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

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CHAPTER 2

Maternal Care Determines Rapid Effects of Stress Mediators on Synaptic Plasticity in Adult Rat Hippocampal Dentate Gyrus

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Abstract

Maternal care in the rat influences hippocampal development, synaptic plasticity and cognition. Previous studies, however, have examined animals under minimally stressful conditions. Here we tested the hypothesis that maternal care influences hippocampal function differently when this structure is exposed to corticosteroid and noradrenergic hormones, which are elevated during the early phase of a stress response. In the adult male offspring of Long-Evans dams characterised as high or low in maternal care (High LG, Low LG) we *i)* examined basal dendritic morphology in the dentate gyrus by Golgi staining; *ii)* investigated rapid modulation of in vitro long term-potentiation (LTP) in the dentate gyrus by glucocorticoid and ß-adrenergic stimulation; *iii)* examined hippocampal and amygdala dependent learning under stress using contextual and cued fear conditioning. We found differences in hippocampal dentate gyrus morphology in adult offspring of High and Low LG mothers, with less dendritic complexity in Low LG offspring. Under basal conditions LTP was lower in slices from Low compared with High LG offspring. Hippocampal LTP was rapidly increased by either corticosterone (100nM) or isoproterenol (1.0μM) in Low LG offspring, suggesting improved dentate plasticity during stress. This was mirrored in hippocampal but not amygdala-dependent learning, as Low LG offspring showed enhanced contextual but not cued fear conditioning. We suggest that decreased pup LG during postnatal life may be adaptive in high-threat environments, potentially enhancing hippocampal function in the offspring under conditions of adversity.

Introduction

The quality of parent – child relationships in humans predicts vulnerability to psychopathology in adulthood, an effect that may be mediated by the effects of parental care on the development of individual differences in stress responses (Parker, 1983; Rapee, 1997; Dozier et al., 1999). In the rat, variations in maternal care directly influence development of corticolimbic systems that regulate endocrine and emotional responses to stress (Liu et al., 1997; Caldji et al., 1998; Francis et al., 1999b; Caldji et al., 2003; Weaver et al., 2004; Zhang et al., 2005; Champagne and Meaney, 2006; Toki et al., 2007). Thus, the adult offspring of mothers that exhibit higher levels of pup licking/grooming (LG; i.e., High LG mothers) show modest hypothalamic-pituitary-adrenal (HPA) responses to acute stress, attenuated fearfulness in response to novelty and reduced expression of active defensive responses to a threatening stimulus (Menard and Hakvoort, 2007). The offspring of Low LG mothers appear more 'reactive' to adversity than those of High LG dams.

Cognitive function in the rat is also affected by maternal care. The adult offspring of High compared to Low LG mothers show improved performance on tests of spatial learning and memory, notably the Morris water maze, and tests of object recognition (Liu et al., 2000b; Bredy et al., 2004; Toki et al., 2007). These tests reflect hippocampal function, and subsequent studies revealed evidence of greater hippocampal expression of glutamate receptor subunits and hippocampal synaptic plasticity in the offspring of High LG mothers (Bredy et al., 2003b; Broadbent et al., 2004; Champagne et al., 2008). However, the improved performance of the High LG offspring on tests of spatial and object recognition learning occurred under relatively low stress conditions in which animals were extensively habituated to the testing conditions to diminish the stress that may otherwise be experienced in such tasks. If the maternal signal associated with enhanced stress reactivity (i.e., decreased pup LG) is associated with an adaptive phenotypic profile that 'prepares' the animal to function under conditions of adversity (Meaney, 2001a; Zhang et al., 2005) then the offspring of Low LG mothers might show enhanced cognitive performance under conditions of increased threat.

Recent findings focusing on synaptic plasticity in the CA1 area support this view (Champagne et al., 2008). Corticosterone, through a delayed (presumably gene-mediated) mechanism, decreased hippocampal LTP in slices prepared from the offspring of High LG mothers, but significantly enhanced LTP in slices from Low LG mothers. However, these studies examined only CA1 responses several hours *after* and not *during* a pulse of corticosterone. Moreover, it is clear that the dentate gyrus is also highly sensitive to stress (Kavushansky et al., 2006; Vouimba et al., 2007) and plays a major role in cognitive performance during stress, such as in contextual fear conditioning (Liu et al., 2000b; Bredy et al., 2004; McHugh et al., 2007; Toki et al., 2007; Hernandez-Rabaza et al., 2008; Nakashiba et al., 2008). We here tested the hypothesis that hormonal conditions such as

occur *during* the early phase of a stress response favor enhanced dentate function in Low LG rats compared to High LG rats using both *in vitro* and *in vivo* approaches. Our initial studies examined the basal state of dentate structure using Golgi staining to define dendritic morphology. Subsequent *in vitro* studies examined rapid modulation of LTP, a model of the cellular mechanism of learning and memory (Bliss and Richter-Levin, 1993; Malenka and Nicoll, 1999), in the dentate gyrus by glucocorticoid and ß-adrenergic stimulation at the time of LTP induction in High and Low LG rats. Glucocorticoid and ß-adrenergic systems are activated by stress and modulate plasticity in the hippocampus (Pugh et al., 1997; Sandi, 1998; Pu et al., 2007; Roozendaal et al., 2008) but also in other limbic areas, most notably the amygdala nuclei (Roozendaal et al., 2006). We used contextual fear conditioning to examine whether the influence of maternal care on stress hormone-LTP interactions in the dentate *in vitro* is translated into effects *in vivo* in a hippocampal-dependent form of learning that occurs under stressful conditions. The dependence of the effect on the hippocampus rather than the amygdala was examined through comparison to cued conditioning, a non-hippocampal, amygdala dependent task (Phillips and LeDoux, 1992).

Materials and Methods

Animals

Long-Evans dams (Charles River) were mated at McGill University, Canada and Leiden University, the Netherlands. After mating, females were singly housed with *ad libitum* access to food and water in a 12h light/dark cycle (lights on at 08:00). Litters were completely undisturbed from the day of parturition until postpartum day 7. Maternal behavior was observed on days 1-6 postpartum, every 3 minutes during 75 minute observation periods, of which two occurred in the dark phase (7:00, 20:00) and three in the light phase (10:00, 13:00, 17:00) as previously described (Champagne et al., 2003). A maternal LG frequency score was calculated for each dam from 750 observations. Pups were designated as High or Low LG based on their mother's LG frequency score and the cohort mean (High and Low LG: LG scores ±1 SD from cohort mean). Previous studies have established that LG frequency is normally distributed across dams and litters of High and Low LG mothers do not differ in weaning weight, pup number or gender ratio (Champagne et al., 2003).

With the exception of weekly cage changing, litters remained undisturbed with the dam until weaning at day 21 when male offspring were group housed with one or more littermates until testing at 3 months of age. Testing occurred during the first half of the animals' light phase. All procedures conformed to the guidelines of the Canadian Council on Animal Care and the local committees on animal bioethics and welfare of the Universities of Leiden and Amsterdam.

Analysis of dendritic morphology

Morphological analysis of dentate gyrus neurons was conducted in hippocampal sections using the Golgi-Cox method (Boekhoorn et al., 2006; Champagne et al., 2008). A minimum of five cells in the upper blade of the dentate gyrus from each of eight High and six Low LG rats were imaged and traced for analysis of apical dendritic length and complexity. Only consistently impregnated cells with soma located in the middle plane of the section with unbroken arbors that did not substantially overlap with neighboring cells were selected for tracing. Neurodraw and Image Pro software (Van Pelt et al., 2005; Boekhoorn et al., 2006) were used for all analyses which were performed by experimenters blind to group identities. For each animal, cell values were averaged to derive a single value per variable (total dendritic length, average branch length, number of branching points and dendritic complexity index [DCI = (Σ branchtip orders + # of branch tips) / (# of primary dendrites) * (total arbor length)]. Moreover, spine density was determined in every cell, in 2 segments of 20 μm each (90-110 μm and 190-210 μm from the soma) on one of the main branches.

Electrophysiology

Adult male offspring of High and Low LG mothers were decapitated between 08:00 and 09:00 hrs during the nadir of the diurnal corticosterone rhythm. Trunk blood was collected and plasma stored at -20°C for later analysis of corticosterone levels by radio-immunoassay (B anti-serum, B3-163; Endocrine Sciences, Tarzana, CA; [^{3}H]corticosterone, 101 Ci/mmol; NEN, Boston, MA) (Weaver et al., 2005). Circulating corticosterone levels at the time of decapitation were low in rats used for electrophysiology and did not differ between High (n=16, mean ± SD: 1.22 ± 0.55 μg/dl) and Low LG rats (n=14, 1.28 ± 0.44 μg/dl).

The brain was rapidly removed and immersed in ice cold artificial cerebrospinal fluid (aCSF) containing 120 mM NaCl, 3.5 mM KCl, 1.3 mM $MgSO_4 \cdot 7H_2O$, 1.25 mM NaH_2PO_4, 2.5 mM $CaCl_2 \cdot 2H_2O$, 10 mM glucose and 25 mM $NaHCO_3$, oxygenated with 95% O_2 and 5% CO_2. Coronal slices 400 μm thich were cut using a vibratome (Leica VT1000S). Slices were kept in oxygenated aCSF at room temperature for at least 2hrs prior to recording (Wiegert et al., 2006) and then transferred to a recording chamber maintained at 30-32°C with a constant flow of oxygenated aCSF. Field excitatory postsynaptic potentials (fEPSP) were recorded in the hippocampal dentate gyrus using a stainless steel bipolar stimulation electrode (60 μm diameter, insulated except for the tip) positioned in the medial perforant pathway and a glass recording electrode (2-5MΩ impedance, filled with aCSF) positioned in the middle third of the molecular layer of the upper blade. An input-output response curve was generated by gradually increasing the stimulus intensity to define a level that generated the half-maximal response that was used for the remainder of the experiment. The magnitude of the response was assessed by

measuring the peak-amplitude of the fEPSP, which in the dentate gyrus *in vitro* is more stable than the fEPSP slope and generally not confounded by a population spike due to the very negative resting membrane potential. Drug perfusion commenced after 10 minutes of stable baseline. The modulation of synaptic plasticity by stress hormones was examined in slices perfused with either corticosterone (Sigma-Aldrich, The Netherlands; 100nM, dissolved in 0.01% ethanol), the ß-adrenergic agonist (-)-isoproterenol (+)-bitartrate (Sigma-Aldrich, 1.0 μM) or vehicle solution; earlier experiments in uncharacterized Wistar rats showed successful modulation of LTP with these concentrations (Pu et al., 2007). Drug perfusion co-terminated with theta-burst stimulation (TBS; four pulses at 100 Hz, followed 200 ms later by another burst of four pulses; this sequence occurred five times with a 30 sec inter-train interval). Synaptic responses were monitored for 60 minutes following TBS at 60 sec intervals. The percent change of evoked responses from the initial pre-drug baseline was calculated for each minute of the drug baseline and the post-TBS period. Drug effects on baseline synaptic transmission were examined by comparing the average of the first (pre-drug) and second (drug) baseline periods. LTP was examined by comparing the average of the pre-TBS baseline and final 10 minutes of the post-TBS period.

QRT-PCR measures

Adult male offspring of High LG and Low LG mothers (n=3) were sacrificed by rapid decapitation and brains were quickly snap frozen. Hippocampal tissue punches were taken from frozen sections (200 μm) on dry ice. Total hippocampal RNA was isolated with an RNeasy mini kit (QIAGEN Science, Maryland, USA) and treated with DNase I (Roche Diagnostics, Laval, Canada). RNA was reverse transcribed using RTAMV (Roche) and subjected to QPCR (Roche LightCycler 480). Samples were used to amplify GR (forward primer, 5'- CTGCTTTGCTCCTGATCTGA -3'; reverse primer, 5'- TTCATAGGATACTGCAATCTTTG -3') and ß1-adrenoreceptor (forward primer, 5'- CCTCGTCCGTCGTCTCCTT-3'; reverse primer, 5'- GCTGTCGATCTTCTTCACCTGTT-3'). A housekeeping gene, B2M (ß-2 micro-globulin), was amplified (forward primer, 5'- CCGTGATCTTTCTGGTGCTT-3'; reverse primer, 5'-AAGTTGGGCTTCCCATTCTC-3') and the concentration of gene transcripts was determined relative to concentration of B2M. Amplification of GR, ß1-adrenoreceptor and B2M was performed in duplicate using a reaction mixture of 2.0 μl synthesized cDNA product, 5 μl SYBR Green Master Mix (Roche), primers (ß1: 0.5μl each of 5μM stock, GR and B2M: 1μl each of 10μM stock) and PCR grade H_20 to a total volume of 10 μl . The real-time thermocycler protocol (Roche LightCycler 480) began with a single pre-incubation (95˚C for 10 mins) followed by 45 amplification cycles (ß1: 95˚C for 20 sec, 68˚C for 15 sec, 72˚C for 15 sec, GR and B2M: 95˚C for 20 sec, 55˚C for 15 sec, 72˚C for 15 sec) with a single fluorescence reading at the end of each elongation step. The specificity of the amplified PCR products was assessed by

performing a melting curve after the PCR amplification (95˚C for 5sec, 65˚C for 30sec, 97˚C with continuous acquisition of fluorescence) to confirm that all amplified fragments for each primer set had a single common melting point. No primer–dimers were detected that might otherwise interfere with quantification of the PCR products.

In vitro ß-adrenoreceptor autoradiography

Adult male High LG and Low LG offspring (n=5) were sacrificed by rapid decapitation and brains quickly snap frozen. Brain sections (15 µm) were thaw-mounted onto poly-L coated slides and stored at -80°C. ß-adrenoreceptor and $ß_1$-adrenoreceptor binding was quantified using Iodopindolol (Vos et al., 1990). Slides were thawed, pre-incubated in assay buffer (0.17 M Tris-HCl, pH 7.4) for 15 min at 4°C and incubated with a saturating 250 pM concentration of [^{125}I]-(-)Iodopindolol (2200 Ci/mmol, New England Nuclear, Boston, MA) in assay buffer for 60 min at 23°C. For quantification of $ß_1$-adrenoreceptors, 50nM ICI 118551 (Tocris, Cedarlane Laboratories, Hornby, Canada) was added to selectively block $ß_2$-adrenoreceptors. Nonspecific binding was determined in parallel sections using 100 µM (-)-isoproterenol (Tocris). Slides were washed (3x 15 min, 4°C) in assay buffer, dipped in dH_20 (4°C), dried at 56˚C and apposed to LKB Ultrofilm (Amersham Canada Inc., Montréal, Canada) for 24h.

Autoradiograms were analyzed by obtaining optical densities using an MCID image analysis system (Imaging Research Inc., St Catherines, Canada). For each animal, 3-4 sections were analyzed and the mean from these values was used in the statistical analysis.

Fear Conditioning

Conditioning and testing occurred in fear conditioning chambers (MED Associates, St Albans VT) controlled by a PC running MED Associates software (MED-PC) and sessions were video taped for later assessment. Animals were habituated to manipulations associated with testing for two days prior to conditioning. For contextual conditioning (High LG n=12, Low LG n=10), five minutes after placement in the conditioning chamber the first of four foot-shocks (1 sec / 0.5mA) was delivered. The interval between the subsequent three shocks varied (average 3 min ISI). Animals were removed from the chamber 10 sec after the final shock and returned to their home-cage. The following day contextual fear memory was assessed by a five minute test in the conditioning chamber, during which no further shocks were delivered. A separate group of experimentally naive animals were used for tone conditioning (High LG n=12, Low LG n=10) which followed the same procedure as contextual conditioning except that each shock was preceded by a tone (6 kHz, 70dB, 10 sec pure tone). Tone conditioning was assessed in a novel context during a two-minute tone presentation preceded by a five-minute habituation period. This procedure was repeated for three days to examine tone extinction. The novel context

was distinguished from the conditioning context by placing white plastic inserts over the metal grid floor and cleaning with orange rather than pine-scented cleaner to provide a distinctive odor. Additionally, the chambers were placed in different locations within the testing room. Freezing, characterised as the absence of all body movements except those required for respiration (Blanchard and Blanchard, 1969) was scored for the duration of context test sessions and during the period of tone presentation. Similar protocols for contextual and tone conditioning have been demonstrated to rely on hippocampal and amygdala function respectively (Wilensky et al., 1999; Hernandez-Rabaza et al., 2008; Schenberg and Oliveira, 2008).

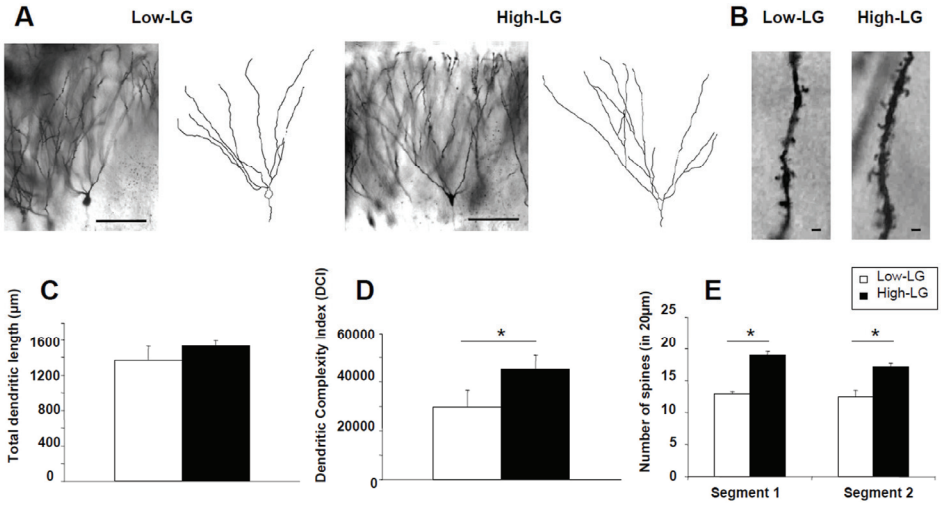

Figure 1. Effect of maternal care on dendritic morphology in the dentate gyrus. (A) Typical examples of Golgi-stained dentate gyrus granule neurons. On the left, a stained section from an adult male offspring of a Low LG mother is represented (left, representative level of focus), as well as the 3-D reconstructed drawing. The pictures on the right show a representative example from offspring of a High LG mother. Calibration bars: 100 µm. (B) Representative examples of spine density in sections obtained in the adult male offspring from a Low (left) and High (right) LG mother. Calibration bars: 20 µm. (C) Total dendritic length was not different in granule cells from adult male offspring of a Low versus High LG mother. Bars represent the mean of averaged values per animal + SEM (n=40 cells from 6 Low LG animals and n=42 cells from 8 High LG animals). (D) Dendritic complexity (DCI), here defined by DCI = (Σ branchtip orders + # of branch tips) / (# of primary dendrites) * (total arbor length), was significantly enhanced in dentate granule cells of High versus Low LG rats. Asterisk indicates significance (p=0.04). (E) Spine density was measured in two dendritic segments on one of the main dendrites of each cell (segment 1=90-110 µm and segment 2=190-210 µm from the soma). The bars represent the mean of values averaged per animal +SEM. In both segments a significantly higher number of spines was observed in granule cells of High versus Low LG offspring (segment 1: $p<0.0001$; segment 2: $p=0.0003$) (n=40 from 6 Low LG rats and n=42 from 8 High LG rats).

Statistical Analysis

All statistical analyses were conducted using SPSS 13.0. Simple comparisons between two groups (High LG and Low LG) on single dependent variables were analyzed by independent Student's *t*-tests. For analyses involving comparisons between more than

two groups and dependent variables with repeated measures, the primary analysis conducted was a repeated measures ANOVA. Significant main effects and interactions were interpreted using Fischer post-hoc tests.

Results

Dendritic Morphology

Morphological analysis of dentate gyrus neurons revealed greater dendritic arborization in adult offspring of High compared to Low LG mothers (typical examples in Figure 1A). Although total dendritic length (Figure 1C) was not different, dendrites of High compared to Low LG rats had significantly more branching points (t=1.88, df=12, p=0.04; data not shown). Moreover, the dendritic complexity index, a measure of dendritic arborization, was greater in High than Low LG rats (t=1.91, df=12, p=0.04; Figure 1D). Dendrites of High LG rats also had significantly more spines than dendrites of Low LG rats (p<0.001, based on summated data for two 20 μm segments per cell; typical examples in Figure 1B; averaged values for the two segments in Figure 1E).

In Vitro Electrophysiology

We examined *i)* if *in vitro* induced LTP in the dentate was different in High versus Low LG offspring and *ii)* if LTP was differentially affected by corticosterone and the ß-adrenergic agonist isoproterenol, mimicking hormonal effects shortly after stress.

Although signals tended to be somewhat decreased during corticosterone and isoproterenol administration in the High LG group (Figure 2B & 2C), three-way repeated measures ANOVA of the pre-drug and drug baseline periods revealed no main effect or interactions (maternal care x drug x baseline period, p=0.103; maternal care x drug, p=0.102). Subsequent analyses of LTP compared post-TBS response amplitudes to the pre-TBS drug baseline. A three-way repeated measures ANOVA revealed a significant three-way interaction between maternal care, drug and period (F(2,24)=7.499, p=0.003; Figure 2). Fischer's post-hoc tests of pair-wise comparisons revealed significantly greater potentiation in vehicle-treated slices from High LG compared to Low LG mothers (p=0.002). Indeed, observation of Figure 2A suggests that TBS produced some long term depression (LTD) in the Low LG vehicle-treated slices, but this did not reach significance (paired comparison between the signal over t=-10 to 0 minutes versus t=50-60 minutes, p=0.2). By contrast, after application of isoproterenol a significantly (p=0.029) greater potentiation was observed in slices from Low LG than in those from High LG offspring (Figure 2B). Rapid effects of corticosterone resulted in a comparable though less prominent shift: compared to the Low LG-vehicle group a clear increase in LTP was seen in the Low LG group after corticosterone treatment (p=0.003); yet, in the High LG group the reduction in LTP after corticosterone compared to vehicle treatment just missed

significance (p=0.051). The latter may have contributed to the fact that a direct comparison of corticosterone-treated slices from Low versus High LG animals did not yield a significant effect (p=0.153). Thus, although LTP was induced under control conditions in High but not Low LG slices, treatment particularly with isoproterenol (and to some extent corticosterone) reversed the effect of maternal care, suppressing LTP in slices from High LG animals and facilitating LTP in those obtained from Low LG rats.

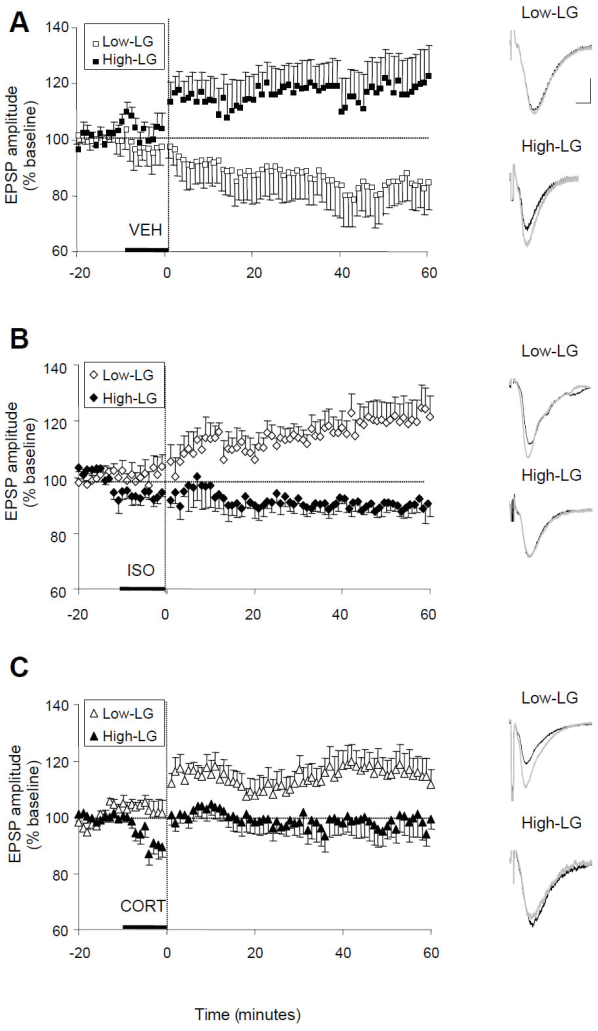

Figure 2. Effects of maternal care and stress hormones on LTP in the dentate gyrus. (A) Slices from adult male offspring of High and Low LG mothers were recorded under baseline conditions and up to 60 minutes after application of theta burst stimulation (t=0 min, here indicated by vertical dotted line). In the absence of exogenous stress hormones (VEH=vehicle administration, from t=-10 to 0 min) significantly greater potentiation was observed in vehicle-treated slices from High LG compared to Low LG mothers (p=0.002). Each point represents the mean +SEM of all recordings in the group (n=7 Low LG and n=5 High LG). Only one vehicle recording was obtained per rat. (B) A significantly (p=0.029) greater potentiation was observed in isoproterenol-treated slices (ISO) from Low LG compared to those from High LG offspring (n=5 Low LG and n=4 High LG). (C) Although LTP was on average increased in corticosterone-treated slices from Low LG relative to High LG offspring this did not reach significance (p=0.153) (n=6 Low LG and n=5 High LG). Insets on the right depict representative fEPSP traces of Low and High LG rats, for each treatment. Black traces represent the response before and grey traces after TBS. Horizontal calibration bar in all cases 100 ms. Vertical calibration bar: 0.5 mV for the Low LG Veh, High LG Veh, High LG CORT and High LG Iso groups; 1.0 mV for the Low LG ISO; and 1.5 mV for the Low-LG CORT group.

Glucocorticoid and ß-adrenoreceptor regulation by maternal care

Consistent with previous reports, GR mRNA expression was significantly greater in the hippocampus of High compared to Low LG rats, as tested in material from a limited

number of rats (n=3; *t*=2.685, *df*=4, p=.05; Figure 3A). In this sample of animals, no effect of maternal care was observed on levels of ß$_1$-adrenoreceptor mRNA (Figure 3B). More importantly, though, a more extensive comparison at the protein level (n=5) also revealed no effect of maternal care on the ß-adrenoreceptor expression. Thus, autoradiographical analysis of total ß (Figures 3C-D) and ß$_1$-adrenoreceptor binding (Figures 3E-F) confirmed that maternal care did not influence regulation of ß-adrenoreceptors in the hippocampal dentate gyrus.

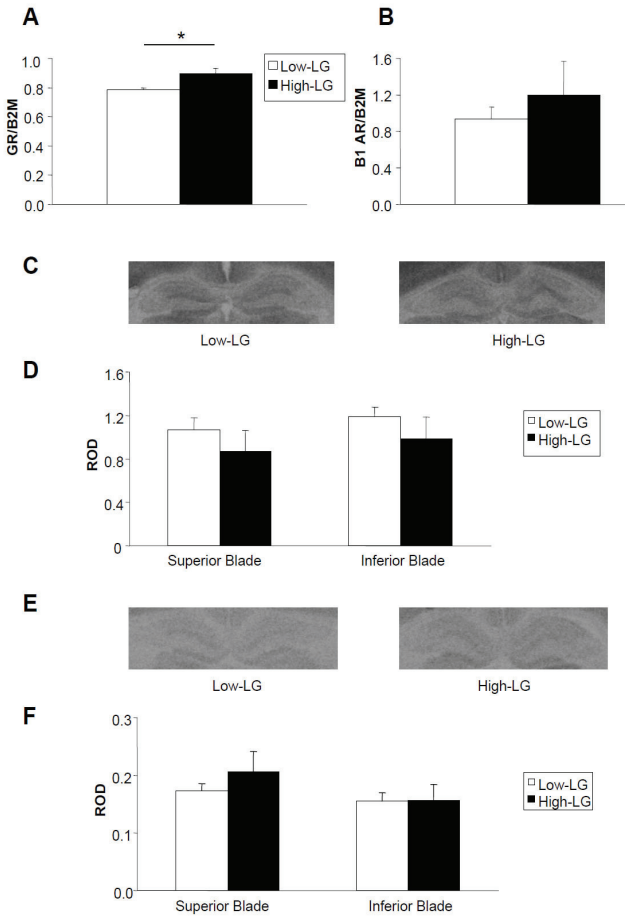

Figure 3. Effect of maternal care on glucocorticoid and ß-adrenergic receptor expression. (A) Glucocorticoid receptor (GR) mRNA expression measured by QRT-PCR was significantly higher in the hippocampus of High LG offspring compared to Low LG offspring. Bars represent group mean +SEM (n=3). (B) ß$_1$ adrenoreceptor (AR) mRNA expression levels in hippocampus did not differ between Low and High LG offspring (n=3). Bars represent group mean +SEM. (C) Typical autoradiograph showing specific [^{125}I]-(-)Iodopindolol binding in hippocampus. (D) Total ß adrenoreceptor expression measured by relative optical density (ROD) of specific [^{125}I]-(-) Iodopindolol binding was not different between Low and High LG offspring in either the superior or inferior blade of the hippocampal dentate gyrus. Bars represent mean of values averaged per animal +SEM (n=5). (E) Typical autoradiograph showing specific [^{125}I]-(-)Iodopindolol binding in presence of ICI 118551 (ß$_2$ cold competitor) in hippocampus of Low and High LG offspring. (F) ß$_1$ adrenoreceptor expression measured by ROD of specific [^{125}I]-(-)Iodopindolol binding in presence of ICI 118551 (ß$_2$ cold competitor) was not different between Low and High LG offspring in either the superior or inferior blade of the hippocampal dentate gyrus. Bars represent mean of values averaged per animal +SEM (n=5).

Fear conditioning

Low LG rats exhibited enhanced contextual conditioning compared to High LG rats (Figure 4A). A Student's *t*-test confirmed that Low LG rats showed significantly higher levels of freezing in the context than High LG rats (*t*=2.18, *df*=20, p=0.04). No freezing in

the context was observed in either group prior to conditioning and all rats exhibited increases in post-shock freezing during the conditioning session that did not differ between groups (post-shock 1: mean ± SD % freezing, High LG 25.0 ± 8.6, Low LG 26.8 ± 9.5; post-shock 2: High LG 74.0 ± 6.2, Low LG 78.8 ± 4.7; post-shock 3: High LG 90.3 ± 3.2, Low LG 90.8 ± 3.5). Maternal care did *not* influence tone conditioning or extinction as revealed by a repeated measures ANOVA which showed no main effect or interaction with maternal care (Figure 4B). A significant main effect ($F(3,48)=25.658$, $p=0.0001$) of test day confirmed that both High LG and Low LG rats exhibited substantial freezing to the tone after conditioning and that this diminished similarly in both groups across extinction tests.

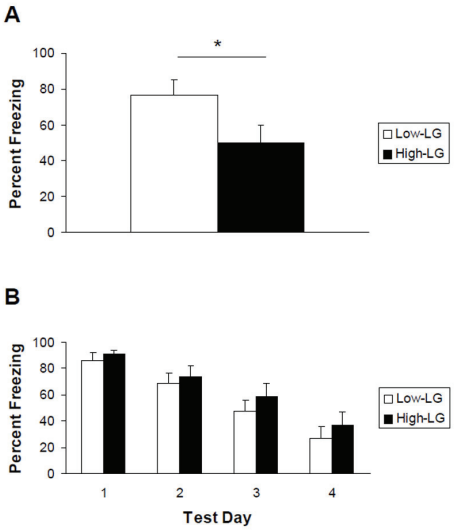

Figure 4. Effect of maternal care on hippocampal learning during stress. (A) Contextual conditioning measured by percent time spent freezing in the conditioned context was significantly ($p=0.04$) greater in Low LG compared to High LG offspring. Following a time-sampling method, every 5 seconds an observation was made and the presence or absence of freezing noted. Bars represent mean of values averaged per animal across the five minute test session +SEM (n=10 Low LG and n=12 High LG). (B) Tone conditioning measured by percent time freezing during tone presentation was not significantly different between Low and High LG offspring on any of the four test days. Freezing to the tone decreased in both Low and High LG offspring across test days ($p=0.0001$). Bars represent mean of values averaged per animal across the two minute tone presentation +SEM on each of the four test days (n=10 Low LG and n=12 High LG).

Discussion

These findings reveal that the effect of stress hormones on dentate plasticity is dependent upon postnatal maternal care. Likewise, variations in maternal care determine hippocampal-dependent learning in a stressful context. Taken together, these findings are consistent with the idea that variations in maternal care produce specific forms of phenotypic diversity in the offspring, the function of which is further determined by the environmental context in later life.

Dentate LTP under 'non-stress' conditions

The results in vehicle-treated slices provide an *in vitro* replication of earlier findings of increased *in vivo* LTP in the medial perforant path of the dentate gyrus in the adult offspring of High compared to Low LG mothers (Bredy et al., 2003b). The observation of

more complex dendritic arbors of dentate granule cells and increased numbers of dendritic spines in High LG offspring infers the presence of a greater number of synapses, which could support the observed enhanced potentiation and might contribute to previously reported enhanced hippocampal learning in adult offspring under relatively low stress conditions (Liu et al., 2000b; Bredy et al., 2003b; Harris et al., 2003; Bredy et al., 2004). Greater dendritic complexity and spine density may favor synaptic plasticity and learning, and both LTP and *in vivo* learning are associated with increases in spine density (Muller et al., 2000; Leuner and Shors, 2004; Diamond et al., 2006). The sensitivity of the hippocampal dendritic morphology to gradual remodeling by environmental influences is well documented and in many instances, morphological alterations are associated with effects on hippocampal learning (McEwen, 1999, 2001; Faherty et al., 2003; Alfarez et al., 2008).

Rapid effects of corticosterone and isoproterenol on LTP

Hippocampal LTP was examined following brief treatment with either corticosterone or the ß-adrenergic agonist, isoproterenol, to pharmacologically mimic the endogenous dynamic corticosteroid or noradrenergic signal during stress. The results revealed differential effects of a fixed concentration of these "stress mediators" on LTP induced in the medial perforant path of the dentate gyrus depending upon postnatal maternal care, particularly in the case of the ß-agonist isoproterenol. A limited survey at the transcript level suggests that the differential responses to isoproterenol cannot be explained by influences of maternal care on adult ß-adrenoceptor mRNA expression. More importantly, in a larger sample size we also observed no difference between High and Low offspring with respect to ß-adrenoceptor binding.

Although rapid modulation of dendritic spine morphology could provide a potential explanation for the effects of corticosterone and isoproterenol, dexamethasone-induced increases in spine density in rat hippocampal neurons require incubation periods of 1 h, well in excess of the treatment period used in our studies (Komatsuzaki et al., 2005). A more likely candidate mechanism involves the effects of corticosterone and isoproterenol on calcium permeability of NMDA receptors (Takahashi et al., 2002; Skeberdis et al., 2006). Although the delayed, genomic effect of glucocorticoids dampens processes downstream of NMDA receptor activation, (Wiegert et al., 2005) a rapid, non-genomic effect (potentially via a putative membrane receptor) enhances NMDA-mediated calcium influx (Takahashi et al., 2002). Isoproterenol acts via protein kinase A (PKA) signaling, and PKA similarly modulates NMDA permeability to calcium (Skeberdis et al., 2006). Thus, both corticosterone and isoproterenol may rapidly increase calcium influx via NMDA receptors. Adult offspring of High LG mothers show increased expression of the NR2B as well as the NR1 subunits of the NMDA receptor and increased NMDA receptor binding (Liu et al., 2000b; Bredy et al., 2003b; Bredy et al., 2004; Toki et al., 2007). These

findings suggest differences in NMDA activity under basal conditions, consistent with the behavioral data cited above, that could support differences in potentiation thresholds (Abraham, 2008). Thus, in hippocampal slices from Low LG offspring, where NMDA activity may be reduced under basal conditions, the effects of corticosterone and isoproteronol on NMDA-gated calcium influx may bring the calcium concentration in the neuron closer to the threshold for inducing potentiation of synaptic responses. In contrast, in High LG rats, where NMDA activity under basal conditions may be elevated, the facilitation of calcium influx may occlude plasticity (Kim et al., 1996). Increased intracellular calcium facilitates the slow afterhyperpolarisation (sAHP) which in turn decreases neuronal excitability and increases the threshold for LTP induction (Borde et al., 2000; Le Ray et al., 2004). Such effects appear to characterize the response of hippocampal slices from the offspring of High LG mothers and this hypothesis should be tested in future experiments. This explanation suggests that maternal care might define the response of the hippocampus to stressful conditions through effects on NMDA receptor expression. However, involvement of other glutamate-related endpoints, e.g. trafficking of AMPA-receptor subunits (Groc et al., 2008), cannot be excluded at this moment.

Functional consequences

Using the same electrophysiological protocols to induce LTP in the dentate gyrus of maternally uncharacterised (Wistar) rats, Pu et al. (Pu et al., 2007) were unable to induce LTP under vehicle conditions or after corticosterone application alone (with treatment at the time of TBS); corticosterone only induced a mild enhancement in LTP when GABAergic transmission was blocked. Treatment with isoproterenol at the time of TBS induced a gradually developing form of LTP (Pu et al., 2007). Overall, this is more comparable to the response of the offspring of Low LG than High LG mothers, although neither group completely conforms to the profile of the uncharacterised Wistar rats. Clearly considerable variation exists among rats in hippocampal plasticity and in the response to stress hormones. The present results suggest that such individual differences could be developmentally defined, associated in part with variations in maternal care.

The effects of corticosterone on hippocampal LTP differ depending upon the timing of corticosteroid exposure and the hippocampal subfield (Joels, 2006; Wiegert et al., 2008). For example, in the CA1, a U-shape relationship exists with respect to slow gene-mediated corticosteroid effects between corticosterone level and cell/network function including synaptic plasticity, whereas in the DG, the relationship is more complex, generally lacking a clear effect of high doses of corticosterone (Joels, 2006). Moreover, short-term exposure to corticosterone at the time of high frequency stimulation, which implies a non-genomic effect, facilitates CA1 LTP, while prolonged exposure prior to stimulation, within a time period that is sufficient for genomic effects, suppresses plasticity (Wiegert et al., 2006). Champagne et al. (Champagne et al., 2008) showed that

previous, prolonged exposure to corticosterone (i.e., 20 minute incubation >1 hour prior to high frequency stimulation) impaired LTP in the CA1 of adult offspring of High LG mothers, but enhanced LTP in animals reared by Low LG dams. We here show in a different hippocampal subregion that *rapid* effects of isoproterenol and corticosterone also facilitate LTP in Low LG offspring, while isoproterenol impairs LTP in animals with a High LG background.

Variations in maternal care were also associated with differences in learning in a stressful hippocampal learning task. The adult offspring of Low LG rats showed greater contextual fear conditioning than High LG rats; the present study did not examine whether maternal care also influences extinction of contextual conditioning. In contrast, maternal care did not affect tone conditioning or extinction, tasks which reflect activity in the amygdala and prefrontal cortex, among other regions. The specificity of the effect of maternal care to contextual conditioning is consistent with a selective effect of maternal care on hippocampal function. In maternally uncharacterised rats, the stress response evoked by the shock stimulus stimulates glucocorticoid and ß-adrenergic activity that modulates hippocampal consolidation of memory for the experience (Roozendaal et al., 2008). Thus, the effect of maternal care on contextual fear conditioning appears consistent with the hippocampal LTP findings. It would be interesting to examine corticosterone and norepinephrine levels in High and Low LG animals after contextual conditioning. Plasma corticosterone levels following restraint stress are greater in Low LG compared to High LG animals and contextual conditioning might also induce differential corticosterone release in Low and High LG animals (Liu et al., 1997). Future experiments should examine the role of glucocorticoid and beta-adrenergic activation in contextual fear conditioning in High and Low LG rats to further explore whether the apparent similarities indeed reflect a common mechanism.

The finding of greater hippocampal learning under stress in Low LG rats in contextual conditioning stands in contrast to the impaired performance of Low LG rats in other hippocampal-dependent tasks such as the Morris water maze and tests of object recognition. We suggest that this discrepancy may be explained by differences in the level of stress encountered in different hippocampal learning tasks. The exposure to stress in the Morris water maze can vary greatly depending on task parameters, such as water temperature (Akirav et al., 2004). Handling and habituation of animals to testing conditions can also reduce exposure to stress during the subsequent learning experience (Beiko et al., 2004). In previous studies of hippocampal learning in High and Low LG animals, animals were extensively habituated to testing conditions and stress was further minimised by using warm water in the Morris water maze and low lighting in the object recognition test (Liu et al., 2000b; Bredy et al., 2003b). In contrast, the shock exposure in contextual conditioning is strongly aversive and stressful. Thus it would seem that stress may critically modulate the effect of maternal care on hippocampal learning. However,

studies directly manipulating stress exposure within the same learning task would provide stronger support for this hypothesis.

Concluding Remarks

Maternal effects on phenotypic plasticity might 'prepare' the offspring for prevailing environmental conditions (Hinde, 1986; Rossiter, 1998; Meaney, 2001a; Zhang et al., 2005). Importantly, chronic stress decreases pup LG in lactating rats (Smith et al., 2004; Champagne and Meaney, 2006). This pattern is consistent with studies performed with simpler organisms showing that environmental adversity decreases parental investment in the offspring and alters phenotypic variation in defensive responses (Cameron et al., 2005). A shift towards enhanced hippocampal learning under conditions of stress might indeed be adaptive for individuals living under persistent and severe adversity (Meaney, 2001a; Zhang et al., 2005). Thus, such phenotypic variation is neither inherently beneficial nor detrimental. Rather, the adaptive virtue of any specific phenotypic variant is determined by the demands of a specific context. Although speculative, the current observations may fit in an emerging concept that adverse conditions during postnatal life may be maladaptive in low-threat but adaptive in high-threat environments later in life.

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