Variations in postnatal maternal care and the epigenetic regulation of metabotropic glutamate receptor 1 expression and hippocampal function in the rat

Rosemary C. Bagot[a,1,2], Tie-Yuan Zhang[a,1], Xianglan Wen[a], Thi Thu Thao Nguyen[a], Huy-Binh Nguyen[a], Josie Diorio[a], Tak Pan Wong[a,b,3], and Michael J. Meaney[a,c,3]

[a]Neuroscience Division, Douglas Mental Health University Institute, and [b]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada H4H 1R3; and [c]The Agency for Science, Technology and Research, Singapore Institute for Clinical Sciences, Singapore 117609

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Variations in maternal care in the rat affect hippocampal morphology and function as well as performance on hippocampal-dependent tests of learning and memory in the offspring. Preliminary genome-wide analyses of gene transcription and DNA methylation of the molecular basis for such maternal effects suggested differences in the epigenetic state and transcriptional activity of the *Grm1* gene in the rat as a function of maternal care. *Grm1* encodes the type I metabotropic glutamate receptor (mGluR1), and we found increased mGluR1 mRNA and protein in hippocampus from the adult offspring of mothers showing an increased frequency of pup licking/grooming (i.e., high-LG mothers) that was associated with a decrease in the methylation of *Grm1*. ChIP assays showed increased levels of histone 3 lysine 9 acetylation and histone 3 lysine 4 trimethylation of *Grm1* in hippocampus from the adult offspring of high-LG compared with low-LG mothers. These histone posttranslational modifications were highly correlated, and both associate inversely with DNA methylation and positively with transcription. Studies of mGluR1 function showed increased hippocampal mGluR1-induced long-term depression in the adult offspring of high-LG compared with low-LG mothers, as well as increased paired-pulse depression (PPD). PPD is an inhibitory feedback mechanism that prevents excessive glutamate release during high-frequency stimulation. The maternal effects on both long-term depression and PPD were eliminated by treatment with an mGluR1-selective antagonist. These findings suggest that variations in maternal care can influence hippocampal function and cognitive performance through the epigenetic regulation of genes implicated in glutamatergic synaptic signaling.

Developmental outcomes are shaped by the prevailing social and economic contexts. Such influences in humans are apparent in studies showing the impact of socioeconomic status (SES) during childhood on health and well-being (1–7). Children reared in poverty show relatively poorer academic achievement and an increased risk for behavioral problems. There is evidence for SES effects on the development and function of brain regions critical for attention, affect regulation, and the processing of emotionally relevant information (8–10). SES effects on individual differences in brain-based developmental outcomes are mediated by parenting and the quality of the home environment (10–13). The demands of economic privation affect the mental health of the parents, increasing forms of parent–child relations that directly affect cognitive and emotional development (12). Importantly, such effects persist into adulthood, which begs the obvious question of how the broader socioeconomic context and associated effects on family function result in stable influences on neural function in the child.

Parental influences on the development of the offspring are not unique to humans (14–16). There are profound effects of variations in parental care on neural development in nonhuman species, including the rat. Naturally occurring variations in maternal care in the rat influence morphology and the capacity for synaptic plasticity in brain regions, such as the prefrontal cortex and hippocampus, that participate in higher cognitive functions (17–19). Thus, the adult offspring of mothers that show a persistently increased frequency of pup licking/grooming (i.e., high-LG mothers) exhibit enhanced hippocampal-dependent learning and memory (17, 19, 20) and increased hippocampal long-term potentiation (LTP), a cellular model for the synaptic plasticity that appears to underlie learning and memory (18, 19, 21, 22). Maternal effects on morphology and synaptic plasticity are apparent in both the dentate gyrus and the CA1 regions. These effects are at least partially reversed with cross-fostering, suggesting a direct effect of postnatal maternal care (17). Moreover, within-litter variation in the frequency of LG directed toward individual pups correlates with hippocampal LTP (23). Thus, in the rat, as in humans, social influences operate during early life to influence the structure and function of brain regions critical for cognitive capacity.

The obvious question concerns the nature of the underlying cellular mechanisms and the processes by which maternal influences exert an enduring effect on neural function. The increased capacity for hippocampal LTP in the adult offspring of high-LG mothers is associated with differential glutamate receptor function. Activity-dependent synaptic plasticity is dependent on glutamate-regulated changes in Ca^{2+} signaling (24). The relation between Ca^{2+} activity and plasticity resembles an inverted U-shaped function, whereby increasing levels of Ca^{2+} signaling enhance, and indeed are obligatory for, synaptic plasticity. However, elevations in Ca^{2+} can inhibit plasticity through the activation of homeostatic mechanisms that protect against Ca^{2+}-induced neurotoxicity (25–27). Patch-clamp studies reveal that hippocampal slices from the adult offspring of low-LG mothers show increased NMDA sensitivity (22) and evidence for NMDA-mediated inhibition of synaptic plasticity. Thus, perhaps somewhat counterintuitively, an NMDA receptor (NMDAR) antagonist enhances hippocampal LTP in slices obtained from the adult offspring of low-LG mothers (22). The same treatment suppresses

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[1]R.C.B. and T.-Y.Z. contributed equally to this work.

[2]Present address: Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, 10029.

[3]To whom correspondence may be addressed. E-mail: takpan.wong@mcgill.ca or michael.meaney@mcgill.ca.

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hippocampal LTP in slices from the offspring of high-LG mothers (22). These differences are associated with altered NMDAR levels; there is increased synaptic expression of NMDAR subunit proteins in the offspring of low-LG mothers (22). Thus, maternal care appears to shape the hippocampal response to excitatory synaptic input in the offspring.

Maternal care appears to influence activity-driven synaptic plasticity through transcriptional influences on genes that encode for proteins implicated in glutamate signaling. We examined this hypothesis using genome-wide profiling of the hippocampal mRNA derived from the adult offspring of high- and low-LG mothers. The results suggested an up-regulation of *Grm1* gene expression in the offspring of high-LG mothers. *Grm1* encodes for the group I metabotropic glutamate receptor (mGluR), mGluR1, which is highly expressed in the hippocampus, including the medial perforant pathway of the dentate gyrus (28, 29). This region is notably sensitive to maternal influences as a result of extensive postnatal development (30). Group I mGluRs, of which there are two, mGluR1 and mGluR5, interact extensively at the postsynaptic membrane with both NMDA and AMPA ionotropic receptors (28–31), and can thus modulate synaptic plasticity, including both LTP and long-term depression (LTD) (28, 29). Genetic deletion of mGluR1 or pharmacological antagonism impairs hippocampal-dependent learning (31–34).

We examined the possible maternal regulation of the *Grm1* gene by first confirming the results of the expression array data using measures of both mGluR1 mRNA and protein in hippocampal tissue from the adult offspring of high- or low-LG mothers. We then examined the potential epigenetic mechanisms that might account for a sustained difference in hippocampal *Grm1* expression, including measures of both DNA methylation and histone modifications. Our findings suggest that maternal care alters the epigenetic state of the *Grm1* gene in a manner that is consistent with an increased expression of *Grm1* in the offspring of high-LG mothers. Finally, we report the results of studies examining the role of mGluR1 in hippocampal synaptic function based on previous studies showing that group 1 mGluR activation induces a form of LTD of synaptic function (28, 29, 34–36) and may modulate presynaptic glutamate release through homeostatic, inhibitory feedback. The findings reveal increased mGluR1-induced LTD and greater mGluR1-mediated inhibitory synaptic regulation in hippocampus from the offspring of high-LG compared with low-LG mothers. Taken together, these findings suggest that maternal care stably influences the epigenetic state of genes that encode for proteins that regulate hippocampal synaptic function.

Results

Hippocampal mGluR1 but Not mGluR5 Gene Expression Is Altered by Maternal Care. We first sought to validate the results of earlier cDNA array studies suggesting differences in hippocampal *Grm1* expression as a function of variations in maternal care. We dissected hippocampal tissue from the adult offspring of high- or low-LG mothers and used quantitative real-time reverse transcription polymerase chain reaction PCR (qRT-PCR) analysis to examine mRNA levels for the two class I mGluRs, mGluR1 and mGluR5 (28, 29). The results (Fig. 1*A*) revealed significantly increased levels of mGluR1 mRNA in hippocampus from the offspring of high-LG compared with low-LG mothers [$t_{(8)} = 2.60$, $P = 0.03$; $n = 5$]. In contrast, hippocampal mGluR5 mRNA levels did not differ as a function of maternal care [$t_{(8)} = 0.21$, $P = 0.84$; $n = 5$]. We then examined whether the differences in mGluR1 mRNA levels were associated with changes in protein levels (Fig. 1 *B–D*). Western blot analysis revealed increased expression of mGluR1 in hippocampus of the offspring of high-LG compared with low-LG mothers [Fig. 1*B*; $t_{(20)} = 2.20$, $P = 0.04$; $n = 10–12$]. In contrast, protein levels of the other group I mGluR, mGluR5, were not affected by maternal care [$t_{(20)} = 1.04$, $P = 0.31$; $n = 10–12$]. These findings suggest a highly selective effect of maternal care on mGluR1 expression but not on mGluR5 expression.

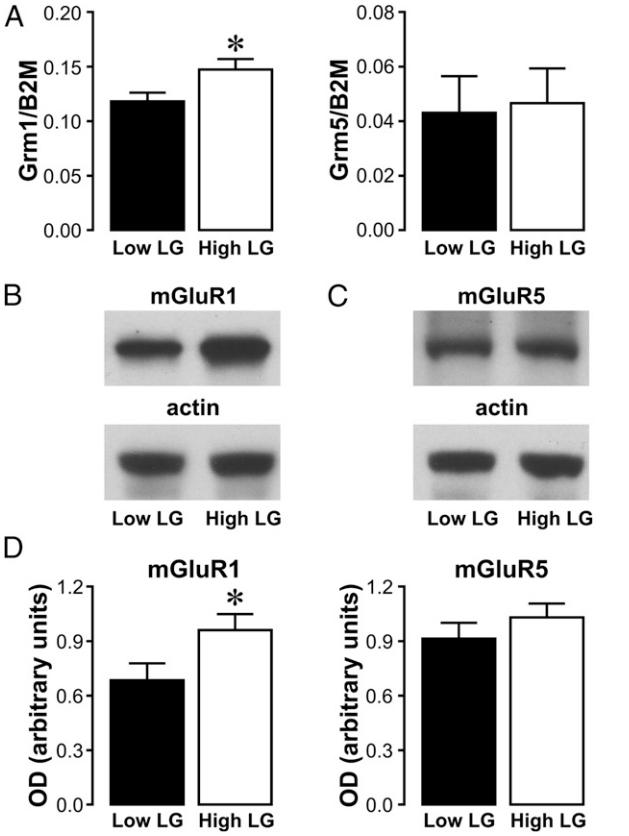

Fig. 1. Group I mGluR expression in the hippocampus. (*A*) qRT-PCR analysis of mGluR1 and mGluR5 mRNA levels in hippocampus from adult offspring of high-LG and low-LG mothers ($n = 5$ per group) expressed as the mean + SEM signal normalized using β_2 microglobulin (B2M). *$P < 0.05$. (*B* and *C*) Protein levels of mGluR1 and mGluR5 were measured by Western blot analysis in hippocampal lysates prepared from high-LG and low-LG offspring ($n = 10–12$ per group) and normalized to actin. Representative blots show the expression of mGluR1 and mGluR5. (*D*) Mean + SEM levels of mGluR1 and mGluR5 immunoreactivity expressed as OD unit in hippocampal samples from the adult offspring or high-LG and low-LG mothers. *$P < 0.05$.

Genome-Wide Analysis Reveals Differences in *Grm1* Promoter Methylation. We performed a genome-wide analysis of DNA methylation with a NimbleGen 2.1 rat DNA methylation promoter array (Roche) that covers 10-kb regions surrounding the transcriptional start site (TSS) of most genes annotated in the rat genome. The array uses 2.1 M oligonucleotide probes (50–75mer) with a median probe spacing of 100 bp covering ~8 kb upstream and ~2 kb downstream of known TSSs, and it informs on the DNA methylation state of ~16,000 CpG islands. DNA was captured using methylated DNA immunoprecipitation (MeDIP) with hippocampal tissue from the adult offspring of high- or low-LG mothers ($n = 4$ per group) using an antibody to 5′methylcytosine and hybridized to the promoter arrays. Following false discovery rate correction, maternal care produced 235 differentially methylated regions across 107 genes. The results revealed apparent differences in methylation across a region in proximity to exon 2 of the *Grm1* gene (Fig. S1 and *SI Materials and Methods*), suggesting increased methylation in samples from the offspring of low-LG mothers compared with high-LG mothers. The differences within this region lie within exon 2 (Fig. 2*A*), which contains one of the few CpG islands across the *Grm1* gene. CpG

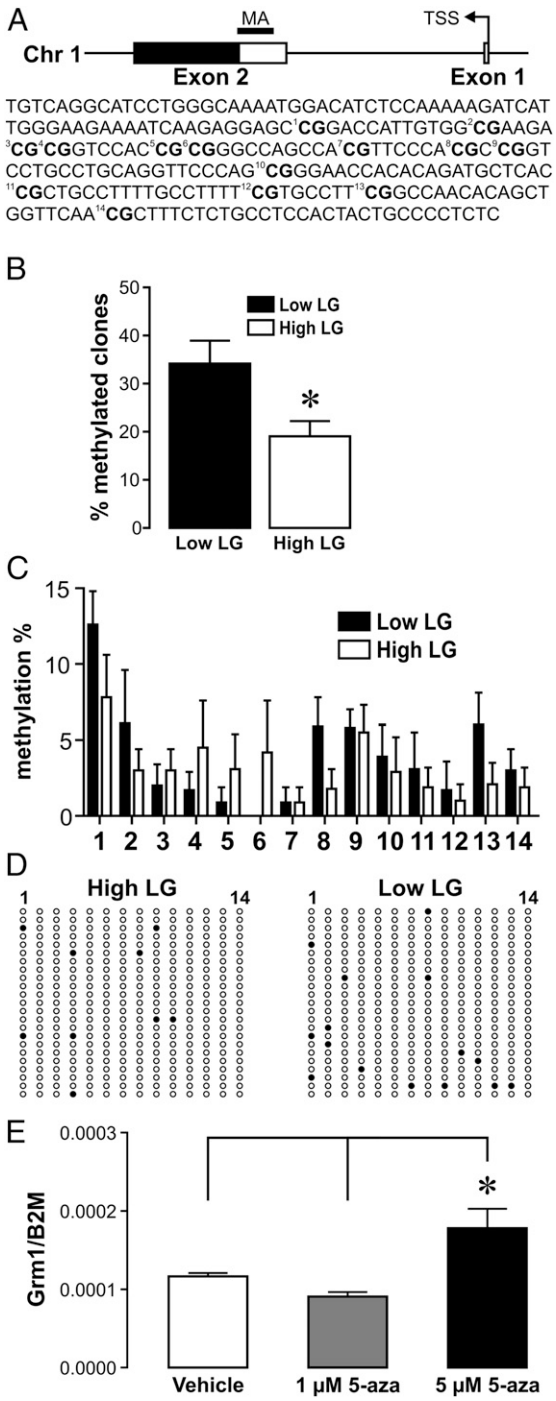

Fig. 2. DNA methylation analysis of the *Grm1* gene. (*A*) Site map depicts the region and sequence (CpG dinucleotides in boldface) of the *Grm1* gene analyzed for DNA methylation using sodium bisulfite sequencing. The bar covers the region in part of the coding and untranslated regions of exon 2 (MA, methylation assay). (*B*) Mean + SEM percentage of methylated clones in hippocampal samples from adult offspring of high-LG and low-LG mothers ($n = 5$ per group). *$P < 0.01$. (*C*) Mean + SEM levels of methylation across the 14 individual CpG sites of the analyzed region of the *Grm1* gene as a function of maternal care ($n = 5$ per group). (*D*) Representative bead-on-string models depict methylated (○) or unmethylated (●) CpG sites across individual clones of one sample from the hippocampus of high-LG or low-LG mothers. The models depict the increased frequency of methylation typical in samples from the low-LG offspring, with a sporadic distribution. (*E*) Mean + SEM level of mGluR1 mRNA determined using qRT-PCR and normalized to β_2 microglobulin (B2M) in cultured primary hippocampal neurons treated with 0, 1, or 5 μM 5-aza-cytosine ($n = 4$–5 per group). *$P < 0.05$.

islands (37) are areas containing a greater than expected density of CpG sites, and they are commonly found in genomic regions that regulate transcription. On the basis of this analysis, we formed a hypothesis concerning potential regions of differential methylation of the *Grm1* gene in the hippocampus as a function of maternal care.

Methylation of mGluR1 Promoter in Hippocampus of Low-LG Offspring. Decreased mGluR1 mRNA levels in the hippocampus of low-LG offspring suggest stable maternal effects on *Grm1* expression and differential methylation of regions that regulate *Grm1* transcription. The results of the genome-wide analysis (Fig. S1) suggested such differences in the methylation state of the *Grm1* gene as a function of maternal care. We focused on the region near exon 2 identified in the methylation arrays (Fig. 3*A* and Fig. S1) and developed primers that permitted analysis of 14 individual CpG sites contained within the target region of exon 2 within the *Grm1* gene (Fig. 2*A*). Exon 2 contains a coding region as well as an untranslated region. The assayed region incorporates 238 bp, starting 1,793 bp from the TSS and covers both coding and UTRs. Sodium bisulfite mapping was used to examine DNA methylation of the *Grm1* promoter with individual CpG resolution and to compare the methylation of multiple clones from hippocampal samples of high- and low-LG offspring (20 clones per sample). The overall percentage of methylated clones (i.e., promoter sequences bearing at least 1 methylated CpG site) was significantly increased in the offspring of low-LG relative to high-LG mothers [Fig. 2*B*; $t_{(8)} = 2.97$, $P < 0.01$; $n = 5$]. Analysis across all 14 CpG sites yielded no evidence for site-specific differences in methylation (Fig. 2 *C* and *D*). Rather, the difference in methylation was distributed across the amplified region.

The results of the sodium bisulfite mapping studies suggested increased methylation. We then examined the relation between DNA methylation and *Grm1* expression in cultured primary hippocampal neurons treated with 5-aza-cytosine, a potent demethylating agent. We found that 5-aza-cytosine treatment significantly increased the levels of mGluR1 mRNA in cultured hippocampal neurons [Fig. 2*E*; $F_{(2,13)} = 8.44$, $P < 0.01$; $n = 4$–5]. Post hoc analysis confirmed that at a concentration of 5 μM, 5-aza-cytosine treatment significantly increased mGluR1 mRNA levels above those of controls ($P < 0.05$). These findings are consistent with the hypothesis that under normal conditions, the level of DNA methylation constrains *Grm1* expression.

Histone Modifications Associated with Hippocampal *Grm1* Expression. The relation between DNA methylation within gene promoter regions and transcriptional activity is commonly mediated by differences in histone posttranslational modifications that regulate access of transcriptional machinery to the TSS (38–40). We obtained primers for two regions (Fig. 3*A*) of *Grm1,* one lying in proximity to the region showing differences in methylation (02A) and one lying within the region of the transcriptional start site (−01A). Previous *in silico* analyses suggested multiple potential transcription factor binding sites within both regions, implying active transcriptional regulation through these sites (41).

We developed a micro-ChIP protocol [adapted from the study by Dahl and Collas (42)] to study multiple histone posttranslational modifications associated with the *Grm1* promoter in hippocampal samples from the same individual animals. We used ChIP assays to examine common histone modifications focusing on two *Grm1* regions in proximity to that showing differential methylation as a function of maternal care (Fig. 3*A*). ChIP assays revealed little evidence for histone 3 lysine 9 trimethylation (H3K4me3) of the *Grm1* promoter in the −01A region (Fig. S2 and *SI Materials and Methods*). In contrast, we found significantly increased association of histone 3 lysine 9 acetylation (H3K9ac; Fig. 3*B*) for both the 02A [$t_{(12)} = 1.86$, $P < 0.05$; $n = 6$–8] and −01A [$t_{(12)} = 1.82$, $P < 0.05$] regions of the *Grm1* gene. Likewise, we found

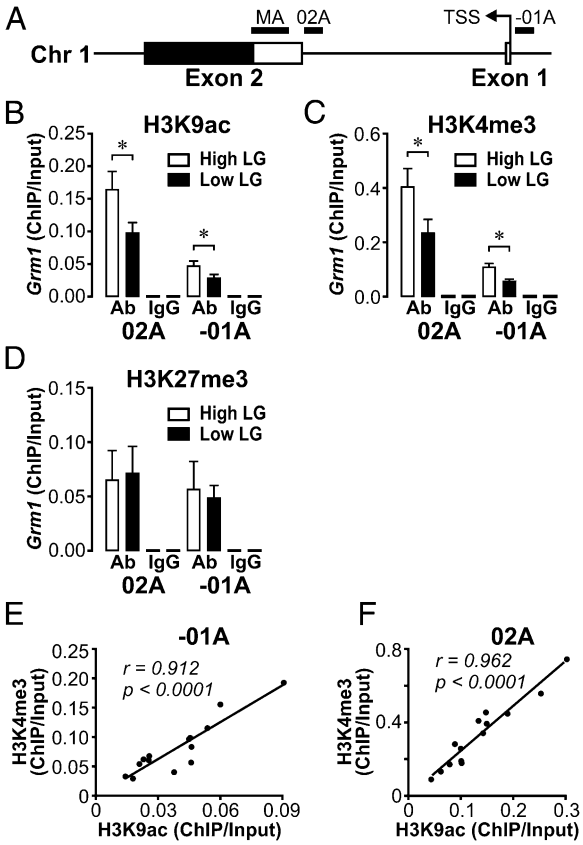

Fig. 3. ChIP analysis of histone posttranslational modifications. (*A*) Schema depicts the regions (02A, 1,393 from TSS) and (−01A, −254 from TSS) of the *Grm1* gene analyzed for various histone modifications. Chr, chromosome. (*B*) Mean + SEM levels of H3K9ac IP of regions 02A and −01A of the *Grm1* gene in hippocampal samples from the adult offspring of high-LG and low-LG mothers (n = 6–8 per group). *$P < 0.05$. (*C*) Mean + SEM levels of H3K4me3 IP of regions 02A and −01A of the *Grm1* gene in hippocampal samples from the adult offspring of high-LG and low-LG mothers (n = 6–8 per group). *$P < 0.05$. (*D*) Mean + SEM levels of H3K27me3 IP of regions 02A and −01A of the *Grm1* gene in hippocampal samples from the adult offspring of high-LG and low-LG mothers (n = 6–8 per group). (*E* and *F*) Scatterplots of the relation between H3K9ac and H3K4me3 across all samples in regions 02A and −01A of the *Grm1* gene in hippocampal samples from the adult offspring of high-LG and low-LG mothers (n = 14 total).

increased association of the H3K4me3 mark (Fig. 3*C*) with both the 02A [$t_{(12)} = 1.89$, $P < 0.05$; n = 6–8] and −01A [$t_{(12)} = 2.05$, $P < 0.05$] regions of the *Grm1* sequences. Both of these histone posttranslational modifications commonly associate with transcriptional activity (37–40, 42). Indeed, we found that the association of these marks across the *Grm1* promoter was very highly correlated (Fig. 3 *E* and *G*; region −01A: $r = 0.94$, $P < 0.0001$; region 02A: $r = 0.96$, $P < 0.0001$). In contrast, we found no differences in the association of trimethylated histone 3 lysine 27 (H3K27me3) with either region of the *Grm1* gene.

Group I mGluR LTD. We then examined the functional significance of the differences in hippocampal *Grm1* expression using mGluR1-induced LTD in hippocampal slices from the adult offspring of high-LG (n = 7) and low-LG (n = 8) mothers. Application of the group I mGluR agonist (R-S)-3,5-dihydroxyphenylglycine (DHPG; Tocris) induces a form of NMDAR-independent LTD that is induced by postsynaptic activation of mGluR1 and mGluR5 (28, 29, 34–36). We hypothesized that reduced mGluR1 levels should associate with an attenuated DHPG-induced LTD in slices from low-LG compared with high-LG offspring. Indeed, 50–60 min after DHPG treatment, there was a significantly greater depression of responses from baseline evident in slices from high-LG compared with low-LG offspring [Fig. 4 *A–C*; $t_{(13)} = 2.96$, $P = 0.01$], reflecting greater mGluR-induced LTD. Application of the mGluR1-specific antagonist JNJ 16259685 (1 μM; Tocris) significantly attenuated the depression at 50–60 min after termination of DHPG treatment in slices from high-LG offspring, confirming the involvement of mGluR1 in DHPG-induced depression [Fig. 4*D*; $t_{(10)} = 2.28$, $P < 0.05$; n = 5–7].

Paired-Pulse Depression. We further examined mGluR function by examining responses to paired-pulse stimulation as a measure of the effect of maternal care on presynaptic function in the dentate gyrus. When two identical stimulations are separated by a short

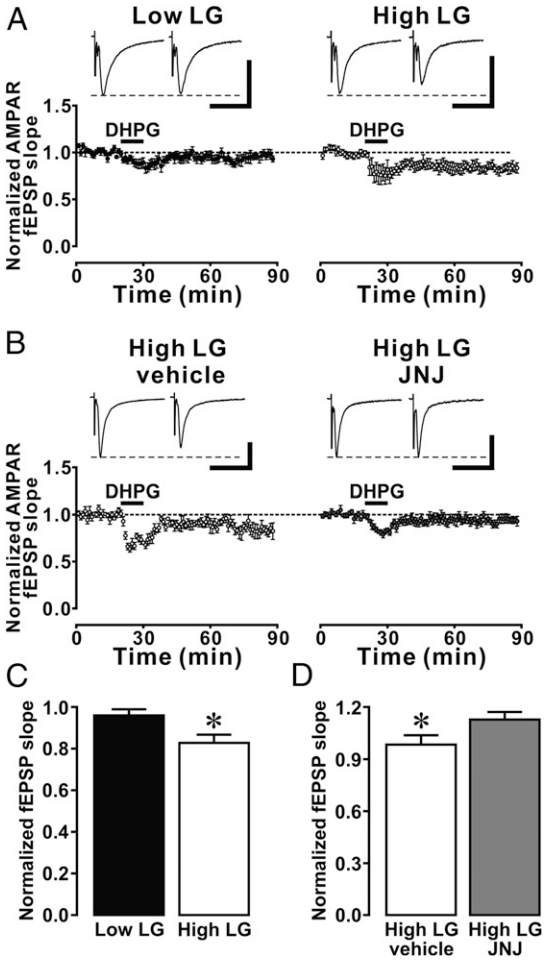

Fig. 4. mGluR1-dependent LTD in high-LG and low-LG offspring. (*A*) Scatterplots of normalized AMPAR- field excitatory postsynaptic potential (fEPSP) slope vs. time before and 1 h after LTD induction in low-LG and high-LG offspring. (*Upper*) Traces show representative examples of AMPAR-fEPSP before (*Left*) and 1 h after (*Right*) DHPG application (50 μM, 10 min). [Adjacent scale bars: 0.4 mV (vertical) and 40 ms (horizontal).] Dashed lines indicate baseline fEPSP amplitude (traces) or fEPSP slope (scatterplot). Note that mGluR-dependent LTD was induced in hippocampal slices from high-LG offspring only. (*B*) Scatterplots of AMPAR-fEPSP slope vs. time before and after LTD induction in high-LG offspring in the presence of vehicle or an mGluR1 antagonist, JNJ 16259685 (JNJ; 1 μM). Note that DHPG-induced LTD was abolished by mGluR1 blockade. (*C*) Mean + SEM changes in fEPSP slope 1 h after DHPG application. Compared with low-LG offspring, high-LG offspring displayed an attenuated fEPSP slope after DHPG. (*D*) DHPG-induced LTD in high-LG offspring was blocked by JNJ 16259685. *$P < 0.05$.

interval (<1,000 ms), modification of the size of the response to the second pulse relative to the first pulse reflects homeostatic control of presynaptic glutamate release (43) and is the measure for paired-pulse depression (PPD). The medial perforant pathway of the dentate gyrus is characterized by a very high glutamate release probability (44), and in contrast to other hippocampal synapses, paired-pulse stimulation results in depression of the second pulse relative to the first (45). We examined regulation of PPD by mGluR1 in slices from high- and low-LG offspring. A two-way ANOVA examining the effect of maternal care (high- or low-LG) and drug treatment (vehicle or JNJ 16259685) found a significant interaction [$F_{(1,38)} = 8.16$, $P = 0.007$]. Fisher's post hoc tests showed that in vehicle-treated slices, the depression of the second pulse was significantly greater in slices from the offspring of high-LG ($n = 11$) compared with low-LG ($n = 12$) mothers (Fig. 5; $P = 0.01$). The specific mGluR1 antagonist JNJ 16259685 (1 μM) significantly attenuated this depression in slices from high-LG offspring compared with vehicle-treated controls (control vs. JNJ 16259685; $P = 0.02$). In slices treated with JNJ 16259685, responses recorded in slices from high-LG offspring ($n = 10$) did not differ from those from low-LG offspring ($n = 9$). Thus, maternal care appears to alter presynaptic glutamatergic function through effects on mGluR1 expression, including effects on homeostatic regulation of presynaptic glutamate release. Importantly, the selective mGluR1 antagonist eliminated the maternal effect on PPD.

Discussion

Preliminary studies using a genome-wide analysis of gene expression suggested differences in hippocampal mGluR1 expression in the adult offspring of high-LG and low-LG mothers that were accompanied by differences in DNA methylation across specific regions of the *Grm1* gene, which encodes for mGluR1. We confirmed the difference in hippocampal mGluR1 expression at the level of both mRNA and protein (Fig. 1). Likewise, we used sodium bisulfite mapping to confirm the maternal effect on the epigenetic state of the promoter for the *Grm1* gene, with increased methylation of the *Grm1* gene in the adult offspring of low-LG compared with high-LG mothers. The results of the epigenetic analyses are consistent with those of the studies of mGluR1 expression: Increased DNA methylation in transcriptional regulatory regions commonly associates with decreased transcriptional activity (37–40, 46).

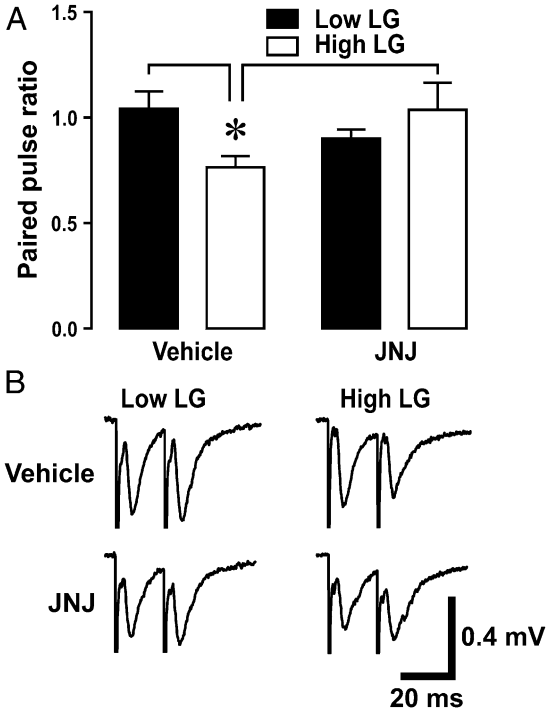

Fig. 5. mGluR1-dependent PPD in low-LG offspring. (*A*) Mean + SEM paired-pulse ratio reflects the effect of an mGluR1 antagonist, JNJ 16259685 (JNJ; 1 μM), on the paired-pulse ratio of the AMPAR-field excitatory postsynaptic potential (fEPSP) slope in high-LG and low-LG offspring. PPD (interpulse interval is 20 ms) of AMPAR-fEPSP was inducible in hippocampal slices from high-LG offspring only. This short-term plastic change was abolished by JNJ 16259685 (JNJ). *$P < 0.05$. (*B*) Representative traces of paired pulse-induced AMPAR-fEPSP recorded from low-LG and high-LG offspring in the presence of vehicle (artificial cerebrospinal fluid; *Upper*) and JNJ 16259685 (*Lower*).

The differences in CpG methylation of the *Grm1* gene were not specific to any individual CpG site. Rather, the region as a whole showed increased cytosine methylation in DNA from low-LG compared with high-LG mothers, with a twofold increase in the percentage of methylated clones in samples from the offspring of low-LG compared with high-LG mothers. These findings are similar to the effect of maternal care on the methylation of the *GAD1* gene promoter that encodes for glutamic acid decarboxylase (47). In both instances, increased methylation across the region was associated with transcriptional regulation associated with decreased gene expression (47), despite the absence of singularly affected CpG sites. It is important to consider this effect in relation to the organization of the underlying DNA (48). DNA methylation within promoter/enhancer regions reliably associates with transcriptional repression (37–40, 46, 49) through one of two pathways (49). The first pathway occurs through the direct interference of densely methylated CpG sites, with transcription factor binding to the DNA sites, thus directly affecting transcriptional activity. The second pathway is more subtle, and probably far more prevalent in euchromatic regions with dynamic variations in gene transcription, particularly with cells, such as neurons and glia, where such a high percentage of the genome is actively transcribed. In this case, the presence of the methylated cytosine attracts a methylated DNA binding protein, such as MeCP2 or MBD2 (37–40, 49). These proteins, in turn, attract repressor complexes that include histone deacetylases (HDACs), which prevent histone acetylation, and thus favor a closed chromatin state that constrains transcription factor binding and gene expression. Note the increased H3K9ac of the *Grm1* gene in samples from high-LG offspring, where there is also decreased DNA methylation. Because DNA is commonly organized into a nucleosome configuration, ~146 bp wrapped around a histone octamer (48–50), the methylation of any sites across this region should be sufficient to promote a closed chromatin configuration and decrease the probability of transcription. Thus, CpG methylation need not necessarily target specific CpG sites to affect local chromatin structure.

The ability of DNA methylation to regulate the capacity for histone modifications, especially histone acetylation, forms a prominent link between methylation and transcription. This is clearly evident for histone acetylation at sites like H3K9, which dynamically regulates the physicochemical properties of chromatin to enhance transcription factor binding (48, 51–56). Cytosine methylation attracts repressor complexes composed of HDACs, such that DNA methylation and histone acetylation are usually inversely related. H3K9ac associates with increased transcription (51–55), and we found increased H3K9ac of both regions of the *Grm1* genes in hippocampus from the adult offspring of high-LG compared with low-LG mothers (Fig. 3). This pattern is similar to maternal effects on hippocampal glucocorticoid receptor or GAD1 expression; in each case, decreased DNA methylation within promoter regions associates with increases in both H3K9ac and gene transcription (47, 57). H3K9ac tends to associate with stably transcribed regions of the genome, which is consistent with the idea of a persistent increase in hippocampal *Grm1*

transcription in the adult offspring of high-LG mothers. Additional histone modifications, notably histone methylation, influence transcription through indirect pathways that involve a complex array of transcriptional mediators (51, 52, 54, 58–60). Moreover, certain histone modifications covary. An example of relevance here is that of H3K9ac and H3K4me. Both marks are generally present at actively transcribed regions of the genome (61, 62). Thus, we find increased H3K9ac and H3K4me3 at both regions of the *Grm1* promoter, and the levels of these individual marks are very highly correlated (Fig. 3*D*).

H3K4me, whether in the mono-, di-, or trimethylated state, appears to protect CpG islands against methylation (63, 64). Thus, genome-wide analyses reveal a negative correlation between H3K4me and CpG methylation. Interestingly, H3K4me3 appears to actively "repel" the binding of the DNA methyltransferase, DNMT3L, which is essential for de novo methylation, and attracts complexes containing histone acetyltransferases, which open chromatin and enhance transcription factor binding (63). Indeed, the absence of H3K4me3 seems to be a prerequisite for the recruitment of de novo DNA methyltransferases and the acquisition of DNA methylation (63–66). The same relation was apparent across the *Grm1* promoter, where the decreased level of DNA methylation was associated with an increased level of H3K4me3 (Fig. 3). H3K4me3 targets the chromatin remodeling factor (NURF) and the Yng1 protein in the nucleosomal acetyltransferase of histone H3 complex to genes increasing the level of histone acetylation and transcriptional activation. This process explains the tight correlation between the levels of H3K4me3 and H3K9ac.

Although differences in histone acetylation are commonly explained in terms of the underlying DNA methylation state, the relation between histone modifications, including H3K9ac, and DNA methylation is bidirectional (56, 58). Thus, HDAC inhibitors, which increase the level of histone acetylation, associate with demethylation of the underlying DNA (56). These findings suggest a model whereby DNA methylation and histone modifications establish a self-perpetuating state (58, 67, 68) that sustains a stable difference in gene transcription. Importantly, treatment with a demethylating agent, 5-aza-cytosine, increased mGluR1 mRNA levels in cultured hippocampal neurons (Fig. 3*E*), suggesting that the methylation state of *Grm1* actively represses transcription.

mGluRs are members of the G protein-coupled receptor superfamily (28, 29), and genes encoding eight mGluR subtypes have been identified. We found clear evidence for an effect of maternal care on mGluR1 but not mGluR5, the other group I mGluR, suggesting selective effects even within an mGluR subgroup. mGluR1 is localized postsynaptically in a perisynaptic zone surrounding the ionotropic NMDARs and AMPA receptors (AMPARs) (69). mGluR-dependent LTP occurs at multiple excitatory synaptic sites. mGluR1 activation is required for LTP of AMPAR in hippocampal interneurons (70). In contrast, mGluR5 activation is required for LTP of NMDAR in principal neurons (71), reflecting both the complexity of the mechanisms underlying various forms of experience-dependent synaptic plasticity as well as the capacity for subtle and context-specific influences of early experience.

The perisynaptic location at excitatory synapses positions mGluR1 as a regulator of synaptic strength through the redistribution of ionotropic NMDARs and AMPARs (29). Thus, mGluRs facilitate or directly induce both LTD and LTP of synaptic strength (72). In vivo, mGluR1 activation is essential for low-frequency stimulation-induced LTD in the dentate gyrus of freely moving rats (73). Maternal effects on mGluR1 expression and presynaptic function may therefore have broad implications for the regulation of synaptic plasticity. mGluR1 receptors appear to operate as sensors of extracellular glutamate, and thus activate negative feedback control over presynaptic function to maintain optimal glutamate release (74). Variations in maternal care were associated with the response to paired-pulse stimulation in the medial perforant path, with a significantly greater depression of subsequent responding in slices from the adult offspring of high-LG compared with low-LG mothers. The medial perforant pathway is characterized by a very high glutamate release probability (44). Regulation of presynaptic glutamate release is therefore a highly effective mechanism in determining synaptic strength and plasticity, especially during repetitive stimulation of this pathway, while inducing synaptic plasticity. Identical stimulations separated by a short interval (<1,000 ms) normally result in depression of the second pulse relative to the first in the medial perforant path (PPD). This is thought to be an inhibitory feedback mechanism to prevent excessive glutamate release during high-frequency stimulation (75, 76). During repetitive stimulation, presynaptic Ca^{2+} influx is inhibited, which, in turn, mediates a reduction in glutamate release to subsequent stimuli occurring in short temporal succession (75, 76, 78). We showed that blockade of mGluR1 using JNJ 16259685 is sufficient to abolish PPD in the dentate gyrus of high-LG rats. The loss of PPD in low-LG rats may indicate an impaired feedback mechanism that would otherwise prevent excessive presynaptic glutamate release in the dentate gyrus and, additionally, may relate to the impairment of LTP in this region. We previously showed that overactivation of NMDAR could be responsible for LTP deficits in low-LG rats (22). The loss of PPD in low-LG rats we report here may favor high glutamate release during LTP induction, resulting in excessive NMDAR activation and LTP impairment.

The best-characterized effect of mGluR1 on synaptic plasticity is that of mGluR-induced LTD of excitatory synaptic strength (28, 29, 72, 79), which might be considered as another form of mGluR-induced synaptic depression. Thus, in addition to differences in PPD, there were maternal effects on hippocampal LTD, with significantly greater DHPG-induced LTD in hippocampal slices from the adult offspring of high-LG compared with low-LG mothers. The maternal effect on hippocampal LTD was eliminated with JNJ 16259685, revealing that the maternal effects are likely associated with the differences in the epigenetically mediated differences in mGluR1 expression. The mGluR-sensitive hippocampal LTD in the dentate gyrus is implicated in the encoding of information concerning novel objects (80, 81). Interestingly, the adult offspring of high- and low-LG mothers differ considerably in tests of object recognition, with stronger evidence of object memory, as well as spatial learning, in the offspring of high-LG mothers (17, 18, 20). Likewise, mGluR1$^{-/-}$mice show impaired performance on tests of hippocampal learning and memory. The mGluR1$^{-/-}$ mouse and the adult rat offspring of low-LG mothers share a common decrease in capacity in prepulse inhibition, an experimental model of sensorimotor gating (82, 83) that is in part hippocampal-dependent.

In summary, these findings suggest that variations in maternal care regulate the epigenetic state and transcriptional activity of the *Grm1* gene that encodes for mGluR1. The increased hippocampal expression of mGluR1 in the offspring of high-LG mothers is associated with decreased DNA methylation and increased H3K9ac and H3K4me3 marks in a *Grm1* region that regulates transcriptional activity. Likewise, we provided evidence for the functional importance of the maternal effect on hippocampal mGluR1 expression in studies of synaptic plasticity (LTD) and synaptic regulation (PPD). The precise mechanism by which the differences in mGluR1 expression regulate hippocampal function and the relationship between the effect on hippocampal physiology and behavior remain to be established. However, these findings are consistent with the "environmental epigenetics" hypothesis (14, 57, 67) suggesting that environmental events regulate the activity of intracellular signaling pathways to modulate the epigenetic state of regions across the genome, thus providing a candidate mechanism for the enduring effects of early social experience.

Materials and Methods

Generation of High- and Low-LG Offspring. Long–Evans dams (Charles River Laboratories) were mated in the animal facility of the Douglas Institute, Montreal, Quebec, Canada and observed for individual differences in maternal behavior (84) (*SI Materials and Methods*). All experiments used the adult (>70 d of age) male offspring of high- and low-LG dams. All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care, with protocols approved by the McGill University Animal Care Committee.

Electrophysiology. Hippocampal slices were prepared, and electrophysiology was performed under $GABA_A$ receptor blockade in the dentate gyrus (96) (*SI Materials and Methods*). mGluR LTD was induced using DHPG (50 μM). For PPD, field excitatory postsynaptic potentials were evoked at 20-s intervals, and once responses stabilized, paired-pulse stimulation (20-ms interpulse interval) was applied. The average of six stimulations was used to calculate the paired-pulse ratio (slope of pulse 2/slope of pulse 1). mGluR1-mediated effects were defined using JNJ 16259685 (1 μM).

Protein and RNA Determinations. Western blotting (*SI Materials and Methods*) was used to examine the expression of mGluR1 (1:5,000; BD Biosciences) and mGluR5 (1:1,000; Millipore).

Quantitative real-time PCR (47) (*SI Materials and Methods*) was used with RT2 PCR primer sets for rat *Grm1* (catalog no. PPR06830A; SABiosciences) and *Grm5* (forward primer TCCAGCAGCCTAGTCAACCT and reverse primer CAGATTTTCCGTTGGAGCTT).

DNA Methylation. Sodium bisulfite sequencing and MeDIP ChIP (46) (*SI Materials and Methods*) were performed with hippocampal tissue from the adult offspring of high- and low-LG mothers. Genome-wide analysis was performed with a custom-designed NimbleGen 2.1 rat DNA methylation promoter array that covers 10 kb of all annotated promoters to examine whole-genome promoter methylation (~27,342 known genes).

ChIP Assay. Assays were performed as previously described (47, 57). Briefly, animals were perfused with 4% (wt/vol) paraformaldehyde and brains stored at −80 °C until dissection. Hippocampi were dissected and sonicated on ice. Aliquoted chromatin from each sample was simultaneously immunoprecipitated using Dynabeads-protein A (catalog no. 100.02D; Invitrogen); rabbit polyclonal antibody to H3K9ac, trimethylated H3K4me3, trimethylated histone 3 lysine 9, and H3K27me3 (all antibodies from Active Motif); and normal rabbit IgG nonimmune antibody (Santa Cruz Biotechnology). DNA was isolated from inputs and immunoprecipitated samples using elution buffer containing proteinase K (2 h at 68 °C) and the phenol-chloroform-isoamyl alcohol extraction method. One-tenth of the lysate was kept before immunoprecipitation (IP) for quantification of DNA present in different samples (input fraction). The rat *Grm1* region of interest (GenBank accession no. NM_017011.1) was examined using primers [GPR1067491 (+) 02A, 1,393 from TSS and GPR1067491 (–) 01A, −254 from TSS; SABiosciences] with isolated DNA subjected to qRT-PCR amplification. All reactions were repeated in triplicate, and the ratio of immunoprecipitated DNA over input was calculated using the formula IP/Input = 2 ^ [Ct(Input)-3.32-Ct(IP)].

Hippocampal Neuronal Cultures. Information on hippocampal neuronal cultures is provided in *SI Materials and Methods*.

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1. Cohen S, Janicki-Deverts D, Chen E, Matthews KA (2010) Childhood socioeconomic status and adult health. *Ann N Y Acad Sci* 1186(Feb):37–55.
2. Hertzman C, Boyce T (2010) How experience gets under the skin to create gradients in developmental health. *Annu Rev Public Health* 31:329–347, 3p following 347.
3. Evans GW (2006) Child development and the physical environment. *Annu Rev Psychol* 57:423–451.
4. Adler NE, Rehkopf DH (2008) U.S. disparities in health: Descriptions, causes, and mechanisms. *Annu Rev Public Health* 29:235–252.
5. Bradley RH, Corwyn RF (2002) Socioeconomic status and child development. *Annu Rev Psychol* 53:371–399.
6. Brooks-Gunn J, Duncan GJ (1997) The effects of poverty on children. *Future Child* 7(2): 55–71.
7. McLoyd VC (1998) Socioeconomic disadvantage and child development. *Am Psychol* 53(2):185–204.
8. Shonkoff JP, Boyce WT, McEwen BS (2009) Neuroscience, molecular biology, and the childhood roots of health disparities: Building a new framework for health promotion and disease prevention. *JAMA* 301:2252–2259.
9. Raizada RD, Kishiyama MM (2010) Effects of socioeconomic status on brain development, and how cognitive neuroscience may contribute to levelling the playing field. *Front Hum Neurosci* 4:3.
10. Hackman DA, Farah MJ, Meaney MJ (2010) Socioeconomic status and the brain: Mechanistic insights from human and animal research. *Nat Rev Neurosci* 11:651–659.
11. Linver MR, Brooks-Gunn J, Kohen DE (2002) Family processes as pathways from income to young children's development. *Dev Psychol* 38:719–734.
12. Repetti RL, Taylor SE, Seeman TE (2002) Risky families: Family social environments and the mental and physical health of offspring. *Psychol Bull* 128:330–366.
13. Conger RD, Donnellan MB (2007) An interactionist perspective on the socioeconomic context of human development. *Annu Rev Psychol* 58:175–199.
14. Kappeler L, Meaney MJ (2010) Epigenetics and parental effects. *Bioessays* 32:818–827.
15. Cameron NM, et al. (2005) The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. *Neurosci Biobehav Rev* 29:843–865.
16. Maestripieri D, Mateo J (2009) *Maternal Effects in Mammals* (Univ of Chicago Press, Chicago).
17. Liu D, Diorio J, Day JC, Francis DD, Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci* 3:799–806.
18. Champagne DL, et al. (2008) Maternal care and hippocampal plasticity: Evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *J Neurosci* 28:6037–6045.
19. Bagot RC, et al. (2009) Maternal care determines rapid effects of stress mediators on synaptic plasticity in adult rat hippocampal dentate gyrus. *Neurobiol Learn Mem* 92: 292–300.
20. Bredy TW, Zhang TY, Grant RJ, Diorio J, Meaney MJ (2004) Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. *Eur J Neurosci* 20:1355–1362.
21. Bredy TW, Humpartzoomian RA, Cain DP, Meaney MJ (2003) Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. *Neuroscience* 118:571–576.
22. Bagot RC, et al. (2012) Maternal Care Influences Hippocampal N-Methyl-D-Aspartate Receptor Function and Dynamic Regulation by Corticosterone in Adulthood. *Biol Psychiatry*, 10.1016/j.biopsych.2012.03.016.
23. van Hasselt FN, et al. (2012) Adult hippocampal glucocorticoid receptor expression and dentate synaptic plasticity correlate with maternal care received by individuals early in life. *Hippocampus* 22:255–266.
24. Malenka RC, Bear MF (2004) LTP and LTD: An embarrassment of riches. *Neuron* 44: 5–21.
25. Frankiewicz T, Parsons CG (1999) Memantine restores long term potentiation impaired by tonic N-methyl-D-aspartate (NMDA) receptor activation following reduction of Mg2+ in hippocampal slices. *Neuropharmacology* 38:1253–1259.
26. Coan EJ, Irving AJ, Collingridge GL (1989) Low-frequency activation of the NMDA receptor system can prevent the induction of LTP. *Neurosci Lett* 105:205–210.
27. Katagiri H, Tanaka K, Manabe T (2001) Requirement of appropriate glutamate concentrations in the synaptic cleft for hippocampal LTP induction. *Eur J Neurosci* 14: 547–553.
28. Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 50:295–322.
29. Lüscher C, Huber KM (2010) Group 1 mGluR-dependent synaptic long-term depression: Mechanisms and implications for circuitry and disease. *Neuron* 65:445–459.
30. Altman J, Bayer SA (1990) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J Comp Neurol* 301:365–381.
31. Tu JC, et al. (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* 23:583–592.
32. Aiba A, et al. (1994) Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell* 79:365–375.
33. Schröder UH, et al. (2008) The potent non-competitive mGlu1 receptor antagonist BAY 36-7620 differentially affects synaptic plasticity in area cornu ammonis 1 of rat hippocampal slices and impairs acquisition in the water maze task in mice. *Neuroscience* 157:385–395.
34. Gil-Sanz C, Delgado-García JM, Fairén A, Gruart A (2008) Involvement of the mGluR1 receptor in hippocampal synaptic plasticity and associative learning in behaving mice. *Cereb Cortex* 18:1653–1663.
35. Palmer MJ, Irving AJ, Seabrook GR, Jane DE, Collingridge GL (1997) The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. *Neuropharmacology* 36:1517–1532.
36. Naie K, Manahan-Vaughan D (2005) Investigations of the protein synthesis dependency of mGluR-induced long-term depression in the dentate gyrus of freely moving rats. *Neuropharmacology* 49(Suppl 1):35–44.
37. Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* 16(1): 6–21.
38. Razin A (1998) CpG methylation, chromatin structure and gene silencing—A three-way connection. *EMBO J* 17:4905–4908.
39. Li E (2002) Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 3:662–673.
40. Cedar H, Bergman Y (2009) Linking DNA methylation and histone modification: Patterns and paradigms. *Nat Rev Genet* 10:295–304.

41. Crepaldi L, Lackner C, Corti C, Ferraguti F (2007) Transcriptional activators and repressors for the neuron-specific expression of a metabotropic glutamate receptor. *J Biol Chem* 282:17877–17889.
42. Dahl JA, Collas P (2009) MicroChIP: Chromatin immunoprecipitation for small cell numbers. *Methods Mol Biol* 567:59–74.
43. McNaughton BL (1982) Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. *J Physiol* 324:249–262.
44. Min MY, Asztely F, Kokaia M, Kullmann DM (1998) Long-term potentiation and dual-component quantal signaling in the dentate gyrus. *Proc Natl Acad Sci USA* 95: 4702–4707.
45. McNaughton BL (1980) Evidence for two physiologically distinct perforant pathways to the fascia dentata. *Brain Res* 199:1–19.
46. Weber M, et al. (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 39:457–466.
47. Zhang TY, et al. (2010) Maternal care and DNA methylation of a glutamic acid decarboxylase 1 promoter in rat hippocampus. *J Neurosci* 30:13130–13137.
48. Turner B (2001) *Chromatin Structure and the Regulation of Gene Expression* (Blackwell Science, Cambridge, MA).
49. Klose RJ, Bird AP (2006) Genomic DNA methylation: The mark and its mediators. *Trends Biochem Sci* 31(2):89–97.
50. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 389:251–260.
51. Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem* 76:75–100.
52. Hake SB, Allis CD (2006) Histone H3 variants and their potential role in indexing mammalian genomes: The "H3 barcode hypothesis." *Proc Natl Acad Sci USA* 103: 6428–6435.
53. Jenuwein T, Allis CD (2001) Translating the histone code. *Science* 293:1074–1080.
54. Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ (2007) How chromatin-binding modules interpret histone modifications: Lessons from professional pocket pickers. *Nat Struct Mol Biol* 14:1025–1040.
55. Lee KK, Workman JL (2007) Histone acetyltransferase complexes: One size doesn't fit all. *Nat Rev Mol Cell Biol* 8:284–295.
56. Szyf M (2009) Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu Rev Pharmacol Toxicol* 49:243–263.
57. Weaver IC, et al. (2004) Epigenetic programming by maternal behavior. *Nat Neurosci* 7:847–854.
58. Berger SL (2007) The complex language of chromatin regulation during transcription. *Nature* 447:407–412.
59. Kouzarides T (2007) Chromatin modifications and their function. *Cell* 128:693–705.
60. Bernstein BE, et al. (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* 120:169–181.
61. Ruthenburg AJ, Allis CD, Wysocka J (2007) Methylation of lysine 4 on histone H3: Intricacy of writing and reading a single epigenetic mark. *Mol Cell* 25:15–30.
62. Pokholok DK, et al. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 122:517–527.
63. Ooi SKT, et al. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* 448:714–717.
64. Thomson JP, et al. (2010) CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* 464:1082–1086.
65. Ciccone DN, et al. (2009) KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* 461:415–418.
66. Nan X, et al. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 393:386–389.
67. Meaney MJ, Ferguson-Smith AC (2010) Epigenetic regulation of the neural transcriptome: The meaning of the marks. *Nat Neurosci* 13:1313–1318.
68. Mohn F, et al. (2008) Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Mol Cell* 30:755–766.
69. Lujan R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *Eur J Neurosci* 8:1488–1500.
70. Ran I, et al. (2009) Persistent transcription- and translation-dependent long-term potentiation induced by mGluR1 in hippocampal interneurons. *J Neurosci* 29: 5605–5615.
71. Jia Z, et al. (1998) Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. *Learn Mem* 5:331–343.
72. Anwyl R (2009) Metabotropic glutamate receptor-dependent long-term potentiation. *Neuropharmacology* 56:735–740.
73. Naie K, Tsanov M, Manahan-Vaughan D (2007) Group I metabotropic glutamate receptors enable two distinct forms of long-term depression in the rat dentate gyrus in vivo. *Eur J Neurosci* 25:3264–3275.
74. Nicoletti F, et al. (1999) Group-I metabotropic glutamate receptors: Hypotheses to explain their dual role in neurotoxicity and neuroprotection. *Neuropharmacology* 38: 1477–1484.
75. Wu LG, Saggau P (1997) Presynaptic inhibition of elicited neurotransmitter release. *Trends Neurosci* 20:204–212.
76. Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. *Annu Rev Physiol* 64: 355–405.
77. Harris EW, Cotman CW (1985) Effects of synaptic antagonists on perforant path paired-pulse plasticity: Differentiation of pre- and postsynaptic antagonism. *Brain Res* 334:348–353.
78. Rausche G, Sarvey JM, Heinemann U (1988) Lowering extracellular calcium reverses paired pulse habituation into facilitation in dentate granule cells and removes a late IPSP. *Neurosci Lett* 88:275–280.
79. Gladding CM, Fitzjohn SM, Molnár E (2009) Metabotropic glutamate receptor-mediated long-term depression: Molecular mechanisms. *Pharmacol Rev* 61:395–412.
80. Kemp A, Manahan-Vaughan D (2008) The hippocampal CA1 region and dentate gyrus differentiate between environmental and spatial feature encoding through long-term depression. *Cereb Cortex* 18:968–977.
81. Massey PV, Bashir ZI (2007) Long-term depression: Multiple forms and implications for brain function. *Trends Neurosci* 30:176–184.
82. Brody SA, Conquet F, Geyer MA (2003) Disruption of prepulse inhibition in mice lacking mGluR1. *Eur J Neurosci* 18:3361–3366.
83. Zhang TY, Chrétien P, Meaney MJ, Gratton A (2005) Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. *J Neurosci* 25:1493–1502.
84. Champagne FA, Francis DD, Mar A, Meaney MJ (2003) Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav* 79:359–371.
85. Tse YC, Bagot RC, Hutter JA, Wong AS, Wong TP (2011) Modulation of synaptic plasticity by stress hormone associates with plastic alteration of synaptic NMDA receptor in the adult hippocampus. *PLoS ONE* 6:e27215.

Supporting Information

Bagot et al. 10.1073/pnas.1204599109

SI Materials and Methods

Generation of High-Licking/Grooming and Low-Licking/Grooming Offspring. Long–Evans dams (Charles River Laboratories) were mated in the animal facility of the Douglas Institute, Montreal, Quebec, Canada. Females were housed singly after mating, with ad libitum access to food and water in a 12-h light/dark cycle (lights on at 0800 h). Maternal behavior was intensively observed on days 1–6 postpartum as previously described (1). Mothers were designated as high-licking/grooming (LG) or low-LG based on the frequency scores for pup LG over the first 6 d postpartum. Observers were trained to a high level of interrater reliability (>0.90). Daily observations occurred during five 75-min sessions, three of which were scheduled during the light phase and two of which were scheduled during the dark phase. Within each of these sessions, mothers were scored 25 times (every 3 min) for the presence of LG behavior (both body and anogenital licking were included). High-LG mothers were defined as females whose LG frequency scores were greater than 1 SD above the cohort mean (~60 lactating females). Low-LG mothers were defined as females whose LG frequency scores were greater than 1 SD below the cohort mean. 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. All experiments used the adult (>70 d of age) male offspring of high-LG and low-LG dams. All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care with protocols approved by the McGill University Animal Care Committee.

Electrophysiology. Animals were decapitated 1–2 h after lights on. The brain was rapidly removed, and coronal sections were prepared as previously described (2). Slices were transferred to a recording chamber with a constant flow of oxygenated artificial cerebrospinal fluid (aCSF) for recording. Bicuculline methobromide (5 μM; Sigma) was used to reduce $GABA_A$ receptor-mediated inhibitory synaptic transmission.

Field excitatory postsynaptic potentials (fEPSPs) were evoked in the hippocampal dentate gyrus using a tungsten bipolar stimulation electrode (FHC) positioned in the medial perforant pathway and recorded by a glass recording electrode filled with aCSF positioned in the middle third of the molecular layer of the upper blade. The magnitude of the response was assessed by measuring the slope of the fEPSPs. Synaptic responses were amplified and digitized by means of Multiclamp 700B and Digidata 1400 (both from Axon), respectively; stored in a personal computer using Clampex (Axon); and analyzed offline using Clampfit (Axon). To induce group I mGluR long-term depression following a 20-min stable baseline, slices were perfused with (R-S)-3,5-dihydroxyphenylglycine (DHPG, 50 μM; Tocris) for 10 min and fEPSPs were recorded every 20 s for a further 60 min after DHPG treatment. To examine paired-pulse depression, fEPSPs were evoked at 20-s intervals, and once responses stabilized, paired-pulse stimulation (20-ms interpulse interval) was applied. The average of six stimulations was used to calculate the paired-pulse ratio (slope of pulse 2/slope of pulse 1).

Western Blotting. Animals were rapidly decapitated, and dissected hippocampi were snap-frozen and later homogenized in radioimmunoprecipitation assay buffer with protease inhibitor mixture and phosphatase inhibitor. Standard Western blotting protocols were followed to determine expression of mGluR1 (1:5,000; BD Biosciences) and mGluR5 (1:1,000; Millipore). OD measurements were normalized using actin (1:1,000; Sigma) to control for protein loading.

Quantitative Real-Time PCR. Whole brains were rapidly removed and stored at −80 °C. The hippocampal tissue was dissected, and RNA and DNA were extracted simultaneously from one hemisphere using an AllPrep DNA/RNA Mini kit (catalog no. 80204; QIAGEN) with on-column DNase digestion (catalog no. 79254; QIAGEN) for RNA exaction and RNase A treatment for DNA extraction. The overall quality and yield of the RNA or DNA preparation was determined using a SmartSpec plus spectrophotometer (Bio-Rad Laboratories). cDNA synthesis was completed using reverse transcriptase avian myeloblastosis virus (AMV) (catalog no. 10109118001; Roche Applied Science). Quantitative real-time PCR was performed with a LightCycler 480 (Roche Applied Science) and RT2 PCR primer sets for rat *Grm1* (catalog no. PPR06830A; SABiosciences) and *Grm5* (forward primer TCCAGCAGCCTAGTCAACCT and reverse primer CAGATTTTCCGTTGGAGCTT). β_2 microglobulin (catalog no. PPR42607A; SABiosciences) reference gene was amplified from the same samples, and the expression of gene transcripts was determined relative to the expression of β_2 microglobulin.

DNA Methylation. Cytosine methylation was determined using sodium bisulfite conversion followed by sequencing (3, 4). Hippocampal genomic DNA (0.5 μg) was treated with sodium bisulfite (EpiTect Bisulfite Kit, catalog no. 59104; QIAGEN). The *Grm1* putative promoter region [Chromosome RefSeq no. NC_005100.2, transcription start site (TSS) position 5744593] 1,800 bp upstream from the TSS of bisulfite-treated DNA (0.5 μg) was PCR-amplified using outside primers (forward primer TTTGTTAGGTATTTTGGGTAAAATG and reverse primer AACAAAACTCTACACCTCTCAAAAC). The PCR protocol included an initial denaturing cycle (5 min at 95 °C), followed by 34 cycles of denaturation (1 min at 95 °C), annealing (2 min 30 s at 55 °C), and extension (1 min at, 72 °°C), followed by a final extension cycle (7 min at 72 °C) terminating at 4 °C. The PCR product was used as a template for subsequent PCR amplification using nested primers (forward primer TGTTAGGTATTTTGGGTAAAATGGA and reverse primer AAAAACAATAATAAAAACAAAAAAAAC). The nested PCR product was cleaned by agarose gel extraction using a QIAEX II Gel extraction kit (catalog no. 20021; QIAGEN), subcloned (PCR Cloning kit, catalog no. 231124; QIAGEN), and transformed to produce 20 different colonies per plate. A total of 20 to 25 plasmids per animal containing the ligated *Grm1* promoter DNA fragment were sequenced at the Genome Québec Innovation Centre (McGill University). The number of clones with one or more methylated sites was counted, and the total was divided by the total number of clones to calculate percentage methylation.

Methylated DNA Immunoprecipitation ChIP Array. We used the Roche NimbleGen methylated DNA immunoprecipitation (IP) ChIP array (custom-designed 2.1 M rat DNA methylation promoter array), which covers 10 kb of all annotated promoters, to examine whole-genome promoter methylation. It covers 27,342 known genes. The array design was based on sequences and gene annotation obtained from the November 2004 rat genome sequence [University of California Santa Cruze (UCSC) rn 4, Baylor Human genome sequencing center (HGSC) build 3.4], RefSeq NM mRNA sequences current to April 3, 2007, GenBank complete coding sequence mRNAs current to January 25, 2007,

and Rat Ensembl transcripts current to April 3, 2007. Genomic DNA and RNA were extracted using an AllPrep DNA/RNA mini kit (catalog no. 80204; QIAGEN). RNA was treated by DNase I and used to run an Affymetrics gene expression array. DNA (6 μg) was treated by RNase A and then purified before being sonicated into 100- to 800-bp fragments. Heat denaturing, at a fraction of 1/20th, was used as an input control. Mouse anti–5-methylcytidine antibody (5 μg, catalog no. Ab10805; Abcam) was used for IP (IP buffer: 100 mM Na-phosphate, 5 M NaCl, 10% Triton X-100). Protein A agarose beads were washed and added to the DNA-antibody mixture and incubated for 2 h on a rotating platform at 4 °C. The mixture was washed once again and the beads were then resuspended in digestion buffer [1 M Tris·HCl, 0.5 M EDTA, 10% SDS, 7 μL proteinase K mix (10 mg/mL)], which was incubated overnight on a rotating platform at 55 °C. DNA was extracted using phenol/chloroform and resuspended in 10 mM Tris·HCl (pH 8.5). The IP sample was labeled with Cy5, and the input sample was labeled with Cy3. Labeled samples were hybridized to a 2.1-M rat DNA methylation promoter chip for 72 h. Arrays were scanned at the Institut de recherché en immunologie et en cancerologie, University of Montreal, Montreal, Quebec, Canada. Data were normalized, and the final $\log_2$ ratio of IP over input signals was analyzed using the Bioinformatics Toolbox in MATLAB (MATLAB 7.11; MathWorks, Inc.).

Hippocampal Neuronal Cultures. Hippocampi were dissected from embryonic day 18–20 embryos, trypsinized, homogenized, and seeded onto 60-mm plates at a density of $\sim 3 \times 10^7$ cells per plate. Media consisted of MEM-α (Invitrogen) supplemented with 10% FBS, 20 mM KCl, 0.25% glucose, 15 mM Hepes, 0.1% penicillin/streptomycin, and 20 μM 5′-fluorodeoxyuridine to prevent glial cell growth. Media were changed again 3 d later. Cells were treated with 0, 1, or 5 μM 5-aza-cytosine (Sigma) and harvested 48 h later in PBS and stored at −80 °C for later quantification of mGluR1 mRNA levels. RNA was isolated using a Roche HiPure RNA extraction kit. RT reactions were then performed on 0.5 μg of RNA using Roche AMV reverse transcriptase and cDNA generated for qRT-PCR analysis of mGluR1 mRNA.

1. Champagne FA, Francis DD, Mar A, Meaney MJ (2003) Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav* 79:359–371.
2. Tse YC, Bagot RC, Hutter JA, Wong AS, Wong TP (2011) Modulation of Synaptic Plasticity by Stress Hormone Associates with Plastic Alteration of Synaptic NMDA Receptor in the Adult Hippocampus. *PLoS One* 6:e27215.
3. Clark SJ, Harrison J, Paul CL, Frommer M (1994) High sensitivity mapping of methylated cytosines. *Nucleic Acids Res* 22:2990–2997.
4. Frommer M, et al. (1992) A genomic sequencing protocol that yields a positive display of 5–methylcytosine residues in individual DNA strands. *Proc Natl Acad Sci USA* 89: 1827–1831.

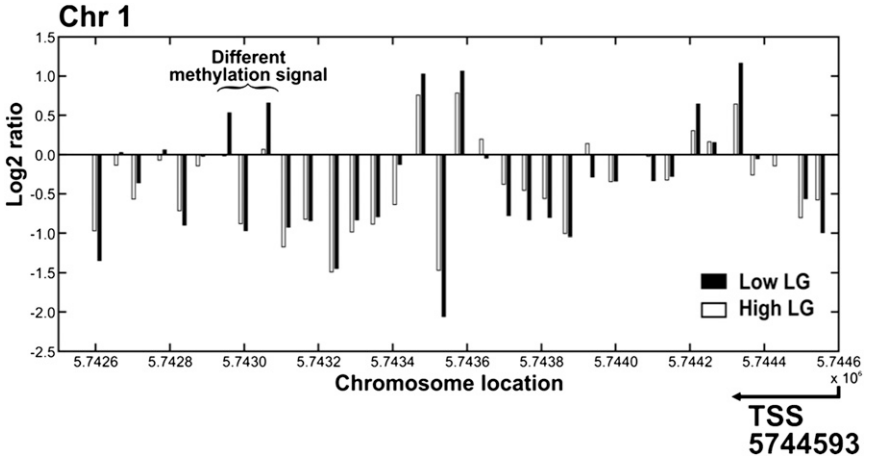

Fig. S1. Differentially methylated region of Grm1 gene. The schema outlines the region of methylation-enriched DNA in the Grm1 gene promoter. The data are plotted as the ratio of methylation relative to input DNA; values above 0 reflect hypermethylated regions, and those below 0 show regions that are hypomethylated relative to input DNA. The differentially methylated region is identified within the frame. The arrow indicates the TSS and direction of transcription.

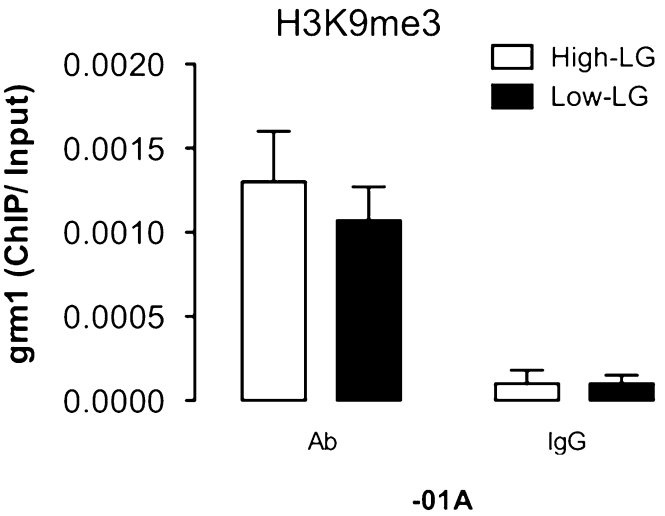

Fig. S2. Mean + SEM levels of trimethylated histone 3 lysine 9 (H3K9me3) IP of region –01A of the *Grm1* gene in hippocampal samples from the adult offspring of high-LG and low-LG mothers (n = 6–8 per group).