Individual differences in maternal care as a predictor for phenotypic variation later in life
van Hasselt, F.N.

Citation for published version (APA):
CHAPTER 6

Effects of Within-Litter Variation in Maternal Care on Hippocampus-Dependent Task Performance in Adult Rats

Felisa N. van Hasselt, Jacintha M. Tieskens, Harm J. Krugers, Marian Joëls
Abstract
Adult stress responsiveness and cognitive performance are profoundly affected by early life environmental circumstances. In rodents, offspring from dams exhibiting high levels of maternal care show enhanced memory performance in hippocampus-dependent learning tasks in later life compared to animals that received a low amount of maternal care in infancy. Conversely, when learning occurs in stressful conditions, Low maternal care offspring perform better than High maternal care offspring, suggesting some kind of adaptation mechanism. In the current study we correlated the amount of individually received licking and grooming (LG, a specific form of maternal behavior) with later-life hippocampus-dependent memory performance in the object location memory task (OLM; non-stressful) and a contextual fear conditioning paradigm (highly stressful). We found that performance on the OLM did not correlate with %LG, in neither of the sexes. Female offspring receiving low amounts of LG display a somewhat stronger fear memory for the conditioned context than High LG offspring. Our data indicate that the variation in individual LG scores is probably too subtle to reliably pick up its effect on hippocampus-dependent behavior. Furthermore, it seems that apart from environmental influences genetic background might also play a role in the development of adult cognitive performance.
Introduction

Early life experience has extensively been shown to influence brain development, stress responsivity and cognitive performance later in life, both in humans and in primates and rodents (McEwen, 2003; Nemeroff, 2004a, b; Pryce et al., 2005; Neigh et al., 2009). Various animal models are currently in use to study these effects and the underlying mechanisms, usually interfering with normal mother-pup interactions (e.g. maternal separation, early handling). However, also paradigms that involve studying normal variations in maternal care have revealed phenotypic alterations in adulthood in response to a differential early life background. Offspring of High licking and grooming (LG) compared to Low LG mothers show greater hippocampal synaptic plasticity in low stress conditions (Bredy et al., 2003b; Champagne et al., 2008; Bagot et al., 2009). Correspondingly, cognitive performance in relatively non-stressful hippocampus-dependent spatial learning tasks (i.e. a Morris water maze task to which animals were habituated, object recognition task) was found to be superior in High compared to Low LG animals (Bredy et al., 2003b).

High versus Low LG offspring express higher levels of glucocorticoid receptor (GR) mRNA in the hippocampus, supposedly contributing to enhanced glucocorticoid negative feedback (Liu et al., 1997; Francis et al., 1999a; Meaney, 2001a). Altered responsiveness to corticosterone also emerged from an in vitro synaptic plasticity experiment, showing strong long-term potentiation (LTP) after corticosterone treatment in Low LG offspring, but reduced LTP in the High LG animals (Champagne et al., 2008; Bagot et al., 2009). In line with this, hippocampus-dependent memory performance under conditions of high stress or high levels of corticosterone was relatively poor in offspring from High compared to Low LG mothers (Champagne et al., 2008; Bagot et al., 2009).

Similar, though much more subtle, findings on hippocampal function have been reported for a variant of the maternal care model, determining individual within-litter licking and grooming scores. In this model, %LG was shown to correlate positively with GR mRNA expression in the hippocampus, and with in vitro long-term potentiation (LTP) particularly in the dentate gyrus (Van Hasselt et al., 2011). In the CA1 area, the maternal effect on synaptic potentiation was partially reversed when high levels of corticosterone were applied at the time of LTP induction (Van Hasselt et al. in preparation).

Therefore, we wondered if individual maternal care scores also predict adult performance in hippocampus-dependent learning tasks, both under non-stressful conditions (tested with an object location memory (OLM) task; Roozendaal et al., 2010) or during stressful contextual fear conditioning (Sanders et al., 2003; Bagot et al., 2009). We present data both on male and female young adult rats.
Materials and Methods

Maternal behavior

Male and female outbred Long Evans rats were obtained from Harlan (Indianapolis, US) and used to breed the animals used for this study in-house (n=34, 17 M / 17 F, from 5 litters). Breeding and subsequent maternal care observations were performed as described previously (Van Hasselt et al., 2011). Briefly, all litters were culled to 8 (preferably 4 male and 4 female), and from postnatal day (PND) 1 to PND7 maternal licking and grooming (LG) scores were determined for each individual pup within each litter. At weaning, on PND 21, pups were ear-punched for later identification and group-housed with their same-sex littermates until testing around 9-10 weeks of age. During the entire experiment animals were maintained on a 12h light/dark schedule (lights on at 8:00 hrs) at a room temperature of 20-22°C and 40-60% humidity. Food was available ad libitum. All experimental procedures were approved by the animal ethical and welfare committee of the University of Amsterdam.

Object location memory task

As previously described (Roozendaal et al., 2010), the object location memory task (OLM) is a non-stressful, hippocampus-dependent spatial learning task. Training and testing occurred during the light phase (between 9:00 and 12:00 hrs), but in dim light conditions. The test apparatus consisted of a black plastic box (40x40x40 cm) with a sawdust-covered floor and an open top to allow videotaping for later offline scoring of exploration behavior.

For four days prior to training, the animals were briefly handled by the experimenter and habituated to the test box by 3 minutes of free exploration (no objects present) per day. For the training session, two identical objects (transparent glass vials; 4.5 cm diameter, 5.5 cm height) were placed in the test apparatus, approximately 5 cm away from the corners, and the animals were then allowed to explore these objects for 10 minutes. Between animals, the objects were cleaned with 70% ethanol and the sawdust was stirred to eliminate possible odor trails. We tested the memory for object location specifically 24 hours later, by using the same objects, but leaving only one in its original location, either left or right, while the other was placed in the middle of the test apparatus. Animals were again allowed to explore both objects, now for 3 minutes only, and a discrimination index was calculated using the times that the animals spent exploring each object [DI = (new-familiar)/(new+familiar)]. Rats that showed a total exploration time of less than 10 seconds in either the training or the test session were excluded from analyses.
Contextual fear conditioning

To assess if individual differences in LG background correlate with fear memory in adulthood, we subjected our animals to a contextual fear conditioning task, which has been shown to be hippocampus-dependent and highly stressful (Phillips and LeDoux, 1992; Hernandez-Rabaza et al., 2008; Schenberg and Oliveira, 2008). Conditioning and testing occurred between 9:00 and 12:00 hrs, during the light phase.

The conditioning chamber consisted of a black plastic box (40x40x50 cm) with a metal grid floor connected to a shock generator and an open top to allow videotaping for later analysis of freezing behavior. For two days prior to conditioning, the animals were habituated to manipulations associated with testing (i.e. transportation to the testing room and brief handling by the experimenter). On the conditioning day, animals were put in the conditioning chamber and allowed to explore the context for 3 minutes before receiving the first of four footshocks (1s/0.6mA; inter-shock interval 1 minute). Thirty seconds after the last shock the animals were removed from the chamber and returned to their home-cage. Twenty-four hours later contextual fear memory was assessed by placing the animals back into the same conditioning chamber for 3 minutes without administering additional footshocks.

For both training and test sessions, we determined a percentage score for freezing behavior, characterized as the absence of all body movements except those required for respiration (Blanchard and Blanchard, 1969), by scoring the presence or absence of freezing every 2 seconds.

Corticosterone assay

Three weeks after fear conditioning, all animals were decapitated at the beginning of the light phase, when corticosterone levels are low. Trunk blood from every animal was collected in EDTA-coated tubes, placed on ice and centrifuged for 20 minutes at 5000 rpm. The plasma was stored at -20°C, until use for determination of circulating corticosterone levels by radio-immunoassay (RIA) (MP Biomedicals, Amsterdam, The Netherlands).

Statistical analysis

Statistical analyses were conducted using SPSS 11.0 for Windows. All correlations were tested using linear regression with %LG as the independent (predictor) variable.

Results

Maternal behavior

All animals used in this study (n=34, 17M/17F) were obtained from 5 different litters. Similar to earlier reports (Van Hasselt et al., 2011, Van Hasselt et al. in preparation, S.E.F.
Claessens et al., personal communication), we found a substantial amount of intra-litter variation in the amount of LG that each individual pup receives. Basal plasma corticosterone levels did not correlate with %LG received, neither in males (n=15, r=-0.064, p=0.821) nor in females (n=17, r=-0.190, p=0.466). As expected, the range of circulating corticosterone concentrations in male offspring was somewhat lower than that observed in female rats. In this cohort of animals, average LG scores did not differ significantly between males and females (Figure 1), contrary to what was described earlier (see chapters 3 and 4).

**Figure 1. Individual licking and grooming scores.** Distribution of LG scores of the animals used in this study (17M and 17F). Average %LG did not differ significantly between males and females, in contrast to what was described before.

**Object location memory task**
The original maternal care model reported an increased performance of High versus Low LG offspring in the object recognition task (Bredy et al., 2003b). However, this task is not considered to be exclusively hippocampus-dependent (Winters et al., 2008; Langston and Wood, 2010). Therefore we chose to use the hippocampus-dependent object location memory task (OLM; Roozendaal et al., 2010; Figure 2A). In this task, the direction of the correlation between %LG and discrimination index was similar for males and females, and average DI values did not differ between sexes. This allowed us to pool data from all animals.

**Figure 2. Object location memory task.** (A) Experimental setup. For the training session, two identical objects were placed in two corners of a 40x40 cm open field. In the test session, one of the objects was moved to a different location. (B) Object location memory performance in relation to %LG in both males and females (n=31, r=0.268, p=0.145). Discrimination Index (DI) = (new-familiar)/(new+familiar).
We did not find an effect of individual differences in maternal care on performance in the OLM. In males and females, there was a non-significant negative correlation between discrimination index and %LG (n=31, r=-0.268, p=0.145; Figure 2B), indicating that animals that received higher amounts of licking and grooming in early life showed a somewhat decreased preference for the object in the novel location. Total object exploration time did not correlate with the %LG received early in life (n=31, r=-0.048, p=0.796; data not shown).

For neither males nor females, basal plasma corticosterone levels correlated with object recognition memory (males: n=13, r=-0.448, p=0.124; females: n=16, r=0.305, p=0.251).

**Contextual fear conditioning**

Previous studies, comparing offspring from extremely High versus Low LG dams, showed an effect of maternal care on performance in hippocampus-dependent, context-based versions of the fear conditioning task. Low LG offspring exhibited better fear memory for the context, which was linked to increased *in vitro* hippocampal synaptic plasticity in the presence of corticosterone in these animals (Champagne et al., 2008; Bagot et al., 2009). Here, we tested if individual differences in licking and grooming correlate with adult learning performance under stressful conditions, in a contextual fear conditioning task. The percentage of freezing was significantly higher in the males than in the females, so for this task we report on males and females separately.

![Figure 3. Contextual fear conditioning.](image)

(A) In females, % Freezing in the training session correlated significantly with %LG (n=17, r=-0.486, p=0.048). (B) The difference between % Freezing in the late stage (II; 90-180 sec) and % Freezing in the early stage (I; 0-90 sec) of the test session, \( \Delta II-I \), showed a positive trend with %LG (n=17, r=0.432, p=0.083).

Somewhat to our surprise, we found a significant negative correlation between %LG and freezing behavior during the training session in the female rats (n=17, r=-0.486, p=0.048; Figure 3A). Thus, it seems that Low LG female offspring are already more anxious to start with, making the subsequent test session freezing data slightly difficult to interpret. In males, there was no correlation between %LG and the percentage of
freezing during training. During the context test session 24 hrs after training, there was no correlation between %LG and freezing behavior, neither in males (n=17, r=-0.178, p=0.495), nor in females (n=17, r=-0.034, p=0.896). We then subdivided the test phase into an early freezing-stage (first 90 sec, I) and a late freezing-stage (last 90 sec, II), in order to examine possible effects over time (Zhou et al., 2010) and found that in females, the difference between early-stage and late-stage freezing (ΔII-I), relative to the total amount of freezing, showed a positive correlation with %LG (n=17, r=0.512, p=0.036; Figure 3B). This indicates that female offspring with lower LG scores reached their maximum level of freezing sooner, presumably because they recognize the adverse context more quickly, suggesting better fear memory in these animals. However, we did not find a correlation between %LG and the amount of freezing in stage I to support this.

No correlations were found between basal plasma corticosterone levels and percentage freezing during the test day, in neither of the sexes (males: n=15, r=0.289, p=0.296; females: n=17, r=0.241, p=0.351).

**Discussion**

It has been postulated that the environment early in life prepares an individual for comparable circumstances later in life, so that if the early environment matches with the situation later in life, the animal possesses the most favorable phenotype (Champagne et al., 2009). However, if there is a mismatch between the phenotype and the environment the individual might not be able to adequately respond to environmental challenges. This was partly based on studies addressing the influence of early life experience on adult hippocampal function, focusing on the extremes (>1SD from the mean) in natural variations of maternal care. Hippocampal long-term potentiation (LTP), the most likely neurobiological substrate for learning, was effectively induced in animals that received relatively high amounts of licking and grooming (LG) compared to offspring of Low LG mothers; yet, application of high levels of corticosterone before or around the time of LTP induction reversed this phenotype (Champagne et al., 2008; Bagot et al., 2009). In parallel, performance in hippocampus-dependent learning tasks was efficient in High versus Low LG offspring under low stress conditions, but the opposite was found under high stress conditions (Bredy et al., 2003b; Champagne et al., 2008; Bagot et al., 2009). We here tested whether similar differences in behavioral performance were also observed when examining animals over the entire range of LG received during the first postnatal week.

Particularly under non-stressful conditions we expected to see a positive correlation between %LG received early in life and behavioral performance in adulthood, since in the DG of non-stressed rats %LG showed a strong correlation with the degree to which LTP could be induced (Van Hasselt et al., 2011); this was less clearly seen in the hippocampal
CA1 area (Van Hasselt et al, *in preparation*). Yet, contrary to our predictions, performance in the OLM test, which is considered to be hippocampus-dependent and non-stressful (Roozendaal et al., 2010), did not correlate with maternal care background. If anything, animals with higher LG scores showed less preference for the novel location than animals with lower LG scores, which is interpreted as these animals having a weaker memory for the original location. It could be argued that in spite of the extensive habituation procedure that was implemented before OLM training, this task did involve a certain amount of stress, tipping the balance towards more advantageous circumstances for the animals on the lower end of the LG scale. However, performance on the OLM task was shown to be enhanced by corticosterone application through activation of the GR (Roozendaal et al., 2010) and since animals with high rather than low LG scores show elevated levels of hippocampal GR expression (Van Hasselt et al., 2011), it seems unlikely that animals on the lower end of the LG scale would have performed better than those with a high %LG even if the task was stressful enough to evoke substantial corticosterone release.

While the between-litter model showed strong contextual memory formation in male offspring from High compared to Low LG mothers, which was paralleled by strong LTP in High compared to Low LG male offspring both in the CA1 hippocampal area and DG after corticosterone administration (Champagne et al., 2008; Bagot et al., 2009), we earlier observed only a marginal effect of corticosterone application on CA1 synaptic plasticity in the within-litter model (Van Hasselt et al. *in preparation*). This suggests that the within-litter model may be too subtle to show a clear correlation between %LG received by male rats early in life and their performance under stressful learning conditions. In agreement, we did not find a significant correlation between %LG and contextual fear memory in males.

Predictions of behavioral performance under stressful conditions were even harder to make in female rats. Females have not been tested in the original between-litter maternal care model, but based on our electrophysiological recordings in the hippocampal CA1 area there was actually reason to expect that females would respond opposite to males (Van Hasselt et al., *in preparation*), i.e. that female rats with high LG scores would outperform those with a low %LG. Yet, no data are available for female rats with respect to the influence of early life environment on corticosterone modulation of LTP in the hippocampal dentate gyrus, which is unfortunate since the DG is considered to be essential for contextual fear conditioning (Hernandez-Rabaza et al., 2008). We here observed that total freezing behavior on the test day (24 hrs after training) did not differ between female animals in relation to %LG, but the animals on the lower end of the LG scale seemed to freeze more quickly compared to animals receiving higher amounts of LG. Interestingly, females already showed differences in freezing behavior during the training session, pointing to a difference in overall anxiety level. The latter is consistent with
previous reports on the between-litter High/Low LG model (Caldji et al., 1998; Francis et al., 1999a).

In general, there seems to be low predictive value of individual LG scores for hippocampus-dependent learning performance in adulthood, which can be explained in a number of ways. From Figure 1 it is apparent that there is not as much variability in individual LG scores, especially for the males, as was reported before (Van Hasselt et al., 2011). This might be due to the fact that for several weeks perinatally, there was construction work going on adjacent to the animal facility, possibly causing prenatal stress to the pregnant dams as well as disturbing normal maternal behavior postnatally. Thus, building noise stress might have disturbed HPA axis development in this cohort and overruled the subtle effects of differential maternal LG (O’Regan et al., 2010). Second, the development and function of the HPA system might be influenced to a larger extent by genetic background than by maternal care. In the individual maternal care model, which completely rules out genetic effects, no differences in either basal or stress-induced plasma corticosterone levels have been reported in relation to differential LG background, despite alterations in GR mRNA expression (Van Hasselt et al., 2011), whereas in the original maternal care model, comparing maternal care effects between litters, High compared to Low LG offspring show lower basal corticosterone levels and stronger negative feedback / normalization of stress-induced rises in corticosteroid hormone level (Liu et al., 1997). Cross-fostering studies have revealed evidence for a direct effect of maternal care on the expression of GR and CRH, but corticosterone responses to stress have not been investigated yet with that paradigm (Francis et al., 1999a). Finally, directly relating individual differences in LG between pups to adult learning behavior might just not be possible due to the subtlety of the variation.

In conclusion, subtle within-litter variation in licking and grooming does not correlate significantly with adult hippocampus-dependent learning behavior, especially not in males, neither under relatively non-stressful nor under stressful conditions. These results indicate that the adult behavioral phenotype in this case does not depend solely on the effects of maternal care, but involves other factors that might influence HPA function, such as genetic background.

Acknowledgements
The authors thank Noortje van der Knaap and Daniëlle de Jong for their help with maternal care observations, Rosemary Bagot and Benno Roozendaal are acknowledged for helping design the fear conditioning and OLM experiments, and Maaike van der Mark for performing the RIA for plasma corticosterone.