfeld P, Gutierrez YA, Martin AM, Mallett HA, Alleva E and Levine S (1991) Maternal regulation of the adrenocortical response in preweanling rats. Physiol. Behav. 50(4): 661-671.
160. Rosenfeld P, Suchecki D and Levine S (1992) Multifactorial regulation of the hypothalamic-

pituitary-adrenal axis during development. Neurosci. Biobehav. Rev. 16(4): 553-568.
161. Rosenfeld P, Sutanto W, Levine S and de Kloet ER (1988) Ontogeny of type I and type II corticosteroid receptors in the rat hippocampus. Brain Res. 470(1): 113-118.
162. Rots NY, de Jong J, Workel JO, Levine S, Cools AR and de Kloet ER (1996) Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. J. Neuroendocrinol. 8(7): 501-506.
163. Sakly M and Koch B (1981) Ontogenesis of glucocorticoid receptors in anterior pituitary gland: transient dissociation among cytroplasmic receptor density, nuclear uptake and regulation of corticotropic activity. Endocrinology 108(2): 591-596.
164. Sanchez MM, Ladd CO and Plotsky PM (2001) Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. Dev. Psychopathol. 13(3): 419-449.
165. Sarrieau A, Sharma S and Meaney MJ (1988) Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors. Brain Res. 471(1): 158-162.
166. Sartor O and Cutler GB, Jr. (1996) Mifepristone: treatment of Cushing's syndrome. Clin. Obstet. Gynecol. 39(2): 506-510.
167. Schapiro S, Geller E and Eiduson S (1962) Neonatal adrenal cortical response to stress and vasopressin. Proc. Soc. Exp. Biol. Med. 109: 937-941.
168. Schmidt MV, Deussing JM, Oitzl MS, Ohl F, Levine S, Wurst W, Holsboer F, Muller MB and de Kloet ER (2006) Differential disinhibition of the neonatal hypothalamic-pituitary-adrenal axis in brain-specific CRH receptor 1-knockout mice. Eur. J. Neurosci. 24(8): 2291-2298.
169. Schmidt MV, Enthoven L, van der Mark M, Levine S, de Kloet ER and Oitzl MS (2003) The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. Int. J. Dev. Neurosci. 21(3): 125-132.
170. Schmidt MV, Enthoven L, van Woezik JH, Levine S, de Kloet ER and Oitzl MS (2004) The dynamics of the hypothalamic-pituitary-adrenal axis during maternal deprivation. J. Neuroendocrinol. 16(1): 52-57.
171. Schmidt MV, Levine S, Alam S, Harbich D, Sterlemann V, Ganea K, de Kloet ER, Holsboer F and Muller MB (2006) Metabolic signals modulate hypothalamic-pituitary-adrenal axis activation during maternal separation of the neonatal mouse. J. Neuroendocrinol. 18(11): 865-874.
172. Schmidt MV, Oitzl MS, Levine S and de Kloet ER (2002) The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. Brain Res. Dev. Brain Res. 139(1): 39-49.
173. Schmidt MV, Oitzl MS, Mueller MB, Ohl F, Wurst W, Holsboer F, Levine S and de Kloet ER (2003) Regulation of the developing hypothalamic-pituitary-adrenal axis in corticotropin releasing hormone receptor 1-deficient mice. Neuroscience 119(2): 589-595.
174. Selye H (1950) The physiology and pathology of exposure to stress: a treatise based on the concepts of the general-adaptation-syndrome and the diseases of adaptation. Montreal, Acta.
175. Skuse D, Albanese A, Stanhope R, Gilmour J and Voss L (1996) A new stress-related syndrome of growth failure and hyperphagia in children, associated with reversibility of growth-hormone insufficiency. Lancet 348(9024): 353-358.
176. Smith MA, Kim SY, van Oers HJ and Levine S (1997) Maternal deprivation and stress induce immediate early genes in the infant rat brain. Endocrinology 138(11): 4622-4628.
177. Spencer RL, Young EA, Choo PH and McEwen BS (1990) Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy and activation with

varying level of steroid. Brain Res. 514(1): 37-48.
178. Stanton ME, Gutierrez YR and Levine S (1988) Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. Behav. Neurosci. 102(5): 692-700.
179. Suchecki D, Duarte PB and Tufik S (2000) Pituitary-adrenal axis and behavioural responses of maternally deprived juvenile rats to the open field. Behav. Brain Res. 111(1-2): 99-106.
180. Suchecki D, Rosenfeld P and Levine S (1993) Maternal regulation of the hypothalamic-pituitary-adrenal axis in the infant rat: the roles of feeding and stroking. Brain Res. Dev. Brain Res. 75(2): 185-192.
181. Suchecki D and Tufik S (1997) Long-term effects of maternal deprivation on the corticosterone response to stress in rats. Am. J. Physiol 273(4 Pt 2): R1332-R1338.
182. Suomi SJ (2003) Gene-environment interactions and the neurobiology of social conflict. Ann. N. Y. Acad. Sci. 1008: 132-139.
183. Suomi SJ (2005) Aggression and social behaviour in rhesus monkeys. Novartis. Found. Symp. 268: 216-222.
184. Sutanto W, Rosenfeld P, de Kloet ER and Levine S (1996) Long-term effects of neonatal maternal deprivation and ACTH on hippocampal mineralocorticoid and glucocorticoid receptors. Brain Res. Dev. Brain Res. 92(2): 156-163.
185. Truss M and Beato M (1993) Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr. Rev. 14(4): 459-479.
186. Underwood RH and Williams GH (1972) The simultaneous measurement of aldosterone, cortisol and corticosterone in human peripheral plasma by displacement analysis. J. Lab. Clin. Med. 79(5): 848-862.
187. Vallee M, Maccari S, Dellu F, Simon H, Le Moal M and Mayo W (1999) Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. Eur. J. Neurosci. 11(8): 2906-2916.
188. Vallee M, Mayo W, Dellu F, Le Moal M, Simon H and Maccari S (1997) Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J. Neurosci. 17(7): 2626-2636.
189. van Eekelen JA, Jiang W, de Kloet ER and Bohn MC (1988) Distribution of the mineralocorticoid and the glucocorticoid receptor mRNAs in the rat hippocampus. J. Neurosci. Res. 21(1): 88-94.
190. van Oers HJ, de Kloet ER and Levine S (1997) Persistent, but paradoxical, effects on HPA regulation of infants maternally deprived at different ages. Stress. 1(4): 249-262.
191. van Oers HJ, de Kloet ER and Levine S (1998) Early vs. late maternal deprivation differentially alters the endocrine and hypothalamic responses to stress. Brain Res. Dev. Brain Res. 111(2): 245-252.
192. van Oers HJ, de Kloet ER and Levine S (1999) Persistent effects of maternal deprivation on HPA regulation can be reversed by feeding and stroking, but not by dexamethasone. J. Neuroendocrinol. 11(8): 581-588.
193. van Oers HJ, de Kloet ER, Li C and Levine S (1998) The ontogeny of glucocorticoid negative feedback: influence of maternal deprivation. Endocrinology 139(6): 2838-2846.
194. van Oers HJ, de Kloet ER, Whelan T and Levine S (1998) Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. J. Neurosci. 18(23): 10171-10179.
195. Vazquez DM (1998) Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. Psychoneuroendocrinology 23(7): 663-700.
196. Vazquez DM, van Oers HJ, Levine S and Akil H (1996) Regulation of glucocorticoid

and mineralocorticoid receptor mRNAs in the hippocampus of the maternally deprived infant rat. Brain Res. 731(1-2): 79-90.
197. Veldhuis HD, van Koppen C, van Ittersum M and de Kloet ER (1982) Specificity of the adrenal steroid receptor system in rat hippocampus. Endocrinology 110(6): 2044-2051.
198. Venerosi A, Cirulli F, Capone F and Alleva E (2003) Prolonged perinatal AZT administration and early maternal separation: effects on social and emotional behaviour of periadolescent mice. Pharmacol. Biochem. Behav. 74(3): 671-681.
199. Wahlsten D, Metten P, Phillips TJ, Boehm SL, Burkhart-Kasch S, Dorow J, Doerksen S, Downing C, Fogarty J, Rodd-Henricks K, Hen R, McKinnon CS, Merrill CM, Nolte C, Schalomon M, Schlumbohm JP, Sibert JR, Wenger CD, Dudek BC and Crabbe JC (2003) Different data from different labs: lessons from studies of gene-environment interaction. J. Neurobiol. 54(1): 283-311.
200. Walker CD, Perrin M, Vale W and Rivier C (1986) Ontogeny of the stress response in the rat: role of the pituitary and the hypothalamus. Endocrinology 118(4): 1445-1451.
201. Warne JM, Harding KE and Balment RJ (2002) Neurohypophysial hormones and renal function in fish and mammals. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 132(1): 231-237.
202. Watamura SE, Donzella B, Kertes DA and Gunnar MR (2004) Developmental changes in baseline cortisol activity in early childhood: relations with napping and effortful control. Dev. Psychobiol. 45(3): 125-133.
203. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M and Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat. Neurosci. 7(8): 847-854.
204. Wise LA, Zierler S, Krieger N and Harlow BL (2001) Adult onset of major depressive disorder in relation to early life violent victimisation: a case-control study. Lancet 358(9285): 881-887.
205. Word Health Organisation (2001) Fact sheet N° 265.
206. Workel JO, Oitzl MS, Fluttert M, Lesscher H, Karssen AM and de Kloet ER (2001) Differential and age-dependent effects of maternal deprivation on the hypothalamic-pituitary-adrenal axis of brown norway rats from youth to senescence. J. Neuroendocrinol. 13(7): 569-580.
207. Workel JO, Oitzl MS, Ledeboer A and de Kloet ER (1997) The Brown Norway rat displays enhanced stress-induced ACTH reactivity at day 18 after 24-h maternal deprivation at day 3. Brain Res. Dev. Brain Res. 103(2): 199-203.
208. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA and Bloom SR (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141(11): 4325-4328.
209. Yi SJ, Masters JN and Baram TZ (1993) Effects of a specific glucocorticoid receptor antagonist on corticotropin releasing hormone gene expression in the paraventricular nucleus of the neonatal rat. Brain Res. Dev. Brain Res. 73(2): 253-259.
210. Zhang LX, Levine S, Dent GW, Zhan Y, Xing G, Okimoto DK, Kathleen GM, Post RM and Smith MA (2002) Maternal deprivation increases cell death in the infant rat brain. Brain Res. Dev. Brain Res. 133(1): 1-11.