Individual differences in maternal care as a predictor for phenotypic variation later in life

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CHAPTER 1

General Introduction
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1. The influence of early life

1.1 Nature versus Nurture

*What contributes more to the area of a rectangle, its length or its width?*

That is what the famous psychologist Donald O. Hebb once answered when he was asked what he thought contributed more to personality, nature or nurture.

A continuously discussed issue in the life sciences has been the question of what determines an individual’s features most, genetic background or environmental factors. This discussion is known as the nature/nurture debate, a term introduced by Francis Galton in 1874, while he was studying trait variations in human populations. The common view at that time was that although nature is by far most important in determining an organism’s traits, nurture might also have a certain influence parallel to that. In other words, both nature and nurture affect the organism, but the two act completely independent from each other (Cowan, 1977; McEwen, 1988).

In the second half of last century, several ethologists and biologists conducted research in favor of ‘nature’, showing the strong contribution of genes to social behavior (see Krubitzer and Kahn, 2003 for review). However, around the same time, the group of Harry Harlow demonstrated the astonishing effects of ‘nurture’. They set up an animal model for depression in monkeys by subjecting them during infancy to various different social rearing conditions, with or without their mother and with or without peers, and showed that early life environment had an enormous impact on the social behavior of these monkeys (McKinney et al., 1971; Young et al., 1973). These findings underlined previous work in humans (Spitz, 1946; Robertson, 1952) and the commonly held view among psychiatrists (Freud, 1917, Mack and Semrad, 1967) that adversity in early life predisposed individuals to adult psychopathology.

Nowadays, many studies have convinced us that it is not either nature or nurture that determines certain individual characteristics, it is the interaction between the two (Lewontin, 1980; Meaney, 2001b).

1.2 Consequences of early-life experience

So how do early life environment and underlying genetic background interact in generating a certain adult phenotype? A large number of studies indicate a relationship between early life adversity and adult chronic disease. For example in humans, unfavorable childhood socioeconomic status has been shown to induce an increased vulnerability to infectious, respiratory, and cardiovascular diseases (Forsdahl, 1977; Cohen et al., 2004; Galobardes et al., 2004; Galobardes et al., 2006; Kittleson et al., 2006), and other studies have linked perturbations in perinatal environment, e.g. undernutrition, to a higher risk of coronary heart disease and metabolic dysfunction in later life (Osmond et al., 1993; Painter et al., 2005; Singhal, 2006). The development of
these effects is underlain by epigenetic processes occurring in response to early life environmental factors (see Box 1) that normally alter the genome to produce a phenotype that suits the predicted later environment best (Gluckman and Hanson, 2004). However, a mismatch between the phenotype and the environment might render the individual (human or rodent) unable to adequately respond to environmental challenges and result in an enhanced susceptibility to various chronic disorders (Pham et al., 2003; Waterland and Jirtle, 2003; Lillicrop et al., 2005; Heijmans et al., 2008; Park et al., 2008).

**Box 1 - Epigenetics: how the environment affects gene expression**

All cells in our body contain identical genetic information but every one of them only expresses a specific subset of genes, resulting in a wide variety of cell types with different functions. Dynamic regulation of gene expression occurs by different combinations of repressing and activating factors that influence transcription, but more persistent modifications to the genome also exist. This so-called epigenetic programming can occur in response to environmental factors, controls gene expression and is stably maintained through cell division.

In the cell nucleus, DNA, together with the histone proteins it is tightly wrapped around, forms the chromatin. Not only higher-order chromatin structure and organization (i.e. intra- and inter-chromosomal interactions) determine the accessibility of the DNA and thus regulate gene expression (e.g. Kosak and Groudine, 2004; Fraser, 2006; Wallace and Felsenfeld, 2007), but also within-chromatin modifications, most importantly DNA methylation (Bird, 1984) and histone acetylation, methylation and phosphorylation (Allfrey et al., 1964; Wade et al., 1997; Taverna et al., 2007; Meaney and Ferguson-Smith, 2010), are essential for transcriptional regulation.

DNA methylation occurs at cytosine residues in CpG dinucleotides and is catalyzed by the enzyme DNA methyltransferase (DNMT). It is generally associated with an inactive state of the chromatin and repressed gene expression (Bird and Wolffe, 1999), either through direct interference with transcription factor binding to the DNA (Watt and Molloy, 1988) or via methyl-cytosine binding proteins such as MeCP2, which attract transcriptional repressor molecules and chromatin-modifying enzymes (Nan et al., 1997). The majority of single CpG sites in the mammalian genome is methylated (Razin and Riggs, 1980), while most of those clustered in so-called CpG islands (containing >60% CpGs) are not (Bird et al., 1987). These CpG islands are often located in gene promoter regions (Bird, 2002), thus suggesting that methylation of these sites might be important in regulating and silencing gene expression.

Inversely related to DNA methylation is the acetylation of histones. Binding of MeCP2 to methylated cytosines leads to recruitment of histone deacetylases (HDACs), thereby ‘closing’ and inactivating the chromatin (Jones et al., 1998; Nan et al., 1998). In contrast, histone acetylation by histone acetyl transferases (HATs) increases gene transcription (Wade et al., 1997; Marmorstein, 2001) and also seems to be one of the mechanisms involved in the demethylation of DNA (Cervoni and Szyf, 2001). Functionally, dynamic DNA methylation and histone acetylation processes have been shown to cooperatively regulate synaptic plasticity and long-term memory formation (Miller et al., 2008).

Chromatin modification and DNA methylation states are maintained by a dynamic and responsive equilibrium between the actions of modifying and demodifying enzymes. However,
epigenetic patterns that are established in (anticipatory?) response to early life environmental factors, during both pre- and postnatal development, have been shown to remain stable into adulthood and can determine an individual’s phenotype for life (Reik and Dean, 2001; Weaver et al., 2004; Murgatroyd et al., 2009).

Figure 1. In the cell nucleus strands of DNA are wrapped around histone proteins, together forming the chromatin. Within-chromatin modifications, such as histone acetylation (depicted here as circled A’s) and DNA methylation (black diamonds), regulate gene expression and are in constant dynamic equilibrium.

Similarly, the risk of mental disorders – including depression – in humans is enhanced by early life adversity (McEwen, 2003; Arnow, 2004; Raikkonen et al., 2010), often against a background of genetic vulnerability. Depression is moderately heritable (about 40%): several gene polymorphisms have been shown to affect the susceptibility to developing depression (Sullivan et al., 2000; Levinson, 2006). Interactions between these gene variants and childhood adversity have been found in patients suffering from depression, and also post-traumatic stress disorder (PTSD) (Caspi et al., 2003; Binder et al., 2008; Bet et al., 2009). Another risk factor for the development of depression is gender. Epidemiological studies have shown that depressive disorders are more prevalent in women than in men (Kessler et al., 1993; Bland, 1997; Ustun, 2000).

Traditionally, studies on the molecular basis of depression focused mainly on disruptions of monoamine neurotransmitter systems, i.e. serotonin, dopamine and noradrenaline (known as the ‘monoamine hypothesis’) (Coppen, 1967; Hirschfeld, 2000). However, the heterogeneity of the disease implies that other biological mechanisms might also be involved (Nestler et al., 2002). Nowadays, more and more evidence points in the direction of a role of the stress system in the development of depression, particularly because exposure to stress, either in early life, as mentioned above, or in adulthood, has been shown to increase the chance of psychopathology (Kessler, 1997; Heim and Nemeroff, 2001; Lupien et al., 2009; Sterner and Kalynchuk, 2010). Several studies have described hyperactivity of the hypothalamus-pituitary-adrenal axis (HPA axis, see box 2) in depressed patients (Young et al., 1991; Holsboer, 2000; Pariante, 2003), leading to increased levels of cortisol (Rubinow et al., 1981; Pariante, 2009) and corticotrophin-releasing hormone (CRH) in the hypothalamus (Raadsheer et al., 1995).
How adverse (early) life events cause these alterations in HPA reactivity is not completely clear yet, but several studies have shown direct epigenetic modulation of the glucocorticoid receptor promoter (Weaver et al., 2004; McGowan et al., 2009), the glutamic acid decarboxylase 1 (GAD1) promoter (Zhang et al., 2010) and the gene encoding brain-derived neurotrophic factor (BDNF; Tsankova et al., 2006) in the hippocampus.

In sections 2 to 4 I will first give a brief overview of what is currently known about stress and its effects on key limbic structures and then proceed by describing the detailed animal models that allow studying the consequences of early life adversity on the structure and function of these same structures (section 5).

2. Stress

Being confronted with a situation that is either emotionally or physiologically challenging can potentially disturb an individual’s bodily homeostasis. If such a so-called stressor is perceived as being a threat this is referred to as ‘stress’.

2.1 Stress, the HPA axis & steroid hormones

Stress, a term that was introduced by Hans Selye in the 1930’s, is a familiar phenomenon to all of us; everyone has had the experience of feeling stressed or encountering a stressful situation. Stress in itself is not a bad thing. Our innate stress response helps us cope with and adapt to (potentially) threatening situations that we experience. When a situation is perceived as stressful, or in other words, as being a threat to the homeostasis of the body, physiological and behavioral responses are triggered that allow the individual to cope with this challenge. First, the sympathetic nervous system is activated, leading to a rapid increase in adrenaline and noradrenaline levels, preparing the body for a so-called ‘fight or flight response’ (Cannon, 1929): heart rate, respiration rate, blood pressure, energy consumption and attention are increased, while digestive activity is inhibited. Additionally, slow activation of the hypothalamus-pituitary-adrenal (HPA) axis is manifested (see box 2), resulting in the release of corticosteroids (reaching peak levels about 15-20 minutes after the onset of the stressor, De Kloet et al., 2005), which replenish energy resources, but also suppress the immune system and inhibit reproductive behavior. Importantly, they enhance cognitive functioning, thereby warranting storage of relevant information for future reference (Oitzl and de Kloet, 1992; McEwen and Sapolsky, 1995; De Kloet et al., 2005; Joels et al., 2006).

However, altered stress reactivity (e.g. due to genetic background or previous experience of the animal), leading to a response that is either excessive or inadequate in magnitude and duration, or chronic exposure to a stressor, can increase an organism’s
vulnerability to develop various mental disorders (Stratakis and Chrousos, 1995; Brown et al., 2004; De Kloet et al., 2005).

Box 2 – HPA axis

When an organism is subjected to a stressful situation, one of the physiological systems that is activated in order to restore the individual’s homeostatic balance is the HPA axis. In response to the stressor, the paraventricular nucleus (PVN) of the hypothalamus produces corticotrophin releasing hormone (CRH) and vasopressin (AVP), and these peptides then cause the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. ACTH is released into the bloodstream and stimulates the adrenal cortex to produce corticosteroids (corticosterone in rodents, cortisol in humans) (Lightman et al., 2002; Volpi et al., 2004). Via the circulation, these corticosteroid hormones reach and affect organs throughout the body, including the brain. Corticosteroids inhibit their own production by downregulating both hypothalamic CRH release and ACTH secretion by the pituitary via binding to glucocorticoid receptors in these areas (De Kloet and Reul, 1987; Sawchenko, 1987; Feldman et al., 1992; Pinnock and Herbert, 2001), consequently dampening the stress response (Sapolsky, 2000; De Kloet et al., 2005). Corticosteroids also affect higher brain areas such as the hippocampus, which indirectly contribute to HPA negative feedback modulation as well (Sapolsky et al., 1985; Magarinos et al., 1987; Jacobson and Sapolsky, 1991; Feldman and Weidenfeld, 1999; Furay et al., 2008).

2.2 Corticosteroids & receptors

The secretion of glucocorticoids occurs in a pulsatile fashion, i.e. more or less every hour a surge of corticosterone (in rodents) or cortisol (in humans) is released into the bloodstream, even in stress-free conditions (Weitzman et al., 1971; Jasper and Engeland, 1991; Lightman and Conway-Campbell, 2010). The peaks of these pulses vary in height during the day, resulting in a circadian rhythm of circulating corticosteroid levels.
In the brain, corticosteroids can act via two receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), which are both abundantly present throughout the brain, but differ in their distribution and affinity for corticosteroids (Reul and de Kloet, 1985; Arriza et al., 1988). The MR is located mainly in the limbic system and has a ten-fold higher affinity for its natural ligand corticosterone (or cortisol in humans) than the GR. Therefore, it remains almost constantly occupied, even during the nadir of the circadian rhythm when corticosterone levels are very low (Reul et al., 1987; De Kloet et al., 1998; Joels et al., 2008). Tonic MR activation in the hippocampus has been shown to be important for maintaining neural integrity and a stable excitatory tone (Wossink et al., 2001; Joels et al., 2007; Krugers et al., 2007) and is implicated in the downregulatory control over HPA axis activity through trans-synaptic inhibition of CRH-producing neurons in the paraventricular nucleus (PVN; Reul et al., 2000; Herman et al., 2003; Kellner and Wiedemann, 2008). Conversely, the GR is present much more ubiquitously in the brain, with the highest density reported in the PVN of the hypothalamus (Fuxe et al., 1985; Herman et al., 2003; De Kloet et al., 2005). Since its affinity for corticosteroids is relatively low, GR occupancy follows the endogenous pulsatile corticosterone secretion pattern (Windle et al., 1998; Lightman et al., 2008) and consequently is only extensively activated when corticosterone levels are increased, i.e. after stress or during the peak of the diurnal rhythm – just before the start of the active period (Reul and de Kloet, 1985; Joels et al., 2008). Also binding of the activated GR to its response elements on the DNA, after translocation of the receptor-ligand complex to the nucleus, dynamically follows the changes in corticosterone concentration. It was reported that there is a rapid turnover of GR in the nucleus, whereas the depletion of nuclear MR occurs at a much slower pace (Conway-Campbell et al., 2007), which is in line with its tonic activation.

These receptors have long been considered to be solely located in the cell cytosol, from where they translocate to the nucleus upon activation where they exert their actions as ligand-driven transcription factors, thus controlling gene expression (Carson-Jurica et al., 1990; Beato et al., 1995; Htun et al., 1996; Reichardt and Schutz, 1998; Schoneveld et al., 2004). However, recently, evidence has come up for the existence of membrane-bound MRs and GRs, that exert rapid, non-genomic effects after activation by corticosteroids (Orchinik et al., 1991; Johnson et al., 2005; Karst et al., 2005; Tasker et al., 2006; Karst et al., 2010; Roozendaal et al., 2010). It is now proposed that in the hippocampus, where MR and (nuclear) GR are colocalized (Van Eekeleen and De Kloet, 1992; Han et al., 2005), membrane MRs, that have a GR-like affinity to corticosterone, implement the initial stress response in concert with other rapid stress-modulating agents such as (nor)adrenalin, CRH and AVP, whereas intracellular GRs regulate the later adaptive phase and the eventual termination of the response (Joels et al., 2006; Joels et al., 2008).
3. The hippocampus

The hippocampus is one of the main targets of corticosterone, due to its high expression levels of both MR and GR. Below the anatomical and functional characteristics of the hippocampus as well as the main effects of stress on this structure will be discussed.

3.1 Anatomy

The hippocampus is located – bilaterally - in the floor of the temporal horn of the lateral ventricles. In rats, this structure begins dorsally close to the septum (at the midline of the brain), and then arches out caudo-laterally, ending in the temporal pole. The hippocampus is closely connected to the amygdala and the (hypo)thalamus, and forms, together with these and a limited number of other structures, the limbic system, which is associated with the regulation of emotions such as fear, and novelty detection (Purves et al., 2004).

The hippocampus belongs to the archicortex, which is phylogenetically older than the neocortex and less complicated. It has at most three cellular layers (instead of six in other cortical regions) and can be divided in different distinct subfields: the cornu ammonis (CA; Ammon’s horn), consisting of areas CA1, CA2 and CA3 and containing pyramidal cells, the hilus and the dentate gyrus (DG), which is arched around the hilus and contains small, densely-packed, granule neurons. Within the hippocampus, information is mainly processed along the so-called unidirectional trisynaptic circuit (Amaral and Witter, 1989; Lopes da Silva et al., 1990). Afferent fibers originating from the entorhinal cortex and conveying polymodal sensory information enter the hippocampus via the perforant path and synapse onto the dentate granule cell dendritic trees. The granule cells then relay this information and send their mossy fibers towards the CA3 area which in turn projects onto the apical dendrites of the CA1 via the Schaffer collaterals. Finally, axons from the CA1 pyramidal cells leave the hippocampal formation through the subiculum and again project to the entorhinal cortex (see Figure 3). It must be mentioned though that the entire repertoire of input to and connections within the hippocampus are in fact more complex and extensive (van Strien et al., 2009).
3.2 Adult neurogenesis

For a long time the mature brain in mammals was assumed to be structurally stable and to remain unchanged throughout adult life. This would be in contrast to most other organs, which we know contain stem cells that maintain the ability to proliferate and thus allow damage repair and continued growth. In the 1960's however, Joseph Altman initiated a shift in this dogma (Gross, 2000). He treated rats with tritiated thymidine, which incorporates into the DNA of proliferating cells, thereby showing the existence of neurogenesis – the birth of new neurons – in the adult brain (Altman, 1962). This phenomenon only occurs in two distinct regions of the brain, the subgranular layer of the hippocampal dentate gyrus and the subventricular zone, from where newborn neurons migrate through the rostral migratory stream to the olfactory bulbs (Altman and Das, 1965; Altman, 1969; Kaplan and Hinds, 1977; Cameron et al., 1993). Studies by Fernando Nottebohm in songbirds illustrated the relevance of adult neurogenesis, by showing that it occurs as a function of different variables, such as season and hormone levels. Neurogenesis in songbirds is modulated by environmental factors, like learning experience, and the newborn neurons are functionally incorporated into the network (Goldman and Nottebohm, 1983; Paton and Nottebohm, 1984; Barnea and Nottebohm, 1994, 1996). Eventually, adult neurogenesis was also reported to exist in primate (Gould et al., 1998; Gould et al., 1999b; Kornack and Rakic, 1999) and human (Eriksson et al., 1998) hippocampus.

In the hippocampal dentate gyrus, the neurogenetic process starts with the proliferation of neural stem cells in the subgranular zone, the innermost part of the granule cell layer. Some of the newly generated cells remain in the progenitor stage, while others enter a post-mitotic stage, undergo fate determination and start to differentiate. Many of these new cells die within one to two weeks after they are generated (Dayer et al., 2003; Lie et al., 2004; Abrous et al., 2005), but the surviving immature neurons migrate into the granular cell layer, start to send axon projections towards the CA3 area and develop extensive dendritic trees (Hastings and Gould, 1999; Markakis and Gage, 1999; Kempermann et al., 2003. Finally, they become functionally integrated into the existing neuronal network, receiving synaptic inputs and being electrically active (Van Praag et al., 2002; Kempermann et al., 2004; Toni et al., 2008; Mongiat et al., 2009).

During the separate phases of the neurogenic process, the new neurons express different unique proteins that can be visualized with immunohistochemical techniques. Three of those were used in the studies described in this thesis. First, the protein Ki-67 is expressed during all active phases of the cell cycle (which lasts about 24 hours), but not during rest, and is therefore used as a proliferation marker. Second, to examine the survival of newborn cells, labeling with the thymidine analogue BrdU (5’-bromo-3’-deoxyuridine) is commonly used. Like tritiated thymidine, BrdU is stably incorporated into the DNA of dividing cells and remains there indefinitely, thus allowing
determination of the survival rate of a cell population born at the time of BrdU application. When the time between injection and sacrifice of the animal is sufficiently short (e.g. 24 hours) BrdU can also be used as a marker for proliferation. Finally, to identify young, immature neurons, the protein doublecortin (DCX) is used, a microtubule associated protein (MAP) that is expressed in migrating and differentiating neurons (Francis et al., 1999c). DCX in rats is transiently expressed in progenitor cells and developing neurons up to two to three weeks old (Brown et al., 2003). Since this temporal expression window is quite large, DCX-labeled cells of different ages and with very different morphological characteristics can be distinguished in the hippocampal dentate gyrus at any point in time (Plumpe et al., 2006; Oomen et al., 2010b).

In young adult rats, about 9000 new neurons are generated in the dentate gyrus each day (Cameron and McKay, 2001), but the rate of neurogenesis rapidly decreases with age (Seki and Arai, 1995; Kuhn et al., 1996; Heine et al., 2004). Whereas in young to middle-aged rodents, simultaneous cell death occurs at a slower pace, resulting in a net addition of new neurons to the dentate granular layer (Bayer et al., 1982; Boss et al., 1985), in old rats the rates of neurogenesis and apoptosis are in equilibrium (Boss et al., 1985; Heine et al., 2004). The continuous turn-over of neurons and their rapid integration into the network presumably contribute significantly to hippocampal structural and functional plasticity.

3.3 Function
In both rodents and humans, the hippocampus is critically implicated in learning and memory processes and involved in encoding, storage and retrieval of information. This became evident from observations that lesions of the hippocampus lead to severe memory impairments in humans, such as in the well-known case of patient Henry Molaison (H.M.), who suffered from intractable epilepsy. As a treatment most of both his temporal lobes (including the hippocampi and amygdala) were removed. Without affecting other cognitive functions or overall intelligence, the surgery resulted in severe anterograde amnesia (a disability to form new memories) and partial retrograde amnesia (loss of recent existing memories) (Scoville and Milner, 1957). Studies in other patients confirmed the importance of the hippocampus in declarative memory, i.e. the storage and recall of events and facts, but not in procedural learning or short-term memory (Scoville and Milner, 1957; Milner, 1972; Zola-Morgan et al., 1986; Rempel-Clower et al., 1996). The sequence of events in declarative memory is now thought to be as follows: the neocortex first processes the incoming information which is then transmitted to the hippocampus where it is temporarily stored (Squire and Alvarez, 1995; Nadel and Moscovitch, 1997). Then, as the memory matures, intercortical connections become stronger, the prefrontal cortex takes over the integrative role and the memory becomes less dependent on the hippocampus (McClelland et al., 1995; Frankland and Bontempi,
This consolidation process can occur surprisingly rapidly, within 48 hours after acquisition, when an associative network or ‘schema’ is present in the neocortex in which the new (relevant) information can be stored (Tse et al. 2007).

The hippocampus was shown to be particularly involved in spatial memory, a specific form of declarative memory, both in humans and in rodents (Winson, 1978; Smith and Milner, 1981; Morris et al., 1982; Maguire et al., 1997; Kessels et al., 2001). This was confirmed by the discovery of ‘place cells’ in the hippocampus: granule cells or pyramidal cells that only fire when the animal is in a specific spatial location, thereby creating a spatial map of the environment in the hippocampus (O’Keefe and Dostrovsky, 1971; Georges-Francois et al., 1999;Ekstrom et al., 2003).

Increased synaptic connectivity (between existing neurons) is considered to be the neurobiological substrate of hippocampus-dependent learning processes. The best-investigated example of such increased connectivity is known as long-term potentiation (LTP; described in box 3). Also structural plasticity like dendritic remodeling and neurogenesis may contribute to hippocampal learning. Structural rewiring, including outgrowth and retraction of dendrites and synapse formation and elimination, has been suggested to play a role in stable memory storage in the brain (Chklovskii et al., 2004; De Roo et al., 2008). Specific ablation or inhibition of dentate gyrus adult neurogenesis is associated with deficits in spatial memory, object recognition memory, contextual fear conditioning and stress-induced social avoidance (Saxe et al., 2006; Winocur et al., 2006; Jessberger et al., 2009; Lagace et al., 2010). Conversely, upregulated neurogenesis, for example in response to exercise, has been shown to correlate with enhanced performance on spatial learning tasks (Gould et al., 1999c; Van Praag et al., 1999; Van der Borght et al., 2007), and in addition the process of learning itself was found to regulate hippocampal cell proliferation and survival (Gould et al., 1999a; Ambrogini et al., 2000; Dobrossy et al., 2003; Leuner et al., 2006), although other studies contradict these results (Van der Borght et al., 2005; Ehninger and Kempermann, 2006, Oomen et al. personal communication).

Finally, there is evidence that different hippocampal subareas are involved in different aspects of memory. The CA1 area seems to play a role in temporal pattern separation, whereas the dentate gyrus is thought to support spatial pattern separation (Gilbert et al., 2001; Leutgeb et al., 2007; McHugh et al., 2007; Bakker et al., 2008; Brun et al., 2008). Pattern separation is necessary to reduce memory interference and refers to the process that transforms similar memories into distinct, non-overlapping representations of the temporal and spatial relationships encompassing different events. The DG has been proposed to play a specific role in hippocampal functioning by acting as a filter or gate for incoming information (for review, see Hsu, 2007): many granule neurons project onto only one CA3 pyramidal cell, thereby selectively scaling their activity to pass on only the most potentiated dentate patterns (i.e. Hebbian LTP, see box 3).
Box 3 – Long-term potentiation (LTP)

In 1949 Donald O. Hebb proposed that connections between neurons might be strengthened when presynaptic input correlates with simultaneous postsynaptic firing and that these synaptic modifications could be the basis of plasticity in the brain (Hebb, 1949). This phenomenon was first experimentally demonstrated by Bliss and Lomo in the hippocampal dentate gyrus (Bliss and Lomo, 1973) and is now known as ‘long-term potentiation’ (LTP): the persistent strengthening of synaptic connections leading to an increased postsynaptic response upon stimulation of a neuron or neuronal field, which can be induced by brief high-frequency stimulation of excitatory afferents.

LTP is considered to underlie learning and memory processes in the hippocampus (Pastalkova et al., 2006; Whitlock et al., 2006), and its mechanisms are therefore extensively studied. Excitatory synaptic transmission in the hippocampus is mainly mediated by glutamate. After release into the synaptic cleft, this neurotransmitter can bind to two ionotropic channels located in the postsynaptic membrane: the N-methyl-D-aspartate (NMDA) receptor and the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor. These ion channels allow the passage of positively charged ions into the postsynaptic cell, consequently depolarizing the membrane. However, physiologically, NMDA receptors are blocked by magnesium, which is only removed when the membrane is already depolarized. Thus, glutamate acts on the AMPA receptor first, causing membrane depolarization which enables the release of the voltage-dependent Mg2+-block of the NMDA receptor, leading to further cell depolarization and calcium influx (Nowak et al., 1984; Coan and Collingridge, 1985). The elevated levels of Ca2+ in the postsynaptic dendrite activate a cascade of Ca2+-dependent kinases, ultimately resulting in long-term potentiation of the synapse by increased insertion and phosphorylation of AMPA receptors into the synapse and enhanced gene expression which may induce structural changes (Neves et al., 2008).

As discussed in section 3.4, stress and stress hormones have been shown to affect learning and memory processes, and accordingly also hippocampal synaptic plasticity. The hippocampus contains abundant low-affinity glucocorticoid- and high-affinity mineralocorticoid- receptors (Reul and de Kloet, 1985; Herman et al., 1989), through which the effects of corticosterone (in rodents) are exerted. Predominant MR activation is generally associated with enhanced LTP induction, whereas additional GR activation by high levels of corticosterone normally impairs the capacity to induce LTP (Pavlidis et al., 1995; Pavlidis et al., 1996; Smriga et al., 1998; Alfarez et al., 2002; Korz and Frey, 2003; Krugers et al., 2010). However, the opposite effect has also been observed, depending on the timing of the stressor, the type of stressor, the life history of the animal and the exact location in the hippocampus (Kim and Diamond, 2002; Wiegert et al., 2006; Joels and Krugers, 2007; Pu et al., 2007; Champagne et al., 2008; Maggio and Segal, 2007).

3.4 The hippocampus, stress and depression

The hippocampus is involved in HPA axis regulation and itself very susceptible to stress and corticosteroid effects as well (for review, see Fuchs and Flugge, 1998; McEwen, 1999); structural and chemical changes occur in response to both acute and chronic stressors (or treatment with corticosteroids). However, whereas a single exposure to a stressful event, i.e. acute stress, induces an adaptive response that promotes survival of the organism (e.g.
improved memory), prolonged or repeated exposure to a stressor, i.e. chronic stress, leads to imbalance in stress regulatory systems and persistent changes in the brain (e.g. impaired memory, increased fear) (McEwen, 2004). In rats, repeated restraint stress or chronic corticosterone administration causes hippocampal dendritic atrophy, mainly in the CA3 (Uno et al., 1989; Woolley et al., 1990; Watanabe et al., 1992; Magarinos and McEwen, 1995; Alfarez et al., 2009) and a decrease in adult neurogenesis in the DG (Gould and Tanapat, 1999; Heine et al., 2004; Mirescu and Gould, 2006). Concomitantly, learning impairments (Luine et al., 1994; McEwen and Magarinos, 1997) and alterations in hippocampal LTP (see box 3) occur.

Therefore it is not surprising that changes in hippocampal morphology and function are often observed in patients suffering from stress-related diseases like depression (for review, see Sapolsky, 2000). Multiple studies have found a decrease in hippocampal volume in depressive patients (Sheline et al., 1996; Bremner et al., 2000; Campbell and Macqueen, 2004; Videbech and Ravnkilde, 2004), which was associated with illness duration (MacQueen et al., 2003) and cognitive deficits (Burt et al., 1995; Sheline et al., 1999). Also in combat veterans suffering from PTSD hippocampal volume reductions and concomitant attenuations in cognitive performance were observed (Bremner et al., 1997; Gilbertson et al., 2002). Interestingly, in a twin study, it was reported that the healthy twin brothers showed similar deficits (Gilbertson et al., 2002; Gilbertson et al., 2007), suggesting that at least for PTSD hippocampal atrophy might be a risk factor and fueling the question what happens in depression: does the reduction in hippocampal volume precede development of depression or is it a consequence of the disease? Several studies have shown that hippocampal volume reduction particularly occurred in depressive patients that experienced childhood adversity (Vythilingam et al., 2002; Woon and Hedges, 2008; McKinnon et al., 2009; Rao et al., 2010), and also genetic polymorphisms have been associated with a smaller hippocampus in depressed patients (Lyons et al., 2001; Frodl et al., 2007), indicating that the volume reduction indeed might be an important predictor for vulnerability to stress-related psychopathology, rather than a consequence of the disease.

What causes this decrease in hippocampal volume has not been completely elucidated. One possibility is the ‘neurogenic hypothesis of depression’ (Jacobs et al., 2000; Malberg, 2004), stating that a decrease in neuronal cell number due to reduced neurogenesis, but not enhanced apoptosis (Lucassen et al., 2001), might underlie the pathophysiology of depression. Indeed, adult neurogenesis is inhibited by stress and glucocorticoids (see above, Cameron and Gould, 1994) and upregulated by many antidepressant treatments (Duman et al., 2001; Malberg and Schechter, 2005; Warner-Schmidt and Duman, 2006; Boldrini et al., 2009). However currently, changes in neurogenesis are increasingly considered to be a derivative rather than a cause of depression, because ablation of hippocampal neurogenesis in rodents does not result in a
depressive-like phenotype and the extent of hippocampal volume loss after experimentally blocking neurogenesis does not match the volumetric changes seen in depression (Sahay and Hen, 2007). Another option is the ‘neurotrophin hypothesis’ (for review, see Chourbaji et al., 2010), which links neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF), with depression. This idea is based on studies reporting that BDNF levels are associated with hippocampal atrophy, downregulated in depressed patients and upregulated by antidepressants (Nibuya et al., 1995; Smith et al., 1995; Altar, 1999; Chen et al., 2001; Hindmarch, 2002; Karege et al., 2002).

4. The prefrontal cortex

4.1 Structure and network connections

A brain region that is closely connected to many cortical and subcortical regions including the hippocampus is the prefrontal cortex (PFC). In humans and other primates, this structure is well-evolved and extends over the entire frontal lobe, whereas in rodents and other lower mammals its volume is much smaller and its cortical fields are less developed and less discernable (Uylings et al., 2003). The prefrontal cortex can be subdivided into anatomically different subfields (see Figure 4) which are thought to exert different functions (see below). In humans these are the dorsal and ventral PFC, which in turn both consist of a lateral and a medial part (Arnsten, 2009); in rodents the two main subregions are the medial prefrontal cortex (mPFC) – further subdivided into the infralimbic (IL), prelimbic (PrL) and dorsal anterior cingulate cortex (ACd or Cg) – and the orbitofrontal cortex (OFC) (Krettek and Price, 1977; Uylings et al., 2003).

It remains elusive to what extent exactly the anatomical and functional organization of the different PFC subareas is homologous in rats and humans (Uylings et al., 2003). In primates, including humans, the dorsolateral PFC is extensively connected to sensory and motor cortex areas, whereas the ventromedial and orbitofrontal cortices are mainly connected to subcortical and limbic structures such as the hippocampus, amygdala, nucleus accumbens and (hypo)thalamus (Barbas and Blatt, 1995; Price et al., 1996; Barbas, 2002; Arnsten, 2009). The PFC also has reciprocal projections to monoamine nuclei in the midbrain and brainstem – i.e. the locus coeruleus (noradrenaline), the substantia nigra and ventral tegmental area (dopamine), and the raphe nuclei (serotonin) (Porrino and Goldman-Rakic, 1982). In rats, it is possible to much more elaborately investigate the anatomical connections between the PFC and other brain areas by means of anterograde and retrograde tracing techniques, and these have thus been described in detail (Dalley et al., 2004; Vertes, 2004; Gabbott et al., 2005; Hoover and Vertes, 2007). In general, although the rodent PFC is not as specialized and differentiated as in primates, its functional and structural segregation seems to be quite
comparable. The functional connectivity from the infralimbic area is largely homologous to the primate orbitomedial PFC, and prelimbic projections resemble those of the dorsolateral PFC in primates (Heidbreder and Groenewegen, 2003; Vertes, 2004; Radley et al., 2006a).

### 4.2 Function

In general, the PFC exerts cognitive control over other brain areas. It plays an important role in various higher cognitive functions, emotional processing and the regulation of stress responses. A famous example of what implications damage to the prefrontal cortex can have is the case of Phineas Gage, a railroad construction worker whose head was penetrated by an iron rod in an accident, thereby causing extensive damage to his frontal lobes. The accident drastically and permanently changed his personality; he went from being efficient, responsible and well-organized to displaying a range of emotional and behavioral problems (e.g. cognitive inflexibility, impulsivity, and socially inappropriate behavior) (Harlow, 1868). Similar changes were later also observed in other patients (e.g. Damasio et al., 1990; Cato et al., 2004) and in animals in lesion-studies (for review, see Heidbreder and Groenewegen, 2003). Behaviors that are affected by PFC damage or inactivation include decision-making (Bechara et al., 1996; Moretti et al., 2009, De Visser et al. personal communication), goal-directed behavior (Fuster, 1985; Balleine and Dickinson, 1998; Tran-Tu-Yen et al., 2009) and anxiety and emotional learning (Morgan and LeDoux, 1995; Shah and Treit, 2003; Farrell et al., 2010).
The anatomically distinct subareas of the PFC described earlier execute different functions, which appear similarly equivalent between primates and rodents (Ongur and Price, 2000). Basically, the PFC can be functionally divided in a dorsal and a ventral part. Based on experimental lesioning studies in rodents, the dorsal part of the medial prefrontal cortex, including the dorsal anterior cingulate and the dorsal prelimbic area, has been shown to be mainly implicated in the temporal patterning of behavioral sequences, memory for motor responses and response selection (Aultman and Moghaddam, 2001; Heidbreder and Groenewegen, 2003; Dalley et al., 2004). Correspondingly, the dorsal PFC in humans and monkeys (consisting of a medial and a lateral part) is involved in executive functions: working memory, error monitoring and regulating attention, thought and action (Goldman-Rakic, 1987; Fuster, 2001; Modirrousta and Fellows, 2008). On the other hand, the ventral part of the mPFC in rats, comprising the ventral prelimbic, infralimbic and medial orbital areas, and largely homologous to the ventral PFC in humans, plays a role in emotional processing (due to its close connection to the amygdala), but is also responsible for behavioral flexibility, attentional set shifting and response inhibition (Robbins, 1996; Ragozzino et al., 1999; Birrell and Brown, 2000; Ongur and Price, 2000; Heidbreder and Groenewegen, 2003; Dalley et al., 2004).

Finally, the prefrontal cortex is important in regulating autonomic and endocrine stress responses and in the modulation of adaptive coping responses to stress (Sullivan and Gratton, 2002; Figueiredo et al., 2003; Sullivan, 2004; Amat et al., 2005; Amat et al., 2006; Radley et al., 2006a; Weinberg et al., 2010). Through the high density of glucocorticoid receptors it contains (Meaney and Aitken, 1985; McEwen et al., 1986; Sarrieau et al., 1988a; Sanchez et al., 2000) it contributes to negative feedback control of the HPA axis. In rats with lesions in the anterior cingulate cortex, plasma ACTH and corticosterone levels after restraint stress were increased (Diorio et al., 1993), whereas lesions in the infralimbic cortex caused an attenuated CORT-response to immobilization stress (Radley et al., 2006a). Not only is there a dorso-ventral dissociation in PFC emotional processing, but there is also evidence for hemispheric specialization, both in humans and in rats (Carlson et al., 1993; Davidson, 1998; Sullivan and Gratton, 1999), with the right mPFC regulating the response to long-term stressful situations and the left mPFC being implicated in the rapid stress response (see Czeh et al., 2008).

4.3 Stress and the PFC

Like the hippocampus, the PFC not only regulates stress responses, it is also very susceptible to stress and corticosteroids itself. Post-mortem and functional imaging studies of patients suffering from stress-related mental illnesses like major depressive disorder (MDD), bipolar disorder or post-traumatic stress disorder (PTSD) revealed alterations in structure, such as volume reduction and decreased cell density, and
impaired activity of the medial PFC (Rajkowska et al., 2001; Bremner, 2002; Liberzon and Phan, 2003; Pizzagalli et al., 2004; Hains and Arnsten, 2008), and thus the effects of stress on the PFC have been extensively studied.

Chronic application of corticosterone to rats resulted in reorganization of the apical dendritic tree in mPFC pyramidal neurons, i.e. dendritic arborisation was increased proximal to the soma, whereas distally the dendritic tree was impoverished compared to intact animals (Wellman, 2001; Cerqueira et al., 2007). Likewise, repeated restraint stress in adulthood has been shown to result in atrophy of the apical dendritic tree (Cook and Wellman, 2004; Radley et al., 2004; Izquierdo et al., 2006; Perez-Cruz et al., 2007; Shansky et al., 2009) and a reduction in spine density (Radley et al., 2006b; Shansky and Morrison, 2009) in layer II/III pyramidal cells of the prefrontal cortex in rodents. Correspondingly, performance on attentional set-shifting, a mPFC-dependent cognitive task, was impaired after the same immobilization stress (Liston et al., 2006). Excessive prefrontal levels of dopamine and noradrenaline, the release of which has been shown to be increased after flattening of the endogenous glucocorticoid rhythm or high stress exposure (Finlay et al., 1995; Minton et al., 2009), likely contributed to this cognitive impairment (Murphy et al., 1996; Arnsten and Goldman-Rakic, 1998; Birnbaum et al., 1999; Mizoguchi et al., 2000; Vijayraghavan et al., 2007). Acute stress or corticosterone application on the other hand, resulted in enhanced synaptic transmission and working memory function in the PFC (Yuen et al., 2010).

During early postnatal life the prefrontal cortex undergoes critical developmental changes, especially concerning the maturation of mesocortical dopamine projections and also GABAergic neurons (Benes, 1995; Vincent et al., 1995; Benes et al., 2000). Therefore, it is not surprising that neonatal maternal separation stress or variations in maternal care have been found to alter dopamine and GABA transmission in the adolescent and adult rodent PFC (Matthews et al., 2001; Caldi et al., 2003; Jezierski et al., 2007; Helmeke et al., 2008). Repeated maternal separation (MS), compared to daily handling, was related to a decrease in PFC glucocorticoid receptor expression, and a concomitant increase in stress-induced plasma ACTH and corticosterone levels (Ladd et al., 2004). Additionally, early life stress has been shown to influence PFC pyramidal cell shape. Basal dendritic atrophy and a reduced spine number in PFC neurons was observed after repeated MS compared to non-disturbed controls in young-adult rats, whereas early handling induced an increase in both spine density and dendritic length (Monroy et al., 2010). A different study showed a timing effect of separation stress on PFC morphology: animals that were maternally separated prior to the stress-hyposresponsive period (SHRP; see section 5.1) exhibited a decrease in dendritic spine density in the anterior cingulate cortex, whereas separation after the SHRP resulted in an increased spine density (Bock et al., 2005). Interestingly, both paradigms were associated with elevated basal CORT levels at PND21.
Separation stress during the SHRP did not affect either spine density or basal CORT levels at weaning.

5. Modeling the effects of early life experience

As argued in section 1, early life adversity – in addition to genetic load – is associated with an increased vulnerability to psychopathology later in life, probably involving dysregulation of the HPA axis (for review, see Tarullo and Gunnar, 2006; Heim et al., 2008), which is assumed to be important for the development of hippocampal and PFC cell connectivity. This leads to the general question of this thesis: what precisely is the influence of early life environment on adult structure and function of both the hippocampus and the PFC?

Environmental factors are hard to control and manipulate in humans, so animal models have been developed to study the underlying regulatory mechanisms. Since in animals the predominant environmental factor in early life is the mother, most of these models interfere with normal mother-offspring interactions. In this thesis I will focus on the different postnatal manipulation paradigms in use, which cause varying amounts of stress in the offspring. A second advantage of such animal models is that one can study in detail the intermediate endpoints for behavior, such as cellular structure, the expression of essential proteins and LTP.

5.1 Maternal deprivation paradigm

A frequently used and robust model for early life stress is depriving an infant from its mother for prolonged and/or repeated periods of time. A lot of this maternal separation work has been done in non-human primates, as their rearing strategy is very similar to that in humans (i.e. a prolonged period of maternal care, complex social environments and close mother-infant relationships). Although a variety of approaches has been used, in general these studies show long-lasting increases in HPA axis responsivity after maternal deprivation and effects of early life adversity on adult emotionality and depression-like behavior (for review, see Pryce et al., 2005; Neigh et al., 2009). In rodents, too, multiple paradigms of maternal separation are being used, either repeatedly for shorter periods of time (i.e. 3 to 12 hours), or only once, but then with a prolonged duration (up to 24 hours; this is often referred to as maternal deprivation). The first two weeks after birth, the presence of the mother is essential for the well-being of the offspring. During this time, rodent pups are not fully developed yet and rely on their mother for food and warmth, but also for maintaining their bodily homeostasis through urination and defecation, which they cannot do without tactile stimulation from the dam. Also, during post-natal development, the presence of the mother is crucial for maintaining HPA axis hyporesponsivity in the offspring, thereby protecting the sensitive...
developing brain from excess levels of corticosterone (De Kloet et al., 1988; Stanton et al., 1988; Levine, 1994; Schmidt et al., 2002). From approximately PND3 to PND14 in rats and from PND1 to PND12 in mice, rodent pups show a diminished reactivity of the HPA axis in response to mild to moderate stressors (Jailer, 1950; Sapolsky and Meaney, 1986). This period is called the stress hyporesponsive period (SHRP) and is characterized by inhibition of the hypothalamus and the pituitary, and by attenuated sensitivity of the adrenals to ACTH (for review, see Rosenfeld et al., 1992).

Disruption of the early life environment and the SHRP by separating pups from their mother has robust and long-lasting endocrine and behavioral effects, although different studies have found seemingly contradictory results, possibly due to the wide variety of protocols used (Lehmann and Feldon, 2000). Usually, early life stress results in HPA axis hyperactivity (Plotsky and Meaney, 1993; Ogawa et al., 1994; Vazquez et al., 1996; Liu et al., 2000a; Lehmann et al., 2002; Ladd et al., 2004), cognitive impairments (Uysal et al., 2005; Aisa et al., 2007; Oomen et al., 2010b) and higher levels of anxiety (McIntosh et al., 1999; Caldji et al., 2000; Kalinichev et al., 2002). Also hippocampal structure is affected: neonatal stress was found to cause a decrease in mossy fiber density (Huot et al., 2002), downregulated neurogenesis (Mirescu et al., 2004; Aisa et al., 2009; Oomen et al., 2009; Oomen et al., 2010b), reduced BDNF expression (Roceri et al., 2002; Kikusui et al., 2009), dendritic atrophy in the CA1 area (Brunson et al., 2005) and sex-dependent alterations in the number of primary dendrites in DG granule cells (an increase was reported in females and a decrease in males, Oomen et al., 2010a; Oomen et al., 2010b).

5.2 Handling
In contrast to prolonged maternal separation or deprivation, separating rat pups from their mother for short periods of time only, i.e. 3-15 min daily, during the first few weeks after birth, has been shown to have a beneficial effect on later-life emotionality and stress responsivity compared to non-disturbed control animals. This so-called neonatal handling alters HPA axis reactivity in such a way that the individual is better able to respond to, cope with and adapt to stressful situations (Levine and Lewis, 1959; Meaney et al., 1985; Meaney et al., 1991; Plotsky and Meaney, 1993). Handled animals show a rapid decline in corticosteroid levels after stress, due to increased negative feedback; GR expression in the hippocampus is upregulated after handling, presumably resulting in a more sensitive feedback loop (Meaney et al., 1993; Avishai-Eliner et al., 2001). Moreover, diminished reactivity of stress-related neuronal circuitries (e.g. the PVN, amygdala and hippocampus) was found in animals that were subjected to early handling (Abraham and Kovacs, 2000). Correspondingly, fearful behavior and stress-induced anxiety in these animals is decreased (Vallee et al., 1997; McIntosh et al., 1999; Meerlo et al., 1999; Caldji et al., 2000), and memory performance is enhanced, both in the Morris water maze (Lehmann
et al., 2002; Stamatakis et al., 2008) and in an object recognition task (Kosten et al., 2007). Handling effects on GR expression and hippocampus-dependent memory performance might involve decreases in CRH receptor activation, which are probably due to lower levels of circulating CRH (Fenoglio et al., 2005).

**5.3 Natural variations in maternal care**

The models described above disturb normal mother-infant interactions in a dual way: first, during separation, the offspring are deprived of maternal care (e.g. grooming, food, warmth) for a shorter or longer period of time, but later, upon reunion with the mother, the pups are subjected to augmented levels of maternal care (Oomen et al., 2009). Multiple studies suggest that the effects of separation and handling paradigms are largely mediated through these alterations of maternal behavior (Barnett and Burn, 1967; Smotherman et al., 1977; Liu et al., 1997; Pryce et al., 2001), but the question remains how this mediation occurs. Moreover, if disturbances in maternal care have long-lasting effects, this might indicate that naturally occurring variations in the amount of maternal care that a dam provides to her litter (Myers et al., 1989) also contribute to individual differences in later-life stress vulnerability. Michael Meaney and coworkers started exploring this behavioral variability in a normal, undisturbed population of Long Evans rats, and not only confirmed the existence of substantial differences in maternal care between dams (Liu et al., 1997), but also showed that this trait was stable over time and over multiple litters (Champagne et al., 2003). Particularly licking and grooming (LG) and arched-back nursing (ABN; an active form of nursing) appeared to correlate closely with each other and were shown to predict later-life phenotypic outcome (Liu et al., 1997). Since then, this new model for early life experience, the maternal care model, has been used extensively to investigate developmental programming of the limbic system, neuroendocrine responsivity to stress, emotionality and cognitive performance in adulthood.

![Figure 5](image)

Based on elaborate observations of maternal behavior in the first week postpartum, each dam can be classified as providing High (>1 standard deviation above the mean), Mid or Low maternal care. The amount of maternal care provided by dams to their litters is normally distributed within a population of rats. Dams exhibiting extreme amounts of maternal care, i.e. >1 SD above or below the mean, are classified as High LG/ABN and Low LG/ABN, respectively.

**Figure 5.** The amount of maternal care provided by dams to their litters is normally distributed within a population of rats. Dams exhibiting extreme amounts of maternal care, i.e. >1 SD above or below the mean, are classified as High LG/ABN and Low LG/ABN, respectively.
Low (>1 SD below the mean) amounts of LG/ABN (Figure 5). The offspring of Low compared to High LG/ABN dams were shown to exhibit exaggerated HPA axis responses to stress in adulthood and an increased expression of CRH mRNA in the hypothalamus (Liu et al., 1997). Moreover they exhibit impaired negative feedback sensitivity presumably due to a decreased expression of GR in the hippocampus compared to High LG/ABN offspring (Liu et al., 1997; Francis et al., 1999a; Meaney, 2001a).

Maternal care persistently affects GR expression through epigenetic programming: in High LG/ABN offspring increased expression levels of the transcription factor nerve growth factor-inducible protein-A (NGFI-A) lead to demethylation of the (hippocampus-specific; McCormick et al., 2000) exon 17 promoter of the GR gene over the course of the first week after birth, which is associated with increased histone 3 - lysine 9 (H3-K9) acetylation. Conversely, Low LG/ABN offspring show decreased NGFI-A binding to the exon 17 GR promoter, and thus hypermethylation of this promoter and a concurrent reduction of H3-K9 acetylation (Weaver et al., 2004). As described in box 1, low levels of DNA methylation and high levels of histone acetylation are usually associated with increased transcription and thus enhanced mRNA expression of that particular gene, and this has indeed been reported for the exon 17 GR promoter (Weaver et al., 2007). Interestingly, although the epigenetic changes induced by early-life environment persist into adulthood, the different GR methylation patterns in High versus Low LG/ABN animals could still be pharmacologically reversed after the sensitive first week of life (Weaver et al., 2004; Weaver et al., 2005).

Also structural parameters were found to be altered in response to differential maternal care. Neuronal survival in the adult dentate gyrus (Bredy et al., 2003a), and dendritic complexity as well as number of spines of CA1 pyramidal cells (Champagne et al., 2008) are increased in offspring from High compared to Low LG/ABN dams, which is likely associated with higher hippocampal mRNA expression levels of brain-derived neurotrophic factor (BDNF) in these animals (Liu et al., 2000b). On the other hand, dendritic complexity and spine density in layer II/III neurons of the somatosensory cortex were reduced in High LG/ABN offspring (Smit-Rigter et al., 2009).

Field potential recordings in the hippocampal CA1 area showed strong synaptic long-term potentiation (LTP) after high-frequency stimulation in High but not in Low LG/ABN offspring, whereas these phenotypes were reversed after application of high levels of corticosterone to the slices (Champagne et al., 2008). Accordingly, Low LG/ABN animals showed spatial learning deficits under relatively non-stressful conditions such as a Morris water maze task to which the animals were habituated (Liu et al., 2000b; Bredy et al., 2003b) and impaired object recognition memory (Bredy et al., 2003b) compared to High LG/ABN offspring. Memory formation in a contextual fear conditioning task, which is also hippocampus-dependent but highly stressful, was strong in Low LG/ABN animals, but attenuated in the offspring of High LG/ABN dams (Champagne et al., 2008). Also, in
line with their increased stress responsivity, adult Low LG/ABN offspring demonstrated higher levels of anxiety in the open field (Weaver et al., 2006), and fearfulness in a novel environment (Caldji et al., 1998). Taken together, these findings suggest a programming mechanism that might be either adaptive or not, depending on the adult environment (Champagne et al., 2009).

The observations described above suggest a certain regional specificity in the effects of early life environment on brain structure and function later in life. This regional differentiation is also known from corticosteroid effects on the hippocampus in adulthood (Van Gemert et al., 2009): for instance, corticosterone changes calcium current properties in CA1 neurons but not in the dentate gyrus. This led to the first experimental question of this thesis.

**Question:** How is structure and function of the hippocampal dentate gyrus affected by differences in maternal care? (chapter 2)

### 5.4 Adjustments to the maternal care model

One of the caveats of the maternal care model is that when comparing entire High and Low litters, the pups from these litters inevitably share a substantial part of their genetic material. To verify that there is indeed a causal relationship between the amount of maternal care received and later-life outcome, and to rule out the influence of different genetic backgrounds between litters, cross-fostering studies have been performed. These demonstrated a direct effect of maternal care on fearfulness in novelty conditions in the offspring as well as on second generation maternal behavior (Francis et al., 1999a). Artificially increasing the amount of maternal care in Low LG/ABN dams to the level of High LG/ABN dams through pup handling suggested that also the effects on HPA axis parameters were non-genomically transmitted (Francis et al., 1999a). However, with respect to cognitive performance the dissociation between genes and environment was less clear (Liu et al., 2000b). A second consideration regarding the model is the fact that it only focuses on the extremes of the distribution in care. Intermediate amounts of care, which involve around 70% of the pups, were not examined.

Interestingly, when establishing the model, people already noticed that there was quite some variation within litters of High and Low LG/ABN dams in terms of their behavioral performance and endocrine responses in adulthood, but at the time no evidence was found for significant intra-litter differences in licking and grooming (Champagne et al., 2003). However, a few years later, this issue was re-evaluated and it was now shown that dams do show preference for certain pups in their litter over others (M.J. Meaney, personal communication). A pilot study confirmed this finding and also showed that this within-litter variation was linked to differences in HPA axis reactivity in adolescence (S.E.F. Claessens et al., personal communication).
The main question of this thesis was to what extent the natural variation in %LG received by individual pups can predict structure and function of the hippocampus and PFC later in life. The existence of intra-litter differences in LG implies that the same %LG might be received by pups from two dams that differ substantially in the overall amount of LG they bestow on their entire litter. Conversely, mothers showing comparable amounts of maternal behavior towards their entire litter might each have pups that received markedly different amounts of LG. Thus, by using individual pup LG scores (rather than the %LG given by the mother to her entire litter) as the predictor variable, we indirectly address the issue regarding genetic influences. An additional benefit is that this paradigm allows us to use every pup from each litter. Apart from the obvious advantage from an ethical point of view, this also means that one can observe a limited number of litters at a time rather than over 40 litters, necessary to select a sufficient amount of High and Low litters. The latter approach always results in working with large cohorts of the exact same age. For electrophysiological experiments, where only one animal per day can be investigated, this is a serious drawback, since each experimental series then involves animals ranging in age from 2-4 months. Given the changes in brain maturation during this period, randomization of the animals then becomes a crucial factor to avoid an experimental bias; this, however, potentially introduces an age-dependent variability in each experimental group.

In view of all these considerations, we here developed and validated a new refined model of maternal care in rats, based on %LG received by individual pups. The previous maternal care model mostly focused on effects of early life environment on male offspring, but taking into account the documented sex-dependent differences in the vulnerability to stress-related psychopathology in humans, we here examined both male and female offspring.

Questions:
- Are there within-litter differences in the amount of licking and grooming that is provided to each individual pup?
- Do these putative individual differences correlate with HPA axis development, brain morphology, function and cognitive performance?
6. Outline of the thesis

The overall aim of this thesis was to investigate the effects of early-life maternal care on 1) stress responsivity, 2) structural and functional plasticity of the hippocampus and PFC and 3) related cognitive performance later in life. In particular, we aimed to develop and validate a new refined model of maternal care in rats, by evaluating whether subtle individual within-litter differences in licking and grooming would result in adult phenotypic differences comparable to those in the ‘original’ maternal care model.

First, in chapter 2, we examined if in the hippocampal dentate gyrus of male High versus Low LG/ABN offspring (using the original maternal care model) similar differences could be found in synaptic plasticity and morphology as reported earlier for the CA1 area. We also examined the effects of corticosterone application on synaptic potentiation, as well as cognitive performance in a stressful fear conditioning task.

In chapter 3, we examined if in our newly developed maternal care model individually characterized licking and grooming scores gave comparable effects on DG structure and function – including dendritic morphology, neurogenesis, neurotrophin expression, synaptic plasticity and stress responsiveness – as reported for the original maternal care model.

In chapter 4, we extended the previous study to the CA1 area of the hippocampus, again validating the new individual maternal care model regarding structural and functional plasticity after differential early life experience.

Chapter 5 describes whether adult DNA methylation status of the brain-derived neurotrophic factor (BDNF) exon IV splice variant correlates with the individual LG background.

In chapter 6, we investigated non-stressful spatial learning as well as contextual emotional learning to elucidate if the findings in chapters 3 and 4 regarding hippocampal structural and functional parameters might be behaviorally relevant.

In chapter 7, we discuss the effects of early life differences in LG on adolescent play behavior, on performance in the rodent Iowa Gambling Task (IGT, a decision-making task that is known to require the prefrontal cortex) and on markers of cellular activity in relevant brain areas.

Finally, in chapter 8, all experimental findings are summarized and discussed.