Individual differences in maternal care as a predictor for phenotypic variation later in life
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CHAPTER 7

Individual Within-Litter Variation in Maternal Care Strongly Correlates with Adolescent Social Play Behavior, Anxiety and Decision-Making Performance in Rats

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In preparation
Abstract
Early life adversity has been reported to increase the vulnerability to develop psychopathology, such as depression, anxiety and schizophrenia, later in life. In the present study we selected two paradigms studying behavioral aspects that are known to be attenuated in psychiatric patients and we examined in rat offspring whether subtle variations in early life environment, i.e. individual differences in maternal licking and grooming (%LG), correlate with these behaviors. Patients suffering from schizophrenia often exhibit disturbed social behavior. Thus, we first investigated adolescent social play behavior in animals with a different history of maternal care. In a paired social play paradigm, male adolescents with higher %LG exhibited more play behavior than those with lower %LG, whereas in females, %LG did not correlate with the amount of social play. Furthermore, early life environmental factors have been shown to affect the function of the prefrontal cortex, disruption of which has been implicated in the pathophysiology of, for example, mood disorders. Therefore we also examined the effects of individual differences in %LG on decision-making processes, in which the prefrontal cortex plays a crucial role. Decision-making performance was tested in a rodent version of the Iowa Gambling Task. In both males and females, %LG correlated significantly with the number of advantageous choices in this task, indicating that a higher amount of early life LG was related to better decision-making performance. This was accompanied by increased c-fos expression (reflecting neural activity) in several regions of the brain, which was mediated by levels of LG only in the lateral orbitofrontal cortex, insular cortex and ventral striatum. Thus, it appears that individual variations in maternal care influence social interactions as well as decision-making processes and function of related brain structures.
Introduction

A large number of psychiatric diseases such as schizophrenia, anxiety disorders and depression involve dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis and disruptions of higher executive processes, both of which are partly under control of the prefrontal cortex (PFC) (Diorio et al., 1993; Fossati et al., 1999; Sullivan and Gratton, 2002; Rogers et al., 2004). The attenuated PFC function was found to be associated with structural remodeling (Bremner, 2002; Holmes and Wellman, 2009) and impaired monoamine neurotransmission, particularly of serotonin and dopamine (Mann et al., 1995; Nutt, 2001; Olvera-Cortes et al., 2008; Nikolaus et al., 2010), modifications that are often induced by stressors (Abercrombie et al., 1989; Murphy et al., 1996; Liston et al., 2006; Lapiz-Bluhm et al., 2008; Shansky and Morrison, 2009). Interestingly, the risk of developing any of the aforementioned disorders is commonly increased after early life adversity (Breier et al., 1988; Agid et al., 1999; Niwa et al., 2010; Pechtel and Pizzagalli, 2010).

The influence of early life adversity on brain structure and neurotransmission can be addressed in detail in appropriate animal models. For instance, repeated neonatal maternal separation (MS) in rodents, a frequently used model for adverse early life experience, was reported to alter dendritic morphology and spine density in PFC pyramidal neurons. However, these reports were not always consistent, due to differences in e.g. species and PFC area examined; MS has been shown to induce dendritic atrophy and loss of spines in the rat PFC (Bock et al., 2005; Pascual and Zamora-Leon, 2007; Monroy et al., 2010) while in the anterior cingulate cortex in *Octodon degus* it induced elevated spine densities and did not affect dendritic mass (Helmeke et al., 2001). In addition, maternally separated rodents showed a reduction in PFC dopamine and serotonin neurotransmission (Matthews et al., 2001; Gartside et al., 2003) and, compared to animals subjected to daily handling, exhibited a decrease in prefrontal glucocorticoid receptor (GR) expression and a corresponding increase in HPA stress-reactivity (Ladd et al., 2004).

In a different model for early life environment in rats, natural variations in maternal care (both between-litter and within-litter) were shown to affect HPA axis responsiveness in the offspring; receiving low amounts of licking and grooming (LG) as a pup was associated with lower levels of hippocampal GR mRNA expression, enhanced HPA axis responses to stress and increased levels of anxiety and fearfulness in adulthood when compared to animals receiving high LG (Liu et al., 1997; Caldji et al., 1998; Francis et al., 1999a; Van Hasselt et al., 2011). Interestingly, several studies suggest that the effect of maternal care on GR expression in the hippocampus is mediated by differences in early life serotonin turnover in this brain area (Mitchell et al., 1990; Meaney et al., 2000; Weaver et al., 2001). In the medial prefrontal cortex, dopamine responses to stress...
were reported to be increased in Low compared to High LG offspring (Zhang et al., 2005). Other prefrontal characteristics have not yet been studied in great detail in this model.

In view of the above, we wondered if variations in the amount of maternal care received by individual pups within a litter correlate directly with two specific forms of behavior that are relevant for psychopathology. Social behavior is often impaired in patients suffering from psychotic disorders such as schizophrenia (Green and Horan, 2010), and depressed patients are known to be less sensitive to reward (Henriques and Davidson, 2000). Therefore, we first investigated adolescent social play behavior in pairs of animals earlier characterized for the amount of LG received during the first postnatal week. In rats, this type of behavior is the earliest form of social behavior an animal exhibits that is not directed towards the mother, and it is crucial for the further social development of the animal. It is accompanied by increases in forebrain dopaminergic neurotransmission and has a large reward value (Vanderschuren et al., 1997, Trezza et al., 2010). Secondly, we examined the relation between LG received by individuals early in life and later performance on the rodent version of the Iowa Gambling Task (IGT) (Van den Bos et al., 2006b), measuring decision-making performance under uncertainty. Both depressive and highly anxious individuals exhibit impaired performance on the IGT (Miu et al., 2008; Cella et al., 2010; De Visser et al., 2010). We here also assessed the underlying neural circuitry using the immediate early gene c-fos as a marker for neural activity, since IGT performance was shown to be associated with reduced medial PFC activity in rats (De Visser et al., in preparation).

**Materials and Methods**

**Maternal care**

The animals used in this study were bred in our facility, and were kept on a 12h light/dark schedule (lights on at 8:00 hrs) until weaning, when they were moved to a different room where the light cycle was reversed (lights on at 21:00 hrs). During the entire experiment, temperature and humidity were maintained at 20-22°C and 40-60% respectively, and food and water were available *ad libitum*, unless otherwise specified.

Male and female outbred Long Evans rats were purchased from Harlan (Indianapolis, US) at approximately 2.5 months of age and allowed to habituate to the animal facility. Then, two females were housed with one male for one week to allow mating, and after another week of paired-housing, the females were placed separately in large observation cages (30x55x45 cm³). Maternal care observations commenced on postnatal day 1 (PND1; with PND0 being the day of birth), after culling the litters to eight pups (preferably four males and four females), and were performed as described before (Van Hasselt et al., 2011). Briefly, maternal behavior was scored every three minutes during five one-hour observation sessions daily (7:00, 10:00, 13:00, 17:00 and 20:00 hrs).
for seven days, resulting in a total of 700 observations for each litter. Several specific maternal behaviors were scored, including licking and grooming (LG), particularly towards individual pups within each litter. In order to be able to identify the pup that underwent licking and grooming, all pups were uniquely marked every morning until weaning with a non-scenting, non-toxic surgical marker (Codman, Johnson & Johnson, Brunswick, NY). We were able to distinguish which pup was being licked and groomed in about 60% of the cases, and since this percentage varied slightly between litters, we corrected for this with the following equation: (% individual LG observed) /(% total LG identified) * 100%. As described before, we did not find any effects of the marking procedure or the markings per se on the licking behavior of the dam (Van Hasselt et al., 2011).

On PND24, the pups were weaned, ear-punched and put on a reversed 12h day/night regimen. They were group-housed with their same-sex littermates until testing in the social play paradigm on PND35. Then, the animals were redistributed and group-housed with same-sex non-littermates until a subset of animals (n=24, 12 males/12 females) entered the decision-making experiment at around eight (females) to ten (males) weeks of age. The remaining animals (n=24, 15 males/9 females) were kept for use in a subsequent study assessing prefrontal dendritic morphology.

All experimental procedures used in this study were approved by the animal ethical and welfare committee of the University of Amsterdam.

Social play behavior
Twenty females and 22 males were selected and paired according to their individual %LG, in such a way that animals with comparable LG scores were tested together. Habituation and behavioral testing were performed as described previously (Trezza and Vanderschuren, 2008), and occurred in the first two to four hours of the dark phase (in red light conditions), when cage activity is high (Parent and Meaney, 2008; Klein et al., 2010). For 10 minutes per day on the two days prior to testing, the animals were individually habituated to the test cage, which consisted of a square black plastic cage (40x40x40 cm³) with sawdust. The animals were tested on PND35, which is considered to fall well into the age span that covers adolescence in the rat and is associated with high levels of social play behavior (Panksepp, 1981; Spear, 2000; Auger and Olesen, 2009; Klein et al., 2010). On the test day, the animals were first socially isolated for three hours to enhance their motivation to engage in play behavior during the test, and subsequently pairs of animals with similar LG scores were put in the test cage for 15 minutes and allowed to interact. In the majority of cases, the animals did not have previous common social experience (i.e. they did not belong to the same litter), and in none of the pairs the animals differed more than 10% in body weight. Each test session was recorded for later offline analysis. Behaviors were assessed per pair using the Observer 3.0 software (Noldus
Information Technology B.V., Wageningen, The Netherlands) and included ‘pouncing’,
which is a characteristic soliciting behavior, ‘pinning’, when one rat holds its play partner
in a supine position, and social exploration, consisting of sniffing and social grooming
(Pellis and Pellis, 1987; Trezza and Vanderschuren, 2008).

**Elevated Plus Maze (EPM)**
All 24 animals that were designated to participate in the decision-making experiment (see
below) were first tested on the EPM to assess their anxiety level. The maze was made of
black PVC, consisted of four arms (50x10 cm) – two open and two enclosed by 30 cm high
walls, forming a cross with the center area – and was elevated 60 cm above the ground.
Animals were tested during the dark phase of their circadian light cycle, though under
bright light conditions. Each rat was put on the center platform facing one of the open
arms and allowed to freely explore the maze for 5 minutes. Between trials the maze was
cleaned with ethanol and water, and dried thoroughly with clean paper towels. All test
sessions were recorded for later analysis of spatiotemporal measures (i.e. the time spent
in the open and closed arms and the number of entries into each arm) using the Observer
5.0 software (Noldus Information Technology B.V., Wageningen, The Netherlands).

**Rodent Iowa Gambling Task (rIGT)**
24 animals (12M / 12F) along the entire range of LG scores (between 0.32% and 2.30%
LG for males; between 0.00% and 1.40% LG for females) were selected for testing in the
rIGT. Before the start of the experiment, rats were habituated to the test apparatus in a
10 min free exploration trial. Two days later, they were mildly food restricted
(approximately 95% of free feeding body weight) and tested during two 5-day periods. On
weekend days food was available *ad libitum*. All testing occurred in red-light conditions
during the dark phase of the day-night cycle, between 11:00 and 16:00 hrs.

The test apparatus was made of grey PVC and consisted of a start box, choice area
and four arms. A trial started by lifting the slide door of the start box, allowing the rat to
freely enter the choice area of the apparatus and choose one of the four arms. The chosen
arm was closed when the rat had entered that arm with its full body, including its tail. At
the end of each arm, rats could either obtain sucrose pellets or quinine-treated sucrose
pellets (baited arms; see below) or no pellets at all (empty arms). Each trial had a
maximum duration of 6 min, the inter-trial interval was 30 s, and each animal received a
total of 120 trials (10-15 trials per day). Sucrose pellets (45 mg; Bioserve Inc, Frenchtown,
NJ, USA) were used as a reward and quinine-treated sucrose pellets, that were unpalatable
but not uneatable, as punishment. As mentioned before, two of the four arms in the maze
were empty. These were included to measure non-reward related exploration (Van den
Bos et al., 2006b; Homberg et al., 2008). The two baited arms consisted of a ‘bad’ arm and
a ‘good’ arm. In the ‘bad’ arm, the rats received occasional big rewards (three sucrose
pellets in 1 out of 10 trials) among frequent punishments (three quinine-treated sucrose pellets in 9 out of 10 trials). In the ‘good’ arm, the rats received frequent small rewards (one sucrose pellet in 8 out of 10 trials) and infrequent punishments (one quinine-treated sucrose pellet in 2 out of 10 trials). This provided the same principle as in the human IGT: an option with a chance of a big reward (3 sucrose pellets), but with little long-term success (3 sucrose pellets per 10 trials; cf. decks A and B; Bechara et al., 1994) and an option with a chance of a small reward (1 sucrose pellet), but with bigger long-term success (8 sucrose pellets per 10 trials; cf. decks C and D). The location of the baited and empty arms, as well as ‘good’ and ‘bad’ arms was counterbalanced across subjects.

To determine the performance of the rats, the number of choices for the most advantageous option were calculated and expressed as a fraction of the total number of trials per block. Choices were calculated in blocks of 10 trials. Scores during the last block of 10 trials (trial 110-120) were taken as a measure of final rIGT performance. The number of sucrose pellets collected during the task (trial 1-120) was used as a measure of overall task performance to reflect the final “budget” (cf. monetary budget in the human IGT, Van den Bos et al., 2006a).

*C-fos immunohistochemistry*

Two hours after the last session in the rIGT, the animals were rapidly decapitated. Their brains were removed, immediately snap-frozen on dry-ice and stored at -80°C. Coronal sections (20 μm) were cut on a cryostat (Leica CM3050S), mounted on Starfrost adhesive slides (Knittel Glaser, Waldemar Knittel, Germany) and stored at −20°C. For the immunohistochemical detection of c-fos, rabbit anti-c-fos (Calbiochem, Darmstadt, Germany) was used. During the staining procedure the sections were rinsed several times after every step in 0.01 M phosphate-buffered saline (PBS; pH 7.4). First, the sections were dehydrated. Endogenous peroxidase was blocked by treatment with H2O2 (0.1%) for 30 min. Sections were pre-incubated with 5% normal donkey serum (NDS) and 1% bovine serum albumin (BSA) in PBS (PBS-BSA 1% + NDS 5%) for 30 min before the rabbit anti-c-fos incubation (1:4000 in PBS-BSA 1% + NDS 5%, 4°C, 24 h). Negative controls were incubated with the PBS-BSA 1% + NDS 5% solution. Next, the sections were incubated with donkey–anti-rabbit IgG Biotin SP conjugate (1:400 in PBS-BSA 1% + NDS 5%, Jackson ImmunoResearch Laboratories, Inc., PA, USA) for 45 min. Subsequently, the sections were incubated with avidin-horseradish peroxidase solution (1:400 in PBS-BSA 1%+ NDS 5% VECTASTAIN® ELITE ABC, Brunwich Chemie, Amsterdam, The Netherlands) for 60 min. Then, slices were pre-incubated with inactive diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St. Louis, MO, USA) solution containing nickel sulphate. To activate DAB for visualization of bound peroxidase complexes, the substrate H2O2 (30%, 1:2000) was added to the DAB solution and incubated for 5 min. Afterwards the sections were dehydrated in alcohol and coverslipped.
The images of brain sections were projected (10× magnification) and digitized using an Olympus BX 51 microscope (Olympus, Tokyo, Japan) with a high-resolution digital camera interfaced with a computer. The anatomical localization was aided by use of adjacent Nissl stained sections and illustrations in a stereotaxic atlas (Paxinos and Watson, 2005). The following brain regions were investigated: orbitofrontal cortex (OFC; +4.20 from bregma), insular cortex (insula; +1.92 from bregma), medial prefrontal cortex, i.e. cingulate cortex (Cg1; +2.52 from bregma), prelimbic (PrL; +2.52 from bregma) and infralimbic cortex (IL; +2.52 from bregma), dorsolateral striatum (DLS; +1.92 from bregma), dorsomedial striatum (DMS; +1.92 from bregma), nucleus accumbens core (NaC; +1.92 from bregma), nucleus accumbens shell (NaS; 1.92 from bregma), basolateral amygdala (BLA; -2.52 from bregma), central nucleus of the amygdala (CeN; -2.52 from bregma), dentate gyrus (DG; -3.36 from bregma) and CA1 regio of the hippocampus (CA1; -3.36 from bregma). For each region at least two overt landmarks were used. For quantitative analysis of c-fos positive cells, the program Leica QWIN (image processing and analysis software, Cambridge, UK) was used. Only right hemispheres were analyzed, using two subsequent sections per animal. The number of positive cells was then averaged for each animal and expressed per mm².

**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. Male and female data were only pooled for a certain correlation if i) the direction of that correlation was similar in both sexes and if ii) neither of the parameters in the correlation (e.g. %LG, IGT performance) differed significantly between sexes (analyzed by an independent Student’s t-test).

**Results**

**Maternal behavior**

We observed six lactating dams from PND1 to PND7 and assessed the percentage of time they spent licking and grooming (%LG) each individual pup in their litters. The %LG received varied considerably between pups within litters (range 0.00% to 2.30% LG; mean %LG=0.92, SD=0.58, n=48; Figure 1). In general males (n=27) received significantly more LG than females.

![Figure 1. Maternal care characterization.](image)

Substantial within-litter variation exists in the amount of licking and grooming that individual pups receive. Each column represents a litter and each data point represents a pup. Error bars depict one SD above and below the litter mean. Black squares represent males, grey triangles represent females.
(n=21; p=0.047), in agreement with earlier reports (Moore and Morelli, 1979; Van Hasselt et al., 2011).

**Social play behavior**

In the current study we tested 10 pairs of females and 11 pairs of males to examine if individual variations in the amount of LG affected adolescent social play behavior. Correlations for all parameters measured were opposite in direction for male and female offspring, so we analyzed the data for both sexes separately.

![Figure 2. Male social play behavior at PND 35.](image)

In females, none of the play behaviors that were assessed correlated significantly with %LG. In males on the other hand, significant positive correlations emerged between %LG and both the frequency (n=11, r=0.637, p=0.035; Figure 2A) and duration (n=11, r=0.763, p=0.006) of pinning (C, D). (E) However, the latency to start social exploration was increased in High LG animals as well (n=11, r=0.724, p=0.012).
r = 0.672, p = 0.023; Figure 2B) of pouncing, and the frequency (n = 11, r = 0.608, p = 0.047; Figure 2C) and duration (n = 11, r = 0.763, p = 0.006; Figure 2D) of pinning. Clearly, male rat pairs with higher average LG scores exhibited more social play behavior during the 15 minute session compared to pairs that received lower levels of maternal licking and grooming. However, males on the high end of the LG scale also showed a significantly increased latency to first social exploration compared to males with a lower %LG (n = 11, r = 0.724, p = 0.012; Figure 2E).

**Elevated Plus Maze**

We tested our animals on the EPM in order to determine their basal anxiety levels. The males and females used in this experiment did not differ in the average amount of maternal care received (%LG; t = 1.959, df = 22, p = 0.066). However, the average time spent on the open arms of the maze did differ between sexes (t = 2.095, df = 21, p = 0.048) and therefore we analyzed them separately. In males, a significant positive correlation emerged between %LG and time spent on the open arms (n = 12, r = 0.690, p = 0.013; Figure 3A), whereas in females this was a positive trend (n = 11, r = 0.511, p = 0.108; Figure 3B). This indicates that animals with higher LG scores are less anxious than animals with lower %LG. Individual LG scores did not correlate with the number of closed arm entries in males (n = 12, r = 0.137, p = 0.672) or in females (n = 12, r = -0.069, p = 0.830), which demonstrates that there was no difference in general activity between animals.

![Figure 3. Elevated Plus Maze.](image-url)

(A) In ten-week-old male offspring a positive correlation emerged between %LG and open arm time on the EPM (n = 12, r = 0.690, p = 0.013), indicating reduced anxiety in animals with higher LG scores. (B) Similarly, in 8-week-old females, there was a positive trend between these parameters (n = 11, r = 0.511, p = 0.108).

**rIGT performance**

The males and females used in this experiment did not differ in the average amount of maternal care received (%LG; t = 1.959, df = 22, p = 0.066) and average rIGT performance (fraction of advantageous choices; t = 0.000, df = 22, p = 1.000) and were thus pooled for further correlational analysis. There was a significant positive correlation between %LG and the number of advantageous choices made in the rIGT during the last session (n = 24,
r=0.521, p=0.009; Figure 4). Thus, rats that received more maternal care showed a better performance in the rIGT. In line with this, a significant negative correlation existed between %LG and the number of visits to either of the empty arms (n=24, r=-0.421, p=0.040), which demonstrates elevated non-reward related exploratory behavior in animals with lower LG scores (Van den Bos et al., 2006b; Homberg et al., 2008). These findings were accompanied by a significant positive correlation between %LG and the total number of sugar pellets obtained during the task (n=24, r=0.420, p=0.021), indicating that rats that received more maternal care had a higher overall yield of reward.

**Figure 4. Performance on the rodent Iowa Gambling Task.** In young-adult males and females there was a positive correlation between task performance on the rIGT, as indicated by the number of advantageous choices made during the last test session, and %LG (n=24, r=0.521, p=0.009). Thus, animals with higher LG scores perform better on a higher cognitive decision-making task.

**Table 1** Correlations between c-fos expression and both individual %LG and rIGT performance (* p<0.05; † p<0.1). Male and female data were pooled (n=24), except for the lateral OFC (n=12).

<table>
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<tr>
<th>Brain area</th>
<th>LG%</th>
<th>r</th>
<th>p</th>
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**C-fos immunohistochemistry**

Significant gender differences in level of c-fos expression were found in the lateral orbitofrontal cortex (OFC; t=-2.444; df=22; p=0.027) but not in any other brain area. Therefore, we only refrained from pooling the male and female data for the lateral OFC. We first established the correlation between rIGT performance and c-fos expression (Table 1). The rIGT performance, expressed as the fraction of advantageous choices during the last session, showed a significant negative correlation with c-fos expression in the mPFC (PrL and IL), the lateral OFC (in females only) and the insular cortex. There was a negative trend between rIGT performance and c-fos expression in both the cingulate cortex (Cg1) and ventral striatum (NaS).

Table II: Correlations between c-fos expression and rIGT performance, corrected for the effect of LG (* p<0.05; † p<0.1). Male and female data were pooled (n=24), except for the lateral OFC (n=12)

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<th>rIGT-advantageous choices</th>
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<td>p</td>
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</tr>
<tr>
<td>Nucleus accumbens core (NaC)</td>
<td>-.430</td>
<td>.124</td>
</tr>
<tr>
<td>Nucleus accumbens shell (NaS)</td>
<td>-.262</td>
<td>.366</td>
</tr>
<tr>
<td><strong>Limbic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala central nucleus (CeN)</td>
<td>.068</td>
<td>.817</td>
</tr>
<tr>
<td>Basolateral amygdala (BLA)</td>
<td>-.118</td>
<td>.688</td>
</tr>
<tr>
<td>Dentate gyrus (DG)</td>
<td>.280</td>
<td>.332</td>
</tr>
<tr>
<td>CA1 region (CA1)</td>
<td>.197</td>
<td>.500</td>
</tr>
</tbody>
</table>

Next, correlations were calculated for %LG and c-fos expression. Significant negative correlations were found between %LG received in early life and c-fos expression levels in the insular cortex and the ventral striatum (nucleus accumbens shell; NaS). There was a negative trend between %LG and c-fos expression in the lateral OFC (in females only) and in the cingulate cortex (Cg1). Finally, partial correlations were calculated between rIGT and c-fos, controlling for %LG, to determine the effect of maternal care on the relationship between rIGT performance and c-fos staining. Table 2 shows that after controlling for %LG, the correlations between rIGT performance and c-fos staining in the cingulate cortex (Cg1) and medial PFC (PrL and IL) became more apparent, whereas
correlations between rIGT performance and c-fos expression in the lateral OFC, insular cortex and ventral striatum (NaS) were now non-significant. This indicates that maternal care significantly contributes to the relationship between rIGT performance and c-fos expression in the lateral OFC, insular cortex and NaS. By contrast, the relationship between rIGT performance and c-fos labeling in the mPFC is independent of maternal care.

**Discussion**

In the present study, we investigated the effects of individual variation in licking and grooming received early in life on higher cognitive function and social behavior in young-adult rat offspring. In a paired social play paradigm, male adolescents with higher LG scores exhibited more play behavior than their siblings with lower %LG, whereas in females, %LG did not correlate with the amount of social play behavior. Adult anxiety levels, as measured on the elevated plus maze, correlated negatively with %LG in both sexes. Additionally, adult decision-making performance in a rodent version of the Iowa Gambling Task was enhanced in both males and females after receiving high levels of LG in early life. Correlations between decision-making performance and c-fos expression in the lateral OFC, insular cortex and ventral striatum were found to be driven by levels of maternal care.

**Relationship between %LG received early in life and adolescent social play behavior**

The effect of early life environment on adolescent social play behavior was described in one earlier study, using the between-litter maternal care model (Parent and Meaney, 2008). These authors reported increased play behavior in offspring from Low LG compared to High LG dams, which contrasts with our observations. However, the paradigm used in that study was very different from ours. Parent and Meaney housed pups with different maternal care backgrounds (i.e. High LG and Low LG) for several weeks in mixed-gender groups of 8, and observed the play behavior for each pup individually, irrespective of its play partner. This protocol undoubtedly created an enrichment of the environment, to which Low LG offspring are known to be more sensitive (Bredy et al., 2003b; Bredy et al., 2004). Indeed this was suggested by the authors as the reason for the elevated levels of play behavior in Low LG offspring. In our case, the animals were behaviorally tested in pairs, after a three-hour social isolation period, thus creating a situation that is ‘rewarding’ rather than ‘enriched’ (Niesink and Van Ree, 1989). Dams exhibiting High LG, compared to Low LG mothers, were shown to have a more reactive mesolimbic dopamine pathway in response to pup licking, which was induced by higher levels of oxytocin in these dams and resulted in increased dopamine levels in the NaS (Shahrokh et al., 2010). Maternal behavior is non-genomically
transmitted (Francis et al., 1999a), which might imply that offspring that received high levels of LG from their mother also show increased activity of the mesolimbic oxytocin-dopamine system. Since oxytocin is implicated in the formation of social bonds (Insel, 2003; Liu and Wang, 2003), this interaction might very well play a role in the observed increase in play behavior in pairs of animals with higher LG scores. Additionally, it is possible that stress plays a role in the reduced social play behavior in rats with lower LG scores (Klein et al., 2010). Despite extensive habituation to the test box, encountering an unfamiliar animal there, and especially being socially isolated prior to testing, might induce a stronger stress response in Low LG animals.

Individual LG correlates with both anxiety and decision-making

Consistent with previous studies in the between-litter maternal care model (Caldji et al., 1998), the amount of LG received in early life correlated negatively with the level of anxiety in our animals, males as well as females. High anxiety was shown before to be related to impaired decision-making on the IGT, both in humans (Miu et al., 2008; De Visser et al., 2010) and in rats (De Visser et al. in preparation), possibly due to the uncertainty of the probability of encountering rewards and punishments that is characteristic for this task. Although we did not find a direct correlation between anxiety and the number of advantageous choices on the rIGT in the current study, the correlation that emerged between %LG and rIGT performance was in fact inversed to those found between %LG and anxiety in both males and females.

The robust positive correlation between individual LG scores and successful decision-making in the rIGT was paralleled by LG-mediated differences in c-fos expression in several brain areas, i.e. the nucleus accumbens shell (NaS), the insular cortex and the lateral OFC (the latter in females only). In these areas, c-fos expression – which is generally regarded as an index for neuronal activity (Morgan and Curran, 1989) – correlated negatively and significantly with rIGT performance, but this correlation disappeared when correcting for %LG. Interestingly, these areas are extensively interconnected (Reynolds and Zahm, 2005) and generally related to reward and motivation (Salamone, 1996; Ragozzino and Kesner, 1999; Todtenkopf et al., 2006; Berridge and Kringelbach, 2008). In our study, the animals with the lowest LG scores, and the worst performance on the rIGT, exhibited an increased activity in the aforementioned brain areas, which might reflect an exaggerated sensitivity to the occasional, unpredictable punishments in the advantageous arm, leading to less efficient choice behavior and increased exploratory behavior (De Visser et al., 2010).

The insula is involved in anticipation and receipt of risky, aversive stimuli, such as switches in a choice task (Clark et al., 2008; Preuschoff et al., 2008; Yu et al., 2010). Damage to the insula in humans has been associated with a decrease in their sensitivity to differences in expected value between choice options and with a concurrent decrease in
the amount of risky choices they make (Weller et al., 2009). In line with this, increased insula activation during risky decision-making was reported after choosing from a disadvantageous card deck and has been associated with switching choices after loss as well as with harm avoidance and neuroticism in healthy humans (Paulus et al., 2003; Lawrence et al., 2009). The insula has also been implicated in evaluating tastes and taste conditioning in rats (Fresquet et al., 2004; Koh and Bernstein, 2005), again suggesting that the elevated activity in animals with low %LG is related to an increased sensitivity to punishment in the rIGT. Alternatively, they might still be in the process of learning the contingencies of the task after the 120 trials presented in the current study, and will show a decrease in insula activity after further training as well. This delay in task acquisition is supported by the finding that Low compared to High LG offspring showed attenuated cognitive performance on an object recognition task (Bredy et al., 2003b), which was demonstrated to be insula-dependent (Roozendaal et al., 2010).

Also NaS activation (based on c-fos expression) was higher in Low LG rats than in High LG rats, which seems contradictory to their putative reduced reward sensitivity. However, opposing effects on the regulation of motivation and reward occur after differential activation of the various glutamate receptor subtypes present in the NaS (Todtenkopf et al., 2006) and importantly, c-fos expression might also reflect the activity of GABAergic neurons in this area. Thus, the neural activation modeled by c-fos expression in this brain area should be examined in further detail. In females, activity in the lateral OFC correlated negatively with rIGT performance and showed a negative trend with %LG as well. Given that this area contributes to decision-making performance by processing information on reward value (Rogers et al., 1999), this again suggests that animals with low %LG have more difficulty with reward appraisal. The strong and LG-independent correlation between mPFC activity and decision-making performance that emerged in our animals corresponds to earlier findings in the rIGT in rats that were not characterized for maternal background (De Visser et al. in preparation).

Apart from investigating the specific neuronal subsets that are activated in each of the aforementioned cortical and striatal areas after the rIGT, it is crucial to obtain information on the morphological composition of these same brain regions. Pyramidal cells in the somatosensory cortex of High LG compared to Low LG offspring were shown to exhibit reduced dendritic complexity and a lower number of spines (Smit-Rigter et al., 2009). Conversely, Monroy et al. (2010) reported that after early handling (which likely promotes maternal care; Liu et al., 1997) neuronal dendritic complexity in the PFC was increased. Therefore, we are currently attempting to determine the dendritic morphology of principal neurons in both the mPFC and insula in naïve adult male and female offspring with varying LG backgrounds.

In conclusion, our data indicate that the amount of care received by an individual rat from its mother can reliably predict adolescent social play behavior (in males) as well
as adult decision-making performance and related brain activity. It seems that particularly the areas involved in reward processing are influenced by variable maternal care, but further studies are necessary to elucidate the mechanistic underpinning.

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