CHAPTER 1

General Introduction

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It is well documented that early-life experiences are involved in shaping later-life phenotypes. Both human and animal studies have reported the impact of (adverse) early experiences on the development of the stress system, and its consequences for the development of stress-related disorders. The aim of the research described in this thesis is to explore the acute and long-lasting consequences of two distinct types of postnatal experience, varying substantially in nature and severity. The impact of both very subtle differences in maternal environment as well as of exposure to synthetic glucocorticoids during the early postnatal period was investigated. Furthermore, two potential intervention strategies will be introduced to prevent the frequently reported adverse effects of glucocorticoid-induced disruption of normal development and brain maturation.

In this introductory chapter, an overview is given of important concepts for the study of developmental programming. Additionally, several animal models used in experiments described in this thesis will be introduced.

1. DEVELOPMENTAL PROGRAMMING

Early perinatal life represents a critical developmental period. Not only the quality of embryonic environment (1), also early postnatal experiences have long-lasting consequences for emotional and cognitive development and functioning in later-life. Exposure to early adversity such as maternal stress during pregnancy, abuse or exposure to extreme poverty has been shown to increase the vulnerability to develop psychopathology in later-life.

The term 'developmental programming' derives from the concept of 'developmental origin of adult disease' introduced by Barker and colleagues and was based on a vast amount of epidemiological research documenting the relationship between low birth weight and an increased risk of developing metabolic and cardiovascular disorders (2-4). These findings led to what is currently known as the Barker Hypothesis (5). This concept has been since extended and is now frequently studied in the context of the hypothalamic-pituitary-adrenal (HPA) axis (6-10). Programming of the stress system is achieved through the actions of environmental cues acting at a specific time during development, resulting in permanent alterations in the functioning of the HPA axis (6-10).

Both preclinical and clinical evidence suggests that this phenomenon has relevance for the etiology of mental disorders triggered by stressful life events, including depression and post-traumatic stress disorder (9-12).

2. DISRUPTION OF NORMAL DEVELOPMENT

2.1 Human studies

Because of ethical considerations, the direct impact of stress on development cannot be investigated in humans. Therefore progress in this field relies on retrospective reports and correlational studies. There are however some *experiments of nature*

in which the impact of prenatal (intrauterine growth restriction, low birth weight) and postnatal (low socioeconomic status, maltreatment) adversity can be studied.

A series of studies have convincingly shown that exposure to early adverse events, such as childhood abuse, results in an increased risk to develop psychiatric dysfunctions (11-14). A dose-response relationship has been described between the number of experienced childhood adversities and mental health score in later-life (i.e. probability of lifetime depressive disorders) (15, 16). Besides these severe forms of adversity such as emotional neglect and abuse, there is also evidence that milder forms of adversity are associated with increased risk for stress related-pathologies. For instance, early-life socioeconomic disadvantage (17) but also subtle differences in parenting style (18-20) appear to affect health status of the individual in later-life. Therefore, there is no doubt that early adversity plays a crucial role in programming the development of a range of physical and psychiatric disorders which is likely to be mediated (at least partially) via the effects of early adversity on the functioning of the HPA axis. Several studies have shown the association between early-life adversity and enduring sensitization of the responsiveness of the HPA axis in humans. For instance, alterations in basal as well as stress-induced HPA axis activity at different life stages have been reported in human subjects exposed to adversity in early-life (13, 14, 21, 22).

2.2 Animal studies

In contrast to human studies, animal studies allow the development of experimental models where individuals are submitted to acute or chronic adversity and the resulting outcome on brain and behaviour can be investigated. Experimental early-life manipulations can be largely subdivided in prenatal and postnatal manipulations. Prenatal manipulations involve stress during pregnancy, maternal synthetic glucocorticoid exposure, or nutrient restriction. For a review on the impact of prenatal manipulations, we refer to previous literature (23-26).

Postnatal manipulations frequently involve manipulating or depriving the infant from maternal behaviour.

2.2.1 Handling

In the 1950's, it was discovered that exposing rat pups to daily handling sessions, which consisted of brief periods of separation from the dam (< 15 min) between postnatal day (pnd) 1 and 21, had a surprising and unexpected outcome (27). Levine, and others, found that handling induced long-lasting changes in adult phenotype such as HPA axis *hypo*-responsiveness (28-30), reduced emotionality (29), and increased cognitive performance (31) when compared to rats raised in undisturbed laboratory conditions, i.e. non handled. However, the use of such 'undisturbed' control groups was recognized to be problematic later; see reviews (32-34). Because the handling procedure was considered at that time to be a stressful experience, these findings challenged the dominant theory stating that early-life stress invariably contributes to the development of 'emotional instability'. Instead, the findings from Levine demonstrated that, in some instances (e.g. via handling), exposure to 'moderate stress' in early-life appeared to be beneficial for

the infant by promoting a greater ability of the organism to adapt to psychological and physiological stressors in adulthood (27). This same principle also serves as the basis for the stress inoculation-induced resilience theory developed several years later (35-39).

→ *In chapter 5 of this thesis the beneficial effects of neonatal handling will be used as a potential rescue strategy to compensate for the adverse effects of neonatal synthetic glucocorticoid exposure.*

2.2.2 Maternal separation

Over the years new paradigms were introduced in an attempt to also study the mechanisms underlying developmental programming following exposure to more 'adverse' experiences (40). Maternal separation consists of prolonged periods of maternal absence ranging from 1h to 24h. The reported effects of maternal separation appear to be more controversial compared to the effects of handling, in part because of the substantial variety in different experimental procedures across different laboratories in terms of duration, frequency, age of onset of the separation, gender and the choice of control group (41-43). Nevertheless, maternal separation appeared to 'program' the functioning of the HPA axis. As expected, this manipulation was reported to yield a more severe outcome, opposing the effects of handling, including HPA *hyper*-responsiveness following stress (40), increased emotionality (44), and impaired cognitive performance (28). For an extensive review of the consequences of postnatal manipulations, see: (28, 45).

3. MATERNAL MEDIATION HYPOTHESIS

The use of the handling model in rodents raised an important question: how can short episodes of maternal absence result in such profound and enduring effects on adult stress-phenotype? The 'maternal mediation hypothesis' was proposed for the fist time as part of the mechanism underlying the lasting effects of handling by Smotherman and Bell (46). This theory postulates that the outcome of postnatal manipulations (such as handling and maternal separation) is mediated by changes in maternal behaviour directed towards the offspring upon reunion after a given period of mother-infant separation (47). It was observed that brief (15 min) episodes of handling resulted in increased levels of maternal care upon reunion between mother and offspring, sometimes reported to remain higher throughout the entire day (48). Longer periods (4 h) of maternal separation yielded an increase in active maternal care only directly after reunion of the dam with the pups but not at any other time point, leading overall to differences in the amount/quality of maternal care received by handled versus maternally separated pups (48). This suggests that the amount and quality of maternal care, at least in part, mediates effects of handling and maternal separation on functioning of the HPA axis in the offspring.

However, certain findings challenged this theory. For instance, Macri and co-workers (32) reported inconsistencies in the maternal mediation hypothesis. They showed an overall increase in maternal care following both handling and maternal separation. Their findings revealed that directly following maternal separation dams increase their care to such an extent that they fully compensate for the separation time and reach a level comparable to dams of handled pups. Since handled and maternally separated offspring display significantly different endocrine and behavioural stress responses in later-life, it was concluded that maternal care cannot be the only mediator driving the effects of the postnatal manipulations (32, 33).

4. NATURALLY OCCURRING VARIATION IN MATERNAL CARE

4.1 Rodent studies

The most compelling set of evidence for the importance of the amount and quality of maternal care on the development of the stress-regulating system came from studies performed by Meaney and colleagues (49). Employing a non-invasive naturalistic approach, they studied the impact of naturally occurring variation in maternal care on the development of the HPA axis in rodents. This model is based on extreme differences among lactating rats in the frequency of licking and grooming (LG) they provide to their pups. It shows that variation in the amount of maternal LG, a form of tactile stimulation, modulates the development of the structure and function of the neural circuitry underlying stress regulation, emotionality, and cognitive processes (49-54). Reminiscent of the outcome of handling, offspring of high, relative to low LG dams, show decreased behavioural and endocrine responsiveness to stress, reduced emotionality, and enhanced performance in tests of spatial learning (49, 51, 54). These effects are largely reversed with cross-fostering, in which the biological offspring of a high LG mother is cross-fostered to a low LG mother or vice versa. This suggests that variation in maternal care transfers phenotypic differences to the offspring in a non-genetic way (55).

→ *This model is based on the assumption that maternal care is equally distributed over individual pups sharing a litter, such that each individual develops a similar phenotype later in life. In chapter 2 of this thesis this assumption is tested. We hypothesize that the distribution of maternal care directed towards individual pups within a litter is homogenous and therefore results in a uniform 'stress phenotype' in later-life.*

4.2 Human studies

As with animal models, the mediating role of the mother (or another caregiver) in the regulation of the HPA axis of the infant has also been demonstrated in humans (56). Several studies show that when children are exposed to adequate

care, they display diminished cortisol responsiveness, an increased threshold to evoke a cortisol response to various stressors (56), and a better cortisol recovery after stress (i.e. enhanced glucocorticoid negative feedback) (57). This is explained by suggesting that children, under high care-giving conditions, anticipate that a caregiver will protect them and therefore they feel able to cope with a threatening situation (56). Additionally, it has been reported that subtle differences in parenting style are associated with the degree of antisocial behaviour in adolescents (19). Moreover, differences in *within-family* parenting style appear to be associated with variation in antisocial behaviour and depressive symptoms in the offspring (18).

5. EPIGENETIC PROGRAMMING OF THE HPA AXIS

The neuro-endocrine (49), behavioural (51, 58, 59), and cognitive alterations (50, 52) observed in response to naturally-occurring variations in maternal care (60) are suggested to be the consequence of alterations in HPA axis activity, hippocampal glucocorticoid receptor (GR) expression and synaptic plasticity.

A major breakthrough in the field of developmental programming came with the discovery of epigenetic modifications in the promoter area of the GR gene, revealing a mechanism underlying these environmentally driven effects on later-life stress phenotype. It was shown that increased levels of maternal LG during the first week of life alter the methylation pattern of the GR gene in the hippocampus of the offspring (61). These changes persist into adulthood and alter the expression of the GR throughout life via modification of the chromatin structure. Cross fostering of the offspring (from a high to a low LG dam or vice versa) shows a complete reversal of methylation patterns, demonstrating that DNA can be structurally modified (without alterations to sequence) through environmental influences, thus leading to changes in gene expression (61, 62).

The significance of these findings in the field of psychiatry is unclear but recent studies in humans revealed that epigenetic programming of the HPA axis via changes in DNA methylation of GR may occur in human infants born to mothers whom experienced depression during pregnancy (63). Additionally, there are indications of epigenetic regulation of GR in the brains of individuals with a history of adverse childhood experiences whom committed suicide following a stressful life event (64).

6. RESILIENCE

Traditionally, research in the field of developmental programming has focused on the detrimental consequences of stress and far less on the ability to develop resilience to stress or stress-related diseases. Recent findings are challenging this view and suggest that the outcome of early experience is not necessarily deterministic and cannot be perceived as *good or bad*.

6.1 Resilience through matching environments

From an evolutionary perspective, biological mechanisms leading to 'programming' effects are generally meant to be adaptive and not necessarily a substrate for diseases. This is the basis of the 'predictive adaptation plasticity hypothesis' (65-69). This theory is based on the concept that a developing organism responds to cues (e.g. maternal care) in its environment by changing certain aspects of its homeostatic regulation (e.g. HPA axis) in order to produce a phenotype that is highly adapted to its current and anticipated future environment. This concept led to the idea that a high degree of 'mismatch' between the early- and later-life environments accounts for an increased risk to develop diseases in adulthood (66-69). There is much evidence to support this view in the field of metabolic and cardiovascular disorders (67, 70). However in psychiatric research, the validity of this concept is uncertain.

Recent evidence from animal studies however suggests that the concept of 'mismatch' can also apply to the development of individual differences in stress sensitivity. It was recently shown that the outcome of early experiences on stress-related parameters is dependent on later-life environmental context (50, 52). Specifically, it was reported that adult offspring of low LG mothers (considered as a form of adversity) show indeed the expected poor cognitive performance in a low-stress context. However, in a high-stress context their performance was better compared to animals that had received high levels of maternal LG, which in turn were impaired under the same stressful conditions (50, 52). Additionally early deprivation of maternal care (a severe form of adversity) has been reported to result in impaired cognition under low stress but enhanced performance under high stress conditions (71). These findings suggest that the influence of environmental experiences during development might serve as a basis for resilience to stressful challenges in later life.

6.2 Intervention

Interventions, when made at a specific time during development, can mediate the developmental programming of a certain phenotype, as is shown by studies on infants raised in orphanages. These infants have been reported to show changes in cognitive performance (72) and neuronal function in the hippocampus, when compared to never-institutionalized children (73). However, these deleterious effects appear to be reversible when intervention occurs within a certain time window. Placement of institutionalized infants in foster families significantly improves long-term learning and memory performance, with earlier intervention leading to better outcome (74).

The impact of interventions has also been described in animal studies. It has been reported that the cognitive impairment in animals either receiving low levels of maternal LG (75) or being exposed to prolonged periods of maternal separation (76) in early-life can be reversed by exposing them in the peri-pubertal period to environmental enrichment, an effect that might be mediated via structural changes in the hippocampus (77). These findings indicate that even 'adversely' programmed individuals can be 'rescued' by environmental interventions.

→ *In chapters 3 and 5 two intervention strategies to overcome the frequently reported adverse effects of neonatal glucocorticoid exposure will be described*

7. THE STRESS HYPO-RESPONSIVE PERIOD

The outcome of early-life experiences largely depends on the timing, frequency and duration an individual is exposed to particular environmental experiences (78-81). For instance, the timing of handling and 24h maternal separation is crucial for the outcome on adult phenotype, with handling effects being more profound if performed during the early postnatal period as compared to later in the postnatal period (78, 80). This is important since the early postnatal period coincides with onset of the stress hypo-responsive period (SHRP). The SHRP begins several days after birth and terminates around pnd 14 in rodents (28, 45). This period is characterized by very low basal corticosterone (CORT) levels and a reduced ability to show an increase in circulating CORT levels in response to mild stressors that are capable of triggering a profound glucocorticoid response in the adult animal (82). While during the SHRP the neonate's pituitary-adrenal axis is mostly hypo-responsive (83), the central component of the HPA axis does respond to stressors as is revealed by activation of hypothalamic paraventricular (PVN) neurons (84). This hypo-responsiveness of the adrenals is time and stressor specific because more severe stressors have been shown to induce a substantial CORT response (85, 86).

Interestingly, the presence of the mother is highly important in maintaining the SHRP (87, 88). Maternal presence in rodents - resulting in active maternal care and feeding - is suggested to actively regulate the responsiveness of the neonate's HPA axis during the SHRP (82, 89). Adrenocorticotropic hormone (ACTH) and CORT levels slowly increase if pups are separated from the mother, reaching peak levels after 8 h (90-92). The SHRP is disrupted and an adrenal CORT response is more easily activated after exposure to mild stressors and exogenous ACTH administration (91, 93, 94). When certain aspects of maternal behaviour are reinstated during separation, by stroking and feeding of the pups, the effects evoked by separation can be reversed (95)(see figure 1). Therefore maternal presence serves to 'buffer' the impact of stressors on the neonate. There is accumulating evidence that a human analogue to the rodent stress hyporesponsive period exists, emerging in infancy and lasting throughout most of childhood (56).

7.1 Social buffering and attachment learning

The importance of the SHRP and the role of the mother were also illustrated in the context of attachment learning. During the first days of life, rodent pups strongly depend on the mother for survival. They must learn to approach her and exhibit certain behaviours to elicit nursing behaviour from the mother. Since pups do not see or hear during the early postnatal period, attachment to the mother is based on odour learning, supported by a circuitry involving the olfactory bulb

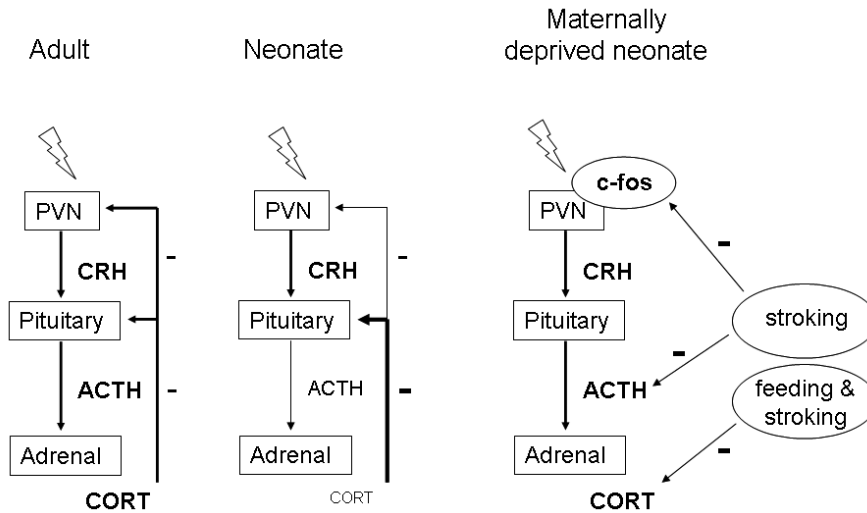

Figure 1. Stress-induced HPA axis activity of an adult, neonate and maternally-deprived neonate rat. During the SHRP the neonate rat shows a central response to stressors, which is not translated to a corticosterone response. The SHRP is characterized by hyporesponsiveness of the adrenals to stress resulting in low and stable levels of circulating corticosterone which is unbound because corticosteroid binding globulin is virtually absent at that age. Also on other levels of the HPA axis there are differences in sensitivity and reactivity compared to an adult animal. Interestingly, the HPA axis of a maternally-deprived neonate is responsive to stressors, showing some resemblance to that of an adult animal and therefore suggesting premature maturation of the stress pathways. However, when certain aspects of the maternal behaviour repertoire are reinstated (by stroking and/or feeding the neonate) during deprivation, several deprivation-induced alterations can be reversed. The size and thickness of symbols and lines represent the magnitude of responsiveness. - depicts suppression of stress-induced activity. PVN: paraventricular nucleus of the hypothalamus; CRH: corticotropin-releasing hormone, ACTH: adrenocorticotropic hormone, CORT: corticosterone.

and locus coeruleus. Pups readily learn to approach the mother based on her smell. Additionally, they learn to approach artificial odours when paired with positive stimuli such as stroking and warmth, and during the very early postnatal period even to negative stimuli such as a shock, indicating that the neonate is programmed for attachment rather than avoidance (96).

Interestingly, this attachment learning only occurs under low CORT conditions. When the neonate reaches the end of the SHRP, and mild stimuli start to elicit a rise in CORT levels, the buffering role of the mother becomes important for maintaining attachment learning. A novel odour paired with a shock will not result in a rise in CORT in presence of the mother, and the pup will learn to approach this odour. However if conditioning takes place in absence of the mother, the pup will display increased CORT levels in response to the shock, which will activate the amygdala, and will result in a shift from odour preference to odour avoidance. Older rodents (weaning age and older), having a mature HPA axis, will always show aversion to odours paired with negative stimuli since they elicit a rise in CORT

levels and subsequently activate the amygdala-fear pathway. Activation of this avoidance learning is obviously an important survival strategy for animals that can no longer depend on their caregiver and have to face the challenges of the world outside the nest (96, 97). However, when this fear pathway is triggered during the very early 'sensitive' postnatal period, due to prematurely elevated CORT levels, there will be long-term consequences for functioning of the amygdala and related pathways with a bias to enhanced activation (Daskakalis et al., submitted).

8. GLUCOCORTICOIDS DURING DEVELOPMENT

The purpose of the SHRP might be to protect the rapidly developing brain from the impact of high levels of glucocorticoids. Appropriate levels of glucocorticoids are necessary for normal development (88, 98, 99). However, not only exposure to high levels of glucocorticoids is disadvantageous, also very low or absent glucocorticoid levels adversely affect development. During normal pregnancy the activity of the maternal HPA axis is dramatically changed, leading to increased circulating glucocorticoid and ACTH levels (100).

The impact of glucocorticoids during development is frequently studied in the context of lung development. Glucocorticoid receptors (GR) are expressed in most foetal tissue and mediate the glucocorticoid action that is essential for survival. GR null mice die several hours after birth because of insufficient lung development and respiratory failure (101). Additionally, animals devoid of the actions of glucocorticoids suffer perinatally from abnormal pulmonary development due to hyper-proliferation and can be rescued by (prenatal) glucocorticoid treatment, a treatment that is obviously ineffective in GR null mice (102, 103).

In prematurely born infants, who frequently display underdeveloped lungs at birth, glucocorticoid treatment can enhance lung maturation (104, 105) by stimulating differentiation of epithelial cells (106). Exogenous glucocorticoid administration during normal development however leads to hypo-proliferation, as well as pulmonary epithelial maturation (107). It appears that glucocorticoid exposure enhances maturation/differentiation at the expense of growth/proliferation, as is reviewed by Bolt (108). These effects can be either beneficial or detrimental depending on the developmental context.

8.1 Synthetic glucocorticoid treatment for prematurity associated respiratory distress syndrome

The initial, and accidental, discovery that antenatal glucocorticoid treatment was associated with accelerated lung maturation (109) led to a first controlled study showing that this treatment prevented respiratory distress syndrome in prematurely born infants (110). Since this important publication numerous reports of randomised controlled trials have been published on this topic (111). Several major health organisations started to recommend the use of antenatal glucocorticoids to reduce the incidence of respiratory distress syndrome (112-114).

Also the postnatal administration of glucocorticoids to attenuate pulmonary inflammation contributing to the pathogenesis of bronchopulmonary dysplasia became common practice (115). By the end of the 1990's glucocorticoid use peaked at around 25% (postnatal) and 60-75 % (antenatal) of all preterm infants (116, 117). However, besides acute beneficial effects leading to reduced mortality and bronchopulmonary dysplasia, there was growing evidence that repeated courses of antenatal (118) and also early postnatal glucocorticoid treatment (119) led to adverse neurodevelopmental effects. Its image of 'magic bullet' changed into 'misguided rocket' (120)(see table 1).

Although long-term follow up studies are scarce (because treated subjects are still relatively young), there are now several reports on the 'long-lasting' outcome of neonatal glucocorticoid treatment showing alterations in cardiovascular, endocrine, immune, motor and cognitive functioning (121-124). Meta-analyses on the lasting effects of this treatment are unfortunately not yet available.

8.2 Impact of neonatal glucocorticoid treatment: rodent studies

To elucidate the neurobiological mechanism underlying the neurodevelopmental side effects reported in human preterm infants, the consequences of neonatal glucocorticoid treatment have been investigated using animal models. The use of rats is especially interesting since rodent pups are born prematurely by nature. The growth spurt of the brain during early postnatal development in rat pups shows similarities with that of human babies during the last trimester of gestation, see Box 1 (137). Since neonatal glucocorticoid treatment is usually administered between 26 and 33 weeks postmenstrual age (last trimester) in the neonatal nursery, the neonate rat pup can be used to study the neurodevelopmental impact of glucocorticoid treatment in the premature infant.

Over the last decade many studies were published on the impact of neonatal dexamethasone treatment in rats. Among the numerous findings were reports on altered social behaviour in adolescence and adulthood (142), impaired spatial learning (144) and hippocampal synaptic plasticity (144, 145), altered endocrine

Table 1. Adverse side effects of glucocorticoid treatment in preterm infants.

	Effect	Reference
Growth	Reduced somatic growth	(125-129)
	Reduced head circumference	(129)
	Reduced gray matter growth	(130)
Motor	Impaired motor performance	(131)
Endocrine	Suppressed HPA activity	(123, 132-134)
Metabolic	Hyperglycaemia	(119, 126, 127, 129, 135, 136)
Immune	Altered Th1-Th2 balance	(123)
Gastrointestinal	Perforation	(119, 126)
Cardiovascular	Hypertension	(119, 126, 129, 135, 136)
Cognition	IQ	(124, 129)

Box 1. Relevance of treatment design for human clinical situation.

Extrapolating findings from animal studies to humans can only be done with great caution. Although there is much variation between species in the complexity of the mature brain and in the timing of neurodevelopmental processes in relation to the timing of birth, there are great similarities between rodents and humans in the sequence of events during brain development. The human brain shows a growth spurt during the last trimester of pregnancy that peaks around birth. In rodents, this growth spurt takes place during the first 10 postnatal days. This suggests that the developmental stage of a rodent brain on postnatal day 1 corresponds to a human brain at the start of the third trimester of pregnancy (137), i.e. a premature infants' brain. Therefore, exposing rodents to glucocorticoids during the first postnatal days can be used as a model to study the neurodevelopmental impact of glucocorticoid treatment in the preterm infant.

Clinical protocols show much variation in timing of treatment initiation (due to timing of preterm birth), duration (1-42 days), as well as cumulative dose (0.2-14 mg/kg) of postnatal dexamethasone treatment. Dosage starts however frequently at 0.5 mg/kg (138, 139). With the current design, a 3-day course of dexamethasone treatment (0.5, 0.3, 0.1 mg/kg), we aimed to deliver a relevant treatment in terms of dosage and timing/duration. Although the cumulative dose seems relatively low, the finding that the rodent is relatively corticosteroid-sensitive compared to man (140), might explain why such severe developmental alterations have been reported in rats using a similar dosage regimen (141-144).

responsiveness to stress (141, 146, 147) and a significant shortening of the lifespan (143, 148, 149). This reduction in lifespan has been associated with heart and kidney failure (143, 149-152). Additionally, immune function has been reported to be affected by neonatal glucocorticoid exposure (153, 154). For an overview of findings in rodents, see table 2.

→ *In chapters 3, 4 and 5 of this thesis the acute and long-lasting impact of neonatal dexamethasone treatment in rats will be described. Additionally, two intervention strategies to overcome the frequently reported adverse effects will be introduced.*

9. SCOPE OF THE THESIS

It is well documented that early-life experiences have an impact on development and aging. The aim of the research described in this thesis is to explore the short- and long-term consequences of two distinct types of early postnatal experiences:

Table 2. Effects of neonatal glucocorticoid treatment in rodents.

	Effect	Drug	Time (days)	Reference
Development				
Body weight	↓	D	1-3 / 1-5 / 3-6 / 4	(142, 145, 146, 155, 156)
Brain weight	↓	D	4 / 3-6 / 7	(147, 155-157)
Cell proliferation	↓	HC	1-4 / 1-7	(158-160)
Adrenal Weight	↓	D	1-5	(146)
Eye opening	↑	D	3-6	(147, 156)
Social behaviour				
Social play	↓	C,D	1-4	(161)
	↑	D	1-3	(142)
Submission	↑	C	3-5	(162)
	↓	D	1-3	(142)
Sexual performance	=	C	1-2	(161)
	↑	D	1-3	(142)
	↓	C	1-3	(163)
Learning and Memory				
Water maze	↓	D	4 / 7 /1-3	(144, 155, 157, 164)
Hippocampal synaptic plasticity	↓	D	1-3	(144, 145, 165)
Passive avoidance	=	D	1-3	(145, 156)
Anxiety				
Elevated plus maze	=	D	1-3	(142)
	Closed arms ↑	D	3-6	(156)
Adult HPA axis				
Basal CORT	=	D	1-3 /1-5	(141, 146)
Stress-induced CORT	Novelty:↓	D	1-3	(141)
	Conditioned fear: =	D	1-3	(141)
	LPS challenge: ↓	D	1-3	(153)
	Crowding: ↑	D	3-6	(156)
	Restraint: ↓	D	1-5	(146)
ACTH-induced CORT	=	D	1-3	(141)
Lifespan				
Survival	↓	D	1-3	(143)

C: corticosterone, HC: hydrocortisone, D: dexamethasone

1) very subtle variations in maternal environment, and 2) exposure to synthetic glucocorticoids. Since the outcome of neonatal glucocorticoid exposure has been reported to be detrimental, we additionally investigated the possibility to reverse these frequently reported adverse effects of glucocorticoid exposure by both pharmacological and behavioural intervention.

Within-litter differences in maternal care
To investigate the consequences of experiencing subtle differences in maternal environment we used an adjusted model of naturally occurring variations in maternal care allowing the study of individual within-litter differences in maternal licking and grooming in Wistar rats. Endocrine responsiveness to an acute novelty stressor was investigated in adolescence and adulthood.

Hypothesis: *Maternal care is equally distributed across littermates, resulting in the development of a uniform stress phenotype within the litter.*

Neonatal synthetic glucocorticoid exposure
To investigate the impact of neonatal exposure to synthetic glucocorticoids, newborn Long Evans rats were injected with dexamethasone. We investigated the consequences of this treatment in early-life, adulthood, middle age and senescence using behavioural and molecular techniques. Additionally, we tested the rescuing potential of behavioural and pharmacological intervention strategies.

Hypotheses:
1. *Neonatal dexamethasone treatment acutely affects brain development*
2. *Neonatal dexamethasone treatment results in long-lasting alterations in endocrine and behavioural reactivity*
3. *These effects can be prevented by*
   I. blocking central GR activation prior to dexamethasone exposure
   II. handling of the neonate during the first 3 weeks of life

10. OUTLINE OF THE THESIS

Chapter 2 describes an adjustment of the original maternal care model as described in section 4.1 which allows the study of individual within-litter differences in maternal care. We report that besides differences in maternal care *between* litters, differences *within* the litter exist. Furthermore, these subtle differences in early maternal environment have long-lasting effects on the offspring's stress phenotype, although in a gender-dependent manner.

Chapter 3 describes the acute central effects of neonatal dexamethasone treatment. We report that hippocampal cell proliferation is acutely, but transiently reduced. The number of astrocytes is reduced one week post-treatment, an effect that can be fully prevented by central GR antagonist pre-treatment, which is proposed as a potential intervention strategy to prevent certain dexamethasone-induced changes in the developing brain.

Chapter 4 describes the long-term effects of neonatal exposure to dexamethasone using several behavioural paradigms. We report that although neonatal dexamethasone treatment leads to developmental alterations, the frequently reported adverse effects on adult phenotype were not observed. It is

suggested that handling of the infant during the postnatal period mediates - and potentially overrides - the outcome of neonatal dexamethasone exposure.

In **chapter 5** this hypothesis is further investigated with the goal to examine the potential of neonatal handling to reverse adverse effects induced by neonatal dexamethasone treatment. We report that the effects of dexamethasone treatment interact with those of neonatal handling in shaping the adult endocrine and behavioural phenotype.

In **chapter 6** all experimental findings are summarized and the relevance of their interactions in shaping the adult phenotype is discussed.

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