Maternal care, hippocampal synaptogenesis and cognitive development in rats

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We report that variations in maternal care in the rat promote hippocampal synaptogenesis and spatial learning and memory through systems known to mediate experience-dependent neural development. Thus, the offspring of mothers that show high levels of pup licking and grooming and arched-back nursing showed increased expression of NMDA receptor subunit and brain-derived neurotrophic factor (BDNF) mRNA, increased cholinergic innervation of the hippocampus and enhanced spatial learning and memory. A cross-fostering study provided evidence for a direct relationship between maternal behavior and hippocampal development, although not all neonates were equally sensitive to variations in maternal care.

Parental care is thought to influence cognitive development in human offspring[1,2], although much of the evidence remains correlational[2], and there is little understanding of underlying mechanisms. Clinically, parental maltreatment or neglect[3,4] or familial strife[5] can compromise cognitive development. Likewise, nonhuman primates and rodents show profound effects of maternal deprivation on cognitive development[2]. Do these findings necessarily imply that under normal conditions maternal care actively contributes to the development of neural systems that mediate cognitive development, however, or simply that the absence of the mother or conditions of abuse are so disruptive to physiology that they inevitably compromise cognitive development? If maternal care is relevant, then what are the relevant features of mother–offspring interactions and how do they influence neural development?

We examined these issues in studies of naturally occurring individual differences in maternal behavior in the rat. Mother–pup contact in the rat occurs primarily within the context of a nest bout, in which the mother approaches the litter, gathers the pups under her, licks and grooms her pups and nurses while continuing to occasionally lick and groom the pups; the bout terminates when the mother leaves the nest[6,7]. Individual differences in two forms of highly correlated maternal behavior, licking/grooming and arched-back nursing (LG-ABN), are stable over the first week of lactation[8–10], and also across multiple litters (D.D.F., A. Mar & M.J.M., unpublished data). The question, then, is whether such naturally occurring variations in maternal behavior might be related to the development of individual differences in cognitive development.

Results

Variations in maternal care

We observed cohorts of lactating female rats, scoring the frequency of maternal licking/grooming and arched-back nursing over the first ten days post-partum. Mothers that showed a high frequency of licking/grooming and arched-back nursing (animals whose scores on both behaviors were 1 s.d. greater than the mean for the entire cohort) were deemed 'high LG-ABN', whereas mothers that were lower on each measure (< 1 s.d. less than the mean) were considered 'low LG-ABN'. The differences in maternal behavior were highly significant ($F_{1,14}$ = 15.6; $p < 0.0001$; Fig. 1a), were apparent over the first 8 days following parturition, and occurred even though high and low LG-ABN mothers spent the same overall time in contact with pups (percentage of observations involving mother-pup contact: high LG-ABN = 54 ± 3%; low LG-ABN = 52 ± 5%, n.s.). Because high and low LG-ABN mothers successfully rear the same number of pups to weaning, and the offspring do not differ in weaning weight (D.D.F., A. Mar & M.J.M., unpublished data), it seems reasonable to assume that the maternal care of these mothers lies within a normal range of maternal behavior.

Hippocampal development

Spatial learning and memory was examined in the adult offspring of high and low LG-ABN mothers using the Morris water maze test[10]. To minimize the influence any potential group differences in emotionality, we habituated the animals to the maze on five consecutive days before testing. The offspring of high LG-ABN mothers showed significantly shorter latencies (data not shown) as well as swim paths ($F_{1,34}$ = 26.07, $p < 0.0001$) to locate the target platform than did offspring of low LG-ABN mothers (Fig. 2a). Although animals did not differ on the first day of testing, significant differences in performance were apparent for each of the next three days (group × day interaction effect, $F_{4,136}$ = 2.93, $p < 0.05$). When the platform was made visible, there were no group differences (all latencies < 10 s), suggesting that these effects are not related to the sensorimotor demands of the task[12]. On day 3 of testing, the animals were given a single 'probe' trial with platform removed. The offspring of high LG-ABN mothers showed significantly increased search-

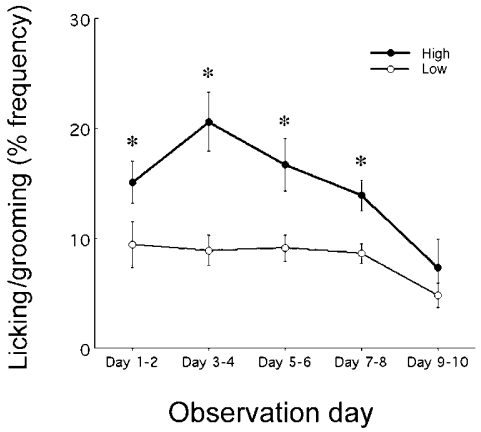

Fig. 1. Maternal behavior of high and low LG-ABN mothers. Mean (± s.e.m.) percentage frequency of licking/grooming in high and low LG-ABN mothers (n = 8 per group) over the first 10 days postpartum. presented in two-day blocks. Data were analyzed as the percentage of observations in which animals were observed to be engaged in licking/grooming a pup. High and low LG-ABN mothers were selected on the basis of individual differences in the expression of licking/grooming as well as arched-back nursing using data derived from each of the first 8 days postpartum (~150 observations per animal per day; *p < 0.0001).

ing in the target quadrant (time, $F_{3,102}$ = 4.6, p < 0.005; swimming distance, $F_{3,102}$ = 7.9, p < 0.001) compared with the offspring of high LG-ABN mothers (Fig. 2b).

Spatial learning and memory in the rat is associated with hippocampal function[10–13], and the first week of life is a period of intense hippocampal synaptogenesis. Thus, we examined expression of two independent synaptic markers, synaptophysin and neural cell adhesion molecule (N-CAM[14]) in western blots with hippocampal samples prepared from day 18 or day 90 offspring of high and low LG-ABN mothers. There were significant group effects for both synaptophysin ($F_{1,33}$ = 26.3, p < 0.0001) and NCAM ($F_{1,36}$ = 32.2, p < 0.0001) immunoreactivity in hippocampal tissue from the offspring of high compared with low LG-ABN mothers (Fig. 2c and d). These findings suggest either increased levels of synaptogenesis or increased synaptic survival in the offspring of high LG-ABN mothers. Despite the magnitude of the differences in synaptic plasticity, there were no differences in hippocampal neuron density at either day 8 or day 90 (Table 1a), nor were there differences in hippocampal volume (Table 1b).

Maternal care in early life was associated with differences in spatial learning and memory that endured even into later phases of aging (Fig. 2b). Thus, at 24 months of age, the offspring of high LG-ABN and low LG-ABN animals differed in their performance in the Morris water maze ($F_{1,15}$ = 12.3, p < 0.005). The results of a probe trial performed on day 3 of testing revealed significantly increased searching in the target quadrant in the aged offspring of high LG-ABN compared with low LG-ABN mothers ($F_{3,45}$ = 4.2, p < 0.01).

Cross-fostering

We examined the relationship between variations in maternal behavior and hippocampal development using a cross-fostering study in which pups born to low LG-ABN mothers were reared by high LG-ABN dams, and vice versa. To avoid affects on maternal behavior[15] and maintain the original character of the host litter, no more than two pups were fostered into or from any one litter[16]. Observations of maternal behavior showed that the behavior of high and low LG-ABN mothers was unaffected and that group differences in maternal behavior were maintained (mean ± s.e.m., frequency of maternal licking/grooming, high pups–high mother, 13.5 ± 0.8; low–high, 14.1 ± 2.5; low–low, 7.5 ± 1.3; high–low, 6.8 ± 0.7; p < 0.01).

As adults, spatial learning and memory of animals born to low LG-ABN mothers but reared by high LG-ABN dams was indistinguishable from that of high LG-ABN pups reared by high LG-ABN mothers (group effect, $F_{3,26}$ = 9.2, p < 0.001; Fig. 3a). Both groups differed significantly from low LG-ABN pups reared by low LG-ABN mothers on days 2 and 3 of testing. In contrast, there was no effect of cross-fostering the biological offspring of high LG-ABN mothers to low LG-ABN dams (Fig. 3a). Hence the spatial learning and memory of the biological offspring of high LG-ABN mothers was unaffected by the nature of the rearing mother.

Likewise, synaptophysin-like immunoreactivity in the adult offspring of low LG-ABN mothers reared by high LG-ABN dams did not differ in measures of synaptic plasticity from the offspring of high LG-ABN mothers (group effect, $F_{3,25}$ = 7.4, p < 0.001; Fig. 3b and c). All groups, including the high-to-low animals, showed higher levels of synaptophysin-like immunoreactivity compared to the offspring of low LG-ABN mothers reared by low LG-ABN mothers.

The septohippocampal cholinergic system

Considering the importance of the septohippocampal cholinergic system for spatial learning and memory[11,17,18], we examined acetylcholine (ACh) release in the dorsal hippocampus using microdialysis in conscious animals under basal and K^+-stimu-

Table 1. Neuron density and cell field volume in various hippocampal fields.

(**a**) Neuron density.		Hippocampal region		
Day 8		DG	CA1	CA3
	High LG-ABN	730113 ± 27494	582070 ± 34339	414236 ± 11891
	Low LG-ABN	682133 ± 34497	544823 ± 27631	367547 ± 19917
Day 90				
	High LG-ABN	545718 ± 6587	365740 ± 34256	186698 ± 7865
	Low LG-ABN	520833 ± 36464	339209 ± 24617	183760 ± 16459
(**b**) Cell field volume.		Hippocampal region		
Day 8		DG	CA1	CA3
	High LG-ABN	1.51 ± 0.06	1.43 ± 0.07	1.47 ± 0.06
	Low LG-ABN	1.45 ± 0.09	1.38 ± 0.09	1.42 ± 0.10
Day 90				
	High LG-ABN	2.45 ± 0.10	2.03 ± 0.08	2.44 ± 0.14
	Low LG-ABN	2.22 ± 0.14	1.92 ± 0.10	2.14 ± 0.13

Mean (± s.e.m.) estimates per mm^3 (n = 4 per group).

lated conditions. There were significantly higher ACh levels in dialysates under both basal and K^+-stimulated conditions in the high LG-ABN offspring ($F_{1,17} = 8.9$, $p < 0.01$; Fig. 4a). To address the question of cholinergic innervation, we assayed levels of choline acetyltransferase (ChAT) activity in two major cholinergic projection sites in the forebrain, the hippocampus and prefrontal cortex. ChAT activity was significantly higher in the hippocampus, but not in prefrontal cortex of adult offspring of high LG-ABN mothers (group × region interaction, $F_{1,18} = 3.10$, $p < 0.05$; Fig. 4b). We also found greater acetylcholinesterase staining in the offspring of the high LG/ABN mothers, a finding consistent with the idea of increased hippocampal cholinergic innervation in these animals (Fig. 4c).

Hippocampal BDNF and NMDA receptor expression

Cholinergic synaptic survival in the hippocampus has been associated with several neurotrophic factors, including nerve growth factor (NGF), brain-derived nerve growth factor (BDNF) and neurotrophin-3 (NT3)[19–21]. We used *in situ* hybridization with oligonucleotides directed against the mRNAs for these neurotrophic factors and found increased expression of BDNF mRNA in the dorsal hippocampus of the day 8 pups of high compared with low LG-ABN mothers (Fig. 5; $F_{1,9} = 8.0$, $p < 0.02$). There were no differences in NGF or NT-3 mRNA levels, nor were there differences in BDNF mRNA levels at 18 or 90 days of age (data not shown).

Activation of glutamate receptors, notably the NMDA receptor, increases BDNF gene expression[22]. *In situ* hybridization studies showed increased levels of mRNAs coding for the NR2A ($F_{1,11} = 5.7$, $p < 0.05$) and NR2B ($F_{1,11} = 7.0$, $p < 0.05$) subunits of the NMDA receptor in the hippocampus of day 8 offspring of high compared with low LG-ABN mothers (Fig. 6b and c). The differences were sustained into adulthood for both the NR2A ($F_{1,17} = 29.7$, $p < 0.0001$) and NR2B ($F_{1,17} = 7.4$, $p < 0.02$) subunits (Fig. 6b and c), and in adults there were also highly significant differences in NR1 ($F_{1,17} = 6.8$, $p < 0.02$) subunit mRNA expression (Fig. 6a). Hippocampal NMDA ([^{3}H]MK801) receptor binding capacity was increased in both the day 8 and adult offspring of high LG-ABN mothers (Fig. 6d), reflecting the functional significance of these changes in gene expression. These results suggest that maternal behavior regulates NMDA receptor expression in the offspring.

The effects of maternal care on NMDA receptor expression are regionally specific. In day 8 animals, there were no differences in NMDA receptor subunit expression in the central or basolateral nucleus of the amygdala. Although there were no differences in NR1 or NR2B mRNA levels in adults, NR2A mRNA expression was significantly higher in the central nucleus of the amygdala ($p < 0.05$) in the offspring of low LG-ABN mothers, with no effect in the basolateral nucleus.

In light of the absence of a cross-fostering effect in the biological offspring of high LG-ABN mothers, we wondered

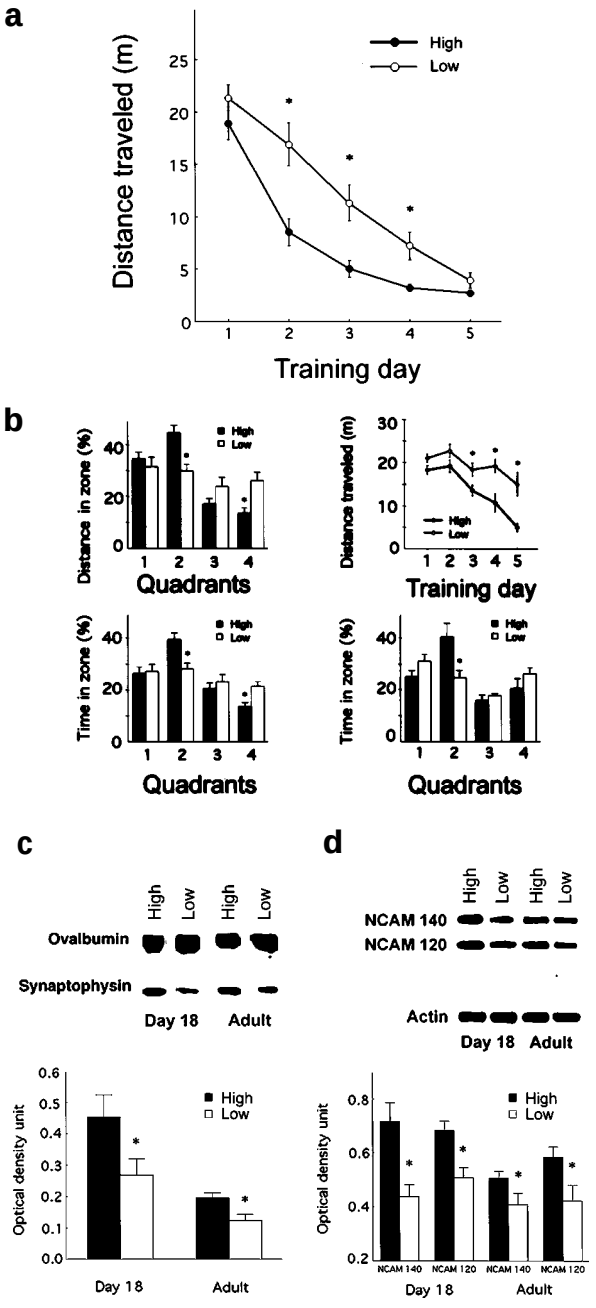

Fig. 2. Spatial learning/memory and hippocampal synaptogenesis in the adult offspring of high compared with low LG-ABN mothers. (**a**) Mean (± s.e.m.) latency (s) to locate and climb onto the submerged platform over five consecutive days of testing in the adult offspring of high and low LG-ABN mothers. The data for each day of testing are derived from three separate trials for a mean value was calculated for each animal. Statistical analysis of groups × day of testing ($n = 20$ animals per group, drawn from 5 high and 5 low LG-ABN litters) showed that group differences are significant for days 2–4 (**$p < 0.001$, *$p < 0.05$), but not for the first or last day of testing. (**b**) Left panels depict the results of the probe trial (target = quadrant 2) for both time and distance. The right panels depict the results of the swim maze and probe trial (as described above) for 24 month-old offspring of high and low LG-ABN animals (*$p < 0.05$). (**c**, **d**) Western immunoblot analysis of synaptophysin ($n = 9$–11 group; **c**) or neural cell adhesion (NCAM; $n = 10$ per group; **d**)-like immunoreactivity from hippocampal homogenates prepared from individual offspring of high or low LG-ABN mothers at 18 or 90 days of age. Top, representative blot (25 μg protein per lane) probed with synaptophysin or NCAM antibody for samples from individual day-18 or day-90 animals. The NCAM antibody recognizes both the 120 and 140 isoforms of NCAM. Ovalbumin or actin staining is presented as a positive control for loading errors. Bottom, mean (± s.e.m.) optical density unit measures across groups ($n = 8$ per group, with animals drawn from 4 high and 4 low LG-ABN litters). *Post-hoc* analysis showed significant (*$p < 0.01$) group effects at each age.

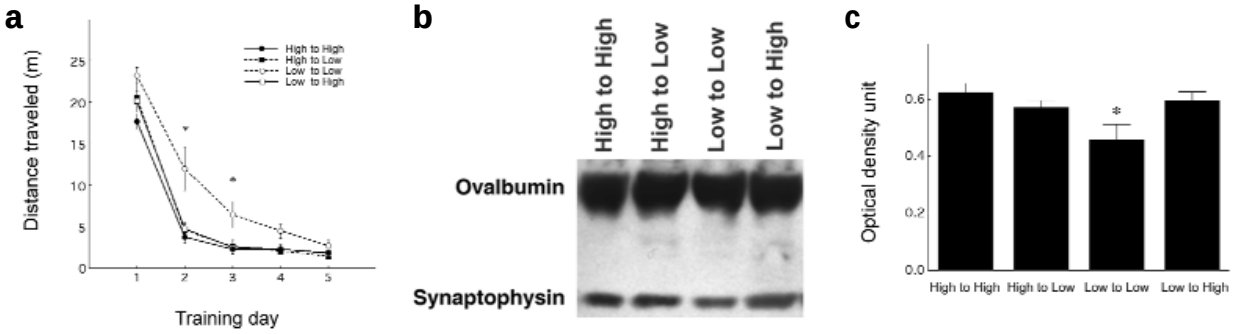

Fig. 3. Cross-fostering reverses differences in spatial learning and hippocampal synaptogenesis in the offspring of low, but not high LG-ABN mothers. (**a**) Mean (± s.e.m.) latency (s) to locate and climb onto the submerged platform over five consecutive days of testing in the adult of offspring born to low LG-ABN mothers and reared either by low (low–low) or high (low–high) LG-ABN dams compared with the offspring of high LG-ABN mothers reared by high LG-ABN mothers (high–high) or Low LG-ABN mothers (high–low). Statistical analysis of groups × day of testing (n = 7–8 animals per group, drawn from 5 litters of each condition) showed significant (*$p < 0.01$) group effects on days 2 and 3 of testing. (**b**) Representative western blots for synaptophysin-like immunoreactivity in hippocampal tissue samples from individual day 90 high–high, high–low, low–low and low–high animals. Ovalbumin staining is presented as a positive control for loading errors. (**c**) Mean (± s.e.m.) optical density unit measures across groups (n = 6–8 per group, drawn from 4 litters from each condition). *Post-hoc* analysis showed that synaptophysin-like immunoreactivity was significantly (*$p < 0.01$) lower in the low–low group by comparison to any of the remaining groups.

whether differences in NMDA receptor subunit expression might be apparent shortly after birth (day 0.5). We found no differences in mRNA for the NR1 subunit (data not shown). NR2B expression was undetectable in animals of this age. However, there was a highly significant increase in NR2A mRNA expression ($F_{1,15} = 12.3$, $p < 0.005$) in the newborn offspring of high LG-ABN mothers compared to those born to low LG-ABN females.

Discussion

These findings suggest that variations in maternal behavior are related to differential expression of genes encoding NMDA receptor subunits, which enhances hippocampal sensitivity to glutamate, and increase BDNF gene expression and thus hippocampal synaptic development. In visual and somatosensory cortex, experience-dependent modifications of synaptic development require NMDA receptor activation[23–25], and there is considerable evidence for common processes of synaptic plasticity in hippocampus and sensory cortex[26]. Thus, variations in maternal care may be considered to cause differential levels of sensory experience for the developing pup, resulting in altered levels of hippocampal synaptic development. Maternal licking/grooming is a major source of tactile stimulation for the developing pup, which affects somatic growth and neural development[27,28]. Maternal deprivation dampens growth hormone release and increases levels of the highly catabolic adrenal glucocorticoids[27–29], which, in turn, reduce BDNF expression[30]. These effects of maternal separation are blocked by artificial 'stroking' of the deprived pups with brushes—a manipulation that mimics the tactile stimulation derived from maternal licking/grooming. Likewise, the arched-back nursing posture of the mother is associated with increased tactile stimulation derived from nipple switching by pups, an activity associated with increased hippocampal volume[6].

The cross-fostering study provides evidence for a direct relationship between maternal care and hippocampal development.

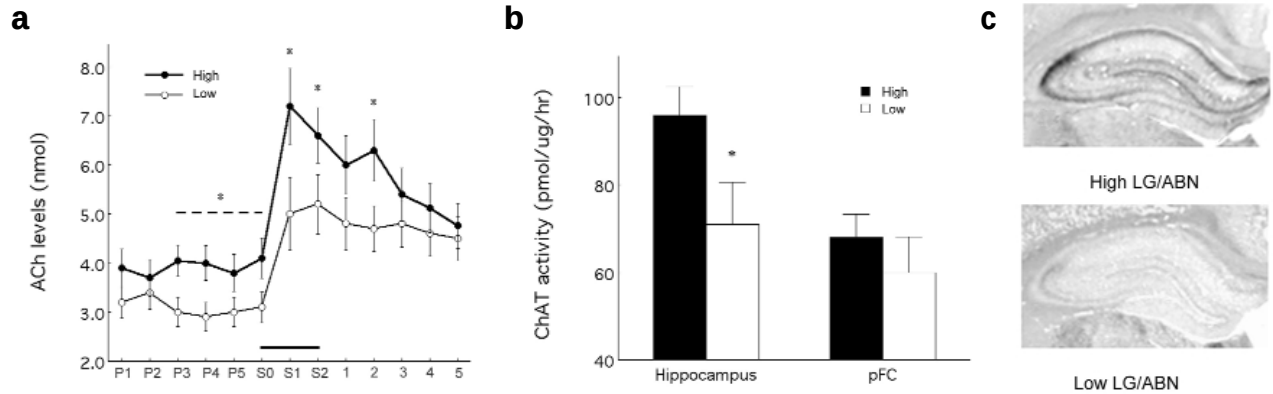

Fig. 4. The adult offspring of high and low LG-ABN mothers differ in hippocampal cholinergic innervation. (**a**) Mean (± s.e.m.) levels of acetylcholine (ACh) in dialysates collected from the dorsal hippocampus of the adult offspring of high or low LG-ABN mothers (n = 9–10 per group with animals drawn from 4 high and 4 low LG-ABN litters) under basal, pre-stimulation conditions (P) or following K^+ stimulation. *Post-hoc* statistical analysis showed a significant group effect at various time points (*$p < 0.05$). (**b**) Mean (± s.e.m.) level of choline acetyltransferase (ChAT) activity in synaptosomal fractions prepared from hippocampal or prefrontal cortex tissue from adult high or low LG mothers (n = 10 per group with animals drawn from 4 high and 4 low LG-ABN litters; *$p < 0.05$). (**c**) Representative coronal sections through the dorsal hippocampus showing acetylcholinesterase staining in the adult offspring of high or low LG/ABN mothers. Note that although staining is apparent throughout the hippocampus in both sections, the intensity is substantially greater in the high LG/ABN offspring.

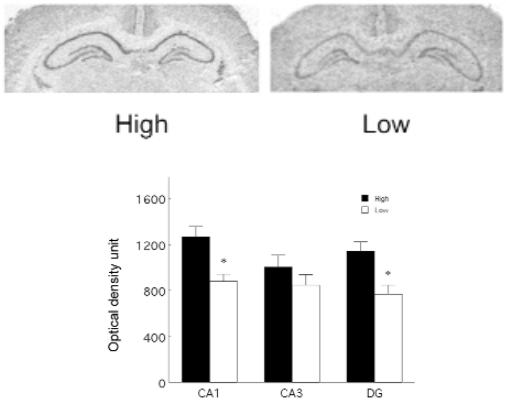

Fig. 5. Brain-derived neurotrophic factor (BDNF) gene expression. (**a**) Representative autoradiograms from *in situ* hybridization examining BDNF mRNA expression in the dorsal hippocampus in the day 8 offspring of high or low LG-ABN mothers. (**b**) Mean (± s.e.m.) levels of BDNF mRNA expression using optical density measures of autoradiograms in the dentate gyrus (DG) as well as the CA1 and CA3 regions of Ammon's horn in the dorsal hippocampus of day 8 pups of high or low LG mothers (n = 4–6 per group, drawn from 3 high and 3 low LG-ABN litters; *p < 0.05). There were no group differences in cell body size or in cell density (data not shown). Note that the effect of maternal care on BDNF mRNA levels was not due to differences in hippocampal neuron density (**Table 1**).

Spatial learning and hippocampal synaptic development in the biological offspring of low LG-ABN dams reared by high LG-ABN mothers were indistinguishable from those in normal offspring of high LG-ABN mothers. Thus, variations in maternal care can directly influence hippocampal development. These findings are consistent with results in the BALBc mouse, a strain that normally shows dramatically impaired hippocampal development and spatial learning deficits compared with C57 mice. As adults, BALBc pups reared by C57 mothers show improved spatial learning, and C57 mothers show increased licking/grooming and arched-back nursing by comparison to BALBc dams[31]. However, there do seem to be constraints, as the biological offspring of high LG-ABN females reared by low LG-ABN mothers resembled the the normal offspring of high LG-ABN mothers. Again the analogy to BALBc and C57 mice is instructive. Although BALBc mice that are reared by C57 mothers resemble C57 mice on measures of spatial learning and memory, C57 pups reared by BALBc mothers are unaffected[31].

The mechanisms underlying such differences in sensitivity to the influence of maternal care are not clear. However, if indeed the differences in NMDA receptor expression are critical for the effect of maternal tactile stimulation on hippocampal synaptic development, then the NMDA receptor subunit data in newborn pups might explain the sensitivity of animals born to low LG-ABN females. Hippocampal development in the low LG-ABN offspring would be enhanced by the increased tactile stimulation associated with a high LG-ABN mother, because of increased NR2A expression and thus greater sensitivity to environmental stimulation. The offspring of the high LG-ABN mothers show enhanced NR2A expression at birth, and this might then reduce their 'reliance' on maternal stimulation—at least with respect to hippocampal development. This hypothesis is certainly speculative, but it does allow us to address a fascinating question: why is parental care more important for certain individuals than for others?

The increased expression of NMDA receptor subunits apparent in the offspring of the high LG-ABN mothers in early postnatal life may serve as a critical mechanism mediating the effect of maternal care on hippocampal development. Alternatively, these receptor differences might reflect different levels of synaptic development. Although this issue remains to be resolved, the existing evidence from various models of cortical development suggests a causal role for such differences in NMDA receptor levels in mediating the effects of naturally occurring variations in maternal care on hippocampal synaptic development[23–25]. The offspring of high LG-ABN mothers showed increased NR2A and NR2B expression as well as increased hippocampal NMDA receptor binding at postnatal day 8. The kinetics of NMDA receptors composed of NR1/NR2A subunits differ from those composed of NR1/NR2B subunits, with NR2A-containing receptors being

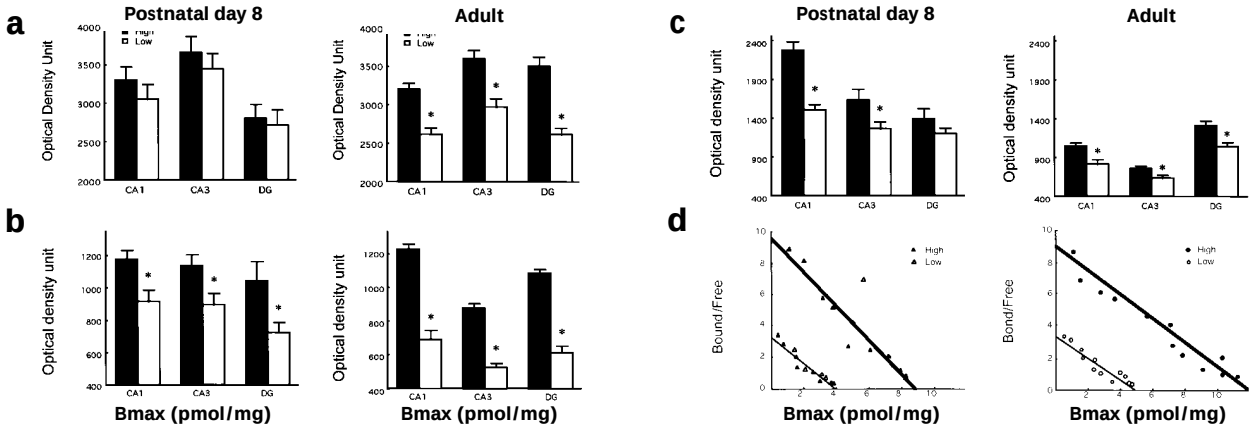

Fig. 6. NMDA receptor subunit gene expression. (**a–c**) Mean (± s.e.m.) optical density unit measures of autoradiograms from *in situ* hybridization studies of the NR1 (**a**), NR2A (**b**) and NR2B (**c**) subunits of the NMDA receptor in day 8 (n = 6–7 per group) and adult (day 100; n = 9–10 per group) offspring of high and low LG-ABN mothers. Data are presented for the dentate gyrus (DG) as well as the CA1 and CA3 regions of Ammon's horn in the dorsal hippocampus (*p < 0.01). (**d**) Representative Scatchard plots from [^{3}H]MK-801 receptor binding assays with hippocampal samples from day 8 or adult offspring of high and low LG-ABN mothers. Statistical analysis (n = 3 animals per group) revealed significant group for B_{max} values for both adult (high LG-ABN, 11.7 ± 0.6; low LG-ABN, 5.1 ± 0.4 pmol per mg; p < 0.001) and day 8 (High LG-ABN, 8.7 ± 0.4; low LG-ABN, 4.0 ± 0.5 pmol per mg; p < 0.001) animals. There were no group differences in the K_d values (range, 0.7 to 1.1 nM).

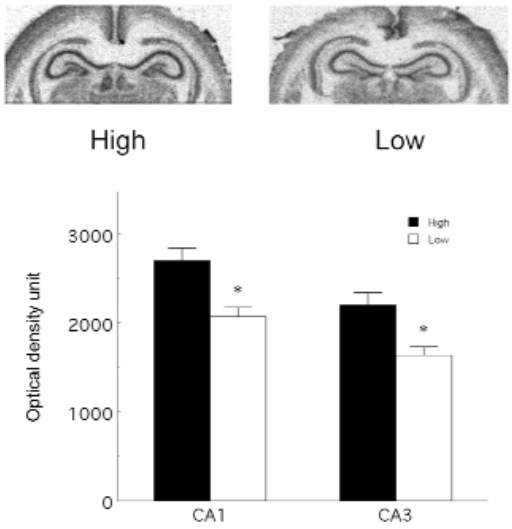

Fig. 7. NMDA receptor subunit gene expression in newborn offspring of high and low LG-ABN mothers. Top, representative photomicrograph of autoradiograms from *in situ* hybridization examining NR2A mRNA expression in the dorsal hippocampus in day 0.5 offspring of high or low LG-ABN mothers. Bottom, mean (± s.e.m.) optical density unit measures of autoradiograms from *in situ* hybridization studies of the NR2A subunit of the NMDA receptor in day 0.5 (n = 8–9 per group) offspring of high and low LG-ABN mothers, for the CA1 and CA3 regions of Ammon's horn in the dorsal hippocampus (*$p < 0.01$).

the mature form. Appropriate sensory stimulation (light) stimulates increased NR2A expression in the visual cortex[32]. We suggest that the increased hippocampal NR2A expression in the offspring of high LG-ABN mothers may derive from increased maternal, tactile stimulation. Tactile stimulation increases NMDA receptor expression in the barrel cortex of mice[33]. These findings also suggest that the effects of maternal care on hippocampal synaptogenesis are mediated through systems involved in the experience-dependent development of several neural systems.

Naturally occurring variations in maternal licking/grooming and arched-back nursing were associated with the development of cholinergic innervation to the hippocampus, as well as differences in the expression of NMDA receptor subunit mRNAs. In adults, there was increased hippocampal NR1 mRNA expression. These findings provide a mechanism for the differences observed in spatial learning and memory in adult animals. In the adult rat, spatial learning and memory depends on hippocampal integrity; lesions of the hippocampus result in profound spatial learning impairments[10–13]. Moreover, spatial learning is impaired by cholinergic or NMDA receptor blockade[11,17,18,34–36] or in mice lacking the NR1 subunit[37]. Likewise, hippocampal long-term potentiation, a potential neural model for learning and memory[36], is enhanced by treatments that increase acetylcholine release[38] or by overexpression of NMDA receptor subunits in the hippocampus[39]. Taken together, these findings suggest that maternal care increases hippocampal NMDA receptor levels, resulting in elevated BDNF expression and increased hippocampal synaptogenesis, and thus enhanced spatial learning in adulthood. These results are also consistent with the idea that maternal behavior actively stimulates hippocampal synaptogenesis in the offspring through systems that mediate experience-dependent neural development.

Methods

Animals. The animals used in all studies were derived from Long-Evans hooded rats obtained from Charles River Canada (St. Constant, Québec) and bred in our facility. No more than two animals per group were drawn from any single litter. Pups were weaned on postnatal day 22 and housed in same-sex, same litter groups of 3–4 animals per cage until day 45, and 2 animals per cage thereafter until the time of testing (~day 100). The mothers and their litters were housed in 46 × 18 × 30 cm Plexiglass cages that permitted a clear view of all activity within the cage. Food and water were provided *ad libitum*. The colony was maintained on a 12:12 light:dark schedule with lights on at 0800 h. All procedures were performed according to guidelines from the Canadian Council on Animal Care and approved by the McGill University Animal Care Committee.

Behavioral observations. Maternal behavior was scored[8–10] for six 100-minute observation periods daily for the first 10 days postpartum (0600, 0900, 1200, 1500, 1800 and 2100 h), during which the behavior of each mother was scored every 4 minutes. The data were analyzed as the percentage of observations in which animals engaged in the target behavior. The following behaviors[8] were scored: mother off pups, mother licking/grooming any pup, mother nursing pups in an arched-back posture, in a 'blanket' posture in which the mother lays over the pups, or in a passive posture in which the mother is lying either on her back or side while the pups nurse.

To define high and low LG-ABN populations, we observed the maternal behavior in a cohort of 32 mothers and devised the group mean and standard deviation for each behavior over the first 10 days of life. High LG-ABN mothers were defined as females whose frequency scores for both licking/grooming and arched-back nursing were more than one s.d. above the mean. Low LG-ABN mothers were defined as females whose frequency scores for both licking/grooming and arched-back nursing were more than one s.d. below the mean. As previously reported[9,10], licking/grooming were highly correlated ($r > 0.90$).

In the cross-fostering study, high or low LG-ABN dams were mated and allowed to give birth. Within 12 hours of birth, dams were removed from the home cage, and 2 animals per litter were cross-fostered. The procedure took less than 15 minutes. The cross-fostered pups, along with two native pups, were labeled with a semi-permanent marker until postnatal day ten and by individual differences in their pelage thereafter. Pups were marked at the time of cross-fostering and again on day five.

The critical groups of interest are animals born to low LG-ABN mothers, and fostered to high LG-ABN mothers (termed low–high) and the high–low reciprocal group. To control for the effects of cross-fostering to another mother, the offspring of high or low LG-ABN dams were fostered to other high or low LG-ABN mothers, respectively (high–high and low–low LG-ABN groups). Maternal behavior of each dam was then observed for the following eight days as described above.

Spatial learning and memory. Spatial learning and memory was examined using the Morris water maze task[10,11,18]. Animals are required to find a submerged (2 cm) platform in a pool (1.6 m diameter) of opaque water using distal spatial cues provided in the testing room[10]. The animals were given 15 trials, 3 trials per day, over five successive days with the platform submerged. Following testing, all animals were run on the sixth day using a visually cued version of the task[10] with the platform elevated 2 cm above the surface of the water to ensure that impairments were not related to sensorimotor defects[10,18].

Morphology. Neuron density estimates were derived using a serial sectioning procedure[47] with 50 μM brain sections prepared from perfused tissue samples. A series of sections was cut through the dorsal hippocampus, and every fifth section was stained and used for counting with an MCID image analyzer (MCID, St. Catherine's, Ontario) using the unbiased optical dissector method[40].

Acetylcholine studies. Dissected frontal cortex and hippocampi from individual adult rats were homogenized and incubated for 15 min in buffer containing [^{14}C]acetyl coenzyme A, as previously described[41]. Data are expressed as mean ± s.e.m. of nmol ACh per mg protein per h.

Protein content was determined as described by Bradford[42].

For *in vivo* microdialysis studies, rats were stereotaxically implanted with a guide cannula in the dorsal hippocampus (0.8 mm posterior to bregma, 2.5 mm lateral to the midline, 2.0 mm ventral to the dura) and allowed to recover for 4–6 d before *in vivo* dialysis. Twelve to sixteen hours before testing, CMA-10 probes (0.5 mm outer diameter, 4 mm length, CMA Microdialysis, Sobna, Sweden) were implanted through the guide cannula. Each animal was dialyzed once. On the day of the dialysis experiments, rats were placed individually in lidless oval cages for 1 h before being connected to a Harvard Microliter syringe pump (Harvard Apparatus, Holliston, Massachusetts) and dialyzed for a 1 h washout period with an artificial CSF that contained 125 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23 mM $NaHCO_3$ and 0.1 M neostigmine bromide (Sigma, St. Louis, Missouri) in aqueous phosphate buffer (1 mM, pH 7.4), at the rate of 5 µl per min. For the K^+ stimulation, 100 mM K^+ was used, and NaCl concentration was decreased to 27.5 mM to maintain equi-osmolarity. Ten-minute dialysate fractions were collected, frozen and stored at –80ºC until assayed.

Acetylcholine was assayed by HPLC with electrochemical detection in conjunction with an enzyme reactor. The samples were separated on a reverse-phase column pretreated with lauryl sulfate. The separated acetylcholine and choline then passed through an enzyme reactor containing acetylcholinesterase and choline oxidase covalently bound to glutaraldehyde-activated Lichrosorb NH2 10 µm (Capital HPLC Ltd., West Lothian, UK) and reacted to give a stoichiometric yield of hydrogen peroxide. The hydrogen peroxide was detected electrochemically by a platinum electrode set at 500 mV (versus Ag/AgCl, Antec Inc., Fremont, California). In the mobile phase, 0.2 M aqueous potassium phosphate buffer pH 8.0, containing 1 mM tetramethylammonium hydroxide, was delivered by a pump (Shimadzu LC-10AD, Shimadzu Scientific Instruments Inc., Columbia, Maryland) at 0.35–0.45 ml per min.

For the acetylcholinesterase study, rats were deeply anesthetized with pentobarbital and perfused through the ascending aorta with 300 ml saline followed by 600 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were dissected, postfixed and cryoprotected in phosphate-buffered 30% sucrose for 24 h. Twenty-five µm sections were then stained for acetylcholinesterase[43]. Briefly, slides were allowed to equilibrate at 4ºC before incubating at room temperature for 3 hours in a solution of copper sulphate (0.05%), glycine (0.075%), ethopromazine (0.0072%) and acetylthiocholine iodide (0.12%) in sodium acetate (0.1 M) buffer, adjusted to pH 5.0. Sections were then rinsed in distilled water and treated for 6 min in a developer solution of sodium sulfide (0.38%, pH 7.8). The staining was finally intensified in a silver nitrate (1%) solution for 2 min, and slices were rinsed in distilled water, and coverslipped.

Western blotting. Following rapid decapitation, brains were removed and placed on ice. The hippocampi were dissected, snap-frozen on dry ice and stored at –80ºC. Hippocampal samples were prepared using Ficoll gradient centrifugation method. Aliquots of the homogenates were taken to determine the levels of synaptic proteins in the whole hippocampus[42]. Equal amounts of proteins (25 mg) were separated by 10–20% SDS-tricine gradient gel electrophoresis[44] and immunoblotted with specific primary antibodies (Sigma). The immunoreactive bands were visualized by enhanced chemoluminescence (ECL, Amersham, Toronto, Ontario). Optical density readings for the synaptophysin (39 kDa) or NCAM (120 and 140 kDa) bands were determined using a computer-assisted densitometry system (MCID Systems). For all studies, single blots were derived from samples from one animal.

***In situ* hybridization.** Animals were killed under resting-state conditions directly from the home cage. *In situ* hybridization was done as described[8,9] using 3´ end [^{35}S]ddATP-labeled oligonucleotide sequences for NR1[45], NR2A[45], NR2B[45], BDNF[46] and NT-3[47] (Sheldon Biotechnology Center, Montréal, Canada). The NGF *in situ* study was done using a [^{35}S]UTP-labeled riboprobe[48] prepared from a rat NGF clone produced by subcloning a 238-bp fragment (bp 532–770) of the rat NGF clone[49] into bluescript. Slides were apposed to Hyperfilm (NR1 for 7 days, NR2A and NR2B for 14 days, BNDF or NT-3 for 21 days) along with sections of [^{35}S]-labeled standards prepared with known amounts of [^{35}S] in a brain paste. The hybridization signal within the dorsal hippocampus was quantified using densitometry with an image analysis system (MCID).

NMDA receptor binding assay. NMDA receptor binding in hippocampal synaptic membranes was measured using the method of ref. 50. Equilibrium saturation binding assays (30 mg membrane protein) were done using 0.38–32 nM [^{3}H]MK-801 (specific activity 22 Ci per mmol). Non-specific binding was determined in the presence of 4 mM MK-801. All assays included 20 mM spermidine, 20 mM glycine and 100 mM glutamate, and following rapid vacuum filtration, bound radioactivity was counted using a liquid scintillation counter.

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