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PARTIAL REVERSAL OF THE EFFECT OF MATERNAL CARE ON COGNITIVE FUNCTION THROUGH ENVIRONMENTAL ENRICHMENT

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Abstract—Maternal care influences hippocampal development in the rat. The offspring of mothers that exhibit increased levels of pup licking/grooming and arched-back nursing (High LG-ABN mothers) show increased hippocampal *N*-methyl-D-aspartate (NMDA) receptor binding and enhanced hippocampal-dependent spatial learning. In these studies we examined whether environmental enrichment from days 22–70 of life might reverse the effects of low maternal care. Environmental enrichment eliminated the differences between the offspring of High and Low LG-ABN mothers in both Morris water maze learning and object recognition. However, enrichment did not reverse the effect of maternal care on long-term potentiation in the dentate gyrus or on hippocampal NMDA receptor binding. In contrast, peripubertal enrichment did reverse the effects of maternal care on hippocampal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor binding. These findings provide evidence for the reversal of the effects of reduced maternal investment in early life on cognitive function in adulthood. Such effects might involve compensatory changes associated with peripubertal enrichment.

Key words: parental care, enriched, cognition, glutamate receptors.

Parental care influences cognitive development in humans, primates and rodents (Tamaroff et al., 1986; Kraemer 1997; Liu et al., 2000). For the rat pup, the dam is the most pervasive and dynamic source of sensory stimulation in the postpartum environment and mother–pup interactions influence hippocampal synaptic development and function (Liu et al., 1997, 2000). The tactile stimulation derived from maternal licking/grooming (LG) and arched-back nursing (ABN) appears to be critical for neural development. The offspring of mothers that show increased LG and ABN (i.e. High LG-ABN mothers) exhibit increased *N*-methyl-D-aspartate (NMDA) receptor and neurotrophic factor expression as well as enhanced spatial learning and memory (Liu et al., 2000; unpublished data). These effects of maternal care are reversed with cross-fostering, such that, as adults, the biological offspring of Low LG-ABN mothers reared by High LG-ABN dams are indistinguishable from the normal offspring of High LG-ABN mothers on measures of hippocampal development or spatial learning and memory (Liu et al., 2000).

Environmental influences on hippocampal development are not limited to the mother-infant interaction during the postnatal period. Hebb (1949) described how environmental enrichment throughout the peripubertal period enhanced maze learning in the rat. Rats exposed to environmental enrichment exhibit increased hippocampal nerve growth factor (NGF) and NGFI-A (an activity-dependent transcription factor) mRNA expression as well as improved performance in the Morris water maze (Pham et al., 1999; Olsson et al., 1994). Environmental enrichment also enhances neuron proliferation in the dentate gyrus (DG) of the hippocampus in rats and mice (Nilsson et al., 1999; Kempermann et al., 1997).

In these studies, the day 22 offspring of High or Low LG-ABN mothers were reared from weaning until day 70 under conditions of environmental enrichment or standard housing and tested between day 120 and day 140 of life. Considering the evidence for the positive effects of environmental enrichment, we hypothesized that the effect of reduced maternal stimulation on hippocampal development and learning and memory could be reversed by environmental enrichment and that such effects might reflect reversal at the level of putative underlying mechanisms, including glutamate receptor expression. In addition to NMDA receptors, we examined α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor binding since activity-dependent stabilization of synaptic contacts and the refinement of neuronal networks are associated with the delivery of functional AMPA receptors to synapses from non-synaptic sites (the "silent" synapse model; Malenka and Nicoll, 1997; Shi et al., 1999; Malinow et al., 2000).

EXPERIMENTAL PROCEDURES

Animals

The mothers were Long-Evans hooded rats born in our colony and derived from females obtained from Charles River Canada (St. Constant, Quebec). Mothers and litters were housed in 46 cm×18 cm×30 cm Plexiglass cages with food and water provided *ad libitum*. The colony was maintained on a 12-h light/

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Abbreviations: ABN, arched-back nursing; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DG, dentate gyrus; EPSP, excitatory post-synaptic potential; EP, field potential; PSA, population spike amplitude; LG, licking/grooming; LTP, long-term potentiation; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclo-hepten-5,10-imine-maleate; NGF, nerve growth factor; NMDA, *N*-methyl-D-aspartate.

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dark schedule with lights on at 0800 h. The animals underwent routine cage maintenance beginning on day 12, but were otherwise not manipulated. All procedures were performed according to guidelines developed by the Canadian Council on Animal Care with protocols approved by the McGill Committee on Animal Care.

Maternal behavior

The behavior of each dam was observed for eight 60-min observation periods, daily, for the first 8 days postpartum using a procedure originally devised by Meyers et al. (1989); also see Liu et al. (1997) and Francis et al. (1999). Observers were trained using videotapes and still photography to a high level of inter-rater reliability (i.e. $>.90$). Observations were performed at six periods during the light phase (0800, 1000, 1100, 1430, 1600 and 1800 h) and two periods during the dark phase of the L:D cycle (2000 and 0600 h). Within each observation period the behavior of each mother was scored every 4 min (15 observations/period$\times$eight periods per day$=$120 observations/mother/day) for the following behaviors: mother off pups, mother carrying pup, mother LG any pup, mother nursing pups in either an arched-back posture, a "blanket" posture in which the mother lies over the pups, or a passive posture in which the mother is lying either on her back or side while the pups nurse (see Meyers et al., 1989; Liu et al., 1997 for a description of behaviors).

The frequency of maternal LG and ABN across a large number of mothers is normally and not bi-modally distributed (Champagne et al., unpublished observations). Hence, the High and Low LG-ABN mothers represent two ends of a continuum, rather than distinct populations. In order to define these populations we observed maternal behavior in large cohorts of mothers (approximately 30–40 dams) and devised the group mean and S.D. for each behavior over the first 8 days of life as previously described (Francis et al., 1999; Liu et al., 2000). High and Low LG-ABN mothers were defined as females whose mean frequency scores over the entire 8-day period for both LG and ABN were greater than 1 S.D. above or below the mean for the cohort.

Postweaning housing conditions

On day 22 of life, the male offspring from five litters each of High and Low mothers were randomly assigned to either environmental enrichment or standard housing conditions. Animals housed under conditions of environmental enrichment were placed into groups of eight animals living within a series of large 60$\times$30$\times$60-cm cages interconnected with a burrow system and filled with toys that were replaced regularly. Standard laboratory conditions were defined as two animals housed in a 20$\times$40$\times$30-cm clear plastic cage. Animals were removed from these conditions on day 70 and housed two per cage until testing began on approximately day 120. During behavioral testing, all subjects were changed to single housing and remained this way until the end of the experiment.

Object recognition test

The object recognition test was performed according to Ennaceur and Delacour (1988) with modification. The testing apparatus was a standard open-field box (50 cm^3), painted black with bedding material covering the floor. The rats were habituated to the testing apparatus with three daily 5-min sessions. The memory test consisted of a sample phase followed by a choice phase with a 15-min inter-trial interval. During the sample phase, the rat was allowed to explore two identical objects for 5 min and then returned to its home cage. Both objects were removed during the 15-min interval and replaced with one identical and one novel object. The rat was then placed back in the box for a 5-min choice phase. Both objects and object location were counterbalanced in order to remove object and location preference effects. The experimenter was blind to the identity of the subjects during the experiment and each trial was videotaped for offline analysis. Exploration of an object was defined as directing the nose toward the object at a distance of 1 cm and/or touching the object with nose and paws. In order to obtain a measure of object discrimination, four measures were recorded; a) time spent exploring each object during the sample phase (T1a, T1b) and b) time spent exploring each object during the choice phase (T2a, T2n). For the result, discrimination was determined using the following formula: (T2n$-$T2a)/T2n$+$T2a) and analyzed by two-way ANOVA (group$\times$housing).

Morris water maze

Rats were required to locate a submerged platform (15$\times$15 cm) that was 1 cm below the water line in a 1.5-m-diameter pool using only ambient spatial cues available within the testing room (Morris et al., 1982). The water was made opaque by a layer of white polypropylene pellets that floated on the surface. Between 1000 and 1600 h, the rats were given 20 trials over 2 consecutive days with reversal of platform location on day 2 (10 trials per day, minimum 20-min inter-trial interval) with the platform submerged. At the end of each testing day, subjects were given a probe trial (60 s) in which the platform was removed to determine spatial bias for platform location. For all tests, search time (s) and time spent in quadrant (s) were recorded using the Poly-Track video tracking system (San Diego Instruments, San Diego, CA, USA). Latency was analyzed by between within ANOVA (group$\times$housing$\times$time).

Tissue preparation

Brains were obtained from adult (approximately 100 days of age) offspring of High and Low LG-ABN mothers by rapid decapitation, snap frozen in -70 °C isopentane and stored at -80 °C. Frozen coronal brain sections (16 μm) were cut on a cryostat, thaw-mounted on polylysine-coated slides and stored at -80 °C until assay.

Electrophysiology

Rats were anesthetized (Somnotol, 65 mg/kg, i.p.) and implanted with chronic stimulating and recording electrodes using stereotaxic techniques under aseptic conditions. Briefly, stimulating electrodes were implanted unilaterally in the medial perforant path with recording electrodes placed in the ipsilateral dentate hilus. Ground screws were placed in the skull along with two anchoring screws. Final positioning of the electrodes was determined during surgery by evoking field potentials using single test pulses. Rats were given a minimum of 1 week to recover.

Field potentials (EP) were evoked in unanesthetized, freely moving rats (in a quiet state of immobility) with single diphasic (0.1 ms/phase) test pulses delivered a minimum of 15 s apart (Hoh et al., 1999). Input-output (IO curve) determination (five to seven test-pulse intensities) was followed by 3 days of stable baseline recording of EPs induced by two test pulses of varying intensity (minimum and 80% of maximum). Long-term potentiation (LTP) was induced via five high-frequency trains, approximately 20 s apart (each train was 50 diphasic pulses, 0.1 ms/phase, 400 Hz, near maximal input-out response) and applied to the perforant path. Averaged responses to the same test pulse (10 sweeps) were obtained immediately, 1 h, and 24 h post-LTP induction. Electrographic activity at the recording site was continuously monitored to detect any after discharge that may have compromised the recording. Between within ANOVA was used to analyze the rising phase of the averaged field excitatory post-synaptic potential (EPSP) (slope) as well as the population spike amplitude (PSA). Upon completion of the experiment, rats were anesthetized (somnotol, 65 mg/kg, i.p.) and transcardially perfused with 10% formalin. The brains were cut in 40-μm sections, counterstained with Cresyl Violet and electrode placement was confirmed.

Glutamate receptor autoradiography

NMDA receptor binding sites were labeled with ^{3}H–MK-801 (21.7 Ci/mmol, RBI) according to Glazewski et al. (1993). Slide-mounted sections were thawed for 30 min at 4 °C and then pre-incubated in 5-mM Tris–HCl, pH 7.4, buffer for 10 min at 5 °C. Incubation was performed for 1 h at 20 °C in the same buffer containing 5-μM spermidine, 5-μM glycine, 5-μM glutamate and 3-nM ^{3}H–MK-801. Non-specific binding was assessed on adjacent sections with incubation buffer containing 100-μM cold MK-801. After incubation, slides were rinsed in 3× ice-cold buffer (30 s), dH_20 and 2.5% glutaraldehyde in acetone and then dried overnight. The incubated slides along with [^{3}H] standards (Amersham, Toronto, Ontario) were exposed to tritium sensitive film (Hyperfilm, Amersham) for 3–4 weeks at 5 °C. Regional NMDA receptor binding was analyzed using an MCID Image Analysis system (MCID, St. Catharines, Ontario) and statistical analysis performed using a three-way ANOVA (maternal care×housing ×region).

AMPA receptor binding sites were labeled with ^{3}H–AMPA (42.2 Ci/mmol, RBI) according to Le Jeune et al. (1996). Slide-mounted sections were thawed for 30 min at 4 °C, and then pre-incubated in 50-mM Tris–acetate, pH 7.2, buffer for 1 h at 4 °C. Incubation was performed for 30 min at 4 °C in the same buffer containing 100-μM potassium thiocyanate and 50-nM ^{3}H–AMPA. Non-specific binding was assessed on adjacent sections with incubation buffer containing 1-mM cold L-glutamic acid. After incubation, slides were rinsed in ice-cold buffer and dH_20 and dried overnight. The incubated slides along [^{3}H] standards (Amersham) were exposed to tritium-sensitive film (Hyperfilm, Amersham) for 6 weeks at 4 °C. Regional AMPA receptor binding was analyzed using an MCID Image Analysis system (MCID) and statistical analysis performed using a three-way ANOVA (maternal care×housing×region).

RESULTS

Object recognition test

There was a significant main effect of maternal care ($F1,34=7.93$, $P<0.01$) and housing ($F1,34=11.04$, $P<0.01$) on performance in the object discrimination test. Overall, the offspring of High LG-ABN mothers spent more time exploring the novel object during the choice phase of the object recognition test than did the offspring of Low LG-ABN mothers. Most importantly, there was a significant maternal care by housing interaction ($F1,34=4.94$, $P<0.05$) with the standard housed offspring of High LG-ABN mothers performing significantly better than the standard housed offspring of Low LG-ABN mothers. This difference was eliminated under conditions of enrichment with a reversal in the performance of the offspring of low LG-ABN mothers such that the offspring of Low LG-ABN mothers reared under conditions of environmental enrichment did not differ from either group of High LG-ABN offspring (Fig. 1).

Morris water maze

Latency to find platform. Overall, there was a significant main effect of maternal care ($F1,64=4.57$, $P<0.05$), housing ($F1,64=34.07$, $P<0.0001$) and day ($F1,64=54.59$, $P<0.0001$) on latency to find the platform. Enriched rats showed significantly shorter latencies than standard-housed rats and the latency to find the platform decreased for all groups across days. Post hoc analysis (Tukey/Kraemer test) showed that enriched rats had a shorter latency to find the platform on trial blocks 2–5 (all Ps <0.01). There was a significant maternal care by housing interaction ($F1,64=3.92$, $P<0.05$). The standard housed offspring of High LG-ABN mothers showing a significantly shorter search time than the offspring of Low LG-ABN mothers. Among animals reared under conditions of enrichment this difference was eliminated (Fig. 2a). Simple effects analysis revealed that in the standard-housed group, the offspring of High LG-ABN mothers had shorter latency to find the platform on trial blocks 2 and 4 (all Ps<0.05). There were no differences in quadrant dwell time during the probe trial at the end of day 1, indicating that all subjects learned to find the escape platform. There was a significant main effect of housing ($F1,32=5.89$, $P<0.05$) on quadrant dwell time during the probe trial at the end of reversal training on day 2. Enriched rats spent more time searching in the quadrant that had previously contained the platform than did standard-housed rats.

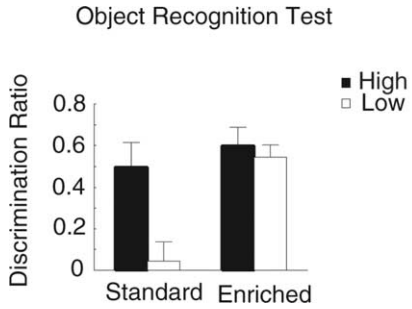

Fig. 1. Mean±SEM discrimination ratio ($n=8$–10/group) between novel and similar objects during the choice phase of the object recognition test. Overall, the offspring of High LG-ABN mothers spent more time exploring the novel object during the choice phase of the object recognition test than did the offspring of Low LG-ABN mothers ($p<0.01$). Under conditions of enrichment, the effect of reduced maternal care was reversed such that performance in the Low LG-ABN offspring did not differ from either group of High LG-ABN offspring ($p<0.05$).

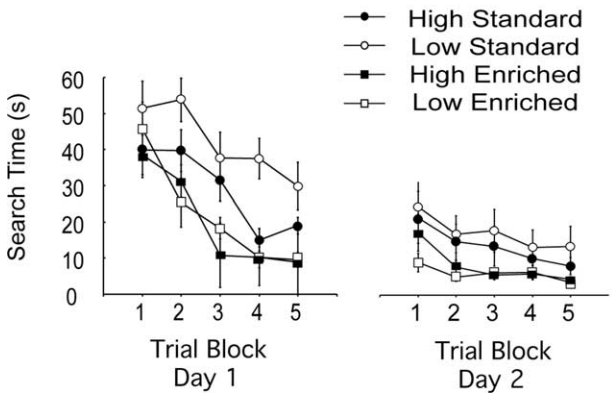

Fig. 2. (a) Mean±SEM latency to find platform (s) ($n=8$–10/group) on days 1 and 2 of Morris water maze training for adult offspring of High and Low LG-ABN mothers reared under standard or enriched housing conditions. Enriched rats showed significantly shorter latencies than standard-housed rats and the latency to find the platform decreased for all groups across days. ($p<0.0001$). Standard housed offspring of High LG-ABN mothers showing a significantly shorter search time than the offspring of Low LG-ABN mothers an effect that was eliminated among animals reared under conditions of enrichment ($p<0.05$).

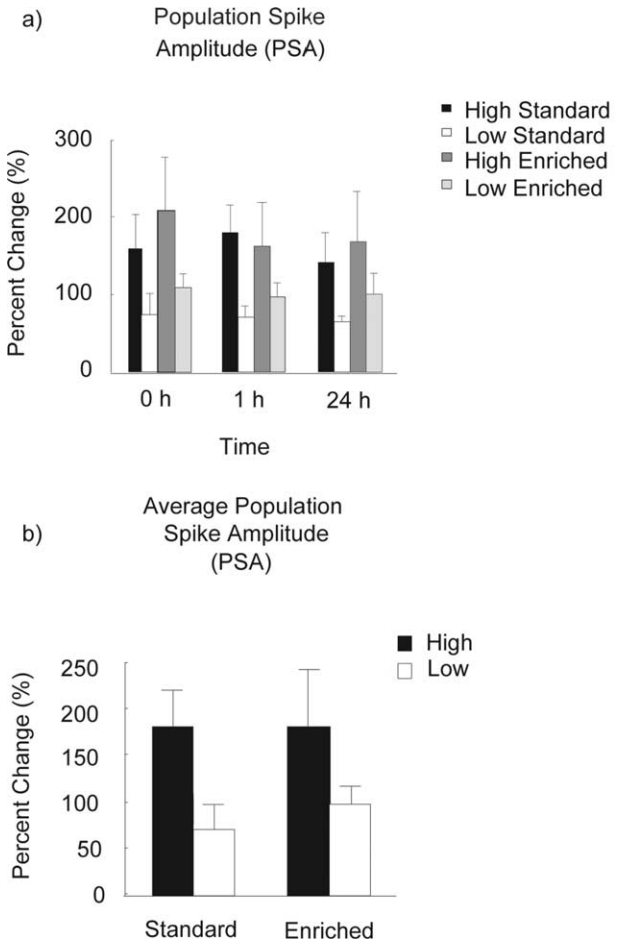

Fig. 3. (a) Mean±SEM percent change from baseline population spike amplitude (PSA) for adult offspring of High and Low LG-ABN mothers reared under standard or enriched housing conditions at 0, 1 and 24 h post LTP induction (n=5/group). The offspring of High LG-ABN mothers showed a trend towards greater population spike amplitude (PSA) ($p<0.10$). (b) Mean±SEM average percent change from baseline population spike amplitude (PSA). The offspring of high LG-ABN mothers had a greater average PSA than the offspring of Low LG-ABN mothers, regardless of housing condition ($p<0.05$).

Electrophysiology. There were no effects of maternal care or housing on the averaged field EPSP (data not shown). The offspring of High LG-ABN mothers showed a trend toward greater PSA at 0, 1 and 24 h post-LTP ($F1,15=3.35$, $P<0.10$) (Fig. 3a). Collapsing the data revealed a significant main effect of maternal care ($F1,15=5.21$, $P<0.05$) on average PSA. The offspring of High LG-ABN mothers had a greater average PSA than the offspring of Low LG-ABN mothers, regardless of housing condition (Fig. 3b).

Glutamate receptor autoradiography.

^{3}H–MK-801 binding. There was a significant main effect of maternal care ($F1,90=79.52$, $P<0.0001$) and region ($F1,90=40.70$, $P<0.0001$) on ^{3}H–MK-801 binding. The offspring of High LG-ABN mothers had higher ^{3}H–MK801 binding in all hippocampal regions, regardless of housing condition and ^{3}H–MK801 binding was highest in the CA1 and lowest in the DG (Fig. 4a). Environmental enrichment had no effect on ^{3}H–MK801 binding in either group.

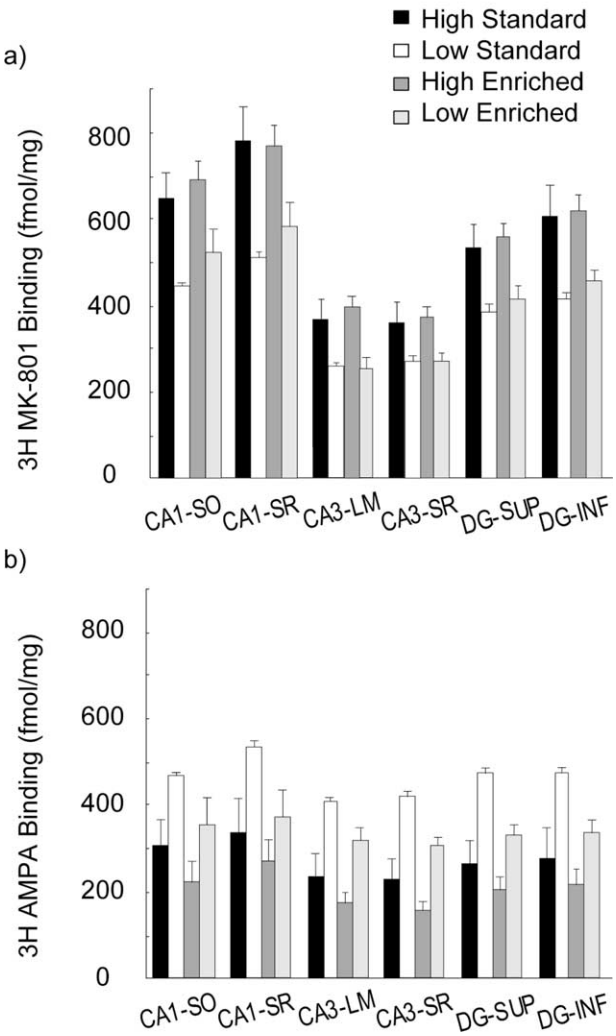

Fig. 4. (a) Mean±SEM hippocampal specific [^{3}H] MK-801 binding (n=4–5/group) for adult offspring of High and Low LG-ABN mothers reared under standard or enriched housing conditions. The offspring of High LG-ABN mothers had higher ^{3}H-MK801 binding in all hippocampal regions, regardless of housing condition ($p<0.0001$). (b) Mean±SEM hippocampal specific [^{3}H] AMPA binding (n=4–5/group). The offspring of Low LG-ABN mothers had higher ^{3}H-AMPA binding in all regions, regardless of housing condition and environmental enrichment decreased ^{3}H-AMPA binding in all regions in both groups ($p<0.0001$). Under conditions of enrichment, ^{3}H-AMPA binding did not differ between the offspring of High and Low LG-ABN mothers in the CA1 stratum radiatum or inferior blade of the dentate gyrus ($p<0.05$) (SO- stratum oriens, SR- stratum radiatum, INF- inferior blade, SUP- superior blade).

^{3}H–AMPA binding. There was a significant main effect of maternal care ($F1,78=70.12$, $P<0.0001$), housing ($F1,78=25.50$, $P<0.0001$), and region ($F1,78=2.53$, $P<0.05$) on ^{3}H–AMPA binding. ^{3}H–AMPA binding was highest in the CA1 and lowest in the CA3. The offspring of Low LG-ABN mothers had higher ^{3}H–AMPA binding in all regions, regardless of housing condition and environmen-

tal enrichment decreased ^{3}H–AMPA binding in all regions in both groups. Simple effects analysis of maternal care at housing/region revealed that under conditions of enrichment, ^{3}H–AMPA binding did not differ between the offspring of High and Low LG-ABN mothers in the CA1 stratum radiatum or inferior blade of the DG ($F1,78=2.50$, $P>0.05$) (Fig. 4b).

DISCUSSION

Maternal care alters hippocampal development and forms a basis for stable, individual differences in learning and memory (Liu et al., 2000). In the present study, we found that post-weaning environmental enrichment reversed the effects of reduced maternal care at the level of cognitive performance (see Figs. 1 and 2). Such effects were apparent on measures of both spatial learning and object recognition where the performance of the enriched offspring of Low LG-ABN mothers was comparable to that of High LG-ABN offspring. Interestingly, while there was no significant effect of enrichment on the offspring of High LG-ABN mothers in the object recognition test, enrichment enhanced performance in both groups in the Morris water maze. However, the significant interaction effect between maternal care and housing in both tests suggests that the offspring of Low LG-ABN mothers were more sensitive to enrichment. These findings resemble the results of child intervention programs in humans (Ramey and Ramey, 1998a,b). Such programs greatly offset the risk for impaired cognitive development associated with family dysfunction, and such effects are most apparent when development was compromised as a function of early life adversity. Thus, the effects of these "early start" programs are most marked in children from families characterized by turmoil and reduced parental education. In the rat, effects of postnatal forms of "enrichment," such as neonatal handling, are most apparent in animals exposed to prenatal stress (Weinberg et al., 1995; Smythe et al., 1994; Vallee et al., 1997). The common theme is that adversity in early life appears to enhance sensitivity to later forms of enrichment.

The obvious question concerns the issue of mechanism: Does functional reversal through enrichment involve the same neural targets that are sensitive to maternal care? Environmental enrichment did not reverse the effects of maternal care at the level of structure. The offspring of High LG-ABN mothers show increased NMDA receptor binding associated with elevated expression of the mRNAs encoding for the NR1, NR2A and NR2B subunits of the NMDA receptor (Liu et al., 2000). In the adult rat, spatial learning/memory is dependent upon hippocampal integrity (Morris et al., 1982). Moreover, spatial learning is impaired under conditions of NMDA receptor blockade or NR1 subunit knock-out (Gage and Bjorklund, 1986; Morris et al., 1986; Bailey et al., 1996; Bliss and Collingridge, 1993; Quirion et al., 1995). Likewise, hippocampal LTP, often considered as a model of neuroplasticity associated with learning and memory, is enhanced by overexpression of NMDA receptor subunits at the level of the hippocampus (Tang et al., 1999), and the High LG-ABN offspring showed enhanced LTP in the DG (see Fig. 3b). We found that environmental enrichment, despite clear affects on spatial learning/memory, did not reverse the effects of low maternal care on LTP or hippocampal NMDA receptor binding.

These findings suggest that environmental enrichment stimulates the development of neural systems that, ultimately, compensate for the effects of reduced maternal investment (i.e. Low LG-ABN) during infancy. Rampon et al., (2000) showed an increase in synaptic density in area CA1 after exposure to environmental enrichment in NR1 knockout mice. They suggest that NR1 activity is not specifically required for structural plasticity in the CA1 region of adult animals induced by enrichment. The findings are consistent with the idea that enrichment could "compensate" for the effects of low maternal LG-ABN on NMDA receptor systems rather than directly reverse the effects.

Interestingly, environmental enrichment significantly decreased AMPA receptor binding in both groups and reversed the difference in AMPA binding in the CA1 stratum radiatum and inferior blade of the DG in the Low LG-ABN offspring (see Fig. 4b). AMPA receptors play an important role in activity-dependent synaptic plasticity and, like NMDAR, are functionally dependent on their subunit composition (Zamanillo et al., 1999; Malinow et al., 2000; Seifert et al., 2000). The maternal care effect on AMPA binding and the learning/memory deficits is perhaps surprising, but is consistent with previous work demonstrating that among aged rats, those exhibiting more pronounced cognitive impairment have higher AMPA binding in the hippocampus (Le Jeune et al., 1996). The apparent contradiction here involves the association between enhanced cognitive performance and decreased AMPA binding in the hippocampus. Receptor binding assays, however, provide a functional measure of receptor activity and do not necessarily reflect absolute receptor levels. Factors such as receptor trafficking (Shi et al., 1999) influence the availability of functional membrane receptor sites. At this point these findings are best interpreted as suggesting an effect of maternal of care on AMPA receptor function and a possible reversal with environmental enrichment. The nature of this effect and its relationship to hippocampal function remains to be clarified.

Environmental enrichment had no effect on either hippocampal NMDA receptor binding or on LTP in the DG, suggesting that the relevant effects of enrichment involve mechanisms that differ from those associated with maternal care. This would suggest that the functional reversal associated with environmental enrichment involves "compensatory" effects rather than a reversal of the neural mechanisms affected by maternal investment. Such effects could involve changes in AMPA receptor activity. Further, environmental enrichment increases the expression of hippocampal NGFI-A mRNA (Pinaud et al., 2002). NGFI-A is associated with the effect of neonatal handling on serotonin expression in the hippocampus (Meaney et al., 2000); thus, serotonergic systems may play an important role in the compensatory effects of enrichment. More-

over, considering its executive role as the interface between emotion and cognitive function, the anterior cingulate cortex may be potential structural target for studies concerning the effect of environmental enrichment (for a review, see Allman et al., 2001). An obvious question that remains concerns the basis for the apparent increased sensitivity to enrichment in animals previously reared under conditions of "impoverished" maternal care.

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