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

van Hasselt, F.N.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 8

General Discussion
Contents

1. Maternal care as a measure for early life environment: rationale 121

2. Effects on HPA axis activity 123

3. Structural and functional implications of differential maternal care 127
   3.1 Maternal care and the hippocampus – structure
   3.2 Maternal care and the hippocampus – function
   3.3 Maternal care and PFC function

4. The influence of early life 134
   4.1 Recommendations for future experiments
   4.2 Nature versus Nurture – concluding remarks

120
1. Maternal care as a measure for early life environment: rationale

A large amount of research has been done on the effects of early life experience on brain development and adult phenotype, both in humans and in animal models. These models usually encompass disruptions of normal mother-infant interactions, thus inducing stress in both the mother and the offspring. In contrast, the models used in this thesis represent a more subtle and naturalistic approach by employing the naturally occurring variation in maternal care between dams in a population of rats as a measure for early life environment. In the between-litter model of maternal care, offspring from dams engaging in extremely high amounts (>1SD above the mean) of licking and grooming (LG) and arched-back nursing (ABN) towards their entire litter are compared to those reared by dams exhibiting extremely low amounts of LG/ABN (>1SD below the mean). By selecting and comparing the extremes within a population, strong effects of maternal care background on later life phenotype have been found (e.g. Francis et al., 1999b; Liu et al., 2000b; Bredy et al., 2003a; Champagne et al., 2008). This appeared to be also the case in our hands. For example, as reported in chapter 2, LTP could be induced in the hippocampal dentate gyrus under control conditions in slices from adult High LG/ABN but not in those from Low LG/ABN offspring, in line with the increased dendritic complexity found in the former compared to the latter group. However, in an environment containing high levels of stress hormones, i.e. either corticosterone or isoproterenol, DG LTP was facilitated in Low LG/ABN slices and suppressed in High LG slices.

Despite the robust and consistent effects of maternal care that have been detected with this model there are some major limitations, as noted in the General Introduction of this thesis. The common genetic background of pups within High and Low litters could potentially influence or mediate the effects of differential maternal care on adult phenotype. Therefore, cross-fostering studies have been performed, eliminating genetic bias and raising evidence for a direct effect of maternal care on certain parameters such as fearfulness (Francis et al., 1999a). However, this cross-fostering procedure is labor-intensive, and requires many litters in order to include all the proper control groups. Also the generally large amount of animals needed to obtain a sufficient amount of High and Low litters, the ethical consideration that most of these animals (from the Mid litters) are often not used in experiments, and the fact that with this approach one is constrained to work with large cohorts of the same age, led us to explore a more refined model for maternal care, to which we here refer as the ‘within-litter’ model.

This within-litter maternal care model in rats takes into account the uneven distribution of LG towards individual pups within each litter and enabled us to much more directly relate differences in maternal care to later-life phenotypic variation. Moreover, in this paradigm all pups could be used, since we determined the %LG for each individual pup and used that as a variable in each experiment. Finally, this new model
allowed us to precisely adjust the size of the cohorts that we bred to the amount of animals required for a certain experiment. Interestingly, in an early study using the between-litter model, Liu et al. (1997) showed that %LG/ABN between dams correlated positively with hippocampal GR mRNA expression and negatively with hypothalamic CRH mRNA expression and stress-induced plasma corticosterone response in the offspring. Similarly, pup exploration time in an open field (reflecting the level of fearfulness) correlated positively with whole-litter maternal care (Caldji et al., 1998). In an in vitro LTP experiment performed in our lab, Mid pup synaptic potentiation values fell exactly between those of High and Low pups (H.J. Krugers and M. Joëls, unpublished observation), again suggesting a linear correlation with maternal care. These studies underline the importance of examining animals over the entire range of LG.

In studies on the influence of maternal care in the between-litter model one of the main regions of interest has been the hippocampus. Strong effects were found on HPA axis responsiveness to stress and glucocorticoid negative feedback efficiency (Liu et al., 1997; Francis et al., 1999a) which has been linked to the decreased DNA methylation status of the GR exon 1/ promoter and the concurrent elevation of GR mRNA expression levels in the hippocampus of High versus Low LG offspring (Weaver et al., 2004). Also performance on hippocampus-dependent tasks was reported to differ in response to varying maternal care backgrounds (Bredy et al., 2003b; Champagne et al., 2008), which we confirmed in the study described in chapter 2 of this thesis. Thus, in order to validate our newly developed individual model of maternal care, we first aimed to replicate the effects of licking and grooming on neuroendocrine parameters, as well as on adult hippocampal structural and functional parameters that were previously found in the between-litter maternal care model (chapters 3, 4, 5 and 6). At the same time, we extended our research to examining if LG exerts its effects in a sex-dependent manner. In humans, males and females are known to be differentially susceptible to developing depressive disorders (Ustun, 2000), the risk of which is strongly related to disruptions in early life (Arnow, 2004). However, to date, the dissociation between sexes has largely been neglected in studies addressing the effects of early life events in animal models, including the maternal care model.

Apart from studying the effects of early life in the hippocampus, we also became interested in the prefrontal cortex (PFC). This brain area is involved in regulating an organism’s behavioral, neuroendocrine and autonomic responses to stress (Diorio et al., 1993; Sullivan and Gratton, 2002), but is also very sensitive to (early life) stress itself (see section 4.3 of the General Introduction of this thesis). The prefrontal cortex plays a crucial role in many behavioral processes, including decision-making and response to reward (De Visser et al. in preparation, Manes et al., 2002; Simmons et al., 2010), both of which are known to be altered in a number of stress-related disorders (Henriques and
General Discussion

Davidson, 2000; Miu et al., 2008; Cella et al., 2010; Simon et al., 2010). Therefore, we decided to slightly shift focus and examine the effects of individual LG on PFC-dependent behavior and corresponding brain activation in chapter 7.

Below, the main findings of this thesis are critically discussed. Those issues requiring further investigation are indicated by an exclamation mark.

2. Effects on HPA axis activity

As mentioned earlier, a system that was reported, by others and by us, to be highly affected by maternal care in the original between-litter model is the HPA axis. Low LG/ABN compared to High LG/ABN animals show more pronounced corticosterone and ACTH responses to stress, lower levels of hippocampal GR mRNA expression and decreased glucocorticoid negative feedback sensitivity, resulting in a slower return to baseline levels of CORT after stress (Liu et al., 1997; Francis et al., 1999a; Champagne et al., 2008, chapter 2).

Which properties of the HPA axis are affected in the maternal care model?

We hypothesized that if HPA axis responsivity is directly affected by maternal care, as suggested by earlier studies (Francis et al., 1999a; Van Oers et al., 1999), we would find comparable effects of individual LG in the within-litter approach. In chapter 3 we first examined the abundance of GR mRNA in the hippocampus of individually characterized offspring and found a positive correlation with %LG received in infancy, in males and females. However, we did not find a corresponding difference in peak CORT levels after stress. It must be noted though that the method used to assess this stress-induced rise in CORT was suboptimal. Due to our experimental design, it was inevitable that some animals remained alone in their homecage for 15 to 20 minutes before being sacrificed. This time window allowed the stress response to develop and CORT levels to rise to peak values (De Kloet et al., 2005), and therefore we excluded these animals from electrophysiological experiments but used their plasma CORT levels at decapitation as an index for stress-induced HPA axis reactivity. Moreover, in this study it was impossible to investigate the recovery rate of plasma CORT levels after cessation of the stressor in relation to individual %LG. This is an important issue because efficient termination of the stress response through glucocorticoid negative feedback is necessary to effectively reinstate homeostasis and thus determines an individual’s vulnerability to stress (De Kloet et al., 2005).

Follow-up studies using the within-litter maternal care model should include proper examination of the CORT response to stress and its subsequent return to baseline levels in individually characterized animals, for example by means of a restraint stress paradigm, as well as determination of ACTH and CRH responses to stress, in
order to draw reliable conclusions on the direct effects of maternal care on HPA axis reactivity.

When and how do differences develop?
A second issue that would need follow-up investigations is the development (over time) of changes in HPA axis reactivity and responsivity. It is very well possible that the differences between High and Low LG offspring with regard to corticosterone and its receptors already start very early after birth. A GR-mediated negative feedback mechanism at the level of the pituitary is responsible for maintaining the stress hyporesponsive period (SHRP) in rodents (Schmidt et al., 2005; Schmidt et al., 2009). Even though brain GR levels are generally low at birth and only slowly rise to adult concentrations around the third week of life, their affinity for corticosterone is higher perinatally than at later ages (Sarrieau et al., 1988b; Rosenfeld et al., 1993). Additionally, due to the low levels of corticosterone-binding globulin (CBG) during development, free corticosterone concentration is sufficient to activate these GRs, despite the very low levels of circulating corticosterone that are characteristic for the SHRP (Henning, 1978; Viau et al., 1996).

It is not known yet if the LG-induced differences in adult hippocampal GR expression are already present during the SHRP (although the fact that GR methylation patterns are already established by PND6 suggests that they might be), nor has it been studied if maternal care affects pituitary GR expression and binding capacity. Yet if it does, one could speculate that the SHRP in offspring receiving low levels of LG is less well controlled, which may cause increased sensitivity of the HPA axis later in life.

Another mediator of the HPA axis that might be directly affected early in life by maternal care is corticotrophin-releasing hormone (CRH). The expression of this hormone in the hypothalamus is reduced in High compared to Low LG/ABN offspring, but also in offspring of Low LG/ABN dams that were handled – and thus exhibited an increased amount of maternal care towards their litters (Liu et al., 1997; Francis et al., 1999a). Other studies reported that handling-induced augmented levels of maternal care in uncharacterized dams resulted in a down-regulation of hypothalamic CRH mRNA expression in the offspring, which preceded and possibly contributed to the elevated hippocampal expression of GR observed in these animals as well (Avishai-Eliner et al., 2001; Fenoglio et al., 2005). Interestingly, during the SHRP, which is maintained by maternal stimulation (Cirulli et al., 2003), pups that are exposed to a mild stressor (e.g. saline injection) generally show only a minimal endocrine response, but the same mild stressor does induce robust transcriptional activation of CRH in the hypothalamus (Dent et al., 2000). This suggests that at least during the SHRP the CRH system is more sensitive to environmental adversity than the rest of the HPA axis and that its
activational threshold is relatively low, thus possibly enabling low levels of maternal care to affect the transcriptional machinery for CRH.

In the maternal care models described in this thesis, neither basal pup CRH levels nor their CRH response to a mild stressor in the first postnatal week (at the time of maternal care observations) have been examined yet.

Assuming that maternal care indeed directly affects the stress responsivity of the HPA axis, the question remains how these effects are exerted. Regarding the hippocampal expression of GR, this was shown to occur through serotonin- and NGFI-A-dependent regulation of the methylation state of the GR exon 17 promoter over the course of the first 6 days after birth (Weaver et al., 2004; see section 5.3 of the General Introduction of this thesis). Although we have not examined GR methylation in the individual maternal care model and thus could not confirm this finding, a recent study by McGowan and colleagues illustrated its relevance. In hippocampal tissue of suicide victims with a history of childhood abuse (i.e. at the level of individuals) increased methylation of the GR exon 1F promoter (analogous to the rodent GR exon 17 promoter) was found, which was associated with alterations in NGFI-A binding and corresponded to a decrease in GR expression (McGowan et al., 2009).

Thus, given that we found maternal care effects on adult hippocampal GR mRNA levels in our within-litter model as well, GR methylation state in relation to individual LG remains to be investigated, as well as the effects of LG on the regulatory factors in the methylation pathway.

To what extent is the corticosteroid level of the dam important?

Another factor that might contribute to pup HPA axis development and which is currently awaiting investigation in our within-litter maternal care model is the CORT level of the dam in the pre-weaning period. Lactating females generally display elevated corticosterone levels compared to virgin rats (Zarrow et al., 1972; Stern et al., 1973), which makes putative differences in hormone level a very relevant parameter. Interestingly, offspring of dams drinking water supplemented with corticosterone during nursing resemble High LG/ABN offspring on several neuroendocrine and cognitive parameters later in life (Catalani et al., 2000; Catalani et al., 2002). Moreover, higher maternal plasma CORT levels are related to higher amounts of maternal care (Rees et al., 2004). If this relationship also holds in the reverse direction, High LG/ABN dams might be expected to exhibit higher levels of CORT than Low LG/ABN dams. Although this seemingly contrasts with earlier findings that High and Low LG/ABN offspring (themselves usually becoming High and Low LG/ABN mothers, respectively; Francis et al., 1999a; Champagne et al., 2003) do not differ in their basal corticosterone levels (Liu et al., 1997), High LG/ABN dams were indeed found to show higher peripartum levels of
basal CORT than Low LG/ABN dams (R.C. Bagot and M.J. Meaney, personal communication), which probably underlie the elevated levels of maternal care they provide.

Conversely, lactating rats not only show elevated basal neuroendocrine activity, they also exhibit a dampened circadian HPA rhythm and an attenuated HPA response to stress (Lightman et al., 2001; Neumann, 2001; Brunton et al., 2008). The latter is at least partially regulated by a decrease in hypothalamic CRH (Lightman et al., 2001). Since high levels of maternal care are associated with low levels of CRH expression (Champagne and Meaney, 2001), the reduction of HPA responsivity to stress might be greater in High compared to Low LG/ABN dams.

Future studies would need to extensively investigate the 24 hrs corticosterone release patterns in dams as well as the (normalization of their) corticosteroid response after stress.

Figure 1. Proposed peripartum corticosterone levels in High and Low LG/ABN dams (based on Lightman et al., 2001). High LG/ABN dams have moderately high basal plasma CORT levels and might not be responsive to stressors during lactation, whereas Low LG/ABN dams exhibit lower basal neuroendocrine activity, but their HPA axis might still be stress-reactive while nursing.

An adequate level of corticosterone is crucial for normal development of the offspring HPA system (Wilcoxon and Redei, 2007) and the main source of CORT in pups is from their mother through the milk. Thus, the possibly moderate and stable increase in maternal CORT levels in High LG/ABN dams may be beneficial for pup development (Lightman et al., 2001), whereas the combination of lower basal CORT levels with a more stress-reactive HPA axis that might occur in Low LG/ABN dams may lead to an inadequate exposure of the offspring to corticosterone and attenuate HPA axis development (Figure 1). Clearly, maternal CORT level is a factor that is intrinsic to the dam, to which all pups within a litter are subjected, and which therefore cannot explain putative individual differences in HPA reactivity. However, it might be the reason why we
have not found any effects of individual LG scores on HPA stress responsiveness in our studies.

What is the role of metabolism?
It is known that the HPA axis is highly intertwined with the metabolic system. For example, glucose injections administered to pups during maternal separation in the SHRP delay their HPA response (Schmidt et al., 2006) and sucrose intake normalizes HPA deficiencies after adrenalectomy in rats (Laugero et al., 2001). In the between-litter model of maternal care, %LG and ABN showed a strong correlation (Liu et al., 1997; Caldji et al., 1998), which makes it hard to make a distinction between the effects of tactile stimulation (LG) and the effects of metabolism (ABN) on pup HPA axis development. Unfortunately, the individual maternal care model does not allow investigation of putative within-litter differences in nursing; it is impossible to determine which pup is suckling at any given timepoint.

In the future, pups should be weighed every day until weaning, to obtain information on nursing distribution and pup development, and to determine if putative within-litter differences in pup metabolic rate or body weight correlate with maternal LG and with brain structure and function in the offspring once they have reached adulthood.

3. Structural and functional implications of differential maternal care

3.1 Maternal care and the hippocampus - structure
In chapters 2 to 5 we studied several structural parameters in the hippocampus in relation to the amount of maternal care received in infancy. In rats, the extensive brain development that is still ongoing during the pre-weaning period is highly sensitive to early life environmental factors. We complemented previous findings that in males extremely high levels of maternal care compared to extremely low levels were related to increased dendritic complexity and spine density in the CA1 area of the hippocampus (Champagne et al., 2008) with similar data on dentate gyrus dendritic morphology (chapter 2). Thus, we report that in the between-litter model, High LG/ABN offspring show a more complex dendritic tree and a larger number of dendritic spines in the hippocampal DG than Low LG/ABN offspring. Additionally, we examined hippocampal dendritic complexity in individually characterized animals (chapter 3 and 4) and found that in males the %LG correlated positively with the dendritic complexity of both CA1 pyramidal neurons and DG granule cells, although the effects were much more subtle than what was found in the between-litter model.
This positive correlation between %LG and hippocampal morphology may (at least in part) be mediated by the differential mRNA expression of brain-derived neurotrophic factor (BDNF) in these animals. Since BDNF is a positive modulator of neuronal development and viability (Henderson, 1996; Binder and Scharfman, 2004) and is also known to be implicated in hippocampal synaptic transmission (Kang and Schuman, 1995; McAllister et al., 1999), the higher expression levels of this neurotrophin found in offspring with LG scores on the higher end of the scale (confirming earlier reports in the between-litter model, Liu et al., 2000b) nicely correspond to their increased dendritic complexity (chapter 3). We further explored the maternal effect on BDNF expression by determining the promoter methylation level of one of its regulatory exons, exon IV, in the hippocampus of adult male offspring (chapter 5). In contrast to what we expected based on the mRNA study, a significant positive correlation emerged between %LG and the degree of DNA methylation, suggesting a more closed chromatin state in animals that received higher amounts of maternal care. Yet, elevated levels of DNA methylation are not always related to a decrease in gene transcription (Weber et al., 2007). Moreover, whereas we examined mRNA expression of total BDNF transcript, our epigenetics study only addressed exon IV methylation status, not taking into account putative effects on other epigenetic modifications or promoter regions of other BDNF exons.

Although our data suggest that LG affects hippocampal dendritic morphology through differential regulation of BDNF expression, the underlying long-lasting epigenetic modifications remain to be explored in further detail.

Putative influence of sex hormones

In females (which were not studied in the between-litter model), the correlations that emerged between %LG and dendritic complexity were opposite to those found in males, both in the CA1 area and the DG (chapter 3 and 4). The most obvious explanation for this that comes to mind is the influence of sex hormones, both in early life and in adulthood. Although adult plasma estradiol levels did not correlate directly with dendritic morphology, they did show a significant positive correlation with hippocampal BDNF exon VI mRNA expression (chapter 3). It has been shown that female offspring from High compared to Low LG/ABN dams show lower expression of the estrogen receptor alpha (ERα) in the paraventricular nucleus of the hypothalamus and decreased plasma levels of progesterone during proestrus (Cameron et al., 2008). This suggests a less reactive hypothalamus-pituitary-gonadal (HPG) axis in High LG offspring. If so, that would be accompanied by a decreased level of hippocampal BDNF expression, possibly leading to reduced dendritic complexity in CA1 and DG primary neurons. The aforementioned study also showed increased testosterone levels on embryonic day 20 in offspring from High compared to Low LG/ABN mothers (Cameron et al., 2008), raising more evidence for the idea that High female offspring might display a more masculinized phenotype. One could
speculate that the above occurs in the individual maternal care model as well; since each fetus has its own placenta and its own amniotic sac, embryonic testosterone levels may vary between individual pups from a single litter. In view of our finding that male LG scores are generally higher than female LG scores (chapter 3, 4, 5 and 7), it is possible that pup testosterone levels contribute to determining the degree of maternal attendance.

Sex hormones, including estrogen, progesterone and testosterone, are neuroprotective and stimulate neurogenesis (Garcia-Segura et al., 2001; Spritzer and Galea, 2007; Zhang et al., 2008). Accordingly, a study in the between-litter maternal care model found an enhanced level of long-term survival of newly born cells in the dentate gyrus of male offspring from High compared to Low LG/ABN dams (Bredy et al., 2003a). However, in our new maternal care paradigm there was no maternal effect on cell proliferation or cell survival, but only a negative correlation between %LG and the number of young neurons in the DG in adult males (as measured by the amount of DCX labeling in chapter 3). Together with the increased dendritic complexity found in male offspring with high LG scores this suggests that these animals possess a more mature pool of dentate granule neurons. In individually characterized females there was no correlation between %LG and any of the adult neurogenic parameters. This corroborates previous findings showing no effects of early maternal deprivation on female adult neurogenesis (Oomen et al., 2010a). However, in line with the detrimental effects of corticosterone on cell proliferation and survival (Mayer et al., 2006; Mirescu and Gould, 2006), we found a significant negative correlation between stress-induced CORT levels and the number of surviving BrdU labeled cells.

The overall lack of maternal effects on neurogenesis in either gender might in part be due to timing issues. We studied the proliferation rate in our animals at PND52, around the age at which our other cohorts of animals were tested for morphology, electrophysiological properties and behavior. At that same timepoint, we determined the remainder of incorporated BrdU – as a measure for cell survival, 4 weeks after administration of this thymidine analogue on PND25 (a few days after weaning). This differs from the earlier study by Bredy et al. (2003) who injected High and Low offspring with BrdU already on PND7 and examined the survival rate both 2 and 8 weeks later.

In the current study we neither distinguished between cells with a neuronal fate and those destined to become glial cells, nor did we take into account the various maturation stages that exist within a population of newborn cells and that might be differentially affected by early life experience (Plumpe et al., 2006; Oomen et al., 2010b).
3.2 Maternal care and the hippocampus - function

Effects in the between-litter model

Research on the between-litter model has shown that the effects of maternal care on hippocampal structure and HPA axis parameters are paralleled by differences in hippocampal function and cognitive performance in adulthood. In electrophysiological studies, both *in vivo* and *in vitro*, male High compared to Low LG/ABN offspring exhibited facilitated long-term potentiation after high-frequency stimulation (HFS) in the CA1 area (Champagne et al., 2008) and the dentate gyrus (Bredy et al., 2003b, chapter 2). Interestingly, *in vitro*, this phenotype in the CA1 could be reversed by application of corticosterone to the slices several hours prior to HFS (Champagne et al., 2008). LTP induction in High LG/ABN offspring was hampered by CORT treatment, which was consistent with previous reports in uncharacterized rats (Wiegert et al., 2005; Joels and Krugers, 2007). However, the normal GR-mediated suppressive effects of corticosterone on LTP induction might be diminished in Low LG/ABN rats due to their lower levels of hippocampal GR (Champagne et al., 2008, and see box 3 of the General Introduction of this thesis).

In the DG we described a similar reversal effect by either CORT or isoproterenol application around the time of HFS (chapter 2). This phenomenon might be explained by the differences in hippocampal glutamate receptor expression that occur in offspring with a different history of maternal care. High compared to Low LG/ABN offspring were reported to show increased NMDA receptor binding associated with elevated hippocampal mRNA expression of NMDA subunits (Liu et al., 2000b; Bredy et al., 2003b; Bredy et al., 2004), overexpression of which is known to enhance LTP in the hippocampus (Tang et al., 1999). Both corticosterone and isoproterenol affect the calcium permeability of these NMDA receptors in a non-genomic manner (Takahashi et al., 2002; Skeberdis et al., 2006).

It remains to be examined if corticosterone application to slices of High LG offspring hippocampus might lead to an overshoot of intra-cellular calcium concentrations, thereby reducing neuronal excitability and increasing the threshold for LTP induction. In contrast, increased calcium influx induced by corticosterone in hippocampal slices of Low LG animals might create ideal circumstances for inducing LTP (see chapter 2).

Additionally, Low versus High LG/ABN offspring show greater AMPA receptor binding in the hippocampus (Bredy et al., 2003b), which might be beneficial for LTP induction particularly under stress conditions, when the release probability of glutamate and the lateral diffusion of AMPA receptors is enhanced (Groc et al., 2008; Krugers et al., 2010). Interestingly, increased AMPA receptor binding has previously been linked to more pronounced cognitive impairments in aged rats (Le Jeune et al., 1996), suggesting that it
is indeed a negative regulator for hippocampal LTP, which is considered to be the cellular substrate for learning (Pastalkova et al., 2006; Whitlock et al., 2006). In line with the above, and as mentioned in the General Introduction of this thesis, performance on hippocampus-dependent spatial learning tasks, both under stressful and non-stressful conditions, was also associated with variations in maternal care. In chapter 2 we confirmed the findings on contextual fear conditioning, a highly stressful hippocampus-dependent learning task; Low LG/ABN offspring showed enhanced conditioned fear memory compared to High LG/ABN rats.

**Effects in the within-litter model**

We examined whether similar effects on hippocampal LTP and cognitive function would arise in the refined maternal care model. Indeed, in the DG, long-term potentiation of field potentials in hippocampal slices was enhanced in animals with higher individual LG scores (chapter 3). This effect of maternal care was present in both males and females, but it only concurred with the aforementioned LG-effects on DG dendritic complexity in males. Thus, in females there is a dissociation between the influence of %LG on structure and its effects on function of the hippocampus. This might be understood by interpreting our data on hippocampal mRNA levels of BDNF and GR. The negative correlation between %LG and dendritic morphology in females is possibly explained by the interaction effects of estrogen and BDNF (see section 3.1), whereas the LG effect on DG function might be related to the differential expression of corticosteroid receptors in our individually characterized animals, which occurs in both males and females. A previous study in the between-litter model reported that High compared to Low LG/ABN rats not only show higher levels of GR mRNA in the hippocampus, but also elevated hippocampal MR mRNA expression (Champagne et al., 2008). The latter was associated with the enhanced synaptic potentiation found in the CA1 area of these High LG/ABN offspring. Since individual %LG correlates with hippocampal GR mRNA expression in both males and females (chapter 3), it might similarly affect hippocampal MR mRNA expression in both sexes, which, in turn, might underlie the positive correlation found between %LG and LTP in the dentate gyrus.

We have not yet been able to investigate the expression of MR in relation to individually received LG in the within-litter model.

No differences in NMDA and AMPA receptor subunit expression have been found in relation to individual LG scores (unpublished observations), thus implying that this cannot explain the maternal effects on LTP in the within-litter model.

In the CA1 area we reported a dichotomy between the sexes for the effects of LG on LTP, similar to what we showed for the morphology (chapter 4), suggesting that in this brain area structure and function are related to each other. Moreover, we examined the
effects of CORT application during high-frequency stimulation on synaptic potentiation in the CA1, and found a subtle phenotypic reversal that is in line with the effects of corticosterone exposure found in the DG in the between-litter model (chapter 2), in both sexes.

At this moment it remains to be established what might explain the differential acute effect of corticosterone on CA1 LTP in animals with different maternal care backgrounds.

Individual variation in LG and hippocampus-dependent behavior

With respect to behavior, the effects of individually scored maternal care were equally subtle (chapter 6). In the object location memory task (OLM), a hippocampus-dependent type of spatial learning, we found a non-significant negative correlation between %LG and discrimination index (which indicates how well the original location of the object was remembered). This was unexpected, given that this task was supposed to be non-stressful and previous studies in the between-litter model showed that in non-stressful learning tasks Highs perform better than Lows (Liu et al., 2000b; Bredy et al., 2003b). If anything, however, in our case, this was the opposite in both males and females, which suggests that i) this task involves brain areas other than the hippocampus that are possibly differentially regulated by maternal care, or ii) there still remained a certain level of stress to the task, despite the extensive habituation procedure that we applied. Taking into account that the object was located in the middle of the arena during the test phase, the latter appears reasonable. However, animals subjected to lower levels of LG are known to be more anxious (Caldji et al., 1998; Francis et al., 1999a, chapter 7), which would attenuate their performance on this task, i.e. the opposite to what was presently observed. In addition, a previous study using this model reported that in uncharacterized rats performance in the OLM was enhanced by high levels of corticosterone through GR activation (Roozendaal et al., 2010), which seems incompatible with the enhanced location memory in Lows compared to Highs observed in our model. We should therefore seriously consider a third explanation: influences of maternal care on spatial learning earlier observed in the between-litter model (Liu et al., 2000b; Bredy et al., 2003b) might not have been caused by maternal care per se but for instance by the genetic background introduced by the dams. However, this would still be at odds with the positive correlation between %LG and LTP induction observed in the present model.

Given the trend towards a negative correlation between %LG and LTP induction in the CA1 area of males under high versus low corticosterone conditions, we expected to see a similar negative correlation between %LG and contextual fear conditioning, a behavioral paradigm that undoubtedly elicits a lot of stress in the animals, and that is in part dependent on the hippocampus (Phillips and LeDoux, 1992). Male Low, but not High, LG/ABN offspring in the between-litter model exhibited strong emotional memory for
the context, which corresponded to the effects of corticosterone application on LTP (Champagne et al., 2008, chapter 2). In the individual model, we did not find a correlation between %LG and fear memory in males (chapter 6). In females, %LG correlated negatively with anxiety level, as measured by the percentage of freezing during training, which is in line with previous studies (Francis et al., 1999a). Additionally, Low %LG females seemed to display a somewhat better fear memory than High %LG females (chapter 6), again suggesting the influence of female sex hormones.

In conclusion, individual LG scores do have a certain predictive value for several hippocampal parameters, which is usually comparable though much more subtle than in the between-litter model. Additionally, hippocampal function does not necessarily match its structure.

It seems that maternal care exerts its effects via multiple regulatory pathways, involving the HPA axis, growth factors and sex hormones. The contribution of each of these factors and putative interactions deserve more in-depth investigations.

3.3 Maternal care and PFC function

Interestingly, while the effects of individual levels of maternal care on hippocampus-dependent learning tasks were subtle, the effects on reward-related behaviors are much stronger. In chapter 7 we describe that in a adolescent social play paradigm, pairs of male offspring with high LG scores engaged in more play behavior than pairs with lower average %LG, whereas females with different LG backgrounds did not differ in their play behavior. These data seem in contradiction with a previous study in the between-litter model, showing that in a group housing environment with access to multiple play partners, male offspring from Low LG/ABN dams in fact exhibited more play initiation, which was proposed to depend on their increased sensitivity to environmental enrichment (Parent and Meaney, 2008). However, it should be emphasized that in the current study we used a different protocol which was more reward-related; it involved social isolation of the animals prior to testing, which presumably increased their social play behavior through the activation of opioid and dopaminergic systems (Niesink and Van Ree, 1989). In dams in the original maternal care model, high levels of pup LG were associated with increased dopamine expression in the nucleus accumbens shell (NaS) (Champagne et al., 2004). Additionally, pups subjected to low levels of LG/ABN showed an asymmetric dopamine response to stress in the medial prefrontal cortex (Zhang et al., 2005), and after early life stress in rodents dopamine levels are decreased in the PFC, but increased in the NaS (Boksa and El-Khodor, 2003). Correspondingly, in human subjects reporting low parental care the release of dopamine in the ventral striatum was increased after a stressor (Pruessner et al., 2004). Thus, both early life environment and glucocorticoids affect dopamine signaling (Rodrigues et al., 2010), and may lead to an increase in reward-sensitivity and motivation, and a concomitant higher amount of play
behavior in animals with High %LG. Furthermore, social isolation probably induces a surge of corticosterone, as mentioned in section 2 of this Discussion, which may alter subsequent play behavior directly.

We have not examined dopamine levels as a function of %LG in these animals, nor their peak CORT level after stress and its rate of decline to baseline. In the literature only the effects of repeated or chronic social isolation on HPA reactivity are described (e.g. Van den Berg et al., 1999; Weintraub et al., 2010).

Another strong correlation with %LG was found for PFC-dependent decision-making performance on the rodent Iowa Gambling Task (rIGT). Both males and females performed better – i.e. made more advantageous choices – when they received higher levels of LG during infancy (chapter 7). Previously, reduced mPFC activity through application of GABA-receptor agonists, was shown to result in high levels of anxiety and a related attenuation in rIGT performance (De Visser et al. in preparation). In the between-litter maternal care model, alterations in GABA A receptor subunit composition were reported, leading to differential activation of anxiety-related corticolimbic brain areas (Caldji et al., 2003). Low LG/ABN offspring, for example, showed reduced GABAergic activity in the PFC. However, in our study, animals with higher LG scores were less anxious on the elevated plus maze, although this did not correlate directly with their performance in the rIGT. Determination of c-fos expression in the brains of animals trained in the rIGT revealed an LG-independent significant negative correlation between decision-making performance and mPFC activation. This suggests decreased neural activity in animals performing better on the task, which is in line with the aforementioned study using this behavioral paradigm (De Visser et al. in preparation). Interestingly, neural activity in two brain regions implicated in reward-related behavior, the insular cortex and the NaS, also showed this negative correlation with rIGT performance, but that disappeared when we corrected for the effects of LG, suggesting a direct mediating effect of maternal care.

It remains to be elucidated which neuronal subset in these brain areas, e.g. dopaminergic, GABAergic or glutamatergic neurons, is altered in function depending on the LG history. Also forebrain dendritic morphology in relation to %LG should be examined, in naïve animals, since early life manipulations have been shown to affect PFC pyramidal cell shape (Monroy et al., 2010) and dendritic complexity in the somatosensory cortex (Smit-Rigter et al., 2009).

4. The influence of early life

In this thesis, we aimed to unravel the influence of early life environment on adult structure and function in both the hippocampus and the PFC, by means of two rat
models, involving between-litter and within-litter variation in maternal care respectively. We found that this natural variation in early environmental experience affected adult phenotypic outcome to a greater or lesser extent, depending on the parameter studied. For those parameters that are clearly influenced by the %LG received early in life, this could offer an explanation for the extensive variability often found within experimental groups. Thus, we emphasize the importance of acknowledging, and potentially even making use of, the variable background of laboratory animals when performing experiments (Koolhaas et al., 2010).

4.1 Recommendations for future experiments
In the previous sections a number of issues were already raised that would require more attention in the future (paragraphs indicated by an exclamation mark). Below are two general remarks about the current set of experiments.

First, the individual model of maternal care, which was the primary focus of this thesis, involves certain difficulties. Although it is astonishing that even slight within-litter variability in LG can predict later-life outcome to a certain extent, the effects often seem too subtle to draw conclusions on the underlying mechanisms. Models such as early handling and maternal separation evidently elicit much more robust effects, but have the disadvantage that they involve substantial manipulation of normal mother-offspring interactions. Yet our maternal care model inevitably entails a certain degree of litter disturbance as well, due to the daily marking of the pups. Although handling has been shown to increase overall maternal behavior in Low LG/ABN dams, while not affecting that of High LG/ABN dams (Liu et al., 1997; Pryce et al., 2001), we do not expect a change in within-litter LG variability. We are aware, though, that putative fragmentation of maternal care in the first observation period after pup marking might not be picked up with our behavioral sampling method. Thus, in future studies with this model, handling should be eliminated as much as possible, for example by using more permanent and/or telemetrically discernable pup identification methods. In addition, to improve the correlations in this model i) the number of maternal care observations should be increased, i.e. once every 2 minutes instead of once every 3 minutes, in order to magnify the existing within-litter variation in LG, and ii) a larger number of animals should be used in each experiment.

Second, as mentioned throughout this discussion, all factors that possibly mediate the effects of individual LG should be thoroughly examined, to elucidate the underlying regulatory processes. Manipulations such as CORT administration to either the dam or the pups during the first postnatal week might further enhance our insight. Finally, the findings in this thesis point out that aside from studying the hippocampus it is also important to convert to other brain areas such as the PFC. This region was shown before
to be sensitive to early life stress (Ladd et al., 2004; Monroy et al., 2010), but now appears to be strongly influenced by natural variations in neonatal environment as well.

4.2 Nature versus Nurture – concluding remarks
As mentioned in the General Introduction of this thesis, the current view in the nature/nurture debate is that genetic background (‘nature’) and environmental factors (‘nurture’) act in concert to establish a certain phenotypic outcome. Our findings underscore this view, showing that differences in early life environment can strongly contribute to long-lasting individual phenotypic variation. This programming property of perinatal environmental factors might exist to produce an adult phenotype that is most advantageous for the animal in the anticipated adult environment (‘match’). If, however, a ‘mismatch’ occurs between the early and later-life environment, or between the predicted and the actual adult environment, this could result in inadequate adaptation and attenuated coping with environmental challenges (Gluckman et al., 2007). Particularly the HPA axis has been shown to be susceptible to this developmental programming (Champagne et al., 2009), with differential early life experience leading to alterations in stress responsivity and corresponding differences in cognitive performance under stress. Indeed, in the between-litter model of maternal care, Low LG/ABN males have a cognitive advantage under acutely stressful conditions over High LG/ABN male offspring (chapter 2), similar to the advantage that females with Low individual LG-scores seem to have (chapter 6). Thus, our data support the match-mismatch hypothesis, though the effects of individual maternal care, particularly on HPA axis parameters, remain generally subtle. Despite its short-term stimulatory effect on learning and memory, cumulative and prolonged exposure to glucocorticoids might eventually have detrimental effects on the brain and increase vulnerability to stress-related psychopathology (De Kloet et al., 2005). This is in line with the fact that in humans, depression is often related to hyperactivity of the HPA axis (Young et al., 1991; Holsboer, 2000; Pariante, 2003). Moreover, impairments in PFC function like we report in animals with Low %LG are also associated with human psychopathology such as schizophrenia and depression.

In conclusion, when taking all of the above into account, individual within-litter differences in LG, as well as between-litter differences, offer a very promising model for the orderly and precise investigation of the effects of early life environment (‘nurture’) on adult phenotype. In order to allow the implementation of LG scores as a predictor for later-life phenotype, it is now important to use our within-litter maternal care model to meticulously separate the influence of factors intrinsic to the dam from the effects that are directly elicited by maternal care.