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Conditional *Dnmt1* deletion in dorsal forebrain disrupts development of somatosensory barrel cortex and thalamocortical long-term potentiation

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The transcriptional mechanisms that govern the development and plasticity of somatopic sensory maps in the cerebral cortex have not been studied extensively. In particular, no studies have addressed the role of epigenetic mechanisms in the development of sensory maps. DNA methylation is one of the main epigenetic mechanisms by which mammalian cells regulate gene transcription. Demethylation results in embryonic lethality, so it has been very difficult to study the role of DNA methylation in brain development. We have used cre-loxP technology to generate forebrain-specific deletion of DNA methyltransferase 1 (Dnmt1), the enzyme required for the maintenance of DNA methylation. We find that DNA hypomethylation of neurons in the cerebral cortex results in the failure of development of somatosensory barrel cortex. We also find that, despite functional thalamocortical neurotransmission, thalamocortical long-term potentiation cannot be induced in slices from Emx1-cre;Dnmt1 mutant mice. These studies emphasize the importance of DNA methylation for the development of sensory maps and indicate that epigenetic mechanisms might play a role in the development of synaptic plasticity.

Keywords: Transcription, plasticity, DNA methylation, NMDA, AMPA, glutamate receptor, cortical column

INTRODUCTION

The mechanisms that underlie the development and plasticity of sensory maps in the cerebral cortex are not well understood and represent a major challenge for developmental neuroscience (Goodman and Shatz, 1993; O'Leary *et al.*, 1994; Katz and Shatz, 1996). In the rodent trigeminal-medial lemniscus system, periphery-related patterns that represent the whiskers develop in the brainstem and thalamus before they develop in the cerebral cortex (Woolsey and Van der Loos, 1970; Van de Loos and Woolsey, 1973; Van der Loos, 1976; Belford and Killackey, 1979). During a critical period in development, these patterns are susceptible to manipulation from the periphery and, therefore, they present an excellent model system for studying the development and plasticity of cortical maps (Van der Loos and Woolsey, 1973; Belford and Killackey 1980; Killackey and Belford, 1980; Ma and Woolsey, 1984). A number of studies have demonstrated the importance of NMDA receptors (Fox *et al.*, 1996; Iwasato *et al.*, 1997; Iwasato *et al.*, 2000; Lee *et al.*, 2005) metabotropic glutamate receptors (Hannan *et al.*, 2001), monoamine oxidase A (MAO-A) (Cases *et al.*, 1996), adenylate cyclase I (Abdel-Majid *et al.*, 1998) protein kinase A R2β (Inan *et al.*, 2006; Watson *et al.*, 2006), phospholipase β1 (Hannan *et al.*, 2001) and the signaling molecule FGF8 (Fukuchi-Shimogori and Grove, 2001). However, only a few studies have attempted to delineate the complex transcriptional networks that mediate the generation of cortical maps (Ince-Dunn *et al.*, 2006). Furthermore, there is no work on whether epigenetic changes either underlie or modulate these transcriptional networks.

Epigenetic changes are mediated in part by DNA methylation, one of the main mechanisms available to mammalian cells to regulate gene transcription. DNA methylation in mammals occurs on cytosine, primarily at CpG dinucleotides, in a spatially and temporally regulated manner. DNA methylation is mediated by a family of DNA methyltransferases (Dnmts) that include *de novo* (Dnmt3a and Dnmt3b) and maintenance methyltransferases (Dnmt1) (Bestor, 2000; Robertson and Wolffe, 2000). Transcriptional downregulation occurs through binding of several proteins to methylated DNA including methyl-CpG-binding protein 2 (MeCP2,) and other methyl CpG binding-domain proteins (MBDs) (Fan and Hutnick, 2005). MeCP2 and MBD2 recruit histone deacetylases (HDACs) to methylated CpG sites, which, in turn, causes deacetylation of histones. These changes promote the formation of a silent chromatin state (Jaenisch and Bird, 2003). Mutations in MeCP2 are the main cause of the neurodevelopmental disorder, Rett Syndrome, underscoring the importance of DNA methylation for brain development (Amir *et al.*, 1999). All three DNA methyltransferases are expressed in the CNS and are dynamically regulated during development and differentiation (Goto *et al.*, 1994; Feng *et al.*, 2005), which indicates that DNA methylation might control transcriptional networks that orchestrate neuronal differentiation, migration and formation of specific neuronal connections. Dnmt1 is responsible for

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maintenance DNA methylation. Without the presence of this maintanance DNA methyltransferase, cells undergoing mitosis become progressively demethylated, leading to embryonic lethality (Li *et al.*, 1992).

In addition to the essential role of DNA methylation in embryonic development, recent studies show that it also can modulate activity-dependent changes in gene expression. For example, the activity-dependent expression of BDNF relies on phosphorylation-dependent detachment of a transcriptional repression complex consisting of MeCP2, HDAC1 and mSin3a from the brain-derived neurotrophic factor (BDNF) promoter. Furthermore, depolarization appears to lead to relative demethylation of CpGs near Ca^{2+}-responsive elements in the BDNF promoter, further re-enforcing transcriptional activation (Chen *et al.*, 2003; Martinowich *et al.*, 2003).

To examine the role of DNA methylation in the CNS directly, we have previously induced DNA hypomethylation throughout the entire CNS by conditional knockout of Dnmt1 in neural precursor cells (Fan *et al.*, 2001). We demonstrated that DNA hypomethylation in the CNS disrupts neural control of breathing at birth, leading to neonatal lethality of mutant mice (Fan *et al.*, 2001). However, the neonatal lethality of these mice prevented us from studying the role of DNA methylation in postnatal neural development.

To circumvent this obstacle, we crossed mice bearing the *Emx1-cre* transgene (Iwasato *et al.*, 2000) to Dnmt1 2lox/2lox mice to induce deletion of Dnmt1 solely in excitatory neurons of the dorsal forebrain. We have shown that, although these mice are viable into adulthood, they develop progressive apoptotic degeneration of the dorsal forebrain. Furthermore, excitatory cortical neurons in these mice have defects in dendritic branching and action potential repolarization (Hutnick *et al.*, unpublished).

OBJECTIVES

Although the above studies highlight the importance of DNA methylation for neuronal survival, morphogenesis and ion-channel expression, no study has yet addressed the role of DNA methylation in development of somatopic sensory maps and in thalamocortical synaptic transmission and plasticity. Here we show that DNA methylation is essential for development of periphery-related anatomical patterns in the somatosensory barrel cortex. We also demonstrate that, although thalamocortical axons functionally innervate the cortex in Dnmt1-mutant mice, thalamocortical long-term potentiation is defective. These results highlight the importance of DNA methylation in activity-dependent remodeling of cortical circuits and synaptic plasticity.

METHODS

Generation of *Emx1-cre;Dnmt1* conditional mutants

All experiments were carried out in accordance with protocols approved by the UCLA Institutional Animal Research committee. Using the cre-loxP binary gene-deletion strategy, we crossed female mice homozygous for the Dnmt1 conditional allele ($Dnmt1^{2lox}$) with male mice carrying the *Emx1-cre* insertion ($Emx1\text{-}cre;Dnmt1^{2lox/+}$), generating both control/heterozygous (either $Dnmt1^{2lox/+}$ or $Dnmt1^{2lox/2lox}$), and mutant ($Emx1\text{-}cre;Dnmt1^{2lox/2lox}$) offspring in the Mendelian ratio expected. Because of the progressive cortical degeneration in mutants (Hutnick *et al.*, unpublished), the brains of mutant animals can be discerned readily from the brains control animals.

Histology

Postnatal day 8 (P8) *Emx1-cre;Dnmt1* conditional mutant mice and control littermates were perfused with 4% paraformaldehyde. Brains were removed and cryoprotected in 30% sucrose overnight. For cytochrome oxidase staining, the cortex was then removed, flattened with a microscope slide on dry ice and sectioned tangentially at 50 μm with a cryostat. Cytochrome oxidase histochemistry was performed as follows. Sections were incubated in 4 g sucrose, 50 mg cytochrome C and 50 mg diaminobenzidine in 100 ml phosphate buffer at 37°C in a shaker incubator for 3–4 hours. Sections were then rinsed in phosphate buffer, mounted on subbed slides and coverslipped with an aqueous medium containing gelatin and glycerol. A second set of control and mutant brains were sectioned in the coronal angle at 10 μm with a cryostat and Nissl stained.

Electrophysiology

Emx1-cre;Dnmt1 mutant mice and control littermates (P3−P6) were anesthetized with halothane and sacrificed by decapitation. The brain was removed quickly and put in chilled artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 26 $NaHCO_3$, 11 glucose, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 1 NaH_2PO_4 bubbled with 95% O_2/5% CO_2. Slices (300−400 μm) containing the somatosensory cortex, the reticular thalamic nucleus (RTN) and the ventroposterior thalamic nucleus (VP) were cut with a vibratome (Leica VT-1000) at an angle that preserves corticothalamic and thalamocortical connectivity (Agmon and Connors, 1991). After incubation at 35°C for 30 min, slices were stored at room temperature (22−24°C) before being transported to a recording chamber. Slices were allowed to recover for at least 1 hour before recording. Electrodes for whole-cell recordings were pulled from borosilicate glass capillaries to a final resistance of 3–6 Mega Ohms. Internal solution for current clamp experiments contained (in mM): 140 K-gluconate, 1.1 EGTA, 0.1 $CaCl_2$, 10 HEPES, 2 Mg-ATP, 1 $MgCl_2$ and 0.3 Na-GTP (pH 7.2). For voltage clamp experiments, the internal solution contained (in mM) 117 Cs-methanesulfonate, 20 HEPES, 0.2 EGTA, 5 TEA-Cl, 3.7 NaCl, 4 Mg-ATP and 0.3 Na-GTP. Biocytin (3 mg ml^{-1}) was also added to the internal solution during some recordings. Whole cell recordings were performed at room temperature (22−24 °C) with an Optopatch patch-clamp amplifier (Cairn Research) in either current clamp or voltage clamp configuration from layer IV neurons in control slices. In thalamocortical slices from control mice, layer IV was readily identified by transillumination of the cortex. Because cortical lamination was disrupted in the cortex of mutant animals, we attempted to record from neurons at similar proportional distances from the pial surface in mutant and in control cortical

neurons. Thalamocortical synaptic responses were evoked by stimulating the thalamic ventrobasal complex or rarely the immediately adjacent internal capsule with a bipolar tungsten electrode. At times, a bipolar stimulating electrode made from theta capillary glass and filled with ACSF was used. Stimuli (100 μs, typically 0.1–1 mA) elicited thalamocortical excitatory postsynaptic potentials (EPSPs). Current and voltage clamp traces were analogue filtered at 5 kHz, digitized at 10 kHz and digitally filtered at 2–5 kHz, and analyzed using custom made software using Lab View (National Instruments) and Igor (Wavemetrics). In experiments to study long-term potentiation (LTP), neurons with accommodating regular spiking action potential firing properties were current clamped using whole-cell patch clamp. Baseline responses were elicited every 30 sec for 10 min. Thalamocortical LTP was induced by delivering 100 stimuli at 1 Hz while depolarizing the neuron to −10 mV (Crair and Malenka, 1995). Thalamocortical responses were elicited and monitored every 30 sec for at least 40 min after the LTP-induction paradigm. The magnitude of LTP was calculated by dividing average amplitude of 20 responses during the baseline period by the average amplitude of 20 responses obtained 30–40 min after pairing protocol. During baseline and after LTP induction, holding current was injected to set the resting membrane potential to −70 mV. A 10 mV junction potential was subtracted post-hoc from the recordings when using a potassium gluconate-based internal solution.

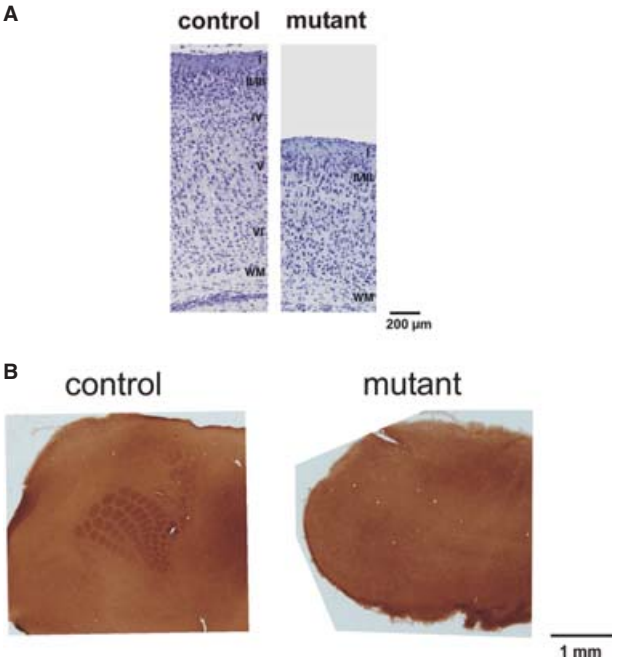

Fig. 1. (A) Photomicrographs of Nissl-stained coronal sections through the dorsal forebrain of control and Dnmt1 emx-cre-mutant mice at P7. Note indistinct development of cortical layers in the mutant cortex. (B) Cytochrome oxidase-stained, flattened, tangential sections of somatosensory cortex from control and Dnmt1 emx-cre-mutant mice at P8. Note the lack of development of periphery related patterns in the section from a *Emx1-cre;Dnmt1* mutant mouse.

RESULTS

Periphery-related patterns fail to develop in the somatosensory barrel cortex of *Emx1-cre;Dnmt1* mutant mice

Nissl-stained coronal sections of control and mutant brains at P7 showed indistinct lamination of the cerebral cortex in mutant animals (Fig. 1A). To determine whether transcriptional regulation of gene expression by DNA methylation is essential for the development of cortical sensory maps, we studied the development of whisker-related barrels in the somatosensory cortex of *Emx1-cre;Dnmt1* mutant mice. The development of barrels at P8 was measured because, at later ages, neurodegeneration of the cortex in *Emx1-cre;Dnmt1* mutants confounds analysis of cortical columnar structure. As expected, cytochrome oxidase staining of flattened sections throughout the cortex of control littermates at P8 showed normal development of forepaw and whisker-related patterns (Fig. 1B, left). In conditional-mutant mice, however, no whisker- or forepaw-related pattern was discerned (Fig. 1B, right). This indicates that DNA methylation is essential for the development of periphery related patterns in the cerebral cortex.

Thalamocortical neurotransmission is intact despite the failure of development of periphery related patterns

To determine whether the failure of development of periphery-related patterns in the barrel cortex results from the failure of either ingrowth of functional thalamocortical afferents or thalamocortical synaptogenesis, we performed whole-cell recordings from cortical neurons in thalamocortical slices of control and *Emx1-cre;Dnmt1* mutants at P3−P6. In slices obtained from control cortices, layer IV was identified readily by transillumination and, in these slices, all recordings were performed in layer IV. Cortical layers were indistinct in *Emx1-cre; Dnmt1* cortex, so recordings were performed at similar proportional distances from the pial surface as in control slices. Stimulation of thalamocortical axons elicited EPSPs/currents (EPSPs/EPSCs) in cortical neurons of both control and *Emx1-cre;Dnmt1* mutants (Fig. 2A). This shows that the mutant cortex develops functional thalamocortical connections. Thalamocortical EPSCs recorded from neurons in mutants at −70 mV had slightly more prolonged onset latencies than EPSCs recorded from neurons in control mice (8.7 ± 0.8 msec in controls, $n = 5$; 11.9 ± 3.0 msec in mutants, $n = 9$; $P < 0.05$) (Fig. 2B). EPSCs recorded in mutant neurons also had more prolonged 20−80% rise times compared to control (0.9 ± 0.2 msec in controls, $n = 5$; 1.4 ± 0.5 ms in mutants, $n = 9$; $P < 0.05$), but the time constant of decay was not statistically different between the two groups (5.6 ± 0.7 msec in controls, $n = 5$; 5.2 ± 1.1 msec in mutants, $n = 9$; $P > 0.05$) (Fig. 2B). This demonstrates that although the thalamocortical synapses are possibly located at an electrotonically more distant location in mutants compared to control, the failure of development of somatosensory barrels does not result from the failure of either ingrowth of functional thalamocortical fibers or generation of thalamocortical synapses in mutant mice.

Because NMDA receptor-mediated responses are essential for development of periphery-related patterns in the barrel cortex (Iwasato *et al.*, 2000), we assessed the ratio of NMDA

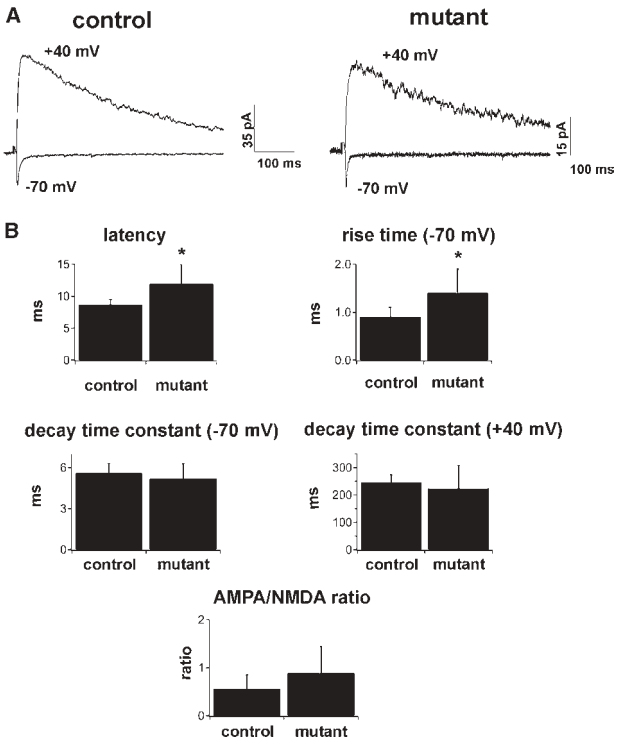

Fig. 2. (A) Thalamocortical EPSCs evoked at −70 mV and +40 mV in slices from control (left) and mutant (right) animals at P4−P5. Stimulation artifacts have been blanked. Note that both slowly decaying NMDA receptor-mediated currents and rapidly decaying non-NMDA receptor-mediated currents are evoked in slices from control and mutant animals. (B) The latency, rise time, decay time constant (at −70 mV and +40 mV), and AMPA:NMDA ratio of thalamocortical EPSCs evoked in slices from control and Dnmt1 emx-cre animals at P4−P6. *, $P < 0.05$. Thalamocortical EPSCs in mutants have slightly more prolonged latencies and rise times, but the decay time constant at −70 mV and +40 mV, and AMPA:NMDA ratio is not statistically different between the two groups.

receptor-:AMPA receptor-mediated EPSCs. Thalamocortical EPSCs were elicited first at −70 mV, where the current is carried largely by AMPA-type glutamate receptors, and then at +40 mV, where, in addition to the fast AMPA component, a slowly decaying, NMDA receptor-mediated current is revealed. As described above, AMPA receptor-mediated currents were observed in both mutant and control animals. In addition, at +40 mV, large, slowly decaying currents that are typical of NMDA receptor-mediated currents were elicited in slices from both control and mutant animals. The time constant of decay of the EPSC at +40 mV was similar in the two groups (246.7 ± 26.7 msec in controls, $n = 5$; 224.1 ± 84.1 msec in mutants; $P > 0.05$) (Fig. 2). The peak amplitudes of the EPSC at −70 mV [−24.8 ± 15.3 pA in control ($n = 5$); −27.3 ± 24.2 pA in mutant ($n = 9$)] and +40 mV [53.0 ± 35.4 pA in control ($n = 5$); 31.2 ± 21.0 pA in mutant ($n = 9$)] were not significantly different in the two groups ($P > 0.05$). We also calculated the ratio of AMPA receptor-:NMDA receptor-mediated currents by dividing the peak amplitude of the EPSC at −70 mV by the amplitude of the EPSC at +40 mV at 50 msec after the stimulus. This ratio was not significantly different in control and mutant neurons (Fig. 2) (0.57 ± 0.28 for control neurons ($n = 5$); 0.88 ± 0.56 for mutant neurons ($n = 9$); $P = 0.27$). These experiments were performed without the blockade of GABA-A receptors and, thus, inhibitory currents might have a confounding effect. However, at this age, GABA-A receptor-mediated currents are not observed commonly after stimulation of thalamocortical fibers (Agmon and O'Dowd, 1992). Furthermore, recordings performed at the glutamate receptor reversal potential showed no evidence of polysynaptic GABA-A receptor-mediated currents (data not shown). Together, our data indicate that the failure of the development of periphery-related patterns is unlikely to result from a lack of NMDA-receptor activity.

Thalamocortical LTP is deficient in Dnmt1 emx-cre cortical neurons

In some mouse strains in which periphery-related patterns do not develop in the barrel cortex are deficient in thalamocortical LTP (Iwasato *et al.*, 2003; Lu *et al.*, 2003). This has prompted investigators to conclude that strengthening thalamocortical synapses through LTP-like phenomena during early development might be essential for development of

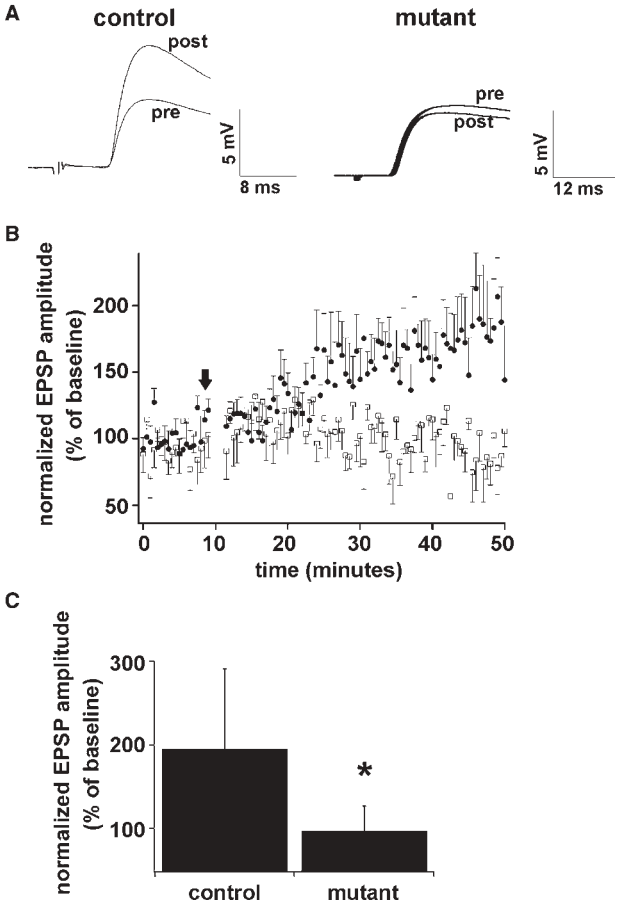

Fig. 3. (A) Thalamocortical EPSPs evoked in slices from control and Dnmt1 emx-cre-mutant animals 0−10 min before (pre) and 30−40 min after (post) pairing 100 stimuli at 1 Hz with depolarization of the postsynaptic cell. Note robust LTP in control but not in mutant cells. (B) Graph demonstrating the amplitude of the EPSP normalized to the average baseline value in slices obtained from control (solid circles) and mutant (open squares) animals before and after the pairing protocol. Error bars are s.e.m. The dark arrow represents the onset of the pairing protocol. (C) The mean normalized EPSP amplitude 30−40 min after the pairing protocol in control and mutant neurons. Error bars are s.d. Note the significant difference between the potentiation induced in control and mutant animals. *, $P < 0.05$.

periphery-related patterns in barrel cortex (Lu *et al.*, 2003; Inan *et al.*, 2006). This enticed us to determine whether thalamocortical LTP occurs in *Emx1-cre;Dnmt1* mutant cortex.

We elicited thalamocortical EPSPs in both control and mutants at P3–P6, during the critical developmental window when thalamocortical LTP is elicited (Crair and Malenka, 1995). After eliciting a stable baseline for 10 min at −70 mV, cortical neurons were depolarized to −10 mV and 100 presynaptic stimuli delivered at 1 Hz to elicit LTP (Crair and Malenka, 1995). Neurons were then hyperpolarized back to −70 mV and thalamocortical responses monitored for ≥40 min after the pairing protocol. Cortical neurons from layer IV in control slices showed robust LTP 30–40 min after the pairing protocol, (195.4 ± 95.4 %; $n = 5$) (Fig. 3A, C). As has been demonstrated before (Crair and Malenka, 1995), the increase in the amplitude of responses developed slowly over 5–15 min before stabilizing for the duration of the recording (Fig. 3B). In contrast, the same pairing protocol in mutant neurons did not elicit significant LTP (97.3 ± 45.6%; $n = 6$) at the same time point (Fig. 3A, C). The magnitude of potentiation was significantly different in control and mutant neurons ($P < 0.05$) (Fig. 3C). This indicates that although thalamocortical transmission is intact in *Emx1-cre;Dnmt1* mutant cortex, thalamocortical synapses in mutant slices are not readily strengthened by pairing synaptic stimulation with neuronal depolarization.

CONCLUSIONS

- Conditional deletion of *Dnmt1* in the dorsal forebrain disrupts the development of periphery-related anatomical patterns in the somatosensory barrel cortex.
- Although thalamocortical axons functionally innervate the cortex in *Emx1-cre;Dnmt1* conditional-mutant mice, thalamocortical LTP is defective.

DISCUSSION

To study the role of DNA methylation on postnatal cortical development we used cre-loxP technology to specifically delete the DNA methyltransferase Dnmt1 in neural precursors of excitatory neurons of the dorsal forebrain. We have previously observed that the cortex is poorly laminated in these mutant mice and undergoes progressive neurodegeneration (Hutnick *et al.*, unpublished). Here, we show that although thalamocortical fibers functionally innervate excitatory cortical neurons of *Emx1-cre;Dnmt1* conditional-mutant mice, periphery-related anatomical patterns do not form in the somatosensory barrel cortex. Furthermore, we show that deletion of Dnmt1 in excitatory cortical neurons abolishes thalamocortical LTP, which highlights the importance of DNA methylation for developmental plasticity at thalamocortical synapses.

Periphery-related anatomical patterns fail to develop in the somatosensory barrel cortex of *Emx1-cre;Dnmt1* mutant mice

The failure of development of periphery-related patterns in the somatosensory cortex of *Emx1-cre;Dnmt1* mice might be a either direct consequence of demethylation on the development of cortical modules or arise as a consequence of early apoptotic cell death and cortical laminar disorganization in these mutant mice. Answering this essential question depends on examining the development of periphery-related patterns in Dnmt1-mutant mice where cell death and cortical migration phenotypes have been rescued by genetic means. Targeting deletion of Dnmt1 to specific subsets of excitatory cortical neurons (such as layer IV progenitors) might allow further dissection of the complexities of this model. Direct effects of demethylation might include inappropriate expression of transcription factors, axon guidance cell-adhesion proteins, and molecules such as BDNF which have been shown to be crucial for activity-dependent development of thalamocortical circuits (Cabelli *et al.*, 1995; Cabelli *et al.*, 1997). Activity-dependent expression of BDNF requires the release of methyl-binding proteins from the BDNF promoter, so BDNF is an important candidate for mediating some of the effects of demethylation (Chen *et al.*, 2003; Martinowich *et al.*, 2003). In addition, increasing the expression of BDNF in Mecp2 mice with a conditional BDNF transgene extends the lifespan, rescues locomotor defects, and reverses an electrophysiological deficit observed in Mecp2 mutants. Again, these effects link transcriptional control of BDNF to DNA methylation binding proteins (Chang *et al.*, 2006). Studies in our laboratory are in progress to characterize genome-wide differences in mRNA expression in specific subsets of cortical neurons in *Emx1-cre;Dnmt1* mice and control littermates to better delineate the specific molecular mechanisms of cortical disorganization in these mice.

It is also possible that disruption of thalamocortical plasticity in *Emx1-cre;Dnmt1* mutants results in disruption of the development of somatosensory cortical barrels. There is considerable controversy about whether thalamocortical LTP is essential for barrel formation. Barrel formation and thalamocortical plasticity are both disrupted by deletion of adenylate cyclase I (Abdel-Majid *et al.*, 1998; Lu *et al.*, 2003). NMDA receptor blockade (Crair and Malenka, 2005), and deletion of protein kinase A RIIβ (PKA RIIβ) both prevent thalamocortical LTP during early development (Watson *et al.*, 2006) but cortical deletion of the crucial NMDA receptor subunit NR1 (Iwasato *et al.*, 2000) and deletion of PKA RIIβ (Watson *et al.*, 2006; Inan *et al.*, 2006) blur barrel boundaries but do not completely disrupt formation of somatosensory cortical barrels. Finally, although studies indicate the importance of GluR1 trafficking for thalamocortical LTP (Lu *et al.*, 2003), barrels form normally in GluR1 KO mice (Watson *et al.*, 2006), again suggesting that thalamocortical LTP might not be essential for the development of somatosensory cortical barrels. This would suggest that the lack of thalamocortical LTP is unlikely to be the sole direct cause of the lack of development of somatosensory cortical barrels in *Emx1-cre;Dnmt1* mice.

Thalamocortical neurotransmission is intact despite the failure of development of periphery related patterns

Although we could elicit both AMPA receptor- and NMDA receptor-mediated EPSCs in Dnmt1 mutant cortex, the rise time of EPSCs at −70 mV were significantly more prolonged in mutant cortex. The small, statistically significant difference in latency might be related to possible changes in either

thalamocortical axonal length or ramifications in mutant cortex. The increased rise time of thalamocortical EPSCs might result from aberrant synapse formation at electrotonically more distant locations in the mutant mice, or from slight desynchronization of release. Our studies show no statistically significant difference in the amplitude EPSCs at −70 mV and +40 mV; however, minimal stimulation or recordings from synaptically connected neurons in thalamus and cortex are needed to quantify changes in unitary EPSC amplitude more accurately.

Impaired thalamocortical LTP in *Emx1-cre;Dnmt1* mice

We elicited both NMDA- and non-NMDA-receptor-mediated mediated responses in *Emx1-cre;Dnmt1* cortical neurons, but pairing postsynaptic depolarization with synaptic stimulation did not elicit thalamocortical LTP in these mice. Several observations indicate that thalamocortical LTP might be affected by demethylation. First, the activity-dependent expression of BDNF (which is essential for conversion of silent thalamocortical synapse to functional synapses) (Itami *et al.*, 2003) relies on the release of methylated CpG binding protein-2 (MECP-2) from the BDNF promoter (Chen *et al.*, 2003; Martinowich *et al.*, 2003); second, either demethylating agents (Levenson *et al.*, 2006) or loss of MECP-2 (Morretti *et al.*, 2006) modulate LTP in the cortex and hippocampus. Each effect might be caused by either an influence of the presynaptic release machinery or the postsynaptic receptor complexes. In Dnmt1-mutant animals only excitatory cortical neurons, which are, by definition, postsynaptic to the thalamocortical inputs are demethylated, so our studies are informative on the locus of the potential plasticity deficits.

Because cortical lamination is disorganized in *Emx1-cre;Dnmt1* mutant mice, one mechanism underlying the lack of thalamocortical LTP might be that thalamocortical axons form synapses with displaced cells that are not destined to become layer IV neurons. These neurons might lack the molecular machinery for thalamocortical LTP during the critical period. Alternatively, transcriptional misregulation through demethylation might impair either the adenylate cyclase/PKA pathway or processes further downstream that are essential for trafficking of AMPA receptors into thalamocortical synapses. Again, definitive answers to these questions will rely on development of techniques to demethylate individual neurons that have already migrated into position and acquired their neuronal identity.

This study highlights the importance of DNA methylation in the development of somatotopic sensory maps and strengthening of thalamocortical synapses during early development. Future studies will address the mechanisms that underlie the developmental defects caused by hypomethylation.

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