Low maternal care exacerbates adult stress susceptibility in the chronic mild stress rat model of depression

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In the present study we report the finding that the quality of maternal care, in early life, increased the susceptibility to stress exposure in adulthood, when rats were exposed to the chronic mild stress paradigm. Our results indicate that high, as opposed to low maternal care, predisposed rats to a differential stress-coping ability. Thus rats fostered by low maternal care dams became more prone to adopt a stress-susceptible phenotype developing an anhedonic-like condition. Moreover, low maternal care offspring had lower weight gain and lower locomotion, with no additive effect of stress. Subchronic exposure to chronic mild stress induced an increase in faecal corticosterone metabolites, which was only significant in rats from low maternal care dams. Examination of glucocorticoid receptor exon 1_7 promoter methylation in unchallenged adult, maternally characterized rats, showed an insignificant tendency towards higher total cytosine methylation in rats from low maternal care dams. Assessment of methylation in the resilient versus anhedonic-like rat phenotypes, revealed only minor differences. Thus, maternal care status seems to be a strong predictor or trait marker for the behavioural phenotype. *Behavioural Pharmacology* 23:735–743 ©

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Introduction

Epidemiological evidence suggests that exposure to negative early life events, such as trauma, abuse and physical or emotional neglect, increases the risk for psychiatric illness in adulthood (Holmes and Robins, 1987; Bifulco *et al.*, 1991; Engert *et al.*, 2010). A theory on the potentially malicious effect of the lack of maternal care or exposure to maternal deprivation originated in the 1940s and 1950s (reviewed by van der Horst and van der Veer, 2008). In the 1940s Spitz studied the effects of hospitalization of infants and found that the isolation, due to hospitalization, was associated with an increase in abnormal behaviour (Spitz, 1945, 1946). In agreement with this finding, studies by Harlow in the 1950s showed that maternal deprivation of infant rhesus monkeys was associated with abnormal behaviour in adult monkeys (Harlow, 1959). In later studies from the 1960s to 1970s, this rationale has been supported in rat studies (Levine, 1967; Wiener *et al.*, 1976; Smotherman *et al.*, 1977). In more recent studies it has been shown that variations in maternal behaviour in rats, particularly licking and grooming (LG), influence the development of endocrine, emotional and cognitive responses to stress in the offspring in adulthood (Meaney, 2001; Champagne *et al.*, 2003).

The recognition of the involvement of the stress system in the aetiology of psychiatric disorders has fostered a vast number of studies focusing on the role of the hypothalamic–pituitary–adrenal (HPA) axis, a unifying hypothesis being that early adverse experiences affect the HPA system, predisposing individuals to develop a disadvantageous stress-coping strategy (Liu *et al.*, 1997; Francis and Meaney, 1999). Activity of the HPA axis is modulated by a glucocorticoid negative-feedback mechanism (De Kloet *et al.*, 1998), which involves hippocampal glucocorticoid receptors (GRs) (Liu *et al.*, 1997). Adult offspring of high LG mothers show increased hippocampal GR expression, enhanced glucocorticoid feedback sensitivity, decreased hypothalamic CRH expression, and lower levels of ACTH and corticosterone after exposure to acute stress, compared with the offspring of low LG mothers (Liu *et al.*, 1997). GR mRNA is transcribed from alternative exon 1 promoters in a tissue-specific manner, with GR exon 1_7-containing mRNA found predominantly in the brain (Weaver *et al.*, 2004). The GR levels correlate to DNA methylation patterns in the GR promoter region (Champagne and Curley, 2009), thus arguing for a potential epigenetic effect of early life environment. Specifically, low levels of GR transcription in adult offspring of low LG mothers have been suggested to be associated with increased methylation across the promoter and particularly at the 5′ CpG dinucleotide of the nerve growth factor-inducible protein A (NGFI-A) binding site (5′-GCG_{16} $GGGGCG_{17}$-3′) (Weaver *et al.*, 2004).

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The aim of the present study was to investigate whether the quality of maternal care in rats affects the response to exposure to the chronic mild stress (CMS) paradigm in adulthood. It is well-established that CMS decreases reward sensitivity in rats, as measured by, for example, a decrease in voluntary sucrose intake (Willner, 2005). We have previously reported that rats show a graduated response to CMS exposure; while some rats show a decrease in sucrose intake (anhedonic-like rats) others show no change in intake (resilient rats) (Bergstrom *et al.*, 2008; Henningsen *et al.*, 2009, 2012). Considering the hypothesis that the quality of maternal care may be predictive of stress responsiveness later in life, we therefore aimed to examine whether resilience or anhedonic-like responsiveness to CMS associates with high, as opposed to low, quality of maternal care, respectively. Besides voluntary sucrose intake, stress sensitivity was determined by measuring faecal corticosterone levels and monitoring weight gain and open field activity. We also investigated whether hedonic status associates with differences in GR promoter methylation.

Methods

Subjects

All animals used in this study were outbred Wistar rats from Taconic M&B (Ry, Denmark). During the period of maternal care characterization rats were housed in transparent cages to promote scoring of dam maternal care activity. Rats were housed singly, food and water were freely available and animals were kept on a standard 12-h light/dark cycle (lights on at 06:00 h), except when one of these parameters was changed because of the stress regime. The animals were weighed once a week. Weight gain was calculated as weight increase indexed to baseline weight. All animal procedures were approved by the Danish National Committee for Ethics in Animal Experimentation (2008/561-447).

Characterization of maternal care

A total of 45 pregnant dams (Wistar) were used for the experiment (Taconic M&B). The dams were 14 days into gestation upon arrival at our facilities. Maternal care behaviour was observed during postnatal week 1 and scored five times a day in 75 min sessions (Champagne *et al.*, 2008). During each session the behaviour of each dam was scored every 3 min, thus 25 times/session. The sessions took place at 06:00, 10:00, 13:00, 17:00 and 21:00 h. Taking into account the light/dark cycle in the animal facilities, the 21:00 h sessions were performed in red light. A score was defined by maternal behaviour and five types of maternal behaviours were distinguished: licking and/or grooming, arched-back nursing (dam shows an obvious arch in her back while nursing), blanket nursing (dam engages in nursing postures with no obvious arch in her back), passive nursing (dam is lying on her side or back while nursing her pups) and no maternal contact. Each dam received a score for a combination of LG behaviour and either one of the three nursing postures, or just the nursing position alone with no LG. The sum of 7 days of LG scores was used as the parameter for dividing pups into groups. Dams were divided in two groups of low ($n = 7$) and high ($n = 7$) LG mothers according to the criteria 1 SD below (low LG, $N_{pups} = 29$) and 1 SD above (high LG, $N_{pups} = 29$) the cohort mean. From weaning (P21) until 5 weeks of age, male offspring of low and high LG mothers were housed in groups (4/cage) with their respective littermates, with free access to food and water.

Sucrose consumption test

The sucrose consumption test (SCT) was a weekly test in which the sucrose intake of individual animals was measured during 1 h exposure to a bottle containing 1.5% sucrose solution, following 14-h food and water deprivation (Jayatissa *et al.*, 2006). Initially, the animals were trained to consume a palatable sucrose solution. The training continued for 5 weeks, with sucrose consumption measured twice a week during the first 3 weeks and once a week during the last 2 weeks. The individual means of the three final sucrose tests were used as baseline values. During a 7-week stress period the sucrose index was calculated as a percentage of the current SCT value relative to the baseline intake. As described by Henningsen *et al.* (2012), the animals were classified as stress susceptible, that is, anhedonic-like (> 30% decrease in sucrose intake, compared with baseline) or resilient (< 10% decrease in sucrose intake compared with baseline).

Chronic mild stress

After 5 weeks of SCT training, animals were divided into two groups and placed in separate rooms. One group was exposed to 7 weeks of chronic mild stressors and the other was left unchallenged (Henningsen *et al.*, 2012). Both groups were food and water deprived for 14 h before the SCT.

The stress protocol consisted of seven different stressors each lasting from 10 to 14 h (Jayatissa *et al.*, 2006). Briefly, a weekly cycle consisted of two periods of intermittent illumination, stroboscopic light, grouping, food or water deprivation, two periods of soiled cage and no stress and three periods of 45° cage tilting.

Faecal corticosterone metabolites

Faecal samples were collected each week on Tuesdays at 09:00 h. Cages were cleaned 24 h before the sampling of faeces, and the corticosterone outcome therefore represents a 24-h release (Christiansen *et al.*, 2012). Following homogenization of the samples, the faecal corticosterone metabolites (FCMs) were extracted with 5 ml of 80% methanol from aliquots of 0.25 g. Extracts were then analyzed with a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (Touma *et al.*, 2003), validated for rats as described by Lepschy *et al.* (2007).

The open field test

It was carried out on Wednesdays between 11:00 and 15:00 h. The open field apparatus consisted of a circular arena 120 cm in diameter divided into 28 circular segments of 400 cm^2 with a 40 cm black wall surrounding the field. The central area was defined as a central segment of about 400 cm^2. Light intensity was ~20 lux. The rats were placed in the testing room 2 h before the test without water and food. In the test apparatus rats were individually placed into the centre of the open field with the head away from the observer. The numbers of horizontal crossings (lines crossed with all four paws) and central crossings (the central area crossed with all four paws) were noted. Each rat was tested for a period of 3 min. After each session the apparatus was cleaned with 70% alcohol and a wet paper towel.

Experimental outline

The study design is shown in Fig. 1.

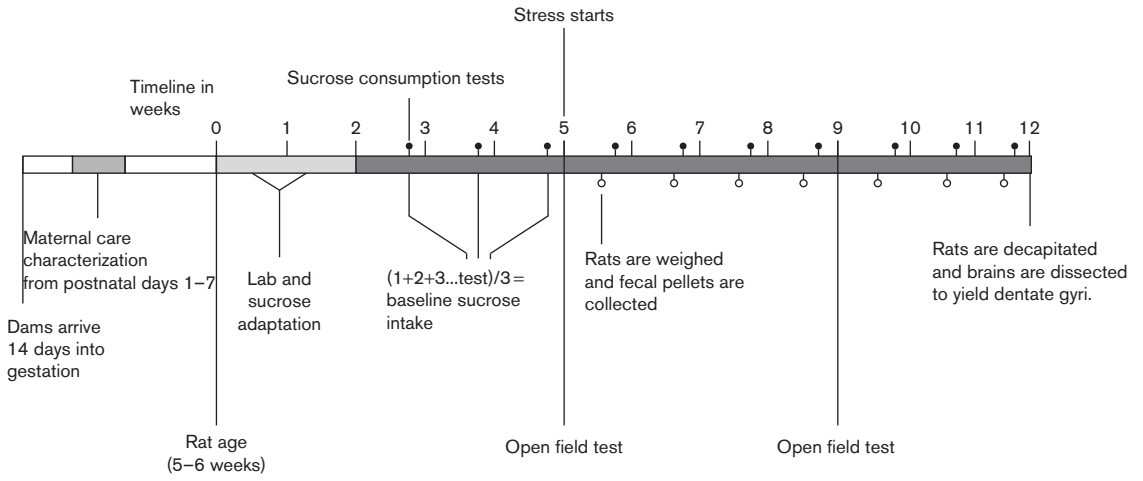
Tissue processing

Following the final SCT, animals were decapitated in a randomized order and the brains were immediately dissected. The dentate gyri were removed and sub-dissected to obtain the ventral dentate gyri. Dissected ventral dentate gyri were snap-frozen and kept at −80°C until further analysis.

Sodium bisulfite mapping

Genomic DNA was isolated from the dentate gyri with a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), following the manufacturer's protocol for purification of total DNA from animal tissues (spin-column protocol). Genomic DNA (1 µg) was bisulfite converted with an EpiTect Bisulfite kit (Qiagen), using the manufacturer's protocol for sodium bisulfite conversion of unmethylated cytosines. The 140 µl reaction mix was run in a Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster city, California, USA). The converted DNA was washed and finally eluted with 2 × 20 µl buffer EB and 15 µl of H_2O was added. The samples were aliquoted (5 × 10 µl) and used immediately for PCR or stored at −20°C. Bisulfite-converted DNA was amplified by PCR using a 10 µl template in a 20 µl reaction mixture. The GR exon 1_7 promoter region (Genbank accession number AJ271870) of bisulfite-converted DNA was PCR amplified using primers (forward, 1731-ttt gta gtt ttt ttg tta gtg tga tat; reverse, 1930-att tct tta att tct ctt ctc cca aa). The reaction mixture consisted of 1x TrueStart Hot Start Taq buffer, 1 mmol/l dNTP, 3.125 mmol/l $MgCl_2$, 0.5 µmol/l of each primer, and 1.25 U TrueStart Hot Start Taq DNA polymerase (Fermentas, Vilnius, Lithuania). The amplification was carried out using a Veriti 96-Well Thermal Cycler (Applied Biosystems) programmed to an initial denaturation cycle (5 min, 95°C), followed by 35 cycles of denaturation (1 min, 95°C), annealing (2 min 30 s, 48°C), and extension (1 min, 68°C), followed by a final extension (5 min, 68°C) terminating at 4°C. The PCR product was visualized using gel electrophoresis and PCR products suited for further processing were excised and isolated from gel slices with a NucleoSpin Extract II kit (Macherey-Nagel, Düren, Germany), using the protocol for DNA extraction from agarose gels. The isolated DNA fragments were ligated using the pGEM-T Easy Vector Systems (Promega, Madison, Wisconsin, USA). The ligation reactions were set up according to the manufacturer's protocol using insert/vector ratios of 3:1. All ligation mixtures were incubated overnight at 4°C before transformation. Subcloning Efficiency DH5α Competent Cells (Invitrogen Life Technologies, Grand

Fig. 1



Study design.

Island, New, York, USA) was transformed according to the manufacturer's protocol with the prepared ligation mixtures. After selecting colonies with correct inserts by colony PCR, the colonies were transferred to 2 ml fresh liquid LB medium for overnight incubation at 37°C. Plasmid DNA was extracted with a NucleoSpin Plasmid kit (Macherey-Nagel), using the protocol for isolation of high-copy plasmid DNA from *Escherichia coli*. A volume of 1.5 μg of each purified plasmid DNA was freeze-dried and the samples were sent to Beckman Coulter Genomics (Hope end, Takeley, Essex, UK) for sequencing.

Data analysis

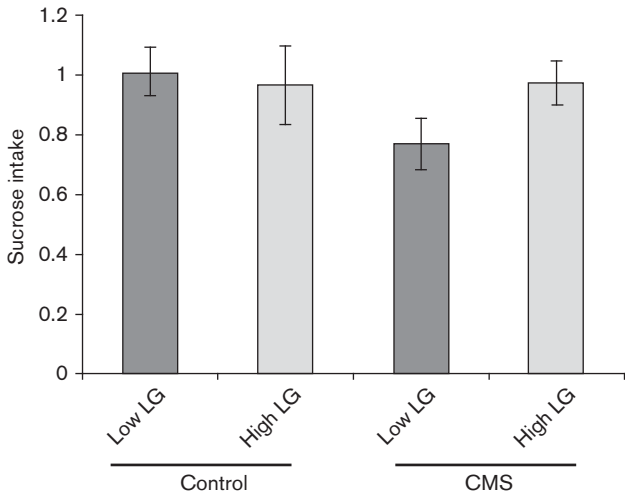
A sucrose intake index was calculated as individual sucrose intake normalized to baseline values. Data were analyzed using a two-way analysis of variance with stress and LG (maternal care) as the two independent factors. Post-hoc pairwise comparisons were done with the Tukey HSD test. The effect of maternal care on stress reactivity (susceptibility or resilience) was analyzed using a χ^2-test.

Results

Sucrose intake

Figure 2 shows sucrose intake, indexed to baseline following 4 weeks of stress. There was no significant main effect of stress or care. As indicated by the graph, there was a trend towards a decreased sucrose intake in the low LG stress group. Accordingly, a pairwise comparison of groups revealed a tendency towards lower sucrose intake in the low LG stress group compared with low LG controls [$F(1,26) = 3.39$; $P = 0.08$] and to the high LG stress group [$F(1,33) = 3.21$; $P = 0.08$] following 4 weeks of stress.

Fig. 2



Sucrose intake (indexed to baseline). Mean (±SEM) sucrose intake following 4 weeks of stress, indexed to baseline values ($n = 10$–18). CMS, chronic mild stress; LG, licking and grooming.

Association of maternal care status and stress susceptibility is shown in Fig. 3. In the low LG group, 50% of the rats were stress susceptible, whereas 17% were resilient. In the high LG group, 17% of the rats were stress susceptible, whereas 53% were resilient. A χ^2-test showed a significant effect of maternal care on stress response [$\chi^2 = 6.01$; $P = 0.049$].

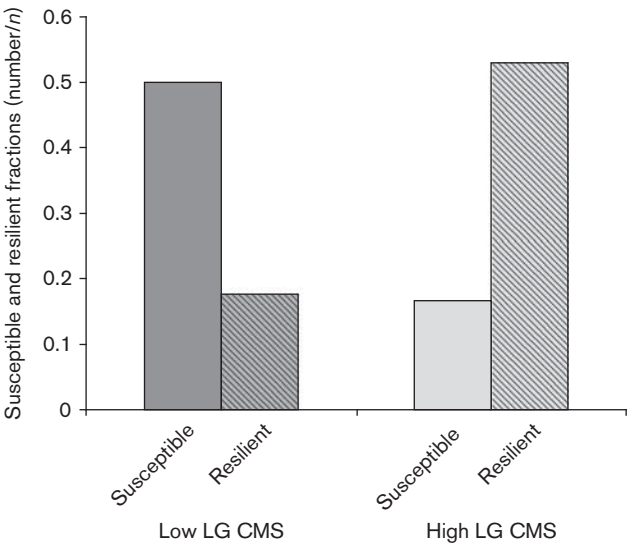
Weight gain

Weight was measured weekly ahead of initiation of the stress protocol and during the stress procedure. There were no significant differences between the groups at baseline. When considering relative weight gain, there were significant main effects of stress [$F(1,50) = 5.86$; $P < 0.02$] and of maternal care [$F(1,50) = 19.54$; $P < 0.001$] after 4 weeks of CMS (Fig. 4). Pairwise comparisons revealed that low LG associates with a significantly lower weight gain, both within control groups ($P = 0.02$) and stress groups ($P = 0.001$). Moreover, the high LG stress group showed significantly lower weight gain than the high LG control group ($P = 0.05$). The statistical analysis applied to results obtained from animals divided in two subgroups according to their stress response (stress susceptible vs. resilient) did not reveal any significant differences (data not shown).

Open field test

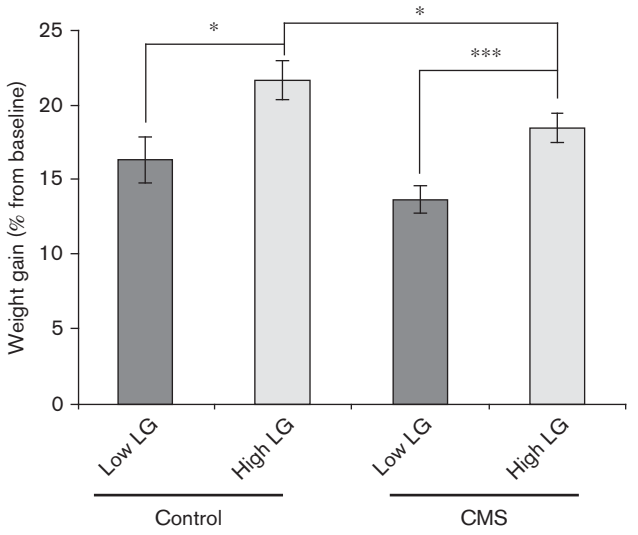
The nature of maternal care extremes did not have any effect on the activity of animals in the open field before

Fig. 3



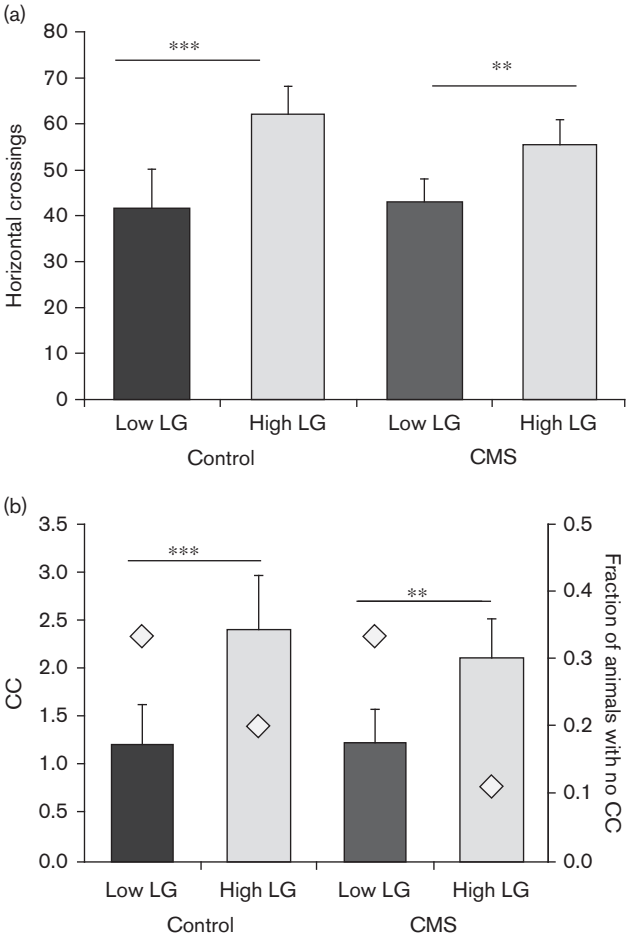
Fractions of susceptible and resilient rats. Rats were defined as stress susceptible or resilient on the basis of an individual decrease of more than 30% in sucrose intake or a decrease of less than 10%, respectively. The histogram shows the susceptible and resilient fractions in the low maternal care (low LG CMS) and high maternal care groups (high LG CMS) ($n = 11$–12). CMS, chronic mild stress; LG, licking and grooming.

Fig. 4



Weight gain. The relative mean weight gain (±SEM) over 4 weeks for control and stress groups, respectively (*$P<0.05$, ***$P<0.001$; $n=10–18$). CMS, chronic mild stress; LG, licking and grooming.

Fig. 5



Analysis of behaviour in the open field arena. (a) The mean (±SEM) group number of total horizontal crossings. (b) The mean (±SEM) group number of central crossings (CC) (left axis) and the fraction of animals that had no CC (right axis) (**$P<0.01$, ***$P<0.001$; $n=10–18$). CMS, chronic mild stress; LG, licking and grooming. The diamonds refer to the right axis.

the exposure to CMS (data not shown). After 4 weeks of CMS, locomotion was 1.4 times higher in the offspring from high LG dams [$F(1,50) = 7.62$; $P = 0.008$] compared with rats raised by low LG dams; however, the exposure to CMS, *per se*, did not have any effect on locomotion (Fig. 5a). Pairwise comparisons revealed a significantly higher activity in the nonstressed control groups compared with the offspring from high and low LG dams [$F(1,17) = 4.5$; $P < 0.05$] and a tendency towards increased locomotion in the high LG–CMS group compared with the low LG–CMS group [$F(1,33) = 3.02$; $P = 0.09$].

Quantification of entries into the inner area of the open field arena, that is central crossings, revealed a reduced anxious-like behaviour of the high LG group, entering the central area of the open field arena 1.9 times more often than the low LG group (Fig. 5b). Although the number of central crossings in this case correlates strongly with general motor activity, the fraction of rats with no central crossings was about twice as high for low LG groups compared with high LG groups (Fig. 5b).

Faecal corticosterone metabolites

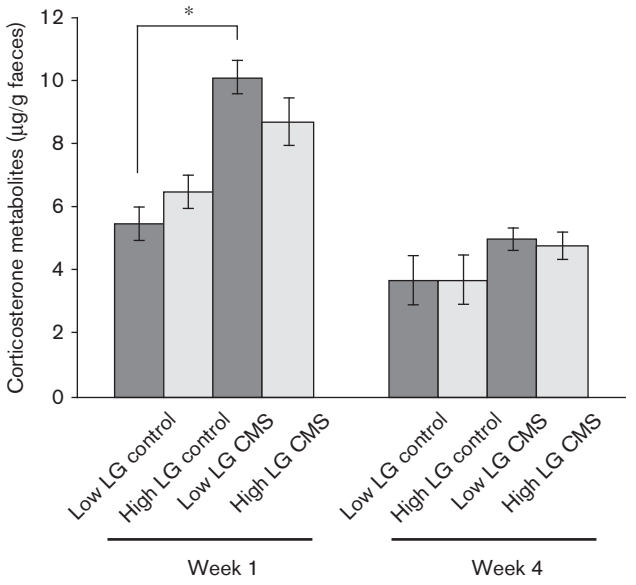
Concentrations of FCM were measured before initiation of the CMS protocol and following 1 and 4 weeks of CMS exposure. No significant differences between maternal care groups were observed at baseline. After 1 week of CMS the two-way analysis of variance revealed a significant main effect of stress on FCM levels [$F(1,47) = 5.86$; $P < 0.02$; Fig. 6], but pairwise analysis revealed only significant differences between low LG groups (Fig. 6). Following 4 weeks of the stress protocol, FCM concentrations in CMS groups were comparable with those in controls (Fig. 6).

Analysis carried out on data sorted according to stress-response phenotypes (stress susceptible vs. resilient) indicated no significant differences between phenotypes (data not shown).

Methylation of the glucocorticoid receptor exon 1_7 promoter

Sodium bisulfite mapping was used to identify DNA methylation of the GR exon 1_7 promoter (Fig. 7a) in unchallenged adult offspring of high or low LG dams. The methylation pattern of the exon 1_7 promoter showed no significant difference between groups (Fig. 7b). However, a small tendency to higher total cytosine methylation in the offspring of low LG dams compared with offspring of

Fig. 6



Faecal corticosterone metabolites. Group mean (±SEM) levels of corticosterone metabolites (*$P<0.05$; $n=10–18$). CMS, chronic mild stress; LG, licking and grooming.

high LG dams was observed (Fig. 7c). The 5′ and the 3′ CpG dinucleotides of the NGFI-A binding site, CpG16–17, were found to be completely unmethylated in both groups (Fig. 7b).

Methylation of the glucocorticoid receptor exon 1_7 promoter after chronic mild stress exposure

DNA methylation of the GR exon 1_7 promoter was examined in adult offspring of low or high LG dams after CMS exposure allowing investigation of the association between DNA methylation and hedonic status. Investigation of the total percentage of methylated cytosines did not reveal any statistical significant differences between the groups (Fig. 8a). However, a tendency of higher total cytosine methylation for adult offspring of low LG dams compared with adult offspring of high LG dams were observed regardless of the hedonic status. The 5′ and the 3′ CpG dinucleotides of the NGFI-A binding site was found to be completely unmethylated in all groups (Fig. 8b).

Discussion

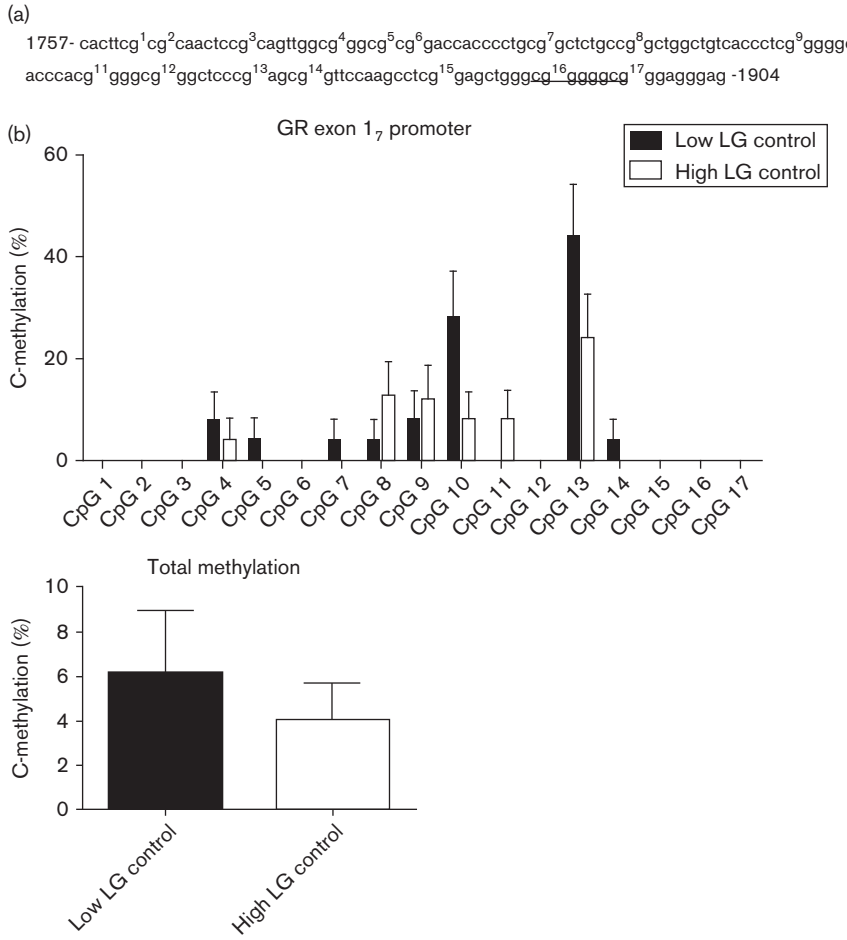
The results reported in the present study indicate that low, as opposed to high, maternal care in early life differentially affects the ability of rats to cope adequately with chronic stress in adulthood. Low LG predisposed rats to become stress susceptible, whereas high LG promoted resilience to stress. In the low LG group CMS induced anhedonic-like responses in 50% of the animals, whereas in the high LG group this fraction was only 17%. Conversely, the fraction of stress-resilient rats was 17% in the low LG group and 53% in the high LG group. This indicates that low maternal care can be evaluated as a risk factor increasing stress susceptibility. The finding that some rats receiving low LG became resilient to stress and some of the rats receiving high LG became stress sensitive indicates that multiple factors are involved in determining stress susceptibility in adulthood.

The impact of maternal care on stress susceptibility is in line with a recent study showing that repeated social defeat induced an anhedonic-like phenotype in rats that were submitted to neonatal maternal separation, whereas nonhandled litters habituated to the repeated stressors (Der-Avakian and Markou, 2010). It should also be noted that, in maternally separated litters, pups were submitted to a significant reduction in LG by the dams, substantiating the suggestion that absence of maternal care or low LG increases stress susceptibility in adulthood.

The results presented here are also consistent with studies showing that maternal behaviour alters the behavioural response to stress in the offspring (Liu *et al.*, 1997; Francis *et al.*, 2000). A comparison of the low LG resilient and anhedonic-like group with the respective high LG groups on related readouts, such as behaviour or molecular analyses, could potentially be used to identify stress-response associated markers as well as maternal care associated markers.

As a standard procedure, the animals' weight was measured once a week throughout the experiment. Both stress and maternal care affected weight gain. However, there was no significant interaction between the two factors. The effect of maternal care on weight gain indicates that maternal care affects the metabolic rate and/or feeding behaviour of the rats, with high LG promoting higher weight gain. To our knowledge the effect of maternal care on weight gain has not been previously reported. Although stress *per se* promoted decreased weight gain, this decrease did not depend on hedonic phenotype; that is, both stress-susceptible and stress-resilient rats had reduced weight gain. In a clinical context, a substantial proportion of depressed patients have a decreased appetite and show weight loss during the course of their illness (Casper *et al.*, 1985). Also, in women with clinically significant levels of depressive symptoms at 5 and 9 months postpartum, higher depressive symptoms at 5 months were associated with less infant weight gain from 5 to 9 months (Gress-Smith *et al.*, 2012). The loss in weight and/or appetite has been suggested to affect HPA-axis function, with weight loss associating with higher plasma cortisol levels (Casper *et al.*, 1987). In the present study, we did not find any correlation between concentrations of FCMs and weight gain, indicating that the difference in weight gain observed in high and low LG groups is not associated with changes in HPA-axis function.

Fig. 7

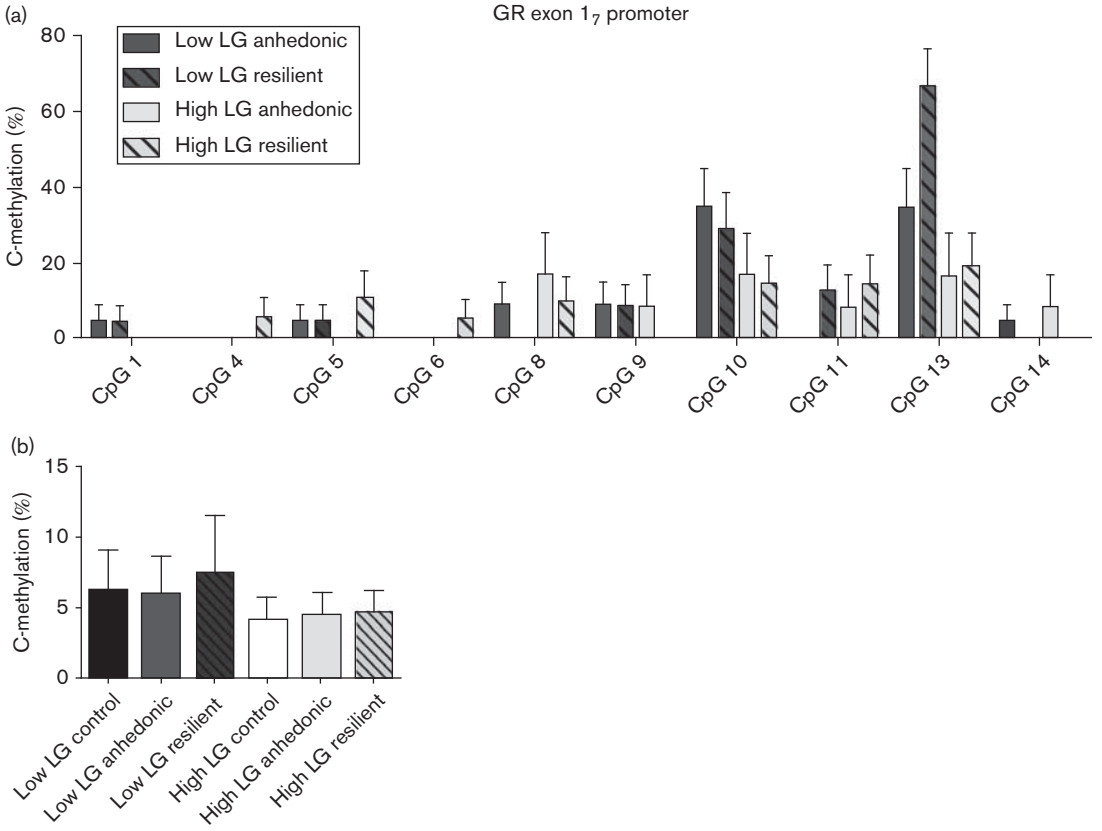


DNA methylation of the glucocorticoid receptor (GR) exon 1_7 promoter. (a) Sequence map of the GR exon 1_7 promoter with each CpG numbered and marked in bold. The nerve growth factor-inducible protein A binding site is underlined. (b) Mean (±SEM) percentage of methylation for each CpG dinucleotide within the GR exon 1_7 promoter. (c) Mean (±SEM) percentage of total C-methylation from cloned GR exon 1_7 promoter sequences (6–7 clones/subject, with $n=4$/group). LG, licking and grooming.

Open field activity was investigated in the course of stress as a measure of general locomotion. Moreover, the number of central crossings in the open field arena was used as a measure of exploratory activity and anxiety-related behaviour. The increase in locomotion observed in high LG rats is consistent with a previous report (Champagne and Meaney, 2007). Notably, the high LG rats also showed a 1.9-fold increase in the number of entries to the inner area when compared with low LG rats. Furthermore, the number of low LG rats with no entries into the central area was about twice as high as for high LG rats. Taken together, this substantiates the suggestion that high LG rats are more fearless.

FCM levels were measured before onset of the stress paradigm and following 1 (subchronic), and 4 (chronic) weeks of stress, respectively. Subchronic stress associates with a significantly increased level of corticosterone in the low maternal LG group. In line with earlier studies, this indicates that low LG associates with an attenuated negative feedback of the HPA axis (Liu *et al.*, 1997). After 4 weeks of CMS, the level of FCM in 24-h samples was similar to the baseline level in all groups. This contradicts an earlier study on rats exposed to CMS, showing increased corticosterone levels following 4 weeks of stress (Ushijima *et al.*, 2006). However, in the previous study corticosterone levels were measured in plasma at specific time points, whereas in the present study we analyzed pooled faecal samples representing the diurnal mean value and compared stress effects according to the maternal care status with stress-unchallenged controls. Measuring FCMs is a noninvasive method that ensures that rats are not submitted to acute stressors during sample withdrawal. The method has been carefully validated and proven to mirror adrenocortical activity in rats (Lepschy *et al.*, 2007). Thus, we consider our results to be consistent with previous findings indicating that an increase in glucocorticoids secretion during the initiation of the CMS

Fig. 8



DNA methylation of the glucocorticoid receptor (GR) exon 1_7 promoter after chronic mild stress exposure. (a) Mean (±SEM) percentage of methylation for each CpG dinucleotide within the GR exon 1_7 promoter. To promote visualization, all positions with no methylation have been excluded from the graph. (b) Mean (±SEM) percentage of total C-methylation from cloned GR exon 1_7 promoter sequences [4–6 clones/subject, with $n=4$ for all groups except high licking and grooming (LG) anhedonic with $n=2$].

protocol is stage dependent and mirrors a natural stress-induced activation of the HPA axis. Maternal care did not affect corticosterone metabolite levels at any time point measured, suggesting that maternal care does not affect corticosterone basal tone following chronic stress.

Examination of DNA methylation in unchallenged adult offspring of high or low LG dams revealed an insignificant tendency to higher total cytosine methylation in the offspring of low LG dams compared with offspring of high LG dams. Remarkably, methylation of the GR exon 1_7 promoter was considerably lower compared with what was reported in a previous study (Weaver *et al.*, 2004). In particular, the 5′ and 3′ CpG dinucleotide positions of the NGFI-A binding site (5′-G**CG**$_{16}$GGGG**CG**$_{17}$-3′) were found to be completely unmethylated. In both studies the assessment of maternal behaviour was conducted as previously described (Champagne *et al.*, 2003); however, some methodological differences could provide potential explanations for the discrepancies between the present data and what has been published previously. First, the tissue used in the present study was restricted to dentate gyrus rather than the entire hippocampi that were used in the previous study (Weaver *et al.*, 2004). Second, in the present study the tissue was collected at postnatal day 140 compared with day 90 in the previous study (Weaver *et al.*, 2004). In agreement with the results presented here, a recent study (Herbeck *et al.*, 2010) reported similar methylation patterns. Herbeck *et al.* (2010) fed pregnant rats a methyl-supplemented diet and found alterations in stress responses. More importantly, the control groups displayed levels of methylation comparable with what is reported in the present study, with the NGFI-A consensus site being almost completely unmethylated. Furthermore, it was inferred from a recent human study that the reduced hippocampal GR expression associated with childhood abuse is caused by increased methylation of the GR exon 1_7 promoter; however, also in this study the NGFI-A site was completely unmethylated (McGowan *et al.*, 2009), which is in line with our findings. It has been suggested that increased DNA methylation of the GR exon 1_7 promoter reduces negative feedback from hippocampal neurons on the HPA axis, consequently increasing stress-induced HPA activity (Liu *et al.*, 1997).

Here we aimed to investigate whether the degree of GR exon 1_7 promoter methylation associates with anhedonic-like and resilient phenotypes. No such relationship was established, although a general tendency to higher methylation was observed for adult offspring of low LG dams regardless of CMS exposure.

Conclusion

Low early maternal care predisposes adult offspring to increased stress susceptibility when exposed to the CMS paradigm. Thus a much higher fraction of CMS-exposed rats adopted an anhedonic-like behaviour indicating an inability to cope adequately with the applied stressors. Conversely, high maternal care promoted higher weight gain, increased locomotion and decreased anxiety-like behaviour, all indicators of a healthy state. Taken together maternal care status seems to be a strong predictor or trait marker for the behavioural phenotype.

Readouts of corticosterone levels indicate that high early maternal care promotes a faster and more adequate subchronic stress response, potentially associating with a slightly higher GR expression, suggesting the negative feedback mechanism is functional.

In contrast to previous studies we did not find a correlation between low maternal care and increased methylation of the NGFI-A site.

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Conflicts of interest

There are no conflicts of interest.

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