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Inhibitory Interneurons and Epilepsy: Cellular Mechanisms and Potential Therapies

by

Daniel Jones

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

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in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

to my wife and son

Inhibitory Interneurons and Epilepsy: Cellular Mechanisms and Potential Therapies

Daniel Jones

Abstract

Epilepsy is a neurological disorder that affects millions of people worldwide, and in it simplest form, is thought to result from an imbalance between excitation and inhibition in the nervous system. Defects in inhibitory systems of the cortex and hippocampus, which can range from disruptions in neural development to impairments in GABA-mediated synaptic transmission, have been associated with epilepsy. In these studies, I explore the complex relationship between inhibitory interneurons and epilepsy in three different systems: (1) a mouse model of type-1 Lissencephaly, in which interneurons are present in normal numbers, but are displaced into inappropriate hippocampal layers; (2) *Dlx1* knockout mice, in which certain populations of interneurons undergo programmed cell death within the first month of life; (3) wild-type mice that have received a graft of interneuron precursor cells from the medial ganglionic eminence. I report significant reorganizations in inhibitory (and excitatory) circuits in these mouse models of epilepsy, and further demonstrate that progenitor cells grafted into the postnatal brain can be used to generate new inhibitory interneurons. Taken together, this body of work illustrates the important role played by inhibitory neurons in regulating network excitability, and highlights the potential of neural progenitor cell transplantation as an effective treatment to reduce seizures.

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Chapter I: Introduction

Epilepsy as a diverse set of neurological disorders

Epilepsy is a neurological disorder defined by the presence of spontaneous, repeated seizures. Worldwide, it is thought that up to 3% of the population suffers or will suffer from a form of epilepsy at some point in their lives (Berkovic et al. 2006), with temporal lobe epilepsy being one of the most common forms of the disease. While some effective treatments exist, including pharmacological therapies and surgical resection of epileptogenic tissue, many forms of epilepsy are medically intractable. Efforts to treat seizures are complicated by the fact that epilepsy can result from a wide array of neurological defects. For instance, an individual might acquire epilepsy after suffering a traumatic brain injury. In contrast, a patient could exhibit severe seizures due to a genetic mutation that alters the function of a channel protein. Because epilepsy can result from such a diverse set of causes, it is perhaps unsurprising that the ways in which seizures respond to medical intervention can vary widely from patient to patient (Cascino 2008). It is for these reasons that the study of cellular and molecular mechanisms of epilepsy in animal models is of great importance. A more complete understanding of the mechanistic underpinnings of different forms of epilepsy is instructive in that it can highlight both differences and similarities in these mechanisms, providing important feedback to those who treat epilepsy patients.

Acquired and genetic epilepsies

The acquired epilepsies, often referred to as symptomatic epilepsies, include a diverse set of seizure disorders that result from brain insults. Brain tumors, stroke, and traumatic brain injury are three common causes of acquired epilepsy (Berkovic et al.

2006). Epilepsy due to traumatic brain injury, for instance, comprises about one-fifth of acquired epilepsy cases, and may rise with the increased incidence of war related injuries (Lowenstein 2009). The risk of developing epilepsy increases with the severity of the head injury, and seizures may not appear until years after the injury occurred. Attempts to treat or prevent seizures in traumatic brain injury cases have met with limited success. Treating patients with antiepileptic drugs (AEDs) after the injury, but before they display seizures, may have little to no effect on whether and when seizures appear (Lowenstein 2009).

Epilepsies caused by genetic mutation make up about one-third of epilepsy cases (Berkovic et al. 2006). These conditions are sometimes called idiopathic epilepsies, in that patients often lack clear anatomical signs of epilepsy in brain tissue. Most commonly, these patients exhibit seizures due to an inherited or spontaneous mutation that impacts the function of a channel protein required for proper neuronal function; these are referred to as "channelopathies" (Mulley et al. 2003). For example, epilepsy can result from mutations in genes that encode subunits of the $GABA_A$ receptor, the principal ionotropic receptor that is activated by the inhibitory neurotransmitter γ-aminobutyric acid (GABA). A mutation that reduces the amplitude of GABA-evoked currents in the $GABA_A$ receptor has been shown to underlie epilepsy, presumably by reducing the efficacy of synaptic inhibition of excitatory circuits (Cossette et al. 2002).

Genetic epilepsies can also result from mutations in genes required for the migration of neuronal precursor cells during embryonic development. Mutations in cytoskeleton-associated proteins such as Lis1, DCX, and ARX have been implicated in Lissencephaly, the hallmarks of which include a smooth cortical surface that lacks gyri

and sulci, enlarged ventricles, mental retardation, and severe epilepsy. These proteins have been shown to play important roles in the process by which microtubules and microtubule-associated motor proteins produce neuronal migration (Kato and Dobyns 2003).

Inhibitory interneurons and epilepsy

Interneurons are the principal source of inhibition within the hippocampus and cortex. This morphologically diverse population of cells shares one important property: they provide inhibitory signals to principal neurons and to each other by releasing GABA. Defects or alterations in the function of inhibitory brain systems are a recurring theme in mechanistic studies of epilepsy in animal models. Given that GABA is the primary inhibitory neurotransmitter in the CNS (Markram et al 2004), it is not surprising that defects in GABAergic transmission can result in hyperexcitability and seizures.

Origins and classification of inhibitory interneurons

In mice, cortical and hippocampal interneurons are born in the medial, lateral, and caudal ganglionic eminences (MGE, LGE, and CGE), which are circumscribed regions within the embryonic subpallium. These precursor cells migrate tangentially toward the developing hippocampus and cortex, where they begin to integrate into local circuits (Marin and Rubenstein 2001). Significant progress has been made in elucidating the cellular and molecular mechanisms that are required for normal interneuron migration (Kato and Dobyns 2003).

Cortical and hippocampal interneurons are often grouped into subclasses according to three broad parameters: (1) expression of neurochemical markers, (2) action potential firing properties in response to current injection, and (3) dendritic and axonal morphology (Flames and Marin 2005). These subclasses provide distinct types of inhibition to pyramidal neurons and other interneurons, and play unique roles in controlling network activity. Interneuron subclasses also have distinct laminar location profiles. For instance, basket cells express the calcium-binding protein parvalbumin (PV), exhibit fast-spiking properties, and innervate cell bodies and proximal dendrites of target cells. Within hippocampus, their cell bodies are located in stratum oriens and stratum pyramidale. Due to their innervation patterns, basket cells express a great deal of control over spike initiation across populations of pyramidal cells (Markram et al. 2004).

In hippocampus, at least three broad classes of interneurons can be defined: (i) the aforementioned, PV-positive, fast-spiking (FS), basket-type cells; (ii) calretinin (CR)-positive, regular-spiking non-pyramidal (RSNP) cells; (iii) somatostatin (SOM)-positive, burst-spiking (BST), oriens-lacunosum-moleculare interneurons (Freund and Buzsaki 1996; Flames and Marin 2005). However, further subdivisions of interneuron classes have been proposed, and other researchers have posited that interneurons exist not in classes but rather along a continuum (Markram et al. 2004). Interestingly, different classes of interneurons appear to arise from distinct locations and at distinct times within the embryonic subpallium (Butt et al. 2005).

By transplanting GFP-labeled neuronal progenitor cells into host mice, Butt and colleagues found that interneurons originating in the MGE at embryonic day 13.5 (E13.5) predominantly mature into fast-spiking basket cells, while many of those that arise in the

E13.5 CGE are regular-spiking, calretinin (CR) positive interneurons with bipolar morphology. It is thought that different spatial expression patterns of certain transcription factors underlies, at least in part, the generation of distinct interneuron subtypes in the ganglionic eminences (Fogarty et al. 2007).

Function of inhibitory interneurons and links to epilepsy

Interneurons mediate synaptic inhibition, in large part, by releasing GABA, which binds to ionotropic $GABA_A$ receptors on the postsynaptic membrane. The binding of GABA causes these receptors to open, passing an inward current that is primarily carried by chloride ions. In the mature brain, this influx of negative current results in a hyperpolarization of the postsynaptic cell, driving its membrane potential away from firing threshold. If this synaptic, or phasic, inhibitory action is disrupted in some way, the resulting imbalance between excitation and inhibition can lead to epilepsy (Kobayashi and Buckmaster 2003; Trotter et al. 2006; Calcagnotto et al. 2005).

Interneurons can also provide tonic inhibition to excitatory neurons. Extracellular levels of GABA can be sufficient to bind and activate extrasynaptic $GABA_A$ receptors, which are often composed of different subunits than are synaptic $GABA_A$ receptors. These tonically open channels provide shunting inhibition, in that they serve to decrease the input resistance of the cell, blunting the impact of excitatory currents that arrive onto the cell (Farrant and Nusser 2005). Disruptions in tonic GABAergic currents have also been observed in animal models of epilepsy (Zhang et al. 2007).

Inhibitory transmission deficits have been associated with epilepsy in a variety of animal models. One such model is the pilocarpine model of temporal lobe epilepsy, in

which rats are injected with the muscarinic agonist pilocarpine and subsequently develop spontaneous seizures (Curia et al. 2008). These mice also display many of the anatomical hallmarks of temporal lobe epilepsy, including cell death and axonal sprouting in the dentate gyrus. Before pilocarpine-injected rats exhibit spontaneous seizures, synaptic inhibition of dentate gyrus granule cells is significantly reduced (Kobayashi and Buckmaster 2003). This reduction in spontaneous inhibitory postsynaptic currents (sIPSCs) coincides with loss of dentate gyrus interneurons, and indicates that inhibitory synapses are lost following pilocarpine injection, but before rats develop epilepsy. These studies strongly support the hypothesis that inhibitory synapse loss is not a result of epilepsy; rather, this work indicates that such a loss, though not necessarily causative, may contribute to the development of epilepsy.

Epilepsy, especially in children, is often associated with cortical malformations (CM), and reductions in inhibition have been noted in numerous rodent and human CM studies. The telencephalic internal structural heterotopia (tish) rat exhibits epilepsy and CM due to a genetic abnormality. Recent studies demonstrate that synaptic inhibition and inhibitory synapse numbers are significantly reduced in the cortex of tish rats before seizures emerge (Trotter et al. 2006). Similar inhibitory defects have been found in tissue resected from human cortical dysplasia patients (Calcagnotto et al. 2005), although in human studies, it is not possible to say whether inhibition loss precedes or follows the onset of seizures.

Reductions in GABAergic transmission can result from loss of interneurons and/or inhibitory synapses, but decreased excitatory drive of interneurons can also contribute to reduced inhibition. In a rat model of cortical dysplasia, Xiang and

colleagues (2006) found a reduction in the frequencies of spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs) onto interneurons. This reduction in excitatory drive of interneurons may contribute to the decreased GABA release observed in the in utero irradiation model of cortical dysplasia-associated epilepsy (Xiang et al. 2006).

Taken together, these studies point to the complicated links between interneuron function and epilepsy. While certain abnormalities in inhibitory systems are thought to be a result of seizures, inhibitory defects precede the onset of seizures in at least some animal models of epilepsy. Moreover, regardless of the relative timing of these events, it is clear that dysfunction of inhibitory networks is associated with epilepsy in a wide variety of animal models, ranging from idiopathic epilepsies to symptomatic epilepsies.

Lis1 and Type-1 Lissencephaly

Lissencephaly is a brain malformation disorder characterized by loss of normal gyri and a disorganized cerebral cortex. In humans, malformations are associated with mental retardation, developmental delay, intractable forms of epilepsy and other serious neurological symptoms (Walsh 1999). Classical, or Type I, Lissencephaly is a haploinsufficiency disorder that results from mutations in one allele of the *Lis1* gene. Lis1 protein is important in microtubule-based motor activity of cytoplasmic dynein (Vallee and Tsai 2006). Because microtubule motility is vital to proper migration of neuronal precursors (Tsai and Gleeson 2005), an insufficient amount of Lis1 protein results in slowed migration and subsequent brain malformation (Wynshaw-Boris and Gambello 2001).

Loss of function mutations in one allele of *Lis1* could also contribute to neurological deficits by impacting synaptic transmission. Research in *C. elegans* indicates that *Lis1* mutations may impair the proper trafficking of GABA-containing synaptic vesicles via its interactions with the cytoplasmic dynein pathway (Williams et al. 2004). As such, *Lis1* deficit may affect inhibitory systems not only by slowing neuronal migration, but also by direct action on synaptic release of GABA.

Mice with one null allele of *Lis1* exhibit brain malformations and slowed migration of interneuron precursors (Fleck et al. 2000; McManus et al. 2004). In these mice, Lis1 deficit is also associated with impaired spatial learning, hyperexcitability of hippocampal circuits, and seizures, mirroring the condition of human Lissencephaly patients (Fleck et al. 2000; Paylor et al. 1999). These mice represent an excellent model in which to study circuit deficits resulting in epilepsy associated with Lissencephaly. Here we hypothesized that inhibitory systems in *Lis1* mutant mice would exhibit functional deficits due to disruptions in interneuron precursor migration and defects in GABA release. These studies are described in detail in Chapter II of this dissertation.

***Dlx1*, interneurons, and epilepsy**

The *Dlx* family of transcription factors is important in the differentiation and tangential migration of interneuron precursors; tangential migration toward the cortex and hippocampus is absent in mice lacking both *Dlx1* and *Dlx2*. Additionally, in mice lacking *Dlx1* and one copy of *Dlx2*, dendritic branching of young interneurons is abnormal, and while tangential migration is not abolished, interneurons settle into inappropriate layers within the cortex (Cobos et al. 2007).

Mice lacking *Dlx1* develop epilepsy by approximately two months of age. Interestingly, though neuronal migration does not appear to be impaired in *Dlx1*$^{-/-}$ mice, certain subtypes of interneurons begin to undergo apoptosis by one month of age. Specifically, the populations of cortical and hippocampal interneurons expressing SOM and CR are reduced by 20-30% at P30, and this is accompanied by a highly significant reduction in GABAergic transmission. In hippocampus and cortex, sIPSC and mIPSC frequencies are reduced by at least 50%, and amplitudes are reduced by about 30% (Cobos et al. 2005). Interestingly, these inhibitory defects appear to be of a much greater magnitude than would be expected based only on modest loss of certain dendrite-targeting interneuron subtypes, suggesting that other circuit alterations or cell-autonomous defects may contribute to the phenotype of *Dlx1*$^{-/-}$ mice.

To date, mice lacking *Dlx1* are the only animals without CM reported to develop epilepsy following interneuron death associated with a genetic defect. Gaining a full understanding of the chain of events that carries these mice from interneuron apoptosis to epilepsy will shed light on how neuronal systems adapt, or fail to adapt, to genetically-mediated epileptogenic phenomena. These studies are discussed in Chapter III of this dissertation.

Therapies for epilepsy

Pharmacological and surgical treatments

Most patients who report having had one or more seizures are prescribed an AED. These drugs share the property of reducing seizures; however, their targets vary widely (Czapinski et al. 2005). Some AEDs, including carbamazepine and phenytoin, block Na^{2+}

channels, reducing the propensity of neurons to fire action potentials. Many AEDs interact with the inhibitory system, promoting GABAergic signaling through various actions. For instance, benzodiazepines bind directly to $GABA_A$ receptors and promote the binding of GABA to the receptor. Tiagabine blocks the reuptake of GABA, leading to increased concentrations of GABA in the extracellular space (Czapinski et al. 2005). Many of these AEDs exhibit undesirable side effects, and nearly one-third of epilepsy patients do not respond to treatment with AEDs (Marson 2008). This highlights the need to explore novel treatment options for epilepsy patients, even those whose seizures are well controlled by AEDs but who might suffer from adverse side effects.

Patients who have epilepsy that does not respond to AEDs are candidates for epilepsy surgery, which normally entails removing an epileptogenic region of brain tissue. Mesial temporal lobe epilepsy (MTLE), for instance, is often treated effectively by removing the affected hippocampus. Using electroencephalography (EEG) and neuroimaging techniques, it is possible to isolate epileptogenic tissue by determining the location of the seizure focus, which is often located within a structurally abnormal, or dysplastic, region of the brain. However, the population of patients who can be successfully treated with surgery is limited (Cascino 2008). Many patients who do not respond well to AEDs will not be candidates for epilepsy surgery because their seizures are generalized rather than partial, or because a seizure focus cannot be identified using EEG and neuroimaging techniques.

Neural progenitor cell transplantation as a potential treatment for epilepsy

Extensive study of human and animal epileptic brains has demonstrated links between epilepsy and inhibitory interneuron defects, including reductions in interneuron number and reductions in GABA release (Calcagnotto et al. 2005; Cobos et al. 2005; Trotter et al. 2005). In agreement with these findings, some of the most highly effective AEDs are those that enhance GABAergic activity, including $GABA_A$ receptor agonists and GABA reuptake blockers (Czapinski et al. 2005). Transplantation of interneuron precursor cells may be an effective, alternative approach to the treatment of epilepsy with AEDs or surgical resection of epileptic tissue (Alvarez-Dolado et al. 2006).

Neuronal transplantation holds a great deal of promise in treating a variety of neurological diseases (Bjorklund and Lindvall 2000). Fortunately, the organization of the embryonic mouse subpallium makes the transplantation of interneuron precursor cells technically feasible. The MGE, where large populations of interneurons are born, can be microdissected from the embryonic mouse brain, and MGE cells can be dissociated and grafted into brains of newborn, adolescent, or adult mice. When cells are transplanted from E13.5 MGE into early postnatal animals, they migrate extensively and mature into inhibitory interneurons. The transplanted interneurons also integrate into local circuits, resulting in significant increases in GABAergic inputs to local excitatory neurons (Wichterle et al. 1999; Alvarez-Dolado et al. 2006).

Because of their high impact on levels of synaptic inhibition, interneuron progenitor cell transplantation is an excellent candidate for alleviating epilepsy. Many antiepileptic drugs work by increasing GABA levels in the brain, but their action cannot be targeted to specific brain regions; in contrast, transplants could be targeted to one hemisphere, or to a particular epileptogenic region of cortex or hippocampus.

Additionally, epilepsy surgery can be an effective treatment for some patients, but many patients, especially those with focal cortical dysplasia, do not benefit from surgery (Cascino 2008). Because many patients with epilepsy have few, if any, viable options for treatment, there remains a pressing need to develop novel therapies like interneuron precursor transplantation. However, many questions remain with respect to the properties of transplanted cells, and with respect to how those cells communicate with endogenous neurons. These studies are described in Chapter IV of this dissertation.

Thesis Summary

In this dissertation, I uncover significant alterations in inhibitory circuitry in two animal models of epilepsy, and I describe basic properties of transplanted interneuron precursor cells and how they integrate into local cortical circuits.

In a mouse model of Lissencephaly, I find that overall levels of synaptic inhibition in the disorganized hippocampus are significantly increased, although the circuit is hyperexcitable. This enhancement in synaptic inhibition is associated with an increase in the excitatory drive of interneurons, which fire action potentials more frequently in *Lis1* mutant mice. Interestingly, inhibitory inputs to $Lis1^{+/-}$ interneurons are altered in a layer-specific fashion, suggesting that interneurons in certain hippocampal layers express abnormal $GABA_A$ receptor subunits. Although overall numbers and intrinsic membrane properties of hippocampal interneurons are normal, basket-type interneurons are shifted into inappropriate layers, contributing to the disorganization of inhibitory circuits in *Lis1* mutant mice.

In mice lacking the transcription factor *Dlx1*, I find significant reorganizations in excitatory inputs to surviving hippocampal interneurons following apoptosis of certain interneuron subtypes by P30. Interneurons lacking *Dlx1* appear to mature more slowly than wild-type interneurons, although they are indistinguishable by P30. Excitatory inputs to pyramidal cells are significantly increased in adult $Dlx1^{-/-}$ mice, prior to epilepsy onset, in agreement with findings in many other animal models of epilepsy. Interestingly, excitatory drive of interneurons in $Dlx1^{-/-}$ mice undergoes significant reorganization after P30, indicating that AMPA receptor number may be reduced in mutants.

Finally, I show that interneuron precursor cells transplanted from the embryonic MGE into early postnatal mice mature into fast-spiking, regular-spiking, and stuttering interneurons. I alo find that, although transplantation of MGE cells increases synaptic inhibition onto host pyramidal neurons (Alvarez-Dolado et al. 2006), it has no effect on levels of inhibition of host interneurons. Importantly, this suggests that transplanted interneuron precursors preferentially target endogenous excitatory neurons, rather than inhibiting interneurons within the host brain. While the reasons for this target preference are unclear, these results encourage further research into neural progenitor cell transplantation as an effective treatment for epilepsy.

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Chapter II: Inhibitory networks in a mouse model of type-1 Lissencephaly

Introduction

Brain malformations are commonly associated with epilepsy, and often result from mutations in genes required for proper neuronal migration. During embryonic development, neural progenitor cells employ microtubule-based molecular machinery to generate tangential and radial migration (Marin and Rubenstein 2001; Metin et al. 2008). In particular, the cytoplasmic dynein pathway plays a central role in the mechanism underlying neuronal migration (Wynshaw-Boris and Gambello 2001). Cytoplasmic dynein is a retrograde-directed microtubule motor protein that is required for translocation of the nucleus during neuronal migration (Tsai and Gleeson 2005). Lis1, which interacts with cytoplasmic dynein, is vital for nuclear translocation in neuronal migration (Wynshaw-Boris and Gambello 2001). Accordingly, interneuron precursor cells exhibit slowed tangential migration in mice with one null allele of *Lis1* (*Lis1*$^{+/-}$ mice). In humans, heterozygous mutation or deletion of *Lis1* causes Type-1 Lissencephaly, a debilitating neurological condition that is associated with cognitive deficits, severe seizures, and an absence of gyri or sulci on the cortical surface (Walsh 1999).

Severe anatomical alterations, including cellular disorganization and multiple principal cell layers in hippocampus, were previously reported in *Lis1*$^{+/-}$ mice (Fleck et al. 2000; Hirotsune et al. 1998). In other rodent models for which disruption of normal brain architecture has been described (e.g., postnatal freeze lesion, in utero exposure to radiation or methylazoxymethanol), studies have noted significant functional disruption of synaptic communication within these disorganized neuronal networks. These phenotypes include, but are not limited to, enhanced N-methyl-D-aspartate (NMDA)

receptor function (Calcagnotto and Baraban 2005), decreased excitatory drive of displaced interneurons (Xiang et al. 2006), increased miniature excitatory postsynaptic current frequency and bursting rate (Zsombok and Jacobs 2007), and reduced IPSC frequency (Trotter et al. 2006). Human brain tissue samples obtained from patients with cortical dysplasia exhibit similar functional deficits (Calcagnotto et al. 2005). Disruption of cognitive function and generation of abnormal seizure discharge—common clinical manifestations of a malformed brain (Guerrini et al. 2003)—are likely to be linked with significant alterations in synaptic transmission.

Consistent with the severe disruption in hippocampal organization seen in *Lis1*$^{+/-}$ mice, acute brain slices from these animals exhibit a reduced threshold for potassium-induced epileptiform bursting, and a modest disruption of excitatory field potentials at the Schaffer collateral-CA1 synapse (Fleck et al. 2000). Because GABA-mediated synaptic transmission plays a critical role in regulating a variety of CNS functions (Farrant and Nusser 2005; Mohler 2006), and because inhibitory interneuron progenitors exhibit slowed migration during embryonic development (McManus et al. 2004), we hypothesized that inhibitory circuits in the *Lis1* mutant hippocampus would be significantly disrupted. We undertook extensive anatomical and electrophysiological studies in order to test this hypothesis, characterizing in detail the state of GABAergic transmission in *Lis1* mutant mice.

Results

Inhibitory input to pyramidal neurons in the Lis1$^{+/-}$ hippocampus

To analyze synaptic inhibition of principal neurons, we obtained voltage-clamp recordings of spontaneous IPSCs from 56 CA1 pyramidal cells in hippocampal slices prepared from wild-type (WT) and age-matched *Lis1* mutant mice (WT, n = 28 cells; *Lis1*$^{+/-}$, n = 28 cells); sIPSCs were recorded at a holding potential of –60 mV. Under IR-DIC, we recorded from *Lis1*$^{+/-}$ pyramidal cells located within the center of a diffuse, but recognizable, "principal" cell layer as well as cells located within clearly heterotopic locations. In all experiments, we did not observe differences between these populations of *Lis1*$^{+/-}$ pyramidal cells and have pooled them for analysis. Here we report a modest, but significant, increase in GABA-mediated synaptic transmission in *Lis1*$^{+/-}$ mice compared with controls. In WT hippocampal slices, mean sIPSC frequency was 3.89 ± 0.44 Hz, whereas in *Lis1*$^{+/-}$ mutants, sIPSC frequency was increased by 32% to 5.14 ± 0.39 Hz ($p < 0.05$; Fig. 1, A–C). Correspondingly, interevent intervals were decreased ($p < 0.05$, Kolmogorov-Smirnov test; Fig. 1E). Mean decay time was unchanged (WT: 4.87 ± 0.20 ms; *Lis1*$^{+/-}$: 4.67 ± 0.15 ms; Fig. 1D), and the overall distribution of decay times was similar (Fig. 1F).

The mean amplitude of sIPSCs was also higher in *Lis1*$^{+/-}$ mice (43.11 ± 2.51 pA) compared with controls (37.01 ± 1.47 pA; $P < 0.05$, Kolmogorov-Smirnov test; Fig. 2A). An amplitude histogram of sIPSCs demonstrated that this increase could not be attributed to a rightward shift in the overall distribution of event amplitudes but instead reflected a threefold increase in the frequency of large (>125 pA) events in *Lis1*$^{+/-}$ mice (Fig. 2B, *).

Only 4% of recorded wild-type sIPSCs were >125 pA, whereas 12% of *Lis1*$^{+/-}$ sIPSCs were >125 pA (Fig. 2C).

Because large events could be due to spontaneous firing of interneurons, we next recorded mIPSCs from 33 pyramidal cells (WT, n = 16 cells; *Lis1*$^{+/-}$, n = 17 cells). With action potentials blocked, these large events were eliminated (Fig. 2C). Analysis of mIPSC kinetics revealed no differences between WT and *Lis1*$^{+/-}$ mice (Fig. 3, A and B). mIPSC frequency was unchanged (WT: 3.40 ± 0.46 Hz; *Lis1*$^{+/-}$: 3.10 ± 0.37 Hz; Fig. 3C); in agreement with these data, an interevent interval plot also showed no differences between WT and mutant animals (Fig. 3F). Similarly, mean mIPSC decay time was unchanged (WT: 4.36 ± 0.15 ms; *Lis1*$^{+/-}$: 4.70 ± 0.17 ms; Fig. 3D), and the distributions of decay times were similar between WT and mutant animals (Fig. 3G). With large events eliminated in the presence of TTX, mean mIPSC amplitude was also unchanged (WT: 28.64 ± 1.68 pA; *Lis1*$^{+/-}$: 32.16 ± 1.73 pA; Fig. 3E) as was the distribution of individual mIPSC amplitudes across all cells (Fig. 3H).

To further assess inhibitory synaptic function in *Lis1* mutant mice, we also recorded tonic inhibitory currents onto disorganized CA1 pyramidal cells. Tonic currents are mediated by extrasynaptic $GABA_A$ receptors that are bound by ambient levels of GABA, providing an important source of shunting inhibition to pyramidal neurons (Farrant and Nusser 2005). We hypothesized that due to an increase in overall GABA release, ambient GABA levels might be higher in mutant mice, leading to an increase in tonic inhibitory currents as compared with WT littermates. To test this hypothesis, we recorded from 25 pyramidal cells (WT, n = 10; *Lis1*$^{+/-}$, n = 15) at –60 mV; at the end of each recording, we bath applied the competitive $GABA_A$ receptor antagonist gabazine

(100 μM). We measured percent change in holding current after gabazine application, and found no difference in mean tonic current size between WT and *Lis1*$^{+/-}$ mice (WT: 10.36 ± 1.63%; *Lis1*$^{+/-}$: 9.92 ± 1.01%; Fig. 4).

Evoked synaptic transmission and paired pulse modulation in the Lis1$^{+/-}$ *hippocampus*

To further characterize inhibitory synaptic transmission in *Lis1*$^{+/-}$ mice, we evoked IPSCs onto 19 CA1 pyramidal cells (WT, n = 8; Lis1+/–, n = 11) using a monopolar electrode placed in s. radiatum. We recorded responses at a holding potential of –60 mV. We measured peak amplitude of each eIPSC and calculated a paired-pulse ratio (PPR) for each cell, examining paired-pulse modulation at interpulse intervals of 40, 100, 400, 500, and 700 ms (Fig. 5, A and B). For all cell types, we observed examples of paired-pulse facilitation (PPF; defined as a PPR >1) and paired-pulse depression (PPD; PPR <1), with some cells showing an absence of any paired-pulse modulation (PPR = 1). The distributions of cells with PPR greater than, less than, or equal to 1 were similar between genotypes at all interpulse intervals tested; for clarity, shown in Fig. 5 is the distribution at a standard interpulse interval of 100 ms (WT, PPR >1: 37.5%; WT, PPR <1: 37.5%; WT, PPR = 1: 25.0%; *Lis1*$^{+/-}$, PPR >1: 36.4%; *Lis1*$^{+/-}$, PPR <1: 45.5%; *Lis1*$^{+/-}$, PPR = 1: 18.2%; $P > 0.05$, χ^2 test; Fig. 5C). A plot of the amplitude of the second response as a percentage of the first response [(eIPSC2/eIPSC1) x 100] reveals that the profile of paired-pulse modulation at different intervals was also similar between WT and mutant animals (for all interpulse intervals, $P > 0.05$, Student's t-test; Fig. 5B). We also examined elPSC decay time constants to determine whether abnormalities in GABA receptor subunit composition or GABA reuptake were functionally evident in *Lis1*$^{+/-}$

mice. We found that values were comparable between WT and mutant mice (WT: 40.79 ± 5.27 ms; *Lis1*$^{+/-}$: 39.76 ± 4.68 ms; Fig. 5D).

Spontaneous firing and excitatory drive of interneurons in the Lis1$^{+/-}$ *hippocampus*

Because of the observed increase in large, action-potential-dependent spontaneous IPSCs in *Lis1*$^{+/-}$ mice, we tested the hypothesis that interneurons in the *Lis1*$^{+/-}$ hippocampus exhibit increased spontaneous firing by recording from 37 CA1 interneurons (WT, n = 20; *Lis1*$^{+/-}$, n = 17) in the cell-attached configuration (Fig. 6). Under IR-DIC, we recorded from fast-spiking (FS), regular-spiking nonpyramidal (RSNP), and burst-spiking (BST) interneurons in s. oriens, s. radiatum, and s. lacunosum-moleculare locations. The mean spontaneous firing rate of interneurons in WT mice was 0.16 ± 0.12 Hz with only 20% of recorded WT interneurons firing at rest. Nonfiring cells were assigned a rate of 0 Hz and included in the overall calculation of mean firing rate. These data confirm that spontaneous firing of interneurons is rare in slices from control animals (Cossart et al. 2001). However, firing activity for interneurons recorded in Lis1+/– slices was more common with 71% of cells exhibiting spontaneous activity ($P < 0.001$, χ^2 test; Fig. 6C). We also assigned a firing rate of 0 Hz to nonfiring Lis1+/– interneurons and found that the mean firing rate of *Lis1*$^{+/-}$ interneurons was significantly increased, at 1.21 ± 0.46 Hz ($P < 0.05$; Fig. 6D). We also compared the firing rates of only those interneurons that exhibited spontaneous firing, excluding all interneurons with a rate of 0 Hz. By this analysis, we found the mean firing rate for WT interneurons to be 0.82 ± 0.50 Hz (n = 4), whereas the rate for *Lis1*$^{+/-}$ interneurons was 1.71 ± 0.60 Hz (n = 12). At the conclusion of cell-attached recording, we ruptured the patch and switched into

current-clamp mode to confirm interneuron identity (Freund and Buzsaki 1996; Flames and Marin 2005) (Fig. 6, A and B, bottom).

To explore the possibility that alterations in intrinsic firing properties of interneurons could underlie their increased activity, we obtained current-clamp recordings from 111 visually identified CA1 interneurons in slices from WT (n = 62 cells) and *Lis1*$^{+/-}$ (n = 49 cells) mice. We injected 1,000-ms current steps to measure resting membrane potential, input resistance, spike width, amplitude of the fast afterhyperpolarization, firing frequency at twice the firing threshold, and firing frequency accommodation (Table 1). In these recordings, we encountered FS, RSNP, and BST interneurons. We found that intrinsic properties were similar for all subclasses of interneurons from WT and *Lis1*$^{+/-}$ mice, indicating that a change in synaptic inputs to interneurons is likely to underlie the increased firing activity we observed in cell-attached recordings of *Lis1*$^{+/-}$ interneurons.

Finally, we hypothesized that an increase in excitatory drive onto GABAergic interneurons could underlie enhanced activity of inhibitory systems in the *Lis1*$^{+/-}$ hippocampus. We recorded spontaneous EPSCs onto 41 CA1 interneurons (WT n = 23, *Lis1*$^{+/-}$ n = 18). The mean sEPSC frequency in mutant mice was significantly increased compared with cells from age-matched WT littermates (WT: 4.19 ± 1.19 Hz; *Lis1*$^{+/-}$: 8.56 ± 1.82 Hz; $P < 0.05$; Fig. 7, A–C). Mean sEPSC amplitude and decay time values were unaltered (Fig. 7, D and E). Interneuron identification was confirmed in current-clamp mode for all cells (Fig. 7, A and B, bottom).

Laminar distribution of interneuron subtypes

We examined interneuron distribution using the neurochemical markers calretinin (CR), parvalbumin (PV), and somatostatin (SOM). In hippocampus, three broad classes of interneurons can be defined: (i) PV-positive, fast-spiking (FS), basket-type cells largely located in stratum oriens (SO) and stratum pyramidale (SP); (ii) CR-positive, regular-spiking non-pyramidal (RSNP) cells; (iii) SOM-positive, burst-spiking (BST), oriens-lacunosum-moleculare interneurons (Freund and Buzsaki, 1996; Flames and Marin, 2005). We counted CR, PV, and SOM-positive somata in hippocampal area CA1 in coronal sections from 3 wild-type mice and 3 age-matched *Lis1*$^{+/-}$ littermates. For each section, we divided area CA1 into 400 μm by 50 μm bins, and counted the number of immunoreactive cell bodies in each bin to illustrate the overall distribution for each interneuron subtype. CR interneuron distribution in *Lis1*$^{+/-}$ mice closely mirrored that of WT littermates (Fig. 1A-D), with CR-positive somata distributed across all layers. Additionally, SOM-positive cells were mostly found in SO in WT and *Lis1*$^{+/-}$ sections (Fig. 8 I-L). However, in agreement with a previous observation that some PV interneurons are displaced or "heterotopic" (Fleck et al., 2000) we observed a significant difference in the distribution of PV-positive somata in *Lis1*$^{+/-}$ mice (Fig. 8 E-H). In sections from WT mice, approximately 50% of PV interneurons are located in SO, with the remaining cells divided evenly between SP and stratum radiatum or stratum lacunosum-moleculare (R/LM). In the *Lis1*$^{+/-}$ hippocampus, about 65% of PV interneurons were located in R/LM, with only ~10% in SO ($p < 0.001$, χ^2 test).

After sorting interneurons into subclasses according to firing properties, we found that the relative proportions of FS, RSNP, and BST cells in *Lis1*$^{+/-}$ animals were comparable to those of WT mice (Fig. 9G). For both mutant and wild-type animals,

RSNP was the predominant interneuron subtype (71.0% in WT, 69.4% in *Lis1*$^{+/-}$). FS cells comprised about one-fourth of the recorded interneurons (22.6% in WT, 24.5% in *Lis1*$^{+/-}$), while BST cells were relatively infrequent (6.5% in WT, 6.1% in *Lis1*$^{+/-}$). However, when we sorted interneuron subtypes by anatomic location, we uncovered a shift in the distribution of FS cells reminiscent of the shift we observed in PV immunostaining studies. While most WT FS cells were located in SO, most *Lis1*$^{+/-}$ FS cells were located in R/LM (WT: 71.4% in SO, 28.6% in R/LM; *Lis1*$^{+/-}$: 25.0% in SO, 75.0% in R/LM; $p < 0.001$, χ^2 test; Fig. 9I). RSNP cells, in contrast, exhibited similar distributions (WT: 29.5% in SO, 70.5% in R/LM; *Lis1*$^{+/-}$: 35.3% in SO, 64.7% in R/LM; Fig. 9H).

GABAergic inputs to stratum oriens interneurons

We hypothesized that disruptions in the distribution of hippocampal interneurons would lead to alterations in GABAergic connectivity. We recorded spontaneous inhibitory postsynaptic currents (sIPSCs) on visually identified CA1 interneurons located in SO, using DNQX and APV in ACSF to block glutamatergic currents (Fig. 10). Interestingly, we found a significant increase in the frequency of sIPSCs on SO interneurons in *Lis1* mutant animals (WT: 6.92 ± 1.48 Hz; *Lis1*$^{+/-}$: 15.96 ± 2.67 Hz; p=0.01; Fig. 10C). However, the mean sIPSC amplitude was unchanged (WT: 29.06 ± 3.05 pA; *Lis1*$^{+/-}$: 33.02 ± 4.58 pA; p=0.48; Fig. 10D), as was mean sIPSC decay time (WT: 1.50 ± 0.10 ms; *Lis1*$^{+/-}$: 1.51 ± 0.12 ms; p=0.97; Fig. 10E).

Next, we repeated these experiments in the presence of TTX, in order to isolate miniature inhibitory postsynaptic currents (mIPSCs) onto SO interneurons (Fig. 11).

Mean mIPSC frequency was not significantly different between WT and *Lis1*$^{+/-}$ littermates (WT: 6.26 ± 2.31 Hz; *Lis1*$^{+/-}$: 8.42 ± 1.76 Hz; p=0.47; Fig. 11C), indicating that the increased sIPSC frequency in mutant SO interneurons is due to action-potential-dependent release of GABA. We also observed no difference in mean decay time (WT: 1.70 ± 0.17 ms; *Lis1*$^{+/-}$: 1.64 ± 0.14 ms; p=0.77; Fig. 11F). However, we found that mean mIPSC amplitude was significantly decreased in *Lis1*$^{+/-}$ animals (WT: 30.58 ± 3.73 pA; *Lis1*$^{+/-}$: 21.06 ± 1.67 pA; p=0.04; Fig. 11D,E). These results show that, in the disorganized *Lis1*$^{+/-}$ hippocampus, interneurons located in SO receive altered GABAergic inputs.

GABAergic inputs to stratum radiatum and stratum lacunosum-moleculare interneurons

The population of interneurons present in R/LM is also significantly different in *Lis1* mutants. We recorded sIPSCs onto interneurons located in R/LM in age-matched WT and *Lis1*$^{+/-}$ littermates in order to determine whether inhibitory connectivity was disrupted in R/LM interneurons (Fig. 12). We found no difference in mean sIPSC frequency (WT: 10.99 ± 2.87 Hz; *Lis1*$^{+/-}$: 8.53 ± 2.22 Hz; p=0.51; Fig. 12C) or amplitude (WT: 40.25 ± 4.12 pA; *Lis1*$^{+/-}$: 43.53 ± 5.07 pA; p=0.62; Fig. 12D). However, we noticed a non-significant trend toward a longer decay time in *Lis1* mutant cells (WT: 1.78 ± 0.07 ms; *Lis1*$^{+/-}$: 2.01 ± 0.16 ms; p=0.18; Fig. 12E,F,H). To analyze this further, we averaged individual sIPSC traces and calculated a decay time constant for each cell. We found that the mean decay time constant was significantly longer in *Lis1* mutant interneurons located in R/LM (WT: 5.46 ± 0.36 ms; *Lis1*$^{+/-}$: 7.74 ± 0.94 ms p=0.03; Fig. 12G).

Finally, we recorded mIPSCs onto interneurons located in R/LM. We again observed no significant differences in mean mIPSC frequency (WT: 6.55 ± 1.87 Hz; *Lis1*$^{+/-}$: 6.51 ± 1.02 Hz; p=0.99; Fig. 13C) and amplitude (WT: 29.52 ± 3.55 pA; *Lis1*$^{+/-}$: 34.99 ± 5.96 pA; p=0.47; Fig. 13D). However, mean mIPSC decay time was significantly increased in *Lis1* mutants (WT: 1.74 ± 0.14 ms; *Lis1*$^{+/-}$: 2.13 ± 0.12 ms; p=0.04; Fig. 13E,F,H). We also found that the mean decay time constant was increased in mutant animals (WT: 5.12 ± 0.51 ms; *Lis1*$^{+/-}$: 7.37 ± 0.59 ms; p=0.01; Fig. 13G). These results suggest that $GABA_A$ receptors in *Lis1* mutant R/LM interneurons may be composed of different subunits than those of WT interneurons.

Discussion

Lis1 haploinsufficiency results in type-I Lissencephaly in humans (Dobyns et al. 1993) and similar neuronal migration disorders in mice (Hirotsune et al. 1998). In humans, Lis1 deficit contributes to profound mental retardation and intractable epilepsy (Dobyns et al. 1993); in mice, Lis1 deficit is associated with impaired spatial learning and hyperexcitability of hippocampal circuits (Fleck et al. 2000; Paylor et al. 1999). To study mechanisms that may contribute to these neurological phenotypes, we analyzed inhibitory systems in *Lis1*$^{+/-}$ mice. We anticipated that GABAergic transmission would be reduced, consistent with the circuit hyperexcitability previously observed in these mice (Fleck et al. 2000) and also consistent with other studies indicating that inhibitory transmission is decreased in cortical malformation disorders (Trotter et al. 2006). Surprisingly, we identified a modest increase in GABA-mediated synaptic inhibition, in that the frequency of large, action-potential-dependent sIPSCs onto disorganized CA1 pyramidal neurons was enhanced compared with age-matched controls. The inhibitory current increase was associated with increased spontaneous firing rates of interneurons, as well as enhanced glutamatergic excitation of these cells. Our findings, therefore, provide evidence that Lis1 deficit is associated with a significant disruption of inhibitory circuitry in the malformed hippocampus.

Potential direct effects of Lis1 deficit on synaptic transmission

In addition to known effects of Lis1 deficit on neuronal migration (McManus et al. 2004), a reduction of Lis1 protein in neurons could also contribute to functional defects via a direct effect on synaptic vesicle trafficking. Research in C. elegans indicates

that *Lis1* mutations may impair the proper trafficking of GABA-containing synaptic vesicles (Williams et al. 2004). Using nematodes expressing a nonsense allele of *lis-1* (i.e., *pnm-1*) Williams et al. noted an uneven distribution of GABA vesicles, some of which were irregularly sized, in *pnm-1* mutants. Similarly, in a Drosophila *Lis1* mutant, large axonal swellings, possibly containing an abundance of synaptic vesicle-associated proteins, were found to "clog" axonal transport (Liu et al. 2000). In a more complex mammalian system, however, we find that Lis1 deficiency does not alter the frequency, amplitude, or decay times of mIPSCs on pyramidal neurons. Similarly, decay kinetics and paired-pulse modulation of evoked IPSCs also appear unaltered in *Lis1* mutant mice as compared with age-matched controls, suggesting that pre- and postsynaptic GABAergic systems are grossly normal.

Spontaneous firing of interneurons in epilepsy

Our recordings of spontaneous firing of hippocampal interneurons support the hypothesis that the increase in large inhibitory events (i.e., sIPSCs >125 pA in amplitude) in $Lis1^{+/-}$ mice is associated with increased firing of hippocampal interneurons in this disorganized circuit. Similar results were reported in other rodent studies featuring hyperexcitable circuits. For example, in kainate- and pilocarpine-treated rats, commonly used animal models of temporal lobe epilepsy, Cossart et al. (2001) observed an increase in cell-attached spontaneous firing rates of interneurons. In the case of $Lis1^{+/-}$ mice, the increased firing of interneurons is most likely attributable to enhanced excitatory inputs arriving from pyramidal cells, as we noted an increase in sEPSC frequency onto these interneurons. Similarly, in kainate- and pilocarpine-treated rats, the increased

spontaneous firing rate of interneurons was associated with an increase in excitatory inputs to interneurons (Cossart et al. 2001). Also consistent with our findings, an increase in sEPSC frequency onto CA1 interneurons was observed in slices bathed in elevated potassium, another commonly studied manipulation eliciting epileptic discharge (McBain 1994).

Increased inhibition of pyramidal neurons in epilepsy

An increase in GABA-mediated inhibition, as reported here for *Lis1*$^{+/-}$ mice, was also recently reported in a mouse model of autosomal dominant nocturnal frontal lobe epilepsy (Klaassen et al. 2006). Increased inhibition appears counter-intuitive in an epileptogenic process but may also contribute to the hyperexcitable phenotype observed. For example, interneurons are known to be critical for the generation and maintenance of oscillatory activity in the CNS (Bartos et al. 2007). Hippocampal parvalbumin-positive basket-type interneurons, in particular, are vital to the generation of gamma oscillations in entorhinal cortex (Cunningham et al. 2006), and GABA-mediated inhibition drives beta/gamma oscillatory activity in hippocampus (Trevino et al. 2007). GABA-mediated oscillations are a physiologically relevant mechanism that modulates the firing activity of large populations of principal glutamatergic neurons. Recent analysis of human electroencephalographic data suggests that high-frequency oscillations, likely controlled by the firing of inhibitory interneurons, may predict the onset of seizure activity (Medvedev et al. 2000; Worrell et al. 2005). Therefore a disruption of the inhibitory system could lead to abnormal synchronization of excitatory circuits and, perhaps, generation of abnormal electrical discharges, i.e., seizures.

Interneurons and cognitive function

Besides temporally linking neuronal firing activity, GABA-mediated oscillations play a critical role in memory consolidation (Bragin et al. 1999; Buzsaki 1989; Grenier et al. 2001). For example, interneuron-generated theta rhythms are thought to gate memory information processing in the hippocampus (Sun et al. 2001). At a cellular level, the neural correlate of learning (spatial learning, in particular) is thought to be encoded by the firing of hippocampal pyramidal (place) cells. Place cell firing patterns are fairly precise, and interneuron-mediated feedback inhibition is hypothesized to maintain this precision (Bose and Recce 2001; Frank et al. 2006; Touretzky et al. 2005). In *Lis1* mutant mice, the enhanced activity of at least some interneurons, and the occasional large-amplitude inhibitory events that result, could disrupt the finely tuned patterning of place cell firing and result in a place field map with limited precision. The known relationship between place cell firing patterns and spatial memory (Hollup et al. 2001; Lenck-Santini et al. 2001) is consistent with the impaired performance of $Lis1^{+/-}$ mice in spatial memory tasks. For example, $Lis1^{+/-}$ mice failed to search in the correct quadrant of a pool during a conventional test of spatial learning performance (Paylor et al. 1999). Most importantly, the observed alteration of GABA-mediated synaptic transmission in hippocampal slices from *Lis1* mutant mice could underlie some of the cognitive deficits associated with type-1 Lissencephaly.

GABAergic inputs to interneurons in epilepsy and brain malformations

Here we also report, to our knowledge, the first study of inhibition of interneurons in a model of cortical malformation and epilepsy. We find significant reorganizations of inhibitory circuits in Lissencephaly mice. Specifically, in *Lis1*$^{+/-}$ mice, we find that the laminar location profile of basket cells is disrupted, and we find that inhibitory connectivity among interneurons undergoes extensive alterations. Specifically, in recordings from *Lis1*$^{+/-}$ SO interneurons, we report a significant increase in sIPSC frequency but a significant decrease in mIPSC amplitude as compared to WT interneurons. In recordings from *Lis1*$^{+/-}$ R/LM interneurons, we find that decay time constants of sIPSCs and mIPSCs are significantly longer than in WT R/LM interneurons.

In the epileptic and malformed brain, significant reorganizations in synaptic transmission take place. Oftentimes, these alterations include increased excitation and decreased inhibition of hippocampal and cortical principal neurons (Cobos et al. 2005; Jacobs and Prince 2005; Trotter et al. 2006), as well as decreased excitatory drive of interneurons (Xiang et al. 2006). Normally, interneurons also receive significant inputs from other interneurons (Markram et al. 2004), and these inhibitory networks are vital to controlling overall levels of excitability in cortical and hippocampal circuits (Cossart et al. 2005). However, no studies have addressed the state of GABAergic inputs to interneurons in models of brain malformation and epilepsy.

The only studies that exist concerning inputs to interneurons and epilepsy are from the kainic acid model of epilepsy. Rats injected with kainic acid develop temporal lobe epilepsy but no malformations, and share many elements of hippocampal pathology with human temporal lobe epilepsy patients, including loss of neurons in the dentate gyrus and hyperexcitability of CA1 pyramidal neurons (Dudek et al. 2002). Morin and

colleagues (1999), using electron microscopy, found layer-specific alterations in GABA synapses on CA1 interneurons in kainic acid injected rats. Specifically, inhibitory synapses on somata of lacunosum-moleculare interneuron were increased in number and length, suggesting an enhancement of perisomatic inhibition of these interneurons. In contrast, no changes were observed in GABA synapses onto SO interneurons. Interestingly, however, the properties of IPSCs on CA1 interneurons are unchanged in rats injected with kainic acid, although layer-specific reorganization of excitatory inputs to interneurons does occur (Morin et al. 1998). GABAergic inputs to certain hippocampal interneurons also undergo reorganization in humans with temporal lobe epilepsy. Anatomical studies demonstrate that calbindin (CB)-positive interneurons in CA1 receive an increased number of GABA synapses from both CB-positive and CB-negative interneurons in the epileptic human hippocampus (Wittner et al. 2002). This evidence, together with our studies, suggests that GABAergic inputs to interneurons may undergo reorganizations in many types of epilepsy, although no previous work has examined such questions in models of brain malformation.

Inhibitory interneurons, brain malformations, and neuronal migration

Brain malformations frequently result from disorders of neuronal migration (Guerrini and Marini 2006). However, it remains unclear how migration defects might affect final laminar positions of interneurons. In fact, the cues that guide interneurons to their final laminar positions are largely unknown. After tangentially migrating from the ganglionic eminences to their final destinations in cortex and hippocampus, interneurons switch to radial migration in order to reach the proper layer (Tanaka et al. 2003; Hevner

et al. 2004). One factor that appears to be vital in allowing interneurons to reach the proper laminar location is p35, an activator of cyclin dependent kinase 5 (Cdk5). Developing interneurons in mice lacking p35, as in *Lis1* mutant mice, exhibit slowed tangential migration. Interneurons in p35 knockout mice also populate the neocortex in an altered distribution (Rakic et al. 2008). Lamination of dentate granule cells is also disrupted in p35 knockout mice, and these mice exhibit spontaneous seizures as well as hyperexcitable hippocampal circuitry (Wenzel et al. 2001; Patel et al. 2004). Whether hippocampal interneurons also exhibit an altered distribution in mice lacking p35 is unknown; in addition, no studies have examined functional consequences of cortical interneuron disorganization in these mice. Indeed, few studies have examined interneurons in the malformed brain, and none have focused on interneurons in a malformed hippocampus. We find a selective disorganization of basket-type, fast spiking interneurons in the hippocampus of *Lis1* mutant mice, and further demonstrate that GABAergic inputs to interneurons are functionally reorganized in this disorganized hippocampal circuit. Importantly, this is the first demonstration of alterations in inhibitory inputs to interneurons associated with a specific lamination defect in a malformed brain, and our results indicate that these inputs may be altered in p35 knockout mice, as well as in other models of brain malformation, including rats exposed to MAM in utero. These circuit alterations may be a specific consequence of the delayed arrival of migrating interneurons to their destinations.

Causes and consequences of altered GABAergic inputs to interneurons

Reorganization of synaptic connectivity between interneurons may be seen as a cause of, or as a response to, hyperexcitability in neuronal circuits. While loss of inhibition of principal neurons is commonly cited as an underlying cause of hyperexcitability and seizures, the fate of synaptic inhibition of interneurons has remained largely unexplored in models of brain malformation and epilepsy. Any understanding of the circuit rearrangements associated with epilepsy and malformations is necessarily incomplete without understanding the state of synaptic inhibition of interneurons, because networks of interneurons are integral in controlling overall network excitability (Cossart et al. 2005).

We find that SO interneurons receive an increased frequency of spontaneous GABAergic events in *Lis1* mutant mice. However, there does not appear to be an increase in inhibitory synapse number onto *Lis1* mutant SO interneurons, because mIPSC frequency is unchanged. This is consistent with our previous finding that *Lis1* mutant interneurons fire more frequently than WT interneurons (Jones and Baraban 2007). Increased inhibition of interneurons may contribute to the epileptic phenotype of Lissencephaly mice in a number of ways. For instance, enhanced inhibitory input may reduce the output of SO interneurons onto principal neurons, and this disinhibition could contribute to hyperexcitability. In addition, augmented inhibition of interneurons is thought to enhance the synchronization of neural networks (Cobb et al. 1995; Wang and Buzsaki 1996), and abnormally high synchronous activity of neuronal populations is associated with seizures (Uhlhaas and Singer 2006). In this way, enhancement of inhibitory inputs to SO interneurons could promote hyperexcitability within the hippocampus. This enhancement of synaptic inhibition, however, is not limited to SO

interneurons in *Lis1* mutant mice. Inhibition of pyramidal cells in *Lis1* mutant mice is enhanced, a possible compensatory response to hyperexcitability. Putative compensatory increases in inhibition of principal neurons have been reported in some epilepsy models (Haas et al. 1996); our results indicate that such enhancements of GABAergic transmission do not only target principal neurons in models of epilepsy and brain malformation.

Interestingly, we also find that mIPSCs on $Lis1^{+/-}$ SO interneurons are significantly reduced in amplitude. In contrast with the presynaptic enhancement of inhibitory input to these interneurons, the reduction in mIPSC amplitude is likely to reflect a postsynaptic modification: a reduction in $GABA_A$ receptor number (Nusser et al. 1997). This could be a compensatory response to the increased frequency of GABA release onto SO interneurons. Along the same lines, Shao and Dudek (2005) observed an increase in mIPSC amplitude onto dentate granule cells in kainic acid injected rats, which the authors argue to be a compensatory response to decreased frequency of IPSCs onto granule cells. In both cases, there appears to be an alteration in postsynaptic $GABA_A$ receptor number in an attempt to counterbalance either an increase or decrease in the frequency of synaptic GABA release. Interestingly, the increase in sIPSC frequency and compensatory decrease in mIPSC amplitude that we report is specific to SO interneurons; sIPSC and mIPSC frequencies and amplitudes are unchanged in $Lis1^{+/-}$ R/LM interneurons.

Finally, we find that the decay time constant of spontaneous GABAergic events is significantly longer in $Lis1^{+/-}$ R/LM interneurons. A similar observation was reported in mIPSCs onto cortical pyramidal neurons in a different line of Lissencephaly mice

(Valdes-Sanchez et al. 2007). Decay times of spontaneous synaptic events are reflective of the postsynaptic receptor subunit composition (Okada et al. 2000), indicating that R/LM interneurons in *Lis1* mutant mice express $GABA_A$ receptors composed of different subunits from those of WT mice. $GABA_A$ receptors are pentameric structures, and the α subunits contribute significantly to decay properties of synaptic events. Specifically, the α1 subunit appears to mediate the rapid decay of synaptic events (Goldstein et al. 2002), while α2 and α3 subunit containing receptors exhibit slower decay properties (Okada et al. 2000). Early in postnatal life, $GABA_A$ receptors in the brain predominantly contain α2 and α3 subunits; in adulthood, α2 and α3 subunit expression is downregulated, and is largely replaced by α1 subunit expression (Heinen et al. 2004). Modifications in $GABA_A$ receptor subunits have been observed in animal models of temporal lobe epilepsy (Poulter et al. 1999), with epileptic animals showing reduced α1 expression (Brooks-Kayal et al. 1998). The increased decay time constant of sIPSCs and mIPSCs on $Lis1^{+/-}$ R/LM interneurons is consistent with the hypothesis that the developmental switch from α2/3 to α1 containing $GABA_A$ receptors is either delayed or absent in *Lis1* mutant mice.

Alterations in $GABA_A$ receptor number and composition in $Lis1^{+/-}$ hippocampal interneurons could also be a direct result of impaired $GABA_A$ receptor trafficking due to reduced levels of functional Lis1 protein. Lis1 interacts with cytoplasmic dynein and dynactin, which are important in the trafficking of synaptic components (Waterman-Storer et al. 1997). *Drosophila* Lis1, through an interaction with cytoplasmic dynein, is required for normal axonal transport (Liu et al. 2000). Gephyrin, a scaffolding protein that clusters $GABA_A$ receptors at postsynaptic zones, interacts with the dynein motor complex (Maas et al. 2006). In this way, Lis1 signaling may be directly linked with

$GABA_A$ receptor trafficking, providing another potential explanation for alterations in $GABA_A$ receptor number and subunit composition in *Lis1* mutant mice. However, it remains unclear why Lis1 deficit would be associated with distinct $GABA_A$ receptor phenotypes in SO versus R/LM interneurons.

An overall picture of inhibitory circuits in Lissencephaly mice

Here we report the first systematic assessment of inhibitory synaptic circuitry in a malformed hippocampus, and provide a clear picture of the extensive inhibitory circuit reorganizations that take place in area CA1 of the *Lis1* mutant hippocampus. Inhibitory interneurons receive increased excitatory drive from CA3 pyramidal cells and fire more frequently than WT interneurons, leading to a significant enhancement in inhibitory input (e.g., sIPSCs) to CA1 pyramidal neurons and nearby SO interneurons. However $GABA_A$ receptor properties and GABA synapse number appear unchanged in CA1 pyramidal cells, although receptor number appears to be reduced in SO interneurons as a potential compensatory response. In contrast, R/LM interneurons do not receive enhanced inhibition, although they may express an abnormal complement of $GABA_A$ receptor subunits, most likely an increase in $\alpha 2/3$ and a decrease in $\alpha 1$. These layer-specific reorganizations in inhibitory circuitry may, in part, be a consequence of the alterations in laminar locations of basket-type interneurons.

In conclusion, we provide the first evidence that inhibitory inputs to interneurons are extensively reorganized in a model of brain malformation. We also report the first detailed examination of interneuron lamination in a malformed hippocampus. These alterations may be seen as compensatory responses to increased excitability. The

cumulative result of these inhibitory changes could be enhancement of the epileptogenic process and/or alteration of cognitive function, two of the primary neurological deficits observed in patients with Type-I Lissencephaly.

Table 1. Intrinsic properties of interneurons are similar between WT and *Lis1*$^{+/-}$ mice.

	FF (2xT) (Hz)	% AC	R_{in} (MΩ)	fAHP (mV)	V_m rest (mV)	Spike width (ms)
FS (WT) (n=14)	31.7 ± 4.2	11.7 ± 2.2	202.4 ± 29.7	21.5 ± 1.6	-58.9 ± 2.2	1.13 ± 0.08
FS (*Lis1* +/–) (n=12)	38.3 ± 9.1	14.0 ± 1.4	218.8 ± 35.3	23.2 ± 1.6	-58.7 ± 2.3	1.07 ± 0.11
RSNP (WT) (n=44)	18.4 ± 1.0	42.9 ± 2.8	202.6 ± 16.0	19.2 ± 0.8	-56.6 ± 0.75	1.19 ± 0.03
RSNP (*Lis1* +/–) (n=34)	18.4 ± 2.4	36.3 ± 1.6	213.5 ± 21.6	19.1 ± 0.9	-57.9 ± 0.92	1.16 ± 0.04
BST (WT) (n=4)	17.2 ± 0.6	45.8 ± 7.9	224.0 ± 69.8	18.3 ± 2.4	-55.8 ± 2.5	1.23 ± 0.13
BST (*Lis1* +/–) (n=3)	15.7 ± 1.5	44.6 ± 2.9	177.0 ± 44.5	20.4 ± 1.3	-61.7 ± 4.6	0.96 ± 0.12

Shown are the mean firing frequency at twice firing threshold, firing frequency accommodation, input resistance (R_{in}), amplitude of the fast afterhyperpolarization (fAHP), resting membrane potential (V_m rest), and spike width for all interneurons recorded in WT and mutant mice. FS, fast spiking; RSNP, regular-spiking nonpyramidal; BST, burst spiking.

Figure 1. Frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) is increased in pyramidal cells recorded from Lis1-deficient mice.

A: sample sIPSC traces from a wild-type (WT) cell. B: traces from a *Lis1*$^{+/-}$ cell demonstrating an increase in the frequency and amplitude of sIPSCs. C: mean sIPSC frequency is increased in *Lis1*$^{+/-}$ mice ($P < 0.05$, t-test). Gray, WT; Black, Lis1+/–. D: mean sIPSC decay time is unchanged. E: mean inter-event intervals are decreased in *Lis1*$^{+/-}$ mice ($P < 0.05$, Kolmogorov-Smirnov test). F: overall distribution of decay times was similar between WT and *Lis1*$^{+/-}$ mice.

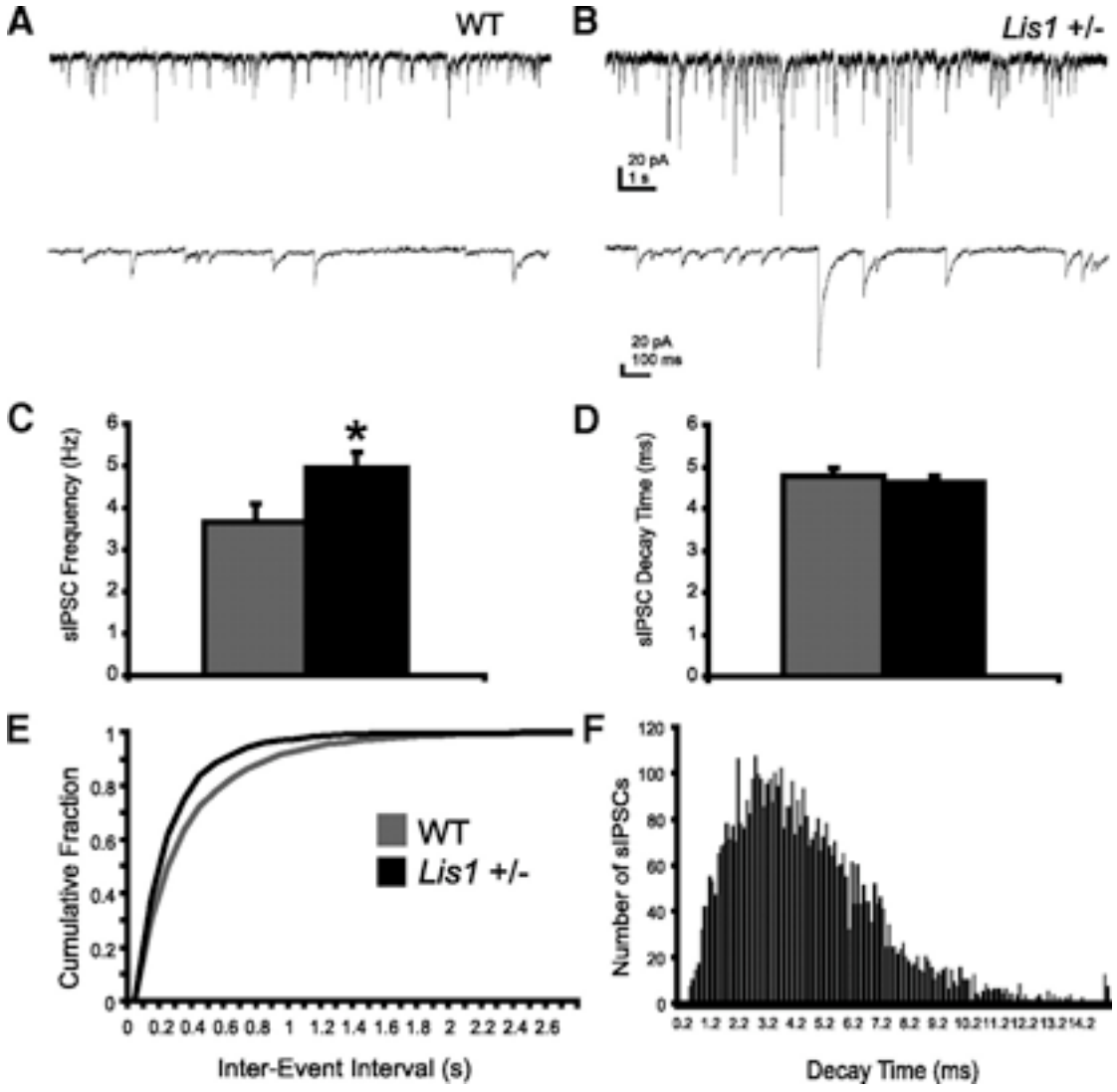

A
WT
B
Lis1 +/-
20 pA
1 s
20 pA
100 ms
C
sIPSC Frequency (Hz)
*
D
sIPSC Decay Time (ms)
E
Cumulative Fraction
WT
Lis1 +/-
Inter-Event Interval (s)
F
Number of sIPSCs
Decay Time (ms)

Figure 2. Increase in mean sIPSC amplitude is due to an increase in the proportion of large, >125 pA, action-potential-dependent sIPSCs in *Lis1*-deficient mice.

A: mean sIPSC amplitude is increased in *Lis1*$^{+/-}$ mice (*, $P < 0.05$, Kolmogorov-Smirnov test). Gray, WT; Black, *Lis1*$^{+/-}$. B: distributions of sIPSC amplitudes appear similar except for a roughly threefold increase in the proportion of >125 pA events in mutant mice (*). Gray, WT; Black, L *Lis1*$^{+/-}$. Inset: sample sIPSC events from a *Lis1*$^{+/-}$ cell. C: events >125 pA are essentially eliminated in the presence of TTX. White, sIPSC data; Black, miniature IPSC (mIPSC) data. D: in WT mice, mIPSC frequency is similar to sIPSC frequency, suggesting little spontaneous firing of interneurons. However, in *Lis1*$^{+/-}$ mice, mIPSC frequency is greatly reduced compared with sIPSC frequency. White, sIPSC data; Black, mIPSC data. *, $P < 0.05$, **, $P < 0.001$, t-test.

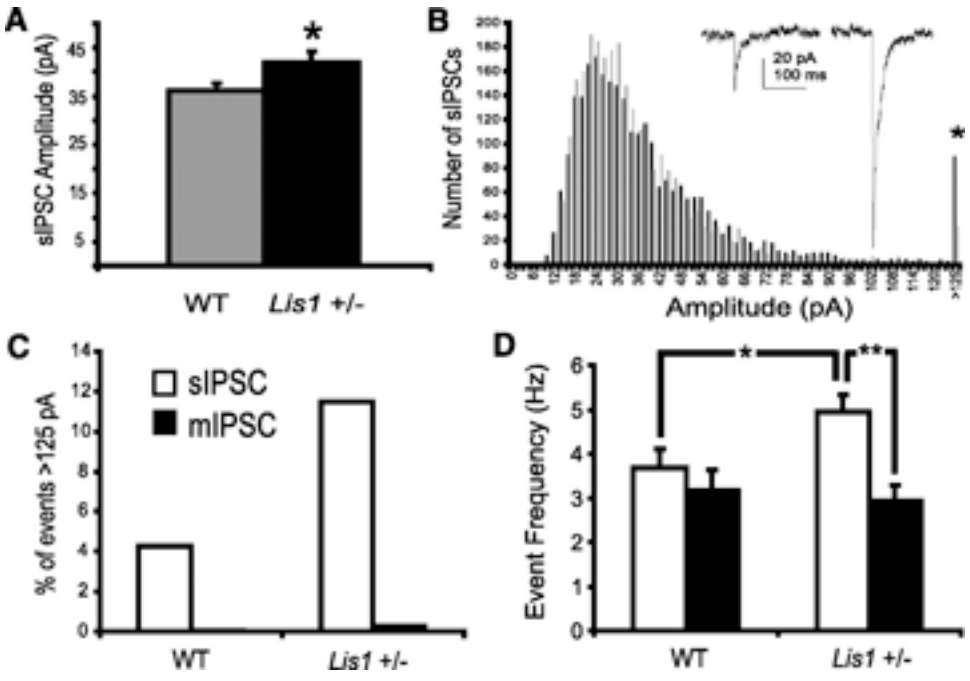

A
sIPSC Amplitude (pA)
45
35
25
15
5
*
WT
Lis1 +/-
B
Number of sIPSCs
20 pA
100 ms
*
Amplitude (pA)
C
% of events >125 pA
14
12
10
8
6
4
2
0
sIPSC
mIPSC
WT
Lis1 +/-
D
Event Frequency (Hz)
6
5
4
3
2
1
0
*
**
WT
Lis1 +/-

Figure 3. mIPSCs do not differ in pyramidal cells recorded from *Lis1*$^{+/-}$ mice as compared with WT mice.

A: sample traces of mIPSCs recorded from a WT cell. B: sample traces from a *Lis1*$^{+/-}$ cell. C: mean mIPSC frequency does not differ. Gray bars, WT; black bars, Lis1+/–. D: mean mIPSC decay time does not differ. E: mean mIPSC amplitude does not differ. F: interevent intervals are normal in *Lis1*$^{+/-}$ mice. G: distribution of decay times is normal. H: distribution of amplitudes is also normal.

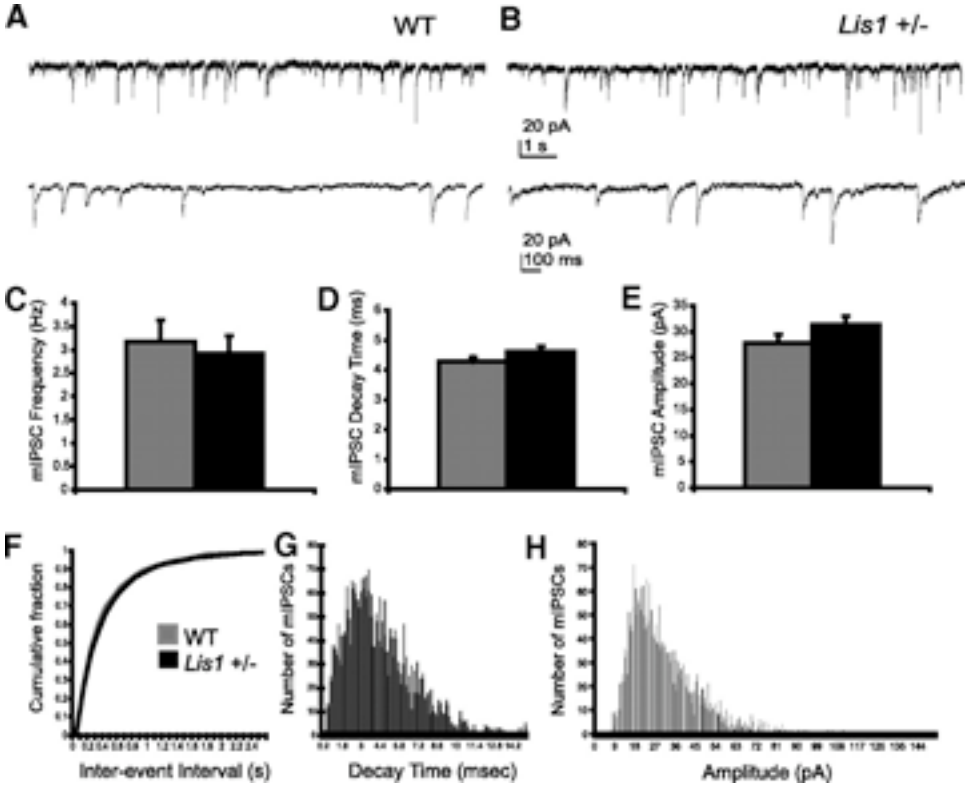

A
WT
B
Lis1 +/-
20 pA
1 s
20 pA
100 ms
C
mIPSC Frequency (Hz)
D
mIPSC Decay Time (ms)
E
mIPSC Amplitude (pA)
F
Cumulative fraction
WT
Lis1 +/-
Inter-event Interval (s)
G
Number of mIPSCs
Decay Time (msec)
H
Number of mIPSCs
Amplitude (pA)

Figure 4. Tonic GABAergic current amplitude is unchanged in Lis1+/– pyramidal cells.

A: representative trace of a WT pyramidal cell recorded in the presence of kynurenic acid to block glutamatergic transmission. After gabazine application, note the shift in holding current; this represents the tonic current amplitude. B: representative trace of a *Lis1*$^{+/–}$ pyramidal cell. C: percentage change in holding current after gabazine application is similar between WT and *Lis1*$^{+/–}$ mice.

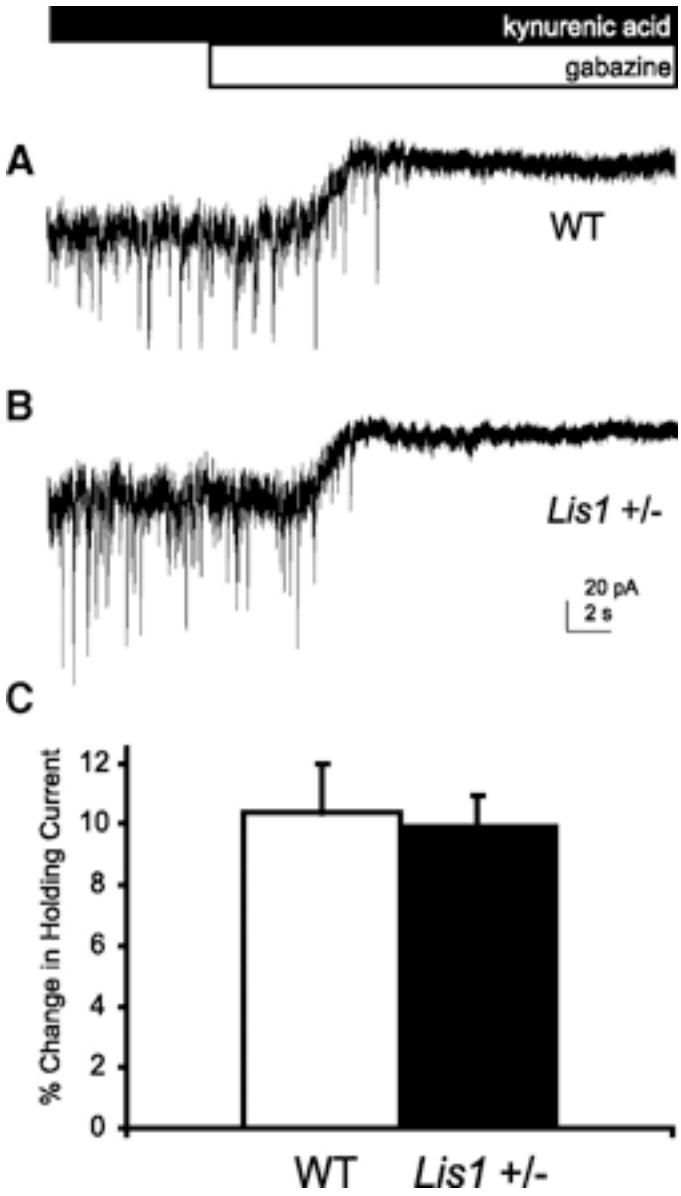

kynurenic acid
gabazine
A
WT
B
Lis1 +/-
20 pA
2 s
C
% Change in Holding Current
12
10
8
6
4
2
0
WT
Lis1 +/-

Figure 5. Evoked IPSCs and paired-pulse modulation appear normal in the *Lis1*$^{+/-}$ hippocampus.

A: representative traces from wild-type (left) and mutant (right) pyramidal cells. Top: traces from cells that underwent paired-pulse facilitation; bottom: from cells that underwent paired-pulse depression. Stimulus artifacts have been removed for clarity; *, time of stimulus. Traces are averaged from 3 trials, and the interstimulus interval is 100 ms. B: summary of paired-pulse modulation at interpulse intervals of 40, 100, 400, 500, and 700 ms. Data are plotted as the mean amplitude of the response to the 2nd stimulus, expressed as a percentage of the amplitude of the response to the 1st stimulus. No significant differences were observed between WT and *Lis1*$^{+/-}$ animals. C: breakdown of different paired-pulse modulation types observed at an interstimulus interval of 100 ms. PPR >1 signifies cells with a paired-pulse ratio greater than one, indicating paired-pulse facilitation. PPR <1 indicates paired-pulse depression, whereas PPR = 1 indicates an absence of modulation. D: decay time constant of evoked IPSCs is similar between WT and *Lis1*$^{+/-}$ mice.

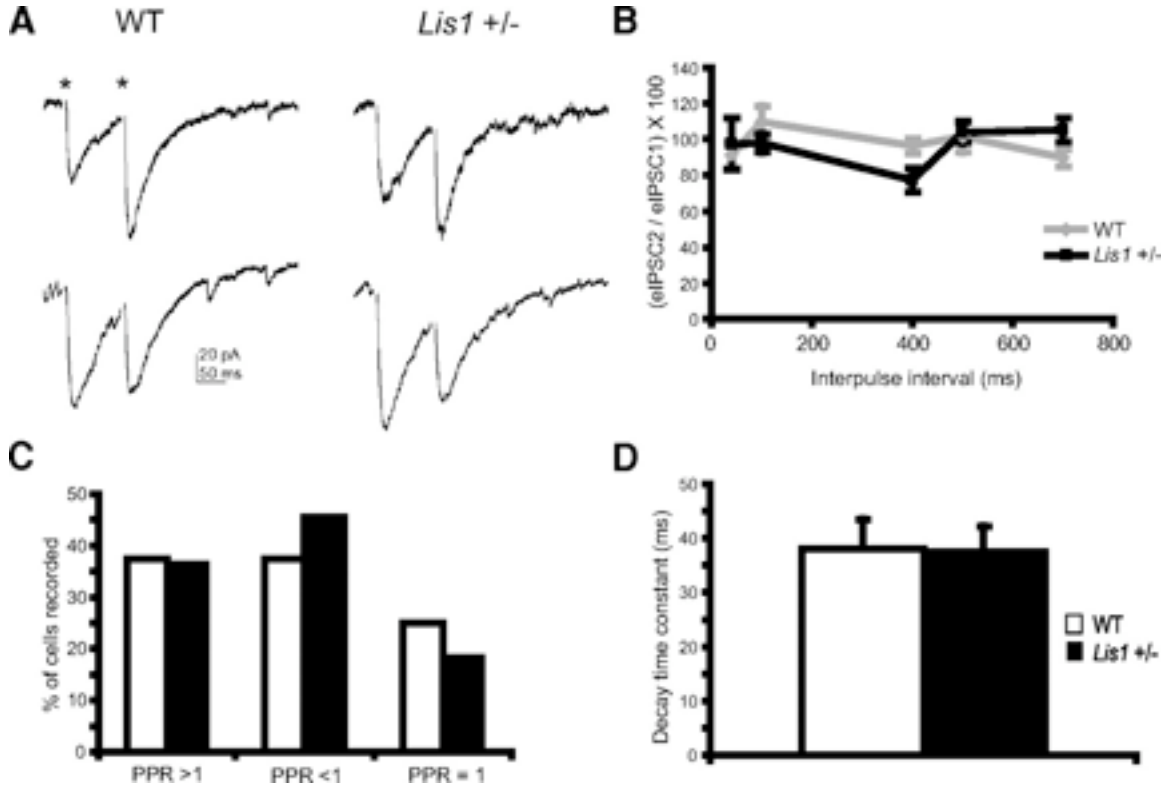

A
WT
Lis1 +/-
20 pA
50 ms
B
(eIPSC2 / eIPSC1) X 100
Interpulse interval (ms)
WT
Lis1 +/-
C
% of cells recorded
PPR >1
PPR <1
PPR = 1
D
Decay time constant (ms)
WT
Lis1 +/-

Figure 6. Interneurons in the *Lis1*$^{+/-}$ hippocampus exhibit increased spontaneous firing.

A, top: representative cell-attached recording from a WT regular-spiking nonpyramidal (RSNP) interneuron demonstrating a lack of spontaneous firing. Bottom: same cell recorded in current-clamp mode after gaining whole cell access to this interneuron. B, top: representative cell-attached recording from a *Lis1*$^{+/-}$ RSNP interneuron demonstrating a high rate of spontaneous firing. Bottom: same cell recorded in current-clamp mode after gaining whole cell access to this interneuron. C: percentage of interneurons that fire in *Lis1*$^{+/-}$ mice is greatly increased ($P < 0.001$, χ^2 test). White, WT; Black, *Lis1*$^{+/-}$. D: mean firing rate of interneurons is increased in *Lis1*$^{+/-}$ mice with a rate of 0 Hz assigned to all nonfiring cells ($P < 0.05$, Student's t-test). Range of firing rates for cells that did fire: WT: 0.11–2.29 Hz; *Lis1*$^{+/-}$: 0.01–5.52 Hz.

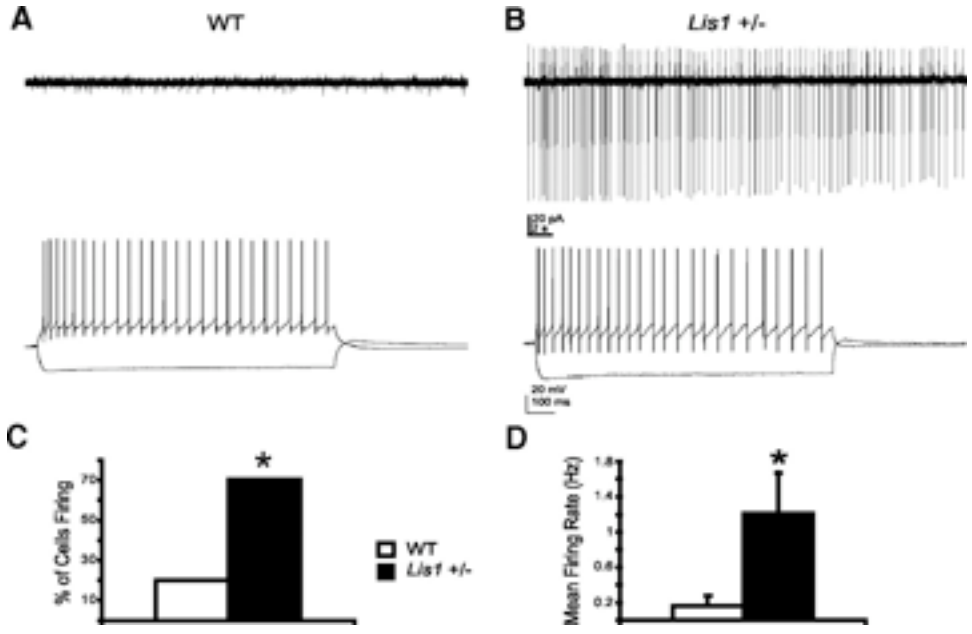

A
WT
B
Lis1 +/-
C
% of Cells Firing
WT
Lis1 +/-
D
Mean Firing Rate (Hz)

Figure 7. Excitatory drive of interneurons is increased in *Lis1*$^{+/-}$ mice.

A, top: representative whole cell voltage-clamp recording of sEPSCs on a WT interneuron. Bottom: same cell recorded in current-clamp mode. B, top: representative sEPSC recording from a *Lis1*$^{+/-}$ interneuron. Bottom: same cell recorded in current-clamp mode. C: mean sEPSC frequency is increased in *Lis1*$^{+/-}$ interneurons ($P < 0.05$, Student's t-test). White, WT; Black, *Lis1*$^{+/-}$. D: mean sEPSC amplitude is similar between WT and mutant mice. E: mean sEPSC decay time is similar between WT and mutant mice.

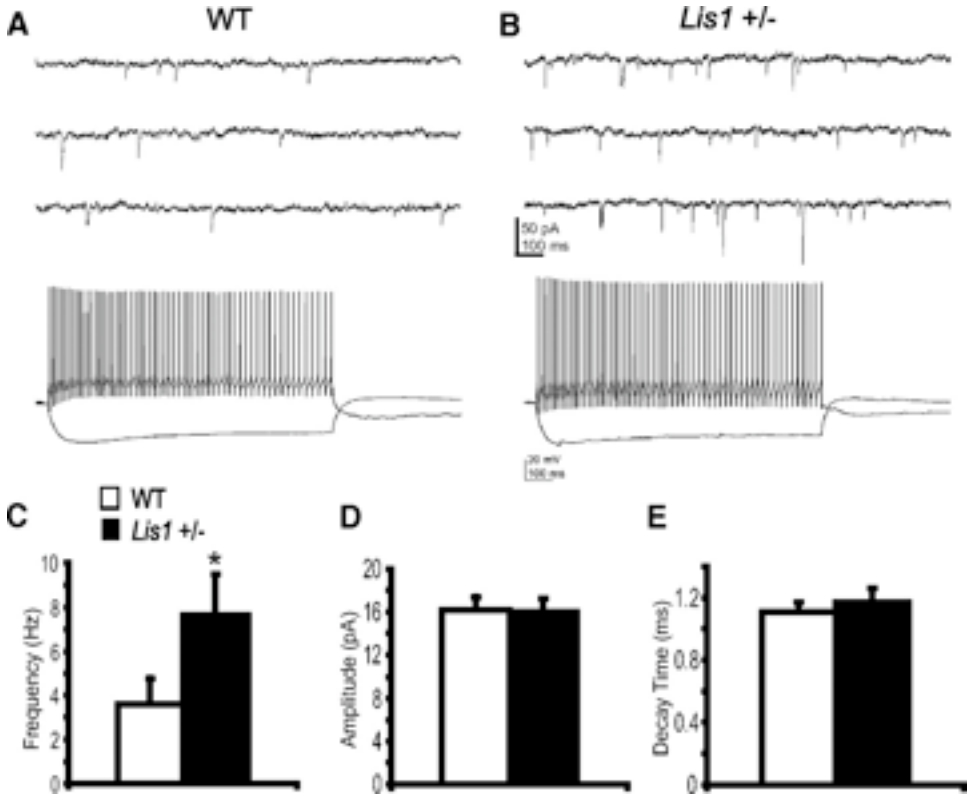

A
WT
B
Lis1 +/-
50 pA
100 ms
C
WT
Lis1 +/-
Frequency (Hz)
D
Amplitude (pA)
E
Decay Time (ms)

Figure 8. Parvalbumin-positive interneurons exhibit an altered distribution in *Lis1* mutant mice.

A, E, I, Hippocampal sections were divided into 400 μm by 50 μm bins (I, inset), and immunoreactive somata were counted in each bin (A, calretinin [CR]; E, parvalbumin [PV]; I, somatostatin [SOM]). White bars indicate % of WT cells in a given bin; red bars indicate $Lis1^{+/-}$ cells. The overall distributions of CR and SOM cells appear normal, while PV cells are shifted from stratum oriens (O) into stratum radiatum and stratum lacunosum-molecuare (R/LM) ($P < 0.001$, χ^2 test), with no change in the pyramidal cell layer (P). B, F, J, Bins are grouped together to represent hippocampal layers. C, D, WT and $Lis1^{+/-}$ section, respectively, stained for CR and focused on area CA1. G, H, WT and $Lis1^{+/-}$ section, respectively, stained for PV and focused on area CA1. K, L, WT and $Lis1^{+/-}$ section, respectively, stained for SOM and focused on area CA1.

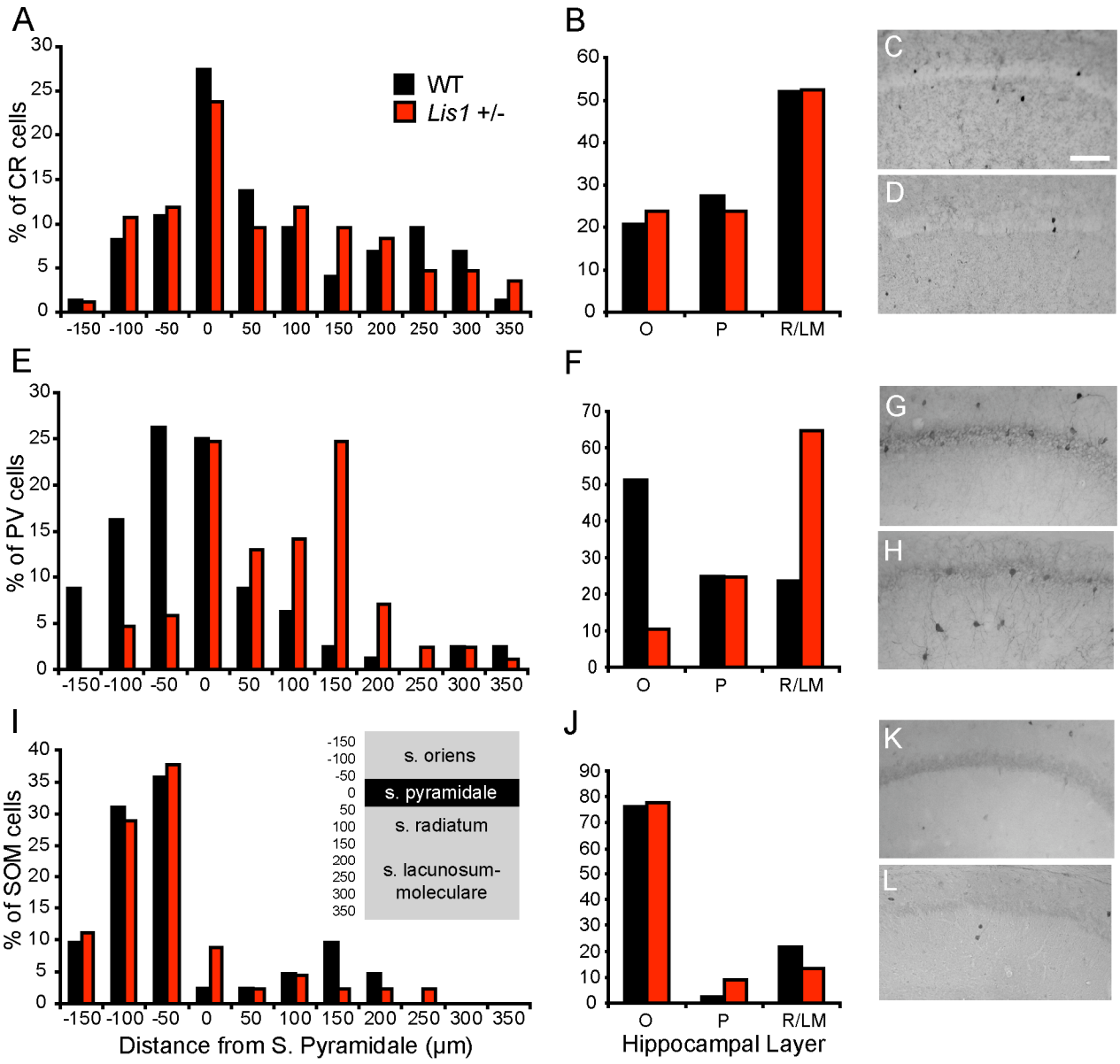

A
% of CR cells
WT
Lis1 +/-
-150 -100 -50 0 50 100 150 200 250 300 350
B
O P R/LM
C
D
E
% of PV cells
F
G
H
I
% of SOM cells
s. oriens
s. pyramidale
s. radiatum
s. lacunosum-moleculare
Distance from S. Pyramidale (µm)
J
Hippocampal Layer
K
L

Figure 9. *Lis1*$^{+/-}$ fast-spiking interneurons exhibit an altered distribution.

A, Sample trace from a WT RSNP interneuron. B, *Lis1*$^{+/-}$ RSNP interneuron. C, WT FS interneuron. D, *Lis1*$^{+/-}$ FS interneuron. E, WT BST interneuron. F, *Lis1*$^{+/-}$ BST interneuron. G, The relative proportions of each subtype encountered were similar between WT and *Lis1*$^{+/-}$ animals. Black bars: WT cells. Red bars: *Lis1*$^{+/-}$ cells. H, The layer distribution of RSNP cells recorded was normal in mutant mice. I, The layer distribution of FS cells was abnormal in *Lis1*$^{+/-}$ mice, with cells shifted from stratum oriens (O) into stratum radiatum and stratum lacunosum-moleculare (R/LM) ($p < 0.001$, χ^2 test).

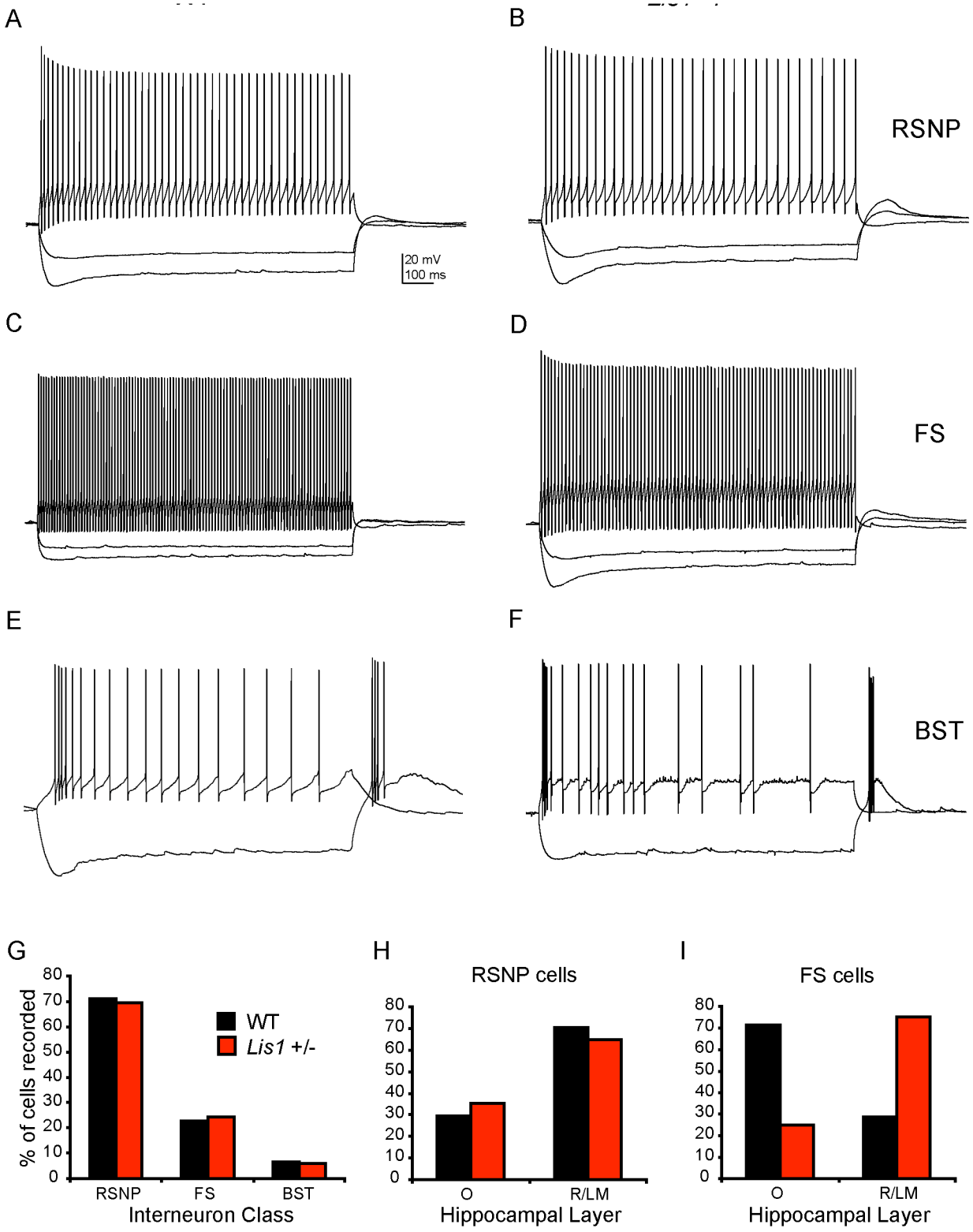

A
B
RSNP
20 mV
100 ms
C
D
FS
E
F
BST
G
% of cells recorded
WT
Lis1 +/-
RSNP
FS
BST
Interneuron Class
H
RSNP cells
O
R/LM
Hippocampal Layer
I
FS cells
O
R/LM
Hippocampal Layer

Figure 10. sIPSC frequency is increased in *Lis1*$^{+/-}$ SO interneurons.

A, Upper trace: sIPSC recording from a WT SO interneuron; Lower trace: current clamp recording from the same interneuron. B, Upper trace: sIPSC recording from a *Lis1*$^{+/-}$ SO interneuron showing an increased event frequency; Lower trace: current clamp recording from the same interneuron. C, Frequency of sIPSCs on SO interneurons is significantly increased in *Lis1*$^{+/-}$ mutant mice ($p = 0.01$, Student's t test). D, Amplitude of sIPSCs is comparable between WT and *Lis1*$^{+/-}$ SO interneurons. E, Decay time of sIPSCs is also unchanged in *Lis1*$^{+/-}$ SO interneurons. Black bars: WT. Red bars: *Lis1*$^{+/-}$.

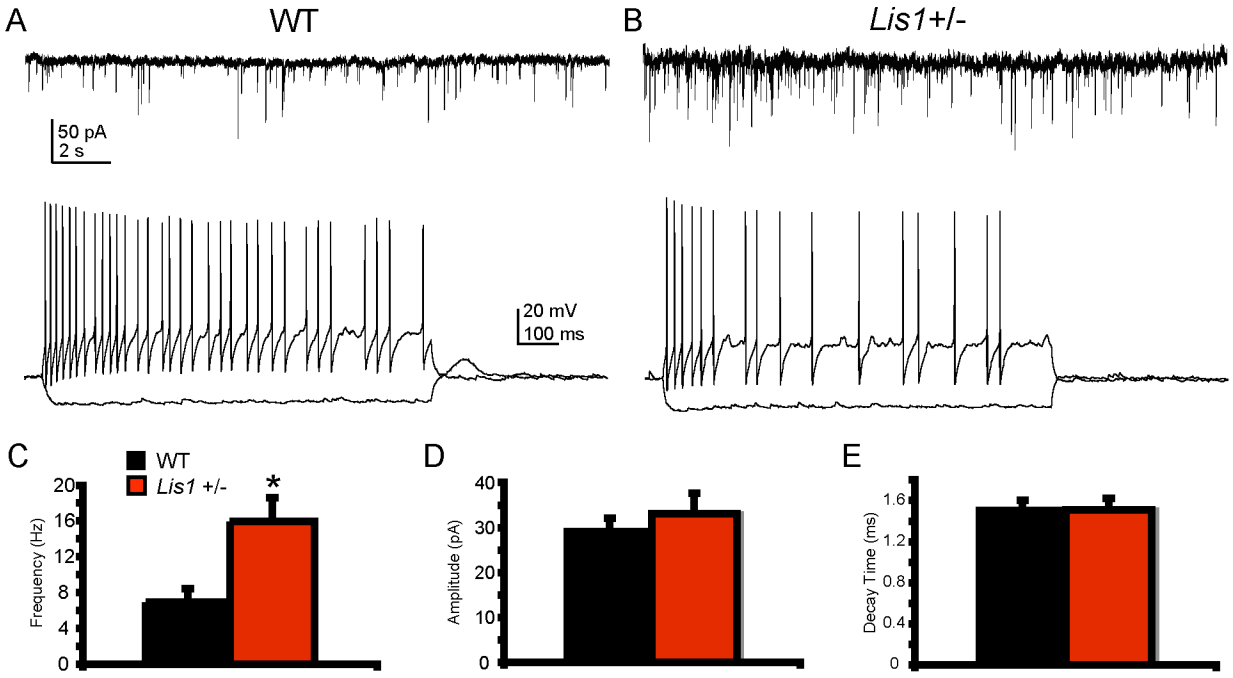

A
WT
B
Lis1+/-
50 pA
2 s
20 mV
100 ms
C
WT
Lis1 +/-
*
Frequency (Hz)
20
16
12
8
4
0
D
Amplitude (pA)
40
30
20
10
0
E
Decay Time (ms)
1.6
1.2
0.8
0.4
0

Figure 11 mIPSC amplitude is decreased in *Lis1*$^{+/-}$ SO interneurons.

A, Upper trace: mIPSC recording from a WT SO interneuron; Lower trace: current clamp recording from the same interneuron. B, Upper trace: mIPSC recording from a *Lis1*$^{+/-}$ SO interneuron showing decreased mean mIPSC amplitude; Lower trace: current clamp recording from the same interneuron. C, Frequency of mIPSCs on SO interneurons is not significantly different in WT and *Lis1*$^{+/-}$ mutant mice. D, Amplitude of mIPSCs is significantly reduced in *Lis1*$^{+/-}$ SO interneurons ($p = 0.04$, Student's *t* test). E, Amplitude histogram showing a leftward shift toward smaller mIPSC amplitudes in *Lis1* mutant animals. F, Decay time of mIPSCs is unchanged in *Lis1*$^{+/-}$ SO interneurons. Black bars: WT. Red bars: *Lis1*$^{+/-}$.

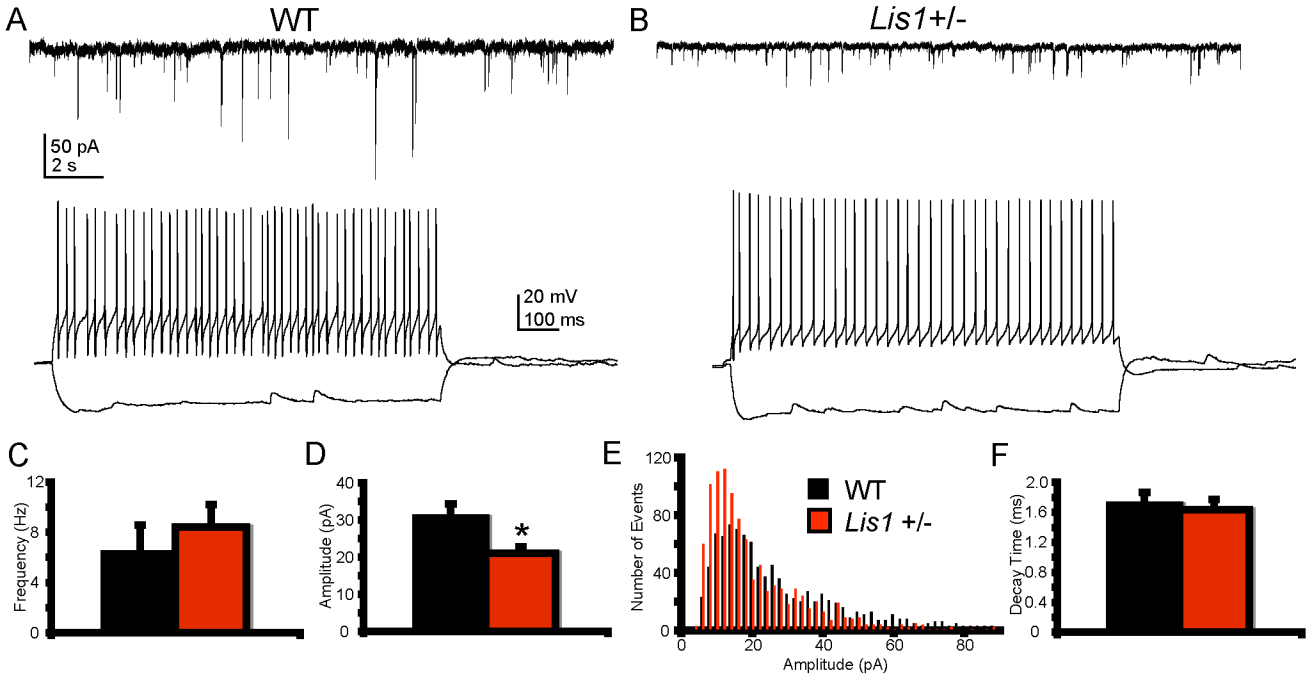

A
WT
B
Lis1+/-
50 pA
2 s
20 mV
100 ms
C
Frequency (Hz)
12
8
4
0
D
Amplitude (pA)
40
30
20
10
0
*
E
Number of Events
120
80
40
0
0
20
40
60
80
Amplitude (pA)
WT
Lis1 +/-
F
Decay Time (ms)
2.0
1.6
1.2
0.8
0.4
0

Figure 12. sIPSC decay time constant is increased in *Lis1*$^{+/-}$ R/LM interneurons.

A, Upper trace: sIPSC recording from a WT R/LM interneuron; Lower trace: current clamp recording from the same interneuron. B, Upper trace: sIPSC recording from a *Lis1*$^{+/-}$ R/LM interneuron; Lower trace: current clamp recording from the same interneuron. C, Frequency of sIPSCs on R/LM interneurons not significantly different between WT and *Lis1*$^{+/-}$ mutant mice. D, Amplitude of sIPSCs is unchanged in *Lis1*$^{+/-}$ R/LM interneurons. E, Decay time of sIPSCs is not significantly different in *Lis1*$^{+/-}$ R/LM interneurons, although there is a trend toward longer decay time in mutant animals. F, Averaged sIPSC traces from representative WT (black) and mutant (red) interneurons showing lengthened decay in *Lis1* mutants. G, Decay time constant is significantly increased in *Lis1* mutant R/LM interneurons ($p = 0.03$, Student's *t* test). H, Decay time histogram showing a trend toward longer decay events in *Lis1*$^{+/-}$ R/LM interneurons. Black bars: WT. Red bars: *Lis1*$^{+/-}$.

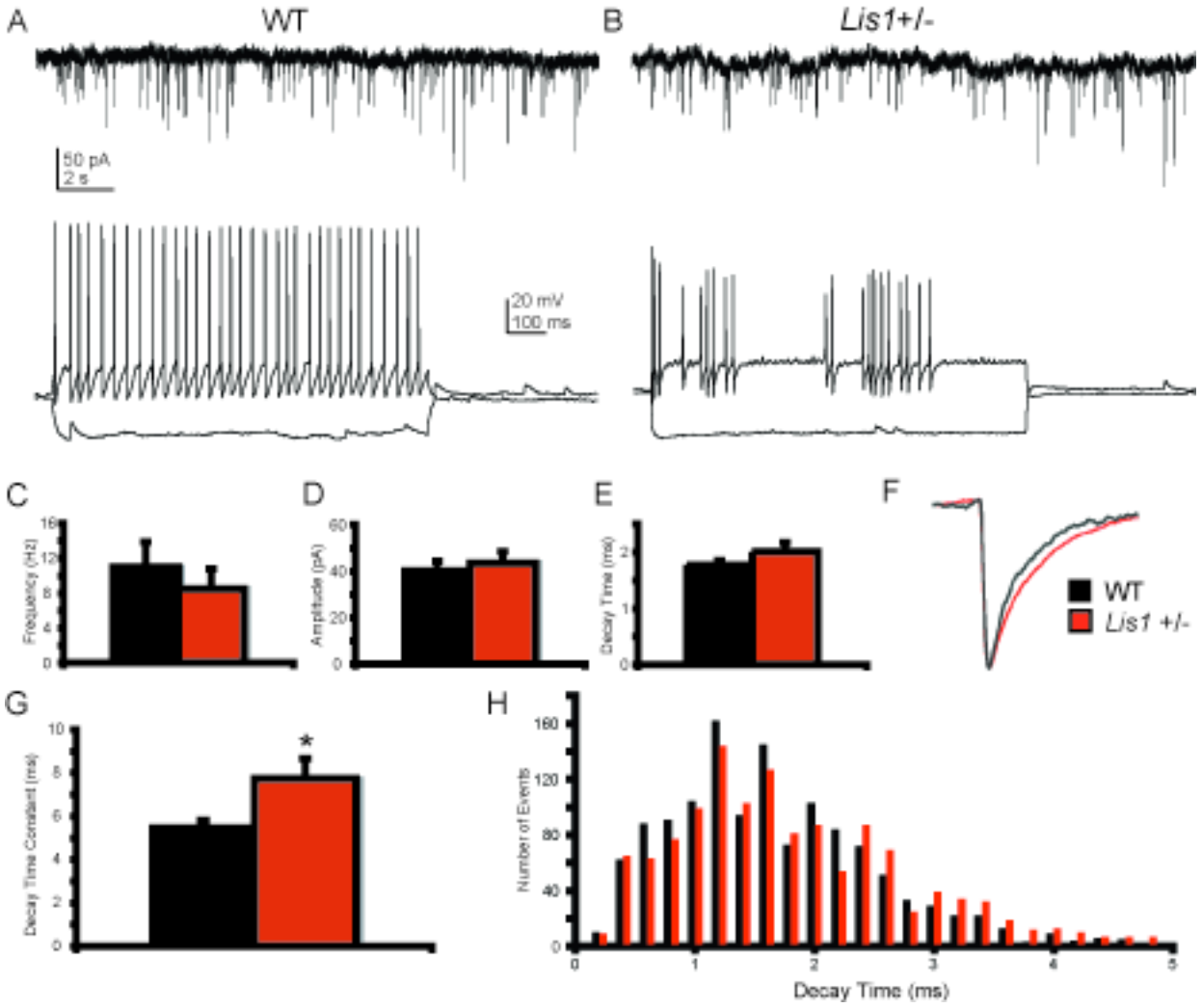
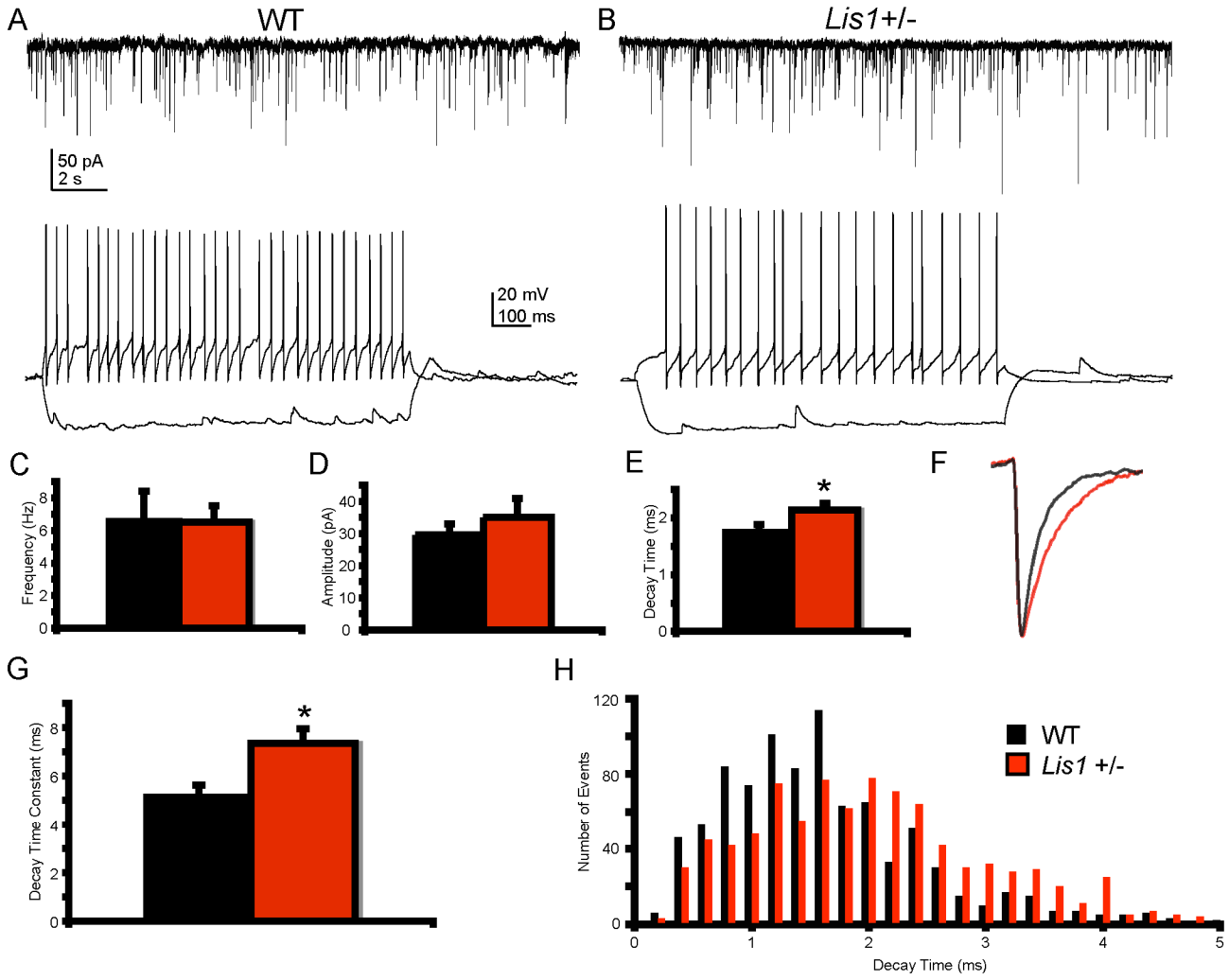

A
WT
B
Lis1+/-
50 pA
2 s
20 mV
100 ms
C
Frequency (Hz)
D
Amplitude (pA)
E
Decay Time (ms)
F
WT
Lis1 +/-
G
Decay Time Constant (ms)
*
H
Number of Events
Decay Time (ms)

Figure 13. mIPSC decay time and decay time constant are increased in *Lis1*$^{+/-}$ R/LM interneurons.

A, Upper trace: mIPSC recording from a WT R/LM interneuron; Lower trace: current clamp recording from the same interneuron. B, Upper trace: mIPSC recording from a *Lis1*$^{+/-}$ R/LM interneuron; Lower trace: current clamp recording from the same interneuron. C, Frequency of mIPSCs on R/LM interneurons not significantly different between WT and *Lis1*$^{+/-}$ mutant mice. D, Amplitude of mIPSCs is unchanged in *Lis1*$^{+/-}$ R/LM interneurons. E, Decay time of mIPSCs is significantly different in *Lis1*$^{+/-}$ R/LM interneurons ($p = 0.04$, Student's *t* test). F, Averaged mIPSC traces from representative WT (black) and mutant (red) interneurons showing lengthened decay in *Lis1* mutants. G, Decay time constant is significantly increased in *Lis1* mutant R/LM interneurons ($p = 0.01$, Student's *t* test). H, Decay time histogram showing a significant shift toward longer decay events in *Lis1*$^{+/-}$ R/LM interneurons. Black bars: WT. Red bars: *Lis1*$^{+/-}$.


A
WT
B
Lis1+/-
50 pA
2 s
20 mV
100 ms
C
Frequency (Hz)
D
Amplitude (pA)
E
Decay Time (ms)
*
F
G
Decay Time Constant (ms)
*
H
Number of Events
WT
Lis1 +/-
Decay Time (ms)

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Chapter III: Reorganization of hippocampal circuitry after interneuron death in mice lacking *Dlx1*

Introduction

Inhibitory interneurons, which release the neurotransmitter γ-aminobutyric acid (GABA), are vital in maintaining appropriate levels of glutamate-mediated synaptic excitation in the brain. Cortical and hippocampal interneurons arise in three regions of the embryonic subpallium: the medial, caudal, and lateral ganglionic eminences (MGE, CGE, and LGE, respectively). Over a period of days, newly generated interneuron progenitor cells migrate tangentially into the developing cortex and hippocampus, where they eventually integrate into local circuits (Marin and Rubenstein 2001).

Members of the *Dlx* family of transcription factors are expressed by developing hippocampal and cortical interneurons, and are important in tangential migration of interneuron precursors during embryonic development. Tangential migration of cortical and hippocampal interneurons is eliminated in mice that lack both *Dlx1* and *Dlx2* (Anderson et al. 1997). *Dlx1* and *Dlx2* normally inhibit a Rho GTPase effector, PAK3; this action supports neuronal migration by impeding neurite growth (Cobos et al. 2007). However, *Dlx* transcription factors also play important roles in differentiation and maturation of interneurons (Cobos et al. 2005).

Mice that lack *Dlx1* (*Dlx1*$^{-/-}$ mice) lose approximately 20-30% of the CR- and SOM-expressing interneuron populations in cortex and hippocampus by approximately one month of age. This modest decrease in interneuron numbers coincides with a drastic reduction in GABAergic synaptic transmission (Cobos et al. 2005). The reductions in IPSC amplitude and frequency are of a magnitude much higher than would be expected given that relatively few dendrite-innervating interneurons undergo apoptosis, with soma-targeting basket cells present in normal numbers. Intriguingly, while primarily dendrite-

innervating interneurons are lost in *Dlx1*$^{-/-}$ mice, IPSC recordings showed a dramatic decrease in large-amplitude events likely to be arriving at or near the soma. This raises the possibility that *Dlx1* expression may be important for the function of other *Dlx1*-negative interneurons, such as the soma-targeting basket cells, even though these cells are present in normal numbers in *Dlx1*$^{-/-}$ mice.

We hypothesized that interneurons lacking *Dlx1* would exhibit alterations in intrinsic membrane properties, e.g. reduced input resistance, which would contribute to the drastic reduction in GABAergic transmission. We also hypothesized that the large decrease in inhibition could be explained by a decrease in excitatory drive to surviving *Dlx1*$^{-/-}$ interneurons. To test these hypotheses, we recorded from hippocampal interneurons in WT and *Dlx1*$^{-/-}$ littermates at two ages: P9-12, before interneuron apoptosis, and >P30, after significant loss of interneurons has occurred.

Results

Interneurons in P9-12 Dlx1 knockout mice

In the first three postnatal weeks, before apoptosis occurs, interneuron numbers are normal in *Dlx1*$^{-/-}$ mice (Cobos et al. 2005). To determine whether *Dlx1*$^{-/-}$ interneurons at these young ages exhibit normal membrane properties, we obtained current-clamp recordings from 37 WT and 35 *Dlx1*$^{-/-}$ interneurons in hippocampal area CA1, recorded at P9-12. We injected steps of depolarizing and hyperpolarizing current to classify interneurons according to their firing properties: fast-spiking (FS), regular-spiking nonpyramidal (RSNP), and burst-spiking (BST). We also used these data to determine spike width, input resistance (R_{in}), resting membrane potential (V_m), and fast afterhyperpolarization amplitude (fAHP) for each interneuron, to ascertain whether intrinsic membrane properties and firing properties of *Dlx1*$^{-/-}$ interneurons are normal (Butt et al. 2005). We used a combination of firing property parameters to determine cell type (Butt et al. 2005); after classifying interneurons according to firing properties, we sorted cells by their laminar location, and also compared relative proportions of interneuron subtypes between WT and mutant animals.

In these experiments, we determined that the relative proportions of interneuron subtypes that could be sampled with a patch-clamp recording electrode were comparable between WT and *Dlx1*$^{-/-}$ mice at P9-12 (Figure 1C). In WT animals, 11% of interneurons recorded were FS cells (n=4), 84% were RSNP cells (n=31), and 5% were BST cells (n=2). In *Dlx1* knockout animals, 11% of interneurons recorded were FS cells (n=4), 83% were RSNP cells (n=29), and 6% were BST cells (n=2). We also found that the laminar distributions of interneuron cell bodies were also comparable between WT and *Dlx1*

knockout mice (Figure 1D,E). All FS cells in WT and knockout mice were located in stratum oriens and alveus (O/A). In addition, most RSNP interneurons were located in stratum radiatum and stratum lacunosum-moleculare (R/LM; WT: 29% in O/A, 71% in R/LM; *Dlx1*$^{-/-}$: 28% in OA, 72% in R/LM). We did not encounter enough BST interneurons in order to make a valid comparison of their distribution across hippocampal layers.

Interestingly, in current clamp recordings, we found that P9-12 *Dlx1*$^{-/-}$ interneurons exhibit immature intrinsic membrane properties (Figure 1A,B and Table 1). Specifically, *Dlx1* knockout interneurons exhibit an increased input resistance, which indicates that interneurons lacking *Dlx1* are immature as compared to age-matched WT interneurons (WT R_{in}: 291.35 ± 18.13 MΩ; *Dlx1*$^{-/-}$ R_{in}: 359.16 ± 27.99 MΩ; $p < 0.05$). Consistent with an increased input resistance, *Dlx1* knockout interneurons at this age also exhibit a relatively depolarized resting membrane potential (WT V_m: -56.6 ± 1.0 mV; *Dlx1*$^{-/-}$ V_m: -51.6 ± 1.3 mV; $p < 0.05$). These data demonstrate, for the first time, that *Dlx1* expression is important in maintaining the normal functional properties of interneurons.

Interneurons in >P30 Dlx1 knockout mice

We repeated these experiments in mice older than P30 (Figure 2), when significant interneuron loss has occurred in the SOM and CR expressing subpopulations of *Dlx1*$^{-/-}$ mice (Cobos et al. 2005). At this age, input resistance was comparable between WT and *Dlx1*$^{-/-}$ mice (WT R_{in}: 187.44 ± 13.25 MΩ; *Dlx1*$^{-/-}$ R_{in}: 212.34 ± 16.47 MΩ; $p = 0.24$), as was their resting membrane potential (WT V_m: -58.5 ± 1.2 mV; *Dlx1*$^{-/-}$ V_m: -

56.8 ± 1.4 mV; $p = 0.35$). All other membrane and firing properties were also normal in *Dlx1* mutant interneurons at >P30 (Table 2). The laminar distribution of interneuron subtypes was also similar between WT and knockout animals (Figure 2D,E). FS cells were primarily located in O/A in both WT and knockout animals (WT: 87.5% in O/A, 12.5% in R/LM; $Dlx1^{-/-}$: 83% in OA, 17% in R/LM); in addition, most RSNP cells were located in R/LM in both WT and knockout animals (WT: 17% in O/A, 83% in R/LM; $Dlx1^{-/-}$: 25% in OA, 75% in R/LM). However, the proportion of BST interneurons encountered in $Dlx1^{-/-}$ mice (7%) was half of the proportion observed in WT mice (14%; Figure 2C). Because BST interneurons are known to express SOM (Markram et al. 2004), these electrophysiological results are consistent with anatomical results from >P30 $Dlx1^{-/-}$ mice, which show significant reductions in the numbers of SOM-expressing interneurons at this age.

Decreased excitatory drive of >P30 interneurons in Dlx1 knockout mice

As a first step in determining why adult $Dlx1^{-/-}$ mice exhibit reduced inhibition with only a modest decrease in interneuron numbers, we recorded spontaneous excitatory postsynaptic currents (sEPSCs) onto 37 CA1 interneurons in >P30 mice (Figure 3). We hypothesized that a reduction in the excitatory input to surviving interneurons might contribute to the inhibitory defect previously reported in these mice (Cobos et al. 2005). For all sEPSC experiments, we obtained voltage clamp recordings at a holding potential of -60 mV and at a temperature of 32 °C. Consistent with our hypothesis, we found that excitatory drive of interneurons in $Dlx1^{-/-}$ mice is significantly reduced. Specifically, mean sEPSC amplitude was reduced by approximately 25% in $Dlx1^{-/-}$ interneurons (WT:

16.87 ± 1.45 pA; *Dlx1*$^{-/-}$: 12.80 ± 1.18 pA; $p < 0.05$), while the frequency (WT: 11.35 ± 2.23 Hz; *Dlx1*$^{-/-}$: 10.77 ± 3.46 Hz; $p = 0.89$) and decay time (WT: 0.86 ± 0.08 ms; *Dlx1*$^{-/-}$: 0.94 ± 0.08 ms; $p = 0.49$) of sEPSCs were unchanged (Figure 3). These data support the hypothesis that inhibition is drastically reduced in *Dlx1*$^{-/-}$ mice due to a combination of factors, rather than being due solely to a modest reduction in interneuron numbers. These results also indicate that there may be a reduction in glutamate receptor number at excitatory synapses onto interneurons.

Excitatory drive of P9-12 interneurons in Dlx1 knockout mice

The reduction in excitatory input to interneurons in adult *Dlx1* knockout mice is consistent with the reduced inhibitory output of these cells previously reported (Cobos et al. 2005). However, before interneurons undergo apoptosis, inhibition is normal in *Dlx1*$^{-/-}$ mice. We hypothesized that, at young ages, excitatory input to these interneurons would also be comparable to that of WT interneurons. To test this hypothesis, we recorded sEPSCs onto 46 CA1 interneurons in P9-12 WT and *Dlx1*$^{-/-}$ mice (Figure 4). At this age, we observed no significant differences in sEPSC amplitude (WT: 16.89 ± 2.01 pA; *Dlx1*$^{-/-}$: 15.35 ± 1.57 pA; $p = 0.55$, Figure 4E), or in any other parameter of sEPSCs, including frequency (WT: 2.94 ± 0.69 Hz; *Dlx1*$^{-/-}$: 3.25 ± 0.81 Hz; $p = 0.77$, Figure 4C) and decay time (WT: 1.12 ± 0.08 ms; *Dlx1*$^{-/-}$: 1.09 ± 0.07 ms; $p = 0.78$, Figure 4D). These data are consistent with the observation that inhibitory output is normal in *Dlx1* knockout interneurons at ages prior to the onset of interneuron apoptosis. Interestingly, a comparison of sEPSC amplitudes across ages but within genotypes shows that sEPSC amplitude appears to decrease as *Dlx1*$^{-/-}$ mice approach adulthood (*Dlx1*$^{-/-}$ P9-12: 15.35

± 1.57 pA; *Dlx1*$^{-/-}$ >P30: 12.80 ± 1.18 pA; $p = 0.20$), while in WT animals, no change occurs from P9-12 to P30 and older (WT P9-12: 16.89 ± 2.01 pA; WT >P30: 16.87 ± 1.45 pA). This indicates that glutamate receptors may initially be present in normal numbers in *Dlx1*$^{-/-}$ interneurons, with removal of receptors occurring throughout the first month of development. However, this trend does not reach statistical significance ($p = 0.20$), so further studies are required to determine whether or not this is the case.

Decreased excitatory drive of pyramidal neurons in Dlx1 knockout mice

In >P30 mice lacking *Dlx1*, sEPSCs on CA1 interneurons are significantly reduced in amplitude, suggesting that glutamate receptor number is reduced in these cells. We next sought to determine whether this reduction was selective for interneurons, or whether CA1 pyramidal cells would also exhibit diminished sEPSC amplitude in *Dlx1* knockout mice. We recorded sEPSCs from 23 CA1 pyramidal cells at a holding potential of -60 mV, and found that sEPSC amplitude was significantly reduced in *Dlx1*$^{-/-}$ mice (WT: 13.23 ± 2.63 pA; *Dlx1*$^{-/-}$: 7.84 ± 0.55 pA; $p < 0.05$, Figure 5D,F), as we found with interneurons. An amplitude histogram confirmed a leftward shift toward smaller events in *Dlx1* knockout mice. In addition, we observed a significant difference in sEPSC frequency (WT: 2.87 ± 0.56 Hz; *Dlx1*$^{-/-}$: 1.43 ± 0.27 Hz; $p < 0.05$, Figure 5C), and we also uncovered a significant reduction in mean decay time of sEPSCs in *Dlx1*$^{-/-}$ pyramidal neurons (WT: 2.15 ± 0.31 ms; *Dlx1*$^{-/-}$: 1.46 ± 0.10 ms; $p < 0.05$, Figure 5E,G). A histogram of sEPSC decay times showed an increase in the proportion of fast decay events in *Dlx1* knockout mice. These results indicate that *Dlx1*$^{-/-}$ pyramidal neurons, like interneurons, may exhibit a reduced number of synaptic glutamate receptors. However,

Dlx1$^{-/-}$ pyramidal neurons receive increased frequency of excitatory input, and they also may express different glutamate receptor subunits, as indicated by altered decay kinetics.

Discussion

In mice that lack *Dlx1*, we have uncovered significant alterations in excitatory circuits of the hippocampus. We also report evidence that *Dlx1* may be important in the functional maturation of interneurons. The glutamatergic circuit reorganizations that we have identified in *Dlx1* knockout mice are consistent with our hypothesis that the significant reduction in GABAergic transmission in $Dlx1^{-/-}$ mice is not simply a direct result of interneuron loss. These alterations also indicate that *Dlx1* may play additional, previously unknown roles in regulation or trafficking of synaptic glutamate receptors.

Excitatory drive of interneurons and reduced inhibition in Dlx1 knockout mice

Mice lacking *Dlx1* exhibit epilepsy and drastically reduced inhibition in cortex and hippocampus, and this reduction is greater than would be expected from modest interneuron loss alone. In addition, while the interneurons that undergo apoptosis are primarily dendrite-targeting, much of the inhibition that is lost in $Dlx1^{-/-}$ mice is thought to be mediated by soma-targeting cells, which are present in normal numbers. Furthermore, $Dlx1^{-/-}$ interneurons show reduced dendrite branching and length, suggesting that dendritic space available for excitatory synapses may be reduced. This led us to hypothesize that, in addition to affecting interneuron survival, loss of *Dlx1* would also affect excitatory inputs to interneurons. In agreement with our hypothesis, we found that glutamatergic drive of interneurons was depressed, in that mean sEPSC amplitude was significantly lower than in WT interneurons. This finding is consistent with a reduced number of glutamate receptors at excitatory synapses on interneurons. However, EPSC frequency was unchanged, suggesting that glutamatergic synapses are present in

normal numbers. One consequence of *Dlx1*$^{-/-}$ interneurons receiving less excitatory drive is that these surviving interneurons would be less active, leading to further reductions in GABA release, consistent with previous findings (Cobos et al. 2005). These results demonstrate for the first time that loss of *Dlx1* results in circuit reorganizations beyond loss of interneurons. The alterations we uncover here are consistent with those observed in other animals with epilepsy. For instance, in a rat model of cortical dysplasia, cortical interneurons receive reduced excitatory input, which contributes to a reduction in GABA release (Xiang et al. 2006).

Interestingly, when we repeated these experiments in *Dlx1*$^{-/-}$ mice at ages before interneuron apoptosis has occurred, we found no significant differences in glutamatergic input to interneurons. In agreement with these findings, synaptic inhibition in young *Dlx1*$^{-/-}$ mice is normal (Cobos et al. 2005) at an age when interneuron number and excitatory drive of interneurons are both at normal levels. Mice lacking *Dlx1* do not develop epilepsy until adulthood, following reductions in interneuron numbers and synaptic inhibition (Cobos et al. 2005). Taken together, these findings demonstrate that P9-12 neuronal circuitry in the cortex and hippocampus appears to be indistinguishable from wild-type circuits, suggesting that the synaptic modifications that we uncovered in adults might be responses to abnormal levels of excitability following interneuron loss.

Excitatory inputs to pyramidal neurons are altered in Dlx1 knockout mice

We found significant reductions in EPSC frequency, amplitude, and decay time in adult hippocampal pyramidal neurons lacking *Dlx1*. Because *Dlx1* is not expressed in pyramidal cells, it is interesting that excitatory neurons appear to exhibit alterations in

glutamate receptor number and subunit composition. Although these alterations cannot be a direct, cell-autonomous result of loss of *Dlx1* signaling, they could be linked to loss of interneurons and inhibition. For instance, loss of inhibition may trigger a compensatory response in pyramidal cells, reducing levels of excitation as an attempt to compensate for depressed inhibition. One way in which is this might occur is the removal of AMPA receptors from pyramidal cell synapses. Consistent with this hypothesis, we found that EPSC amplitude was reduced in pyramidal neurons. Excitation could also be dampened through removal of glutamatergic synapses. Accordingly, we uncovered a significant reduction in EPSC frequency on pyramidal neurons, which is consistent with a decrease in excitatory synapse number. This reduction in EPSC frequency could also result, in part, from reduced spontaneous firing of CA3 pyramidal cells. However, this is unlikely to be the case, as inhibition of CA3 pyramidal neurons is greatly reduced (Cobos et al. 2005); if there were an alteration in spontaneous firing of CA3 neurons, then, it would most likely be an increase. Additionally, less active CA3 pyramidal neurons would be inconsistent with the epilepsy phenotype of these animals. However, further experiments are needed to answer this question definitively.

Dlx1 and glutamate receptors

A depression in EPSC amplitude generally results from a reduction in postsynaptic glutamate receptor number (Katz 1962; Cormier and Kelly 1996). Because these recordings were obtained at a holding potential of -60 mV, at which NMDA receptors are blocked by Mg^{2+} ions, the currents that we obtained are mediated by AMPA receptors (Cormier and Kelly 1996). A postsynaptic modification in adult interneurons

and pyramidal cells, such as a reduction in AMPA receptor number, is thus likely to underlie the depression in excitatory current amplitude that we uncovered in adult *Dlx1*$^{-/-}$ animals. Postsynaptic modifications of synaptic AMPA receptors in *Dlx1*$^{-/-}$ neurons could be a response to abnormal levels of excitability in hippocampal circuits, or a direct result of removal of *Dlx1* signaling.

Dlx1 normally inhibits PAK3, an effector of Rho GTPases that modulates actin networks and neurite extension (Cobos et al. 2007). Interestingly, mice lacking PAK3 exhibit abnormalities in AMPA receptor properties (Boda et al. 2004). This supports the hypothesis that loss of *Dlx1* may directly lead to synaptic alterations. However, *Dlx1* expression in adult basket-type interneurons is extremely low, with only 5% of parvalbumin-positive interneurons expressing detectable levels of *Dlx1* (Cobos et al. 2005). In addition, pyramidal neurons do not express *Dlx1*, which also argues against the hypothesis that loss of *Dlx1* directly affects the number or properties of synaptic AMPA receptors. This lends support to the hypothesis that alterations in AMPA receptor number and composition are a response to abnormal levels of activity in hippocampal circuits. Consistent with this hypothesis, EPSC properties are normal in young *Dlx1* knockout mice, at ages before interneuron apoptosis and significant loss of synaptic inhibition.

Indeed, significant changes in neurotransmitter receptor number and subunits have been reported in a number of animal models of epilepsy. For example, $GABA_A$ receptor subunit composition is altered in mice with temporal lobe epilepsy (Poulter et al. 1999). Glutamate receptors also undergo reorganizations in epileptic circuits For instance, epileptiform activity in neuronal circuits early in life has been linked to extensive alterations in AMPA receptor subunit composition (Jiang et al. 2008).

Intrinsic properties of interneurons lacking Dlx1

Surviving *Dlx1*$^{-/-}$ interneurons in adult mice could have intrinsic properties that lead them to release less GABA onto pyramidal neurons. For instance, *Dlx1*$^{-/-}$ interneurons could have a more hyperpolarized resting membrane potential, or decreased input resistance, meaning that a given excitatory input would be less likely to lead to a spike, and thus GABA release, from the interneuron. To test this hypothesis, we obtained current-clamp recordings from >P30 *Dlx1* knockout interneurons, and we determined that their intrinsic membrane properties are comparable to those of WT interneurons. These results demonstrate that interneurons lacking *Dlx1* are not abnormally excitable, ruling out the possibility that this could be a potential contributor to further reductions in synaptic inhibition than would be expected from interneuron loss alone.

However, we repeated these experiments in younger mice, and found that P9-12 *Dlx1*$^{-/-}$ interneurons are abnormally excitable, in that resting membrane potential was relatively depolarized, while input resistance was increased. This is the first direct evidence that loss of *Dlx1* results in a functional defect in interneurons. However, as previously described, intrinsic properties of adult *Dlx1*$^{-/-}$ interneurons are normal. Two alternate possibilities might explain the fact that young *Dlx1*$^{-/-}$ interneurons appear abnormally immature, while adult *Dlx1*$^{-/-}$ interneurons are indistinguishable from WT interneurons. First, loss of *Dlx1* could result in slowed maturation of interneurons, with cells reaching full maturity by the time *Dlx1*$^{-/-}$ animals reach adulthood. Second, loss of *Dlx1* could lead to abnormal membrane properties only in those interneurons fated to die. These cells could skew the mean resting membrane potential and input resistance values

at P9-12, but by P30, those cells would have undergone apoptosis, leaving only normal interneurons to be recorded. Our data support the former hypothesis. The immature membrane properties that we observed in P9-12 *Dlx1*$^{-/-}$ interneurons were not limited to a subpopulation of cells that we recorded, but rather were present across all interneuron types (data not shown).

Abnormal excitability of young *Dlx1*$^{-/-}$ interneurons could result in functional reorganizations of hippocampal circuits. Interestingly, immature membrane properties of young *Dlx1* knockout interneurons might lead, in part, to the reduction in excitatory drive of interneurons that we uncovered. A depolarized resting membrane potential and increased input resistance would combine to significantly enhance excitability of young *Dlx1*$^{-/-}$ interneurons. With abnormally excitable interneurons present at young ages, AMPA receptors could be removed from glutamatergic synapses in *Dlx1*$^{-/-}$ interneurons as a compensatory response to increased excitability, as an attempt to prevent excessive GABA release during the first few postnatal weeks. However, as interneurons undergo apoptosis in the following weeks, the continued presence of this compensatory decrease in excitation may act in concert with reduced interneuron numbers, increasing the severity of the inhibition defect previously observed in *Dlx1*$^{-/-}$ mice (Cobos et al. 2005).

How loss of *Dlx1* might affect intrinsic membrane properties of young interneurons is unclear. *Dlx1* is known to exert influence over neurite extension during migration through regulation of cytoskelton-associated factors (Cobos et al. 2007), and adult interneurons that lack *Dlx1* display cell-autonomous reductions in total dendrite length and branch number (Cobos et al. 2005). Cell size (e.g., total surface area) is one determinant of input resistance; if interneurons lacking *Dlx1* are significantly smaller, this

may in part lead to the increased input resistance that we observed. However, adult *Dlx1*$^{-/-}$ interneurons exhibit normal membrane properties, indicating that reductions in dendrite length do not translate into appreciable reductions in input resistance. Whether or not dendrite length and branching are reduced in P9-12 *Dlx1*$^{-/-}$ interneurons, however, remains unknown. It is more likely that a lack of *Dlx1* affects developmental programs in interneurons that leads to general a slowing of maturation, although the mechanisms by which this might occur (e.g., through regulation of trophic factors) have not been identified.

Glutamatergic drive and intrinsic properties of hippocampal neurons in Dlx1 knockout mice

We uncovered significant reorganizations of excitatory circuits in the hippocampus of mice lacking *Dlx1*, and also found a novel role for *Dlx1* in the electrophysiological maturation of interneurons. The alterations in glutamatergic synapse number and function that we identified in hippocampal neurons are likely to be compensatory responses to loss of inhibition, and could also result in part from transient, increased excitability of young, P9-12 *Dlx1*$^{-/-}$ interneurons. Finally, loss of *Dlx1* could also directly affect glutamatergic synapse properties, including AMPA receptor number and subunit composition, although this appears to be an unlikely possibility. In conclusion, these findings confirm the hypothesis that loss of *Dlx1* results in reorganizations of synaptic transmission and hippocampal connectivity beyond limited loss of SOM and CR interneurons, and raises the possibility that *Dlx1* may play novel roles in the maturation and function of inhibitory interneurons.

Table 1. *Dlx1* knockout interneurons are functionally immature at P9-12.

	FF (2xT)	**Rin**	**fAHP1**	**AP width**	**Vm**
WT	15.08 ± 1.69	291.35 ± 18.13	19.50 ± 0.81	1.54 ± 0.04	-56.59 ± 0.95
Dlx1 KO	19.51 ± 2.02	**359.16 ± 27.99**	21.62 ± 1.28	1.67 ± 0.08	**-51.57 ± 1.25**

Shown are mean values ± SEM for firing frequency at twice the firing threshold, input resistance, amplitude of fast afterhyperpolarization, action potential width, and resting membrane potential. Recordings were obtained from hippocampal CA1 interneurons in P9-12 wild-type and mutant mice. Bold indicates $p < 0.05$, Student's t-test.

Table 2. *Dlx1* knockout interneurons at >P30 exhibit normal membrane and firing properties.

	FF (2xT)	Rin	fAHP1	AP width	Vm
WT	43.08 ± 8.96	187.44 ± 13.25	25.32 ± 1.55	1.05 ± 0.05	-58.50 ± 1.19
Dlx1 KO	43.14 ± 11.11	212.34 ± 16.47	28.01 ± 1.26	1.13 ± 0.07	-56.79 ± 1.38

All parameters tested were indistinguishable between WT and mutant mice (see Table 1 for abbrevation definitions).

Figure 1. Young *Dlx1*$^{-/-}$ interneurons exhibit immature membrane properties compared to young WT interneurons.

A, Sample current clamp recording from a WT RSNP interneuron. B, Sample trace from a *Dlx1*$^{-/-}$ RSNP interneuron. Note increased input resistance (dashed line). C, The overall proportions of interneuron subtypes encountered were comparable between WT and mutant mice. D, In both WT and *Dlx1*$^{-/-}$mice, all FS cells were found in stratum oriens/alveus (O/A). E, In both WT and *Dlx1*$^{-/-}$ mice, most RSNP cells were recorded in stratum radiatum and stratum lacunosum-moleculare (R/LM). Red bars: WT. Black bars: *Dlx1*$^{-/-}$.

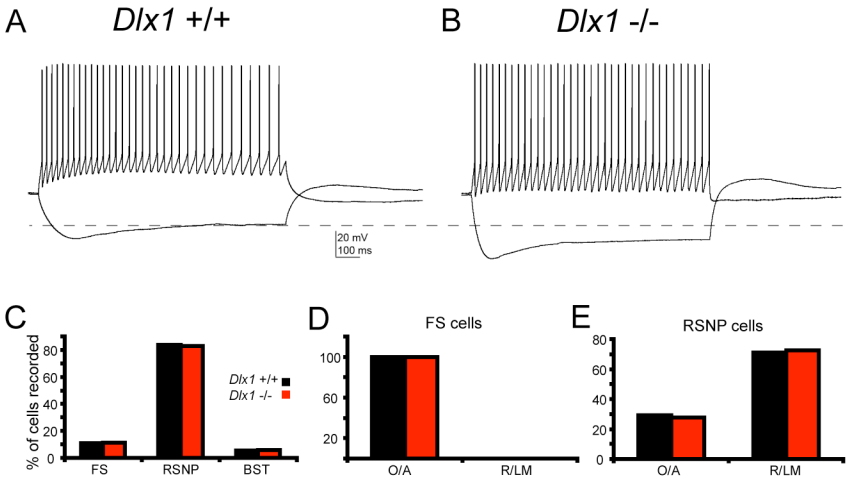

A
Dlx1 +/+
B
Dlx1 -/-
20 mV
100 ms
C
% of cells recorded
80
60
40
20
0
FS
RSNP
BST
Dlx1 +/+
Dlx1 -/-
D
FS cells
100
60
20
O/A
R/LM
E
RSNP cells
80
60
40
20
0
O/A
R/LM

Figure 2. Adult *Dlx1*$^{-/-}$ interneurons are indistinguishable from WT interneurons. A, Sample traces from WT FS, RSNP, and BST interneurons. B, Sample traces from *Dlx1*$^{-/-}$ FS, RSNP, and BST interneurons, showing that *Dlx1*$^{-/-}$ interneurons older than P30 exhibit normal membrane properties. C, The proportion of BST interneurons recorded in *Dlx1*$^{-/-}$ mice was approximately half of the proprtion observed in WT mice (WT: 14% of interneurons recorded were BST cells; *Dlx1*$^{-/-}$: 7% were BST cells). D, FS cells showed a normal laminar location profile in *Dlx1*$^{-/-}$ mice. E, RSNP cells are also show a normal laminar distribution in *Dlx1*$^{-/-}$ mice. Red bars: WT. Black bars: *Dlx1*$^{-/-}$.

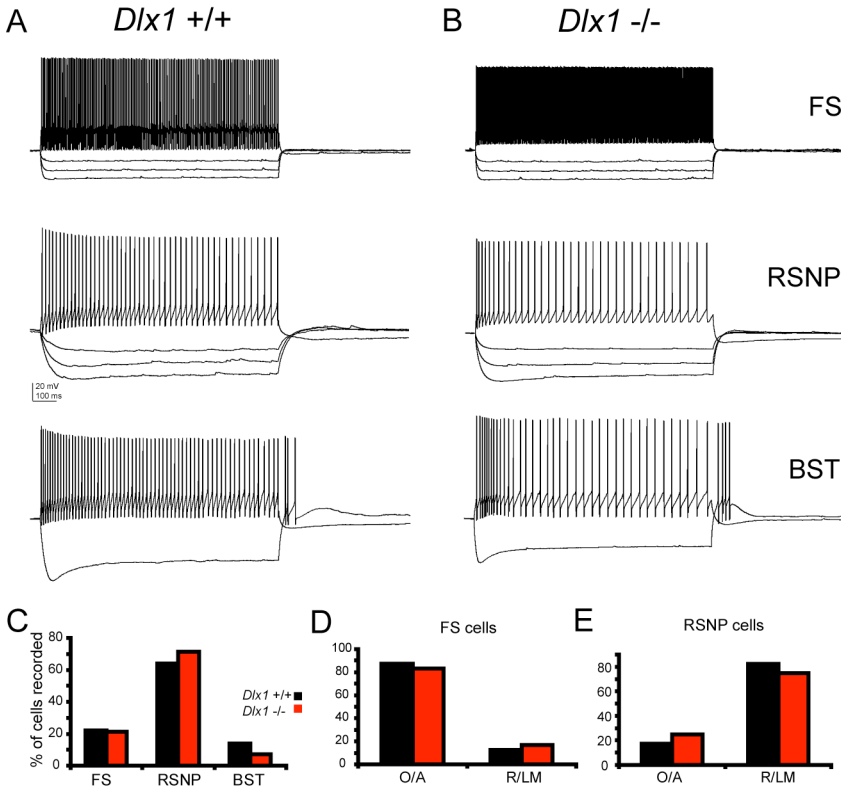

A
Dlx1 +/+
B
Dlx1 -/-
FS
RSNP
BST
20 mV
100 ms
C
% of cells recorded
80
60
40
20
0
FS
RSNP
BST
Dlx1 +/+
Dlx1 -/-
D
FS cells
100
80
60
40
20
0
O/A
R/LM
E
RSNP cells
80
60
40
20
0
O/A
R/LM

Figure 3. Excitatory drive of interneurons is decreased in >P30 *Dlx1*$^{-/-}$ mice.

A, Upper trace: representative voltage-clamp recording from a WT RSNP interneuron showing sEPSCs (downward deflections). Lower trace: the same cell recorded in current-clamp mode, confirming its identity as a RSNP interneuron. B, Upper trace: representative sEPSC recording from a *Dlx1*$^{-/-}$ RSNP interneuron demonstrating reduced excitatory input. Lower trace: the same cell recorded in current-clamp mode. C, Mean sEPSC frequency is comparable between WT and *Dlx1*$^{-/-}$ mice. D, sEPSC decay time is also comparable. E, sEPSC amplitude is significantly decreased ($p < 0.05$, Student's t-test) in *Dlx1*$^{-/-}$ mice as compared to WT mice. F, Amplitude histogram of sEPSCs shows a shift toward smaller events in *Dlx1*$^{-/-}$ mice. Black bars, WT. Red bars, *Dlx1*$^{-/-}$.

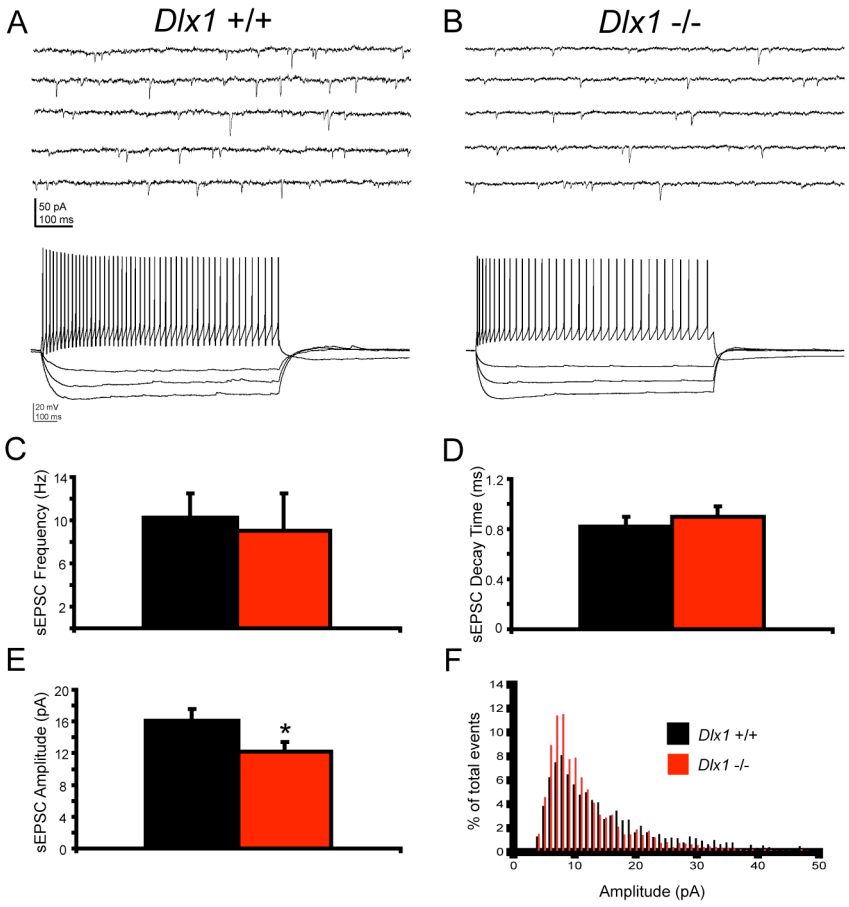

A
Dlx1 +/+
B
Dlx1 -/-
50 pA
100 ms
20 mV
100 ms
C
sEPSC Frequency (Hz)
D
sEPSC Decay Time (ms)
E
sEPSC Amplitude (pA)
*
F
% of total events
Dlx1 +/+
Dlx1 -/-
Amplitude (pA)

Figure 4. Excitatory drive of R/LM interneurons is normal in young Dlx1-/- mice.

A, Upper trace: representative voltage-clamp recording from a WT RSNP interneuron. Lower trace: the same cell recorded in current-clamp mode, confirming its identity as a RSNP interneuron. B, Upper trace: representative sEPSC recording from a *Dlx1*$^{-/-}$ RSNP interneuron. Lower trace: the same cell recorded in current-clamp mode. C, Recordings of sEPSCs in P9-12 WT and *Dlx1*$^{-/-}$ interneurons show that mean sEPSC frequency is unchanged in young *Dlx1* mutant mice. D, Mean sEPSC decay time is also similar between WT and mutant mice. E, Mean sEPSC amplitude is unchanged. Black bars, WT. Red bars, *Dlx1*$^{-/-}$.

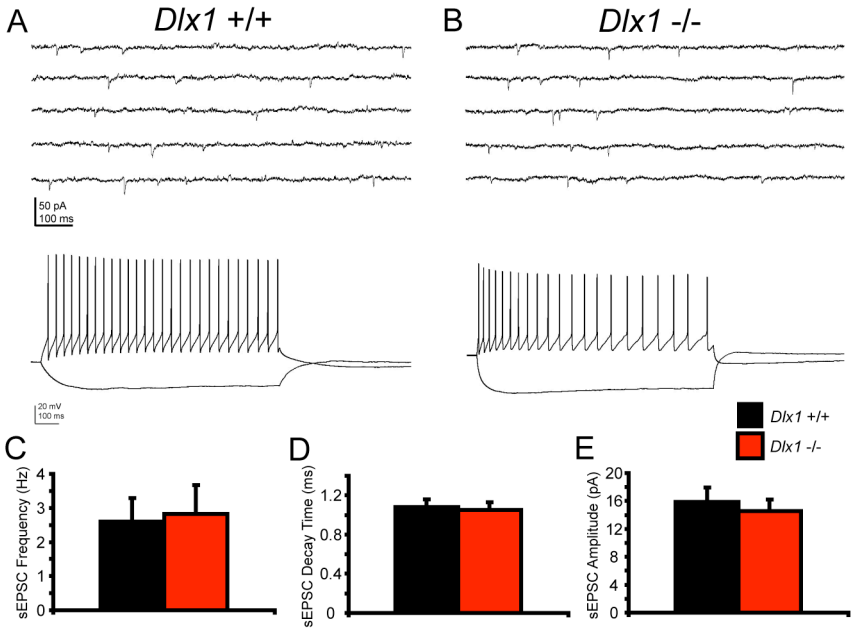
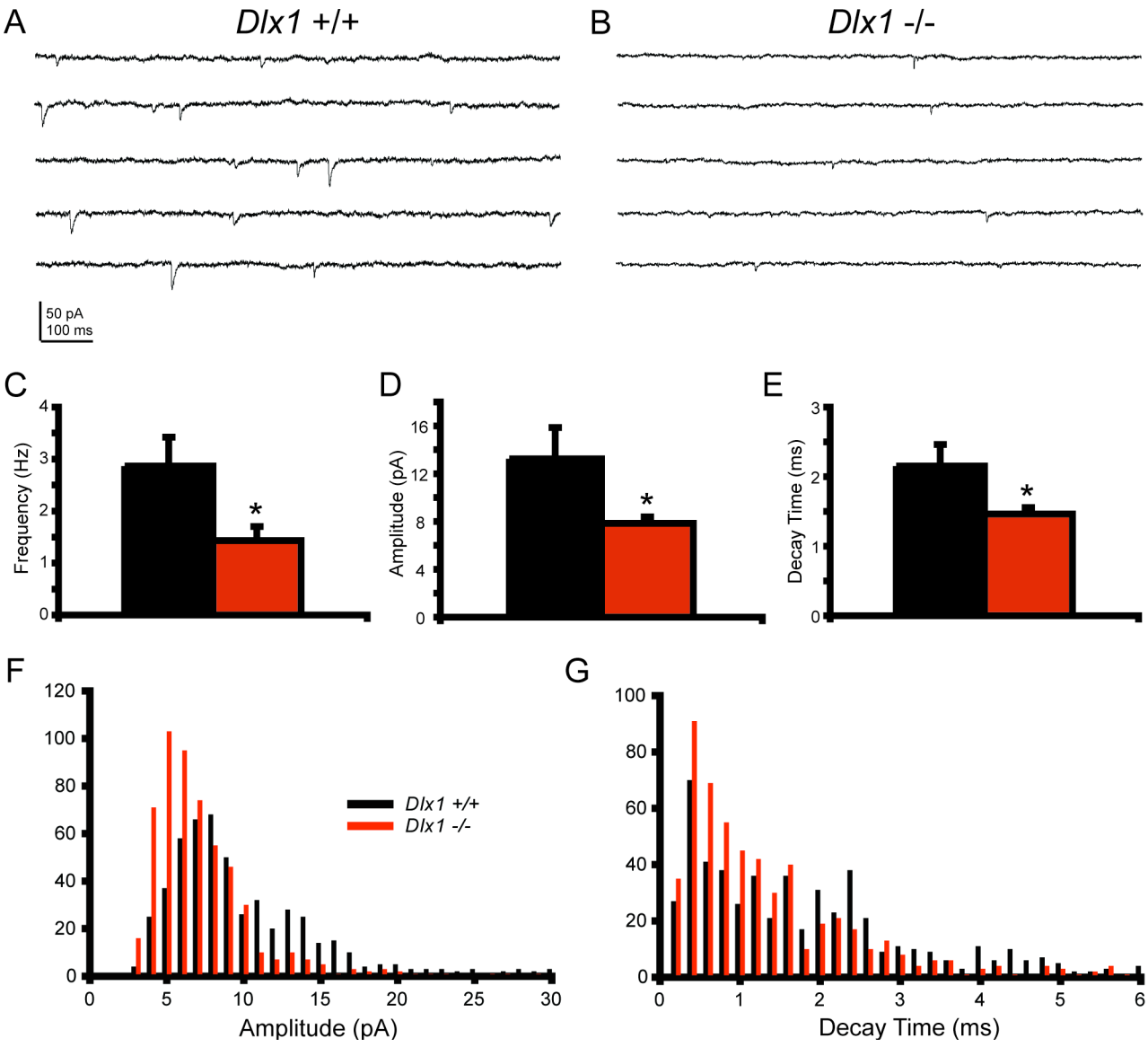

A
Dlx1 +/+
B
Dlx1 -/-
50 pA
100 ms
20 mV
100 ms
Dlx1 +/+
Dlx1 -/-
C
sEPSC Frequency (Hz)
0
1
2
3
4
D
sEPSC Decay Time (ms)
0
0.4
0.8
1.2
E
sEPSC Amplitude (pA)
0
4
8
12
16
20

Figure 5. Reorganization of excitatory inputs to pyramidal neurons in *Dlx1* knockout mice.

A, Sample trace of sEPSCs recorded from a WT pyramidal neuron in CA1. B, Sample trace of sEPSCs recorded from a *Dlx1*$^{-/-}$ pyramidal neuron. C, Mean sEPSC frequency is significantly reduced in *Dlx1*$^{-/-}$ pyramidal neurons ($p < 0.05$, Student's t test). D, Mean sEPSC amplitude is significantly depressed in *Dlx1*$^{-/-}$ pyramidal neurons ($p < 0.05$, Student's t test). E, Mean sEPSC decay time is shorter in *Dlx1*$^{-/-}$ pyramidal neurons. F, Histogram of sEPSC amplitudes showing a leftward shift toward smaller event amplitudes in knockout mice. G, Histogram of sEPSC decay times showing an increase in the proportion of fast decay events in *Dlx1* knockout mice. Black bars, WT. Red bars, *Dlx1*$^{-/-}$.


A
Dlx1 +/+
B
Dlx1 -/-
50 pA
100 ms
C
Frequency (Hz)
D
Amplitude (pA)
E
Decay Time (ms)
F
Dlx1 +/+
Dlx1 -/-
Amplitude (pA)
G
Decay Time (ms)

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Chapter IV: Integration of transplanted neural progenitors into cortical circuits and implications for treatment of epilepsy

Introduction

Epilepsy can result from a diverse set of insults, ranging from genetic mutation to traumatic brain injury. Seizures are typically treated pharmacologically, often with drugs that enhance function of inhibitory systems or that reduce neuronal excitability. Epilepsy patients with medically refractory seizures but with a well-defined seizure focus, such as those with tuberous sclerosis complex, are candidates for surgical resection of epileptogenic brain tissue (Jansen et al. 2007). However, in many patients with epilepsy, pharmacotherapy and surgery are ineffective at reducing seizures, although the reasons for this are unclear. Finding alternative therapies is an important goal of epilepsy research, as attempts at treatment of seizures are unsuccessful in a significant proportion of patients (Cascino et al. 2008).

Synaptic inhibition, mediated by the release of GABA from interneurons, is important in dampening excitability in neuronal circuits. In human and animal studies, epilepsy is frequently associated with impairments in function of cortical and hippocampal inhibitory systems. These deficits include significant reductions in synaptic GABA inputs to hippocampal neurons in rodents with temporal lobe epilepsy (Kobayashi and Buckmaster, 2003), as well as decreased inhibition in tissue resected from patients with focal cortical dysplasia (Calcagnotto et al. 2005). In addition, both humans and animals with epilepsy frequently exhibit significantly reduced numbers of interneurons (Spreafico et al. 1998; Cobos et al. 2005).

These lines of evidence support the hypothesis that increasing interneuron number may reduce seizures, as well as hyperexcitability of neuronal circuits, in animal models of epilepsy. During embryonic development, interneurons arise from the MGE, CGE, and

LGE of the subpallium (Marin and Rubenstein 2001). When microdissected from mouse embryos and grafted into the cortex of neonatal recipient mice, MGE interneuron precursor cells migrate extensively from the injection site and increase inhibition onto host pyramidal neurons, indicating that MGE cells hold a great deal of promise in the treatment of epilepsy (Alvarez-Dolado et al. 2006). However, important questions remain regarding transplanted MGE cells and how they integrate into local circuits within the host brain. First, do MGE grafts generate a functionally normal, diverse set of interneurons in the host brain? Second, grafted MGE cells could also significantly increase inhibitory input to host interneurons. Such inhibition of interneurons could result in decreases in overall GABAergic output, which would be counterproductive, as MGE grafts are intended to reduce hyperexcitability and thus seizures. In this chapter, we attempt to shed light on these questions by recording inhibitory synaptic inputs to host interneurons in mice that have received MGE grafts, and by recording membrane and firing properties directly from grafted neuron.

Results

MGE grafts produce multiple subclasses of interneurons

As a first step in exploring the potential of interneuron progenitor cell transplantation in the treatment of epilepsy, we grafted P2 mice with MGE cells from E13.5 *GFP*$^{+/+}$ mouse embryos (Fig. 1A). After allowing at least 30 days for transplanted cells to migrate and integrate into local circuits, we prepared 300 μm coronal slices of neocortex from grafted mice. We obtained current-clamp recordings from visually identified cells expressing GFP, and injected steps of depolarizing and hyperpolarizing current in order to determine intrinsic membrane and firing properties of grafted interneurons (Fig. 1B). After each recording, we confirmed that the target cell was in fact derived from the *GFP*$^{+/+}$ mouse embryo by aspirating the contents of the cell into the patch pipette, ensuring that the cell from which we recorded was fluorescent.

In current-clamp recordings, we determined that grafted, *GFP*$^{+/+}$ cells developed into interneurons with normal intrinsic and firing properties. We recorded from 33 GFP-expressing cells, of which 32 exhibited the typical membrane and firing properties of interneurons (Fig. 1C). 12 cells were FS cells (36%; Fig. 1B, upper trace), 11 were RSNP cells (33%; Fig. 1B, middle trace), and 9 were stuttering/irregular spiking (STUT) cells (27%; Fig. 1B, lower trace). One cell exhibited a hyperpolarized resting membrane potential, and did not fire action potentials upon injection of strong depolarizing current; these properties are typical of an astrocyte (Adermark and Lovinger 2008). In agreement with our results, previous studies have found that the largest proportion of MGE-derived interneurons consists of FS cells (Butt et al. 2005).

Inhibition of host interneurons is not increased in grafted mice

Previous work from our laboratory has shown that MGE grafts significantly enhance inhibitory input to cortical pyramidal neurons in the host brain (Alvarez-Dolado et al. 2006). However, whether or not MGE cells increase GABAergic inputs to host interneurons is not known. In order to answer this question, we assessed synaptic inhibition of host interneurons in mice that have received an MGE graft. We recorded sIPSCs from 50 visually identified interneurons in slices from grafted mice or from mice that had received a media-only control injection. All cells were recorded between 30 and 40 days after transplantation (DAT). We obtained sIPSCs at a holding potential of -60 mV, with DNQX and APV in the bath to block glutamatergic currents mediated by AMPA and NMDA receptors, respectively. We also filled cells with biocytin in order to confirm their identity as interneurons after recordings were complete.

Interestingly, we found no evidence of increased inhibitory input to host interneurons in mice that had received an MGE graft as compared to media-injected control mice (Fig. 2A). We compared frequency of sIPSCs, and found that it was unchanged between grafted and control mice (Grafted: 16.83 ± 1.47 Hz; Ungrafted: 16.58 ± 1.59 Hz; $p = 0.91$; Fig. $2B_1$). Additionally, we found no difference in mean sIPSC amplitude (Grafted: 55.71 ± 2.59 Hz; Ungrafted: 48.93 ± 3.43 Hz; $p = 0.13$; Fig. $2B_2$). Decay time constants of sIPSCs were also unchanged in grafted animals as compared to ungrafted controls (Grafted: 4.26 ± 0.31 ms; Ungrafted: 3.64 ± 0.30 ms; $p = 0.16$; Fig. $2B_3$). Finally, rise time of sIPSCs was also comparable (Grafted: 0.68 ± 0.02 ms; Ungrafted: 0.65 ± 0.03 ms; $p = 0.34$; Fig. $2B_4$). These data demonstrate that transplantation of MGE-derived interneuron precursors does not lead to enhanced

inhibition of endogenous interneurons, even though inhibitory input to pyramidal neurons is significantly increased.

Discussion

Neural progenitor cell transplantation holds a great deal of promise in the treatment of epilepsy (Bjorkland and Lindvall 2000; Alvarez-Dolado et al. 2006). However, important questions remain about the properties of cells grafted from the MGE, and about their synaptic targets within the host brain. Our results demonstrate that, in our hands, MGE grafts almost exclusively generate interneurons in host mice, and that these grafts do not increase levels of synaptic inhibition onto host interneurons. These results clarify and elaborate on the basic properties of MGE grafts, and provide further encouragement that this technique may serve as an effective therapy for seizures in patients with medically intractable epilepsy.

Origins of interneuron subtypes within the ganglionic eminences

Interneuron precursors are born in the MGE, CGE, and LGE, which are structures within the embryonic subpallium. After birth, these cells migrate tangentially into the developing hippocampus and neocortex (Marin and Rubenstein 2001). Until recently, little was known about how and when distinct subtypes of interneurons were generated. It is now known that, within the ganglionic eminences, the location of birth dictates, at least in part, the subtypes that newly born interneurons are fated to become. For instance, at embryonic day 13.5 (E13.5), the majority of cells generated in the MGE are parvalbumin-positive FS cells or somatostatin-positive BST cells, while the CGE generates calretinin-positive RSNP cells (Butt et al. 2005). Much of this spatial specificity is mediated by the transcription factor *Nkx2.1*, which is expressed in the MGE but not in the CGE (Butt et

al. 2005). When *Nkx2.1* is deleted at various points in embryonic development, the subtype fates of newly born interneurons are shifted (Butt et al. 2008).

In our experiments, we observed that the largest proportion of interneurons generated from the E13.5 MGE were FS cells, consistent with previous reports from another laboratory (Butt et al. 2005). However, we also noted significant proportions of RSNP and STUT cells in MGE grafts. These cell types were also observed to originate from the MGE in previous studies (Butt et al. 2005), although the relative proportions were different from that which we observed. These discrepancies may result from minor variations in dissection techniques, or they may result from genetic differences in the strains of mice used. Further study is required to confirm whether MGE grafts, in our hands, consistently produce a different complement of interneuron subtypes from those reported by Butt and colleagues (2005). Importantly, we do confirm that the MGE generates a significant proportion of FS interneurons, consistent with these previous reports.

The differences in interneuron subtypes generated by the MGE and CGE may prove to be valuable in treatment of seizures. In many forms of epilepsy, from cortical dysplasia to temporal lobe epilepsy, significant loss of interneurons has been reported (Spreafico et al. 1998, Cobos et al. 2005; Knopp et al. 2008). In some cases, interneuron loss is specific to certain subtypes. For instance, in mice lacking *Dlx1*, which develop epilepsy by approximately two months of age, cortical and hippocampal interneurons expressing SOM and CR are preferentially lost (Cobos et al. 2005). By contrast, in the pilocarpine rodent model of temporal lobe epilepsy, hippocampal interneuron loss is confined to PV and SOM interneurons (Dinocourt et al. 2003). By transplanting only the

specific region of the ganglionic eminences known to generate the interneuron subtypes missing in the target animal, neural progenitor cell grafts may be able to selectively replace interneurons that are lost in epilepsy, which may increase their efficacy at reducing seizures. Alternatively, it is possible that one type of graft may always prove to be most effective, regardless of the types of interneurons lost in the target animal. Further experiments are needed to test these alternative possibilities.

Integration of transplanted MGE cells into local circuits within the host brain

Remarkably, transplanted interneuron progenitors migrate extensively from graft sites (Wichterle et al. 1999) and lead to enhanced synaptic inhibition of cortical pyramidal cells in the host brain (Alvarez-Dolado et al. 1999). These significant increases in GABAergic inputs to pyramidal cells suggest that grafted MGE cells not only mature into functional interneurons, but also that they make functional synaptic contacts onto host pyramidal neurons. In agreement with this hypothesis, we have demonstrated that grafted MGE cells exhibit membrane and firing properties typical of interneurons. Additionally, recent electron microscopy studies have confirmed the presence of GFP^+ inhibitory synapses present on host pyramidal neurons in grafted mice, demonstrating that transplanted interneurons form synapses onto cells in the host brain (Baraban, Verdugo, and Alvarez-Buylla, personal communication). These synapses do not appear simply to replace pre-existing GABA synapses; IPSC frequency is significantly increased in grafted animals (Alvarez-Dolado et al. 2006), which supports the hypothesis that new synapses are formed in addition to pre-existing synapses.

However, grafted MGE cells could also make synapses onto host interneurons, and this could prove to be counter-productive in attempts to treat epilepsy. If grafted MGE cells increase inhibitory inputs to endogenous interneurons, overall GABA release in a grafted brain could actually be unchanged or even reduced, which may limit effectiveness of grafts at preventing seizures. The finding that host pyramidal neurons receive enhanced inhibition argues against this possibility, but it remains possible that MGE cells could innervate host interneurons. To test this, we recorded sIPSCs on host interneurons at 30DAT in mice that had received MGE grafts, and compared sIPSC frequency, amplitude, and decay properties to those recorded from interneurons in mice that had received a control, media-only injection. Interestingly, we found that host interneurons in grafted animals do not receive enhanced GABAergic input, in that sIPSC frequency and amplitude were similar to those in control animals. Decay properties were also unchanged, indicating that GABA receptor subunit composition in interneurons is also comparable between grafted and ungrafted animals.

These results stand in stark contrast to the observation that MGE grafts increase inhibition of pyramidal neurons. However, enhanced inhibition of pyramidal cells, and thus increased GABA release, is consistent with unchanged inhibition of host interneurons in grafted mice; if interneurons were found to receive more inhibition, the expected consequence of this would be less GABA release on pyramidal neurons, rather than more. The most straightforward interpretation of our results is that grafted interneurons do not form synapses on endogenous interneurons. However, our results do not rule out the possibility that grafted interneurons may communicate with host interneurons. Grafted cells could form new GABA synapses on host interneurons, with

removal of endogenous GABA synapses as a form of homeostatic compensation. Based on our data, we cannot distinguish between these two alternate possibilities. If it is found that grafted interneurons communicate with host interneurons, with homeostatic removal of endogenous GABA synapses, future studies could focus on why homeostatic compensation occurs in host interneurons, but not pyramidal neurons.

As an additional line of evidence supporting the hypothesis that grafted interneurons do not form synapses in significant numbers on host interneurons, we have found that CR-expressing interneurons are largely absent from MGE grafts (Estrada, Southwell, Alvarez-Buylla, and Baraban, personal communication). In agreement with these results, CR interneurons are generated in CGE, rather than MGE (Butt et al. 2005). CR interneurons are interneuron-selective interneurons, in that they preferentially innervate other interneurons, rather than pyramidal neurons (Markram et al. 2004). An absence of interneuron-targeting interneurons from MGE grafts, then, is consistent with the observation that inhibition of interneurons is unchanged in grafted animals. Because neurons from CGE can also be transplanted (Butt et al. 2005), this hypothesis could be tested by comparing inhibition of host interneurons and host pyramidal neurons in mice receiving CGE grafts.

Neural progenitor cell transplantation as a therapy for epilepsy

In summary, we show that neurons transplanted from MGE mature into functional interneurons of multiple subclasses, and that MGE grafts do not result in enhancement of inhibitory input to host interneurons. These results highlight the promise of MGE cell transplantation as a potential treatment for epilepsy.

Seizures associated with cortical malformations are often medically intractable, in that they do not respond to pharmacological treatments. Along the same lines, less than half of cortical malformation patients who undergo epilepsy surgery are rendered seizure-free (Sisodiya 2000). There is a pressing need for additional treatment options for these and other epilepsy patients with intractable seizures, including traumatic brain injury patients (Lowenstein 2009). Drugs that target GABA systems might fail to work in some patients because they increase inhibition nonspecifically. We have found that one benefit of MGE transplantation appears to be that inhibition enhancement is targeted solely to excitatory neurons, without a potentially counterproductive enhancement of inhibition to interneurons. For this reason, progenitor cell transplants may successfully reduce seizures in epilepsy patients for whom pharmacological interventions have met with limited success.

Unpublished results from our laboratory show that MGE grafts are able to virtually eliminate seizures in mice lacking Kv1.1, the voltage-gated potassium channel α subunit. These *Kcna1*$^{-/-}$ mice exhibit frequent, spontaneous seizures, as well as hyperexcitability of neuronal circuits (Smart et al. 1998; Lopantsev et al. 2003). Chronic electroencephalography (EEG) studies in our laboratory show that mice grafted with MGE cells, but not with a media-only injection, are almost completely seizure-free when assessed 30 days after transplantation (Estrada, Southwell, Alvarez-Buylla, and Baraban, personal communication). Taken together, our data demonstrate the great promise that neural progenitor cell transplantation holds as a potential treatment for otherwise intractable epilepsy.

Figure 1. Characterization of MGE cell grafts.

A, Schematic of experimental protocol. MGE was removed from E13.5 *GFP*$^{+/+}$ donor mice, and cells were bilaterally transplanted into P2 recipient mice. Experiments were done at 30DAT or later. B, Sample traces of grafted MGE cells recorded in current clamp mode at 30DAT or later, showing mature firing properties typical of FS, RSNP, and STUT interneurons. C, FS cells constitute the largest proportion of MGE cells recorded in recipient mice, followed by RSNP and STUT cells. One cell exhibited membrane properties typical of an astrocyte.

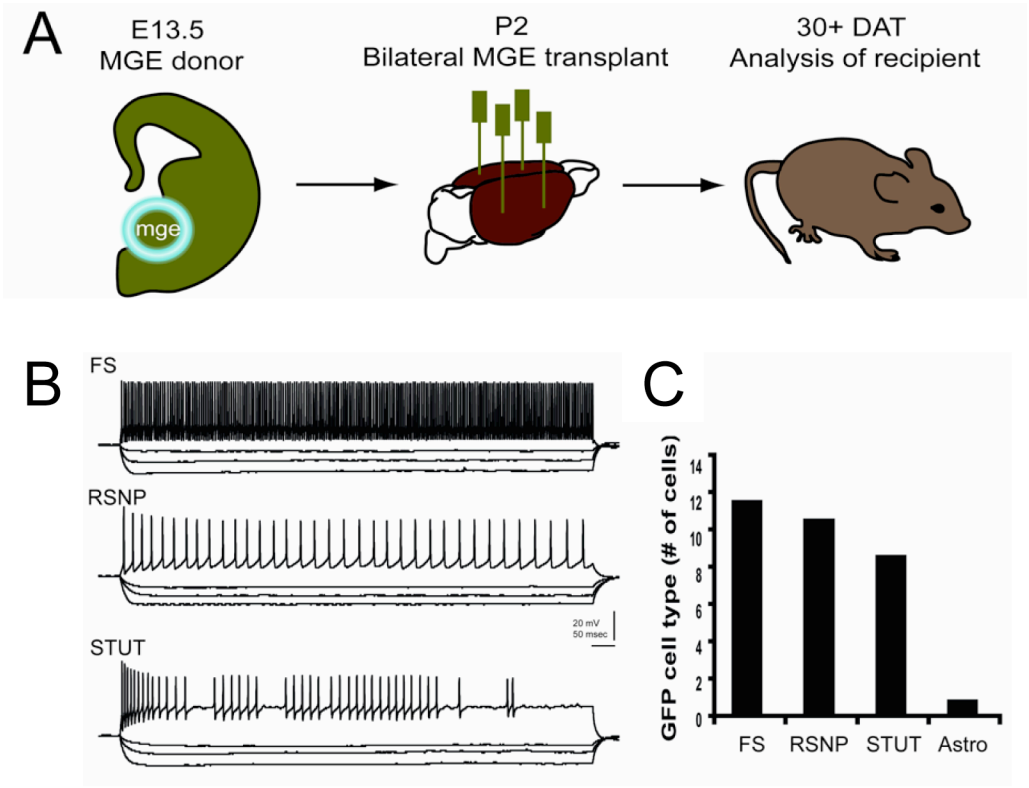
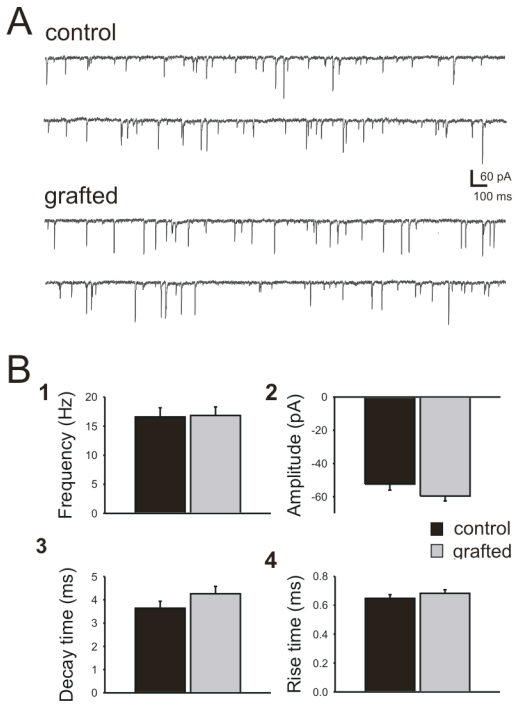

A
E13.5
MGE donor
P2
Bilateral MGE transplant
30+ DAT
Analysis of recipient
mge
B
FS
RSNP
STUT
20 mV
50 msec
C
GFP cell type (# of cells)
0
2
4
6
8
10
12
14
FS
RSNP
STUT
Astro

Figure 2. MGE grafts do not enhance inhibition of host interneurons.

A, Upper traces: Representative sIPSC recording from an interneuron recorded in an ungrafted control mouse. Lower traces: Representative sIPSC recording from an interneuron recorded in a mouse that received an MGE graft. B_1, sIPSC frequency is similar in ungrafted (black bars) and grafted (gray bars) mice. B_2, sIPSC amplitude is not significantly different in ungrafted and grafted mice. B_3, sIPSC decay time is similar in grafted and ungrafted mice. B_4, sIPSC rise time is also unchanged.


A
control
60 pA
100 ms
grafted
B
1
Frequency (Hz)
20
15
10
5
0
2
Amplitude (pA)
0
-20
-40
-60
control
grafted
3
Decay time (ms)
5
4
3
2
1
0
4
Rise time (ms)
0.8
0.6
0.4
0.2
0.0

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Chapter IV: Conclusions and future directions

Key conclusions

Understanding the myriad circuit modifications associated with epilepsy is vital to the development of better treatments for patients. One challenging aspect of studying neuronal circuits in the epileptic brain is that epileptic brains vary widely. An extremely diverse set of neurological insults, from genetic mutation to brain injury, can lead to seizures. In addition, the time point at which the epileptic brain is examined matters a great deal. Seizures result from changes in the brain, but also cause changes in the brain; understanding the direction of causal relationships, if any exist, is a difficult task. However, extensive research has uncovered a number of common threads in studies of brain tissue from humans and animals with epilepsy. One common thread is the importance of inhibitory interneurons in controlling levels of excitability within the brain. Many studies of diverse animal models, as well as studies of tissue resected from humans, have demonstrated that interneuron function is impaired or altered in an epileptic brain (Calcagnotto et al. 2005; Cobos et al. 2005; Kobayashi and Buckmaster 2003; Trotter et al. 2006). Recent research has also highlighted the emerging importance of genetic models of epilepsy, as the number of newly identified "epilepsy genes" continues to increase (Jacobs et al. 2009; Weber and Lerche 2008).

For these reasons, we chose to focus our studies on gaining a better understanding of how inhibitory circuits are reorganized in genetic epilepsies. We focused on two dissimilar animal models: (1) *Lis1* mutant mice, a mouse model of Classical Lissencephaly, a neuronal migration/brain malformation disorder; (2) *Dlx1* knockout mice, in which neuronal architecture is preserved, but epilepsy develops after subpopulations of interneurons are lost during the first month of life. In a novel approach

for the field, we studied synaptic transmission onto interneurons in detail, uncovering significant reorganizations of excitatory and inhibitory inputs to interneurons in epileptic mice.

In *Lis1* mutant mice, we showed that hippocampal malformation was associated with a selective shift of parvalbumin positive, basket-type interneurons into inappropriate hippocampal layers in area CA1, although intrinsic properties of all *Lis1* mutant intereneurons are normal. We found that inhibition of pyramidal neurons was elevated, although $GABA_A$ receptor number and subunit composition appeared normal. In contrast, we found layer-specific alterations in inputs to interneurons. While all interneurons in CA1 receive increased excitatory drive and fire more frequently, interneurons in stratum oriens receive enhanced inhibition, but appear to exhibit reduced $GABA_A$ receptor number as a potential compensatory response. In contrast, inhibitory currents recorded from interneurons in stratum radiatum and lacunosum-moleculare exhibited increased decay time constants, consistent with the hypothesis that $GABA_A$ receptor subunit composition is altered in these cells. Our results demonstrate the extensive reorganization of inhibitory circuits, not just excitatory circuits, that occurs in neuronal migration disorders and epilepsy. These experiments constitute the first in-depth exploration of inhibitory circuits in an animal model of a neuronal migration disorder associated with epilepsy, as well as the first extensive study of inhibition of interneurons in any form of epilepsy.

In *Dlx1* knockout mice, we tested the hypothesis that excitatory circuit rearrangements and alterations in intrinsic properties of interneurons, in addition to the previously described interneuron loss (Cobos et al. 2005), underlie the drastic depression

of inhibition in *Dlx1*$^{-/-}$ hippocampal circuits. Confirming our hypothesis, we found a reduction in excitatory drive of *Dlx1*$^{-/-}$ interneurons in area CA1, and we observed that *Dlx1*$^{-/-}$ interneurons mature more slowly than WT interneurons, with respect to intrinsic membrane properties. In these experiments, we uncovered novel roles for *Dlx1*, and demonstrated for the first time that loss of *Dlx1* signaling affects excitatory circuits in the hippocampus.

Finally, we examined the intrinsic properties and synaptic integration of interneuron progenitor cells grafted into the early postnatal brain. Our recordings showed that MGE grafts generate multiple subclasses of functionally normal interneurons, with the largest proportion being fast-spiking cells. These data are consistent with a previous report concerning the timing and location of fast-spiking cell generation (Butt et al. 2005), and demonstrate that grafted cells from MGE generate functional inhibitory interneurons. We also found that, although MGE grafts enhance inhibitory inputs to host pyramidal cells, host interneurons do not receive increased GABAergic inputs. These data show that MGE cells either do not form new synapses on endogenous interneurons, or that new synapses are formed but old ones are removed as a form of homeostatic compensation. In either case, these results contrast with the effects of MGE grafts on pyramidal neurons, and show that distinct mechanisms must underlie integration of grafted neurons with excitatory versus inhibitory circuits in the host brain. These findings also provide further assurance of the promise that neural progenitor cell grafts hold in the treatment of epilepsy. Unlike antiepileptic drugs that target the GABA system, MGE grafts target inhibition to excitatory neurons, and do not lead to enhanced inhibition of

inhibitory cells. In addition, MGE grafts do not require the removal of brain tissue, but as with epilepsy surgery, could be targeted to epileptogenic regions of the brain.

Homeostasis and epilepsy

Homeostasis is the tendency of neuronal circuits to undergo compensatory changes in response to abnormal levels of activity (Burrone and Murthy 2003). Synaptic scaling is a typical form of homeostatic plasticity, with alterations in receptor trafficking occurring downstream of changes in activity levels (Turrigiano 2008). Examples of homeostatic remodeling have been reported in epileptic circuits. For instance, Shao and Dudek (2005) observed an increase in mIPSC amplitude onto dentate granule cells in kainate-treated rats, which the authors argue to be a compensatory response to decreased frequency of IPSCs onto granule cells. Additionally, $GABA_A$ receptor $\alpha 4$ subunits in pilocarpine-treated rats are upregulated in response to hyperexcitability, due to increased expression of inducible early growth response factor 3 (Egr3; Roberts et al. 2005). However, the cellular mechanisms that mediate homeostatic remodeling in neurons, as well as the rules that govern these compensatory changes, remain poorly understood (Burrone and Murthy 2003).

Homeostatic changes in neural circuits are thought to be triggered by variations in firing rate, which may be detected by calcium-biding sensor proteins such as CamKIV. It is currently thought that these calcium sensors regulate trafficking of receptors, although the evidence for this hypothesis is limited to studies of glutamatergic synapses (Turrigiano 2008). Additionally, variables besides firing rate, such as resting membrane potential, could be relevant to eliciting homeostasis (Burrone and Murthy 2003). In

culture, depolarization of neurons leads to homeostatic alterations that restore firing rate, not resting membrane potential, to previous levels, which supports the hypothesis that firing rate is the relevant variable (Turrigiano and Nelson 2000). A host of major issues remain unaddressed in the field of homeostasis. For instance, it is unknown whether or not the rules that govern homeostasis are consistent across different types of neurons. In addition, while homeostasic alterations of input to excitatory neurons have been described in epileptic circuits, whether or not inputs to interneurons undergo such reorganizations has remained unaddressed. Our studies have contributed to a greater understanding of both of these issues.

In *Lis1* mutant mice, we found that increased excitatory drive and firing rates of inhibitory interneurons in the hippocampus trigger compensatory changes in GABAergic inputs to those interneurons. This is the first evidence that inhibitory inputs to interneurons are capable of undergoing homeostatic remodeling. Interestingly, the changes we uncovered were layer-specific, in that SO interneurons appear to exhibit reduced $GABA_A$ receptor number, while R/LM interneurons exhibit alterations in $GABA_A$ receptor subunit composition. This may be, in part, a result of distinct patterns of inputs to interneurons in different layers. While both SO and R/LM interneurons receive enhanced excitation, only SO interneurons also receive enhanced inhibition. We also found that, although pyramidal neurons in *Lis1* mutant mice receive enhanced inhibition, the number and composition of $GABA_A$ receptors on pyramidal neurons do not appear to undergo compensatory changes.

In mice lacking *Dlx1*, we found homeostatic depression of excitatory circuits in response to loss of inhibition. Interneurons in *Dlx1* knockout mice remove AMPA

receptors from glutamatergic synapses after loss of inhibition in the hippocampus. At ages prior to interneuron loss, when inhibition is normal, AMPA receptor number also appears to be normal. We also found extensive reorganization of excitatory inputs to pyramidal neurons in mice lacking *Dlx1*. In addition to an apparent reduction in AMPA receptor number in pyramidal neuron synapses, we also found reductions in excitatory event frequency, as well as evidence that glutamate receptor subunit composition is altered.

Taken together, our results from *Lis1* mutant mice and *Dlx1* knockout mice highlight an aspect of neuronal homeostasis that remains unappreciated: the rules that govern compensatory remodeling of neural circuits appear to be different in distinct cell types within the same region. For instance, while both SO interneurons and pyramidal neurons receive enhanced inhibition in *Lis1* mutant mice, $GABA_A$ receptor number is reduced only in SO interneurons. A variety of mechanisms could underlie these differences between cell types in the hippocampus. For instance, the relevant variable for homeostasis could be different across cell classes. Some cells, for example, might sense alterations in resting membrane potential and implement molecular changes in order to correct these deviations. By contrast, other types of neurons might be sensitive to firing rate changes. Indeed, there is little reason to suspect that the "homeostasis sensor" (Burrone and Murthy 2003) must be uniform throughout all neurons in the brain, so this possibility remains intriguing.

Alternatively, different cell classes could employ the same homeostasis sensor (e.g., firing rate), while expressing different molecular mechanisms to carry out compensatory changes in response to deviations from the set point. Many signaling

molecules are activated downstream of neuronal activity, with widespread changes in gene expression occurring. These signaling cascades are likely to be different in distinct cell types, which could also underlie differences in the way in which homeostasis is expressed between types of neurons. Indeed, a variety of signaling pathways have been identified as playing a role in glutamatergic synaptic scaling. For instance, the AMPA receptor regulatory protein Arc plays a role in synaptic scaling. Arc promotes endocytosis of AMPA receptors and is expressed in an activity-dependent fashion; reduced levels of Arc lead to compensatory increases in synaptic strength by reducing the removal of AMPA receptors from synapses (Shepherd et al. 2006).

Intriguingly, brain-derived neurotrophic factor (BDNF) appears to play a role in both glutamatergic and GABAergic synaptic scaling. BDNF is released at higher levels as neuronal activity increases, and its release is reduced with low levels of neuronal activity. AMPA receptor number is increased as a compensatory response to reduced BDNF release, and thus reduced neuronal activity (Rutherford et al. 1998). However, BDNF exerts an opposite effect on $GABA_A$ receptors: when BDNF levels are increased, additional $GABA_A$ receptors are inserted into postsynaptic membranes (Swanwick et al. 2006). However, these studies have focused exclusively on cultured excitatory neurons. The molecules that regulate homeostatic plasticity of GABA and glutamate synapses in interneurons have not been identified. While our results indicate that homeostatic processes are different across different cell types, the mechanistic underpinnings for these differences are elusive.

In addition, an important step in understanding the diverse mechanisms that underlie homeostasis in epileptic brains is obtaining a detailed timeline of when

compensatory alterations occur. Do these homeostatic alterations occur after the onset of epileptiform activity or seizures, or do they take place prior to seizure onset? In *Lis1* mutant mice, our results do not distinguish between these possibilities, because the timeline of seizure onset in these mice is unknown. However, in *Dlx1* knockout animals, epilepsy develops at approximately two postnatal months (Cobos et al. 2005), while our recordings were obtained at P9-12 and again between P30-40. Our results show that inhibition loss and compensatory reductions in glutamatergic activity occur at approximately one month of age, with normal inhibition and excitation at P9-12. Seizures follow at approximately two months, after these homeostatic alterations occur.

It remains unclear why are compensatory reductions in glutamatergic activity are insufficient to prevent the development of epilepsy in *Dlx1* knockout mice. Because not all interneurons that normally express *Dlx1* die in $Dlx1^{-/-}$ mice, surviving interneurons may exhibit functional defects that have not been uncovered. Additionally, a simple imbalance between excitation and inhibition is not the only circuit mechanism by which seizures may be generated. Loss of interneurons in mice lacking *Dlx1* likely results in further reorganizations of inhibitory circuits among surviving interneurons, which could alter the timing and properties of oscillatory activity in hippocampal networks, potentially giving rise to seizures (Uhlhaas and Singer 2006). This may also explain why compensatory enhancement of inhibition in $Lis1^{+/-}$ mice is also insufficient to prevent the development of epilepsy, although the relative temporal order of these events is not known.

Future directions

Our focus on interneurons and their synaptic inputs in genetic models of epilepsy is a novel approach for the epilepsy field, illustrating the importance of asking these types of questions in any model of epilepsy or cortical malformation, genetic or acquired. For instance, temporal lobe epilepsy is commonly modeled in mice and rats by injection of pilocarpine (Curia et al. 2008) or kainate (Williams et al. 2007), and while a great deal of experimental work has focused on uncovering physiological and anatomical circuit alterations in these animals, little is known about how inhibitory connections among interneurons might be reorganized in these hyperexcitable circuits. Similarly, as attention on neuronal migration disorders intensifies (Metin et al. 2008; Guerrini and Marini 2006), there is an acute need for in-depth studies of inhibitory circuits in these difficult-to-treat disorders.

Lissencephaly mice and progenitor cell transplantation

With the studies reported here, more is known about inhibitory circuits in *Lis1* mutant mice than in any other mouse model of a neuronal migration disorder. However, a number of important questions remain with respect to the anatomy and physiology of circuits in *Lis1* mutant mice. First, why are fast-spiking, basket-type interneurons selectively displaced into inappropriate layers in *Lis1*$^{+/-}$ mice, while other interneuron subtypes are normally distributed? Lis1 protein appears to be required for proper migration in all neurons (McManus et al. 2004; Tsai et al. 2005), suggesting that basket cells are displaced not due to impaired migration but rather to alterations in the extrinsic cues that instruct the final stages of their migration. The cues that guide interneurons to their final laminar positions within the hippocampus and cortex remain unknown,

complicating attempts to understand the reasons underlying the displacement of basket cells. However, this is an important question both from the perspective of understanding neuronal migration disorders, as well as understanding the basic mechanisms of migration and laminar specification.

Second, how do the inhibitory circuit modifications that we describe translate into hyperexcitability and seizures in *Lis1*$^{+/-}$ mice? We found that *Lis1*$^{+/-}$ interneurons receive enhanced excitatory drive, release more GABA onto pyramidal neurons, and fire more frequently, with extensive alterations in connectivity between interneurons. While no apparent imbalance between excitation and inhibition exists in *Lis1* mutant mice, seizures can be a result of oscillatory network activity, which is mediated by interneurons (Medvedev et al. 2000; Worrell et al. 2005). It is likely that disorganized inhibitory networks in the *Lis1* mutant hippocampus generate abnormal oscillations in the hippocampus, and these oscillations could drive seizures. The study of cortical and hippocampal oscillations in neuronal migration disorders could shed a great deal of light on the consequences of neuronal disorganization on network activity, helping to explain why seizures and cognitive deficits are common in these disorders (Guerrini and Marini 2006).

Third, is the homeostatic remodeling of inhibitory circuits in *Lis1* mutant mice a response to seizures? Furthermore, is the enhancement of inhibition in these mice anticonvulsant, in that increased GABA tone reduces excitability, or proconvulsant, in that increased connectivity among interneurons increases seizure-generating oscillations (Uhlhaas and Singer 2006)? An important first step in addressing these questions will be an in-depth exploration of the relative timing of inhibition enhancement and seizure

onset. In addtion, the previously proposed oscillation studies will determine whether or not rhythmic population activity is enhanced in *Lis1* mutant mice.

Finally, *Lis1* mutant mice also present an interesting opportunity in which to test the seizure-reducing potential of neural progenitor cell grafts in the treatment of epilepsy. While MGE grafts have been proposed as a way to replace inhibition that is missing in an epileptic brain (Richardson et al. 2008), the lissencephalic brain is epileptic but does not display a loss of inhibition. However, patients with Lissencephaly typically exhibit severe, often fatal seizures that are medically intractable (Walsh 1999). As a result, there is a great need to develop novel, viable treatments for Lissencephaly patients. By transplanting *Lis1* mutant mice with MGE cells and monitoring them for seizure activity following receipt of MGE grafts, it will be possible to gain a clearer understanding of the potential benefits, as well as limitations, of progenitor cell grafts in the treatment of epilepsy affiliated with brain malformation.

Dlx1 knockout mice and progenitor cell transplantation

Although the transcription factor *Dlx1* is expressed only in interneurons, we have demonstrated that excitatory circuits in mice lacking *Dlx1* undergo extensive reorganizations. We have also uncovered a potentially novel role for *Dlx1* in the electrophysiological maturation of interneurons. *Dlx1* knockout mice present an intriguing opportunity to study the relationship between inhibition and epilepsy, because interneuron loss prior to epilepsy onset is subtype-specific. 20-30% of cortical and hippocampal SOM and CR interneurons undergo apoptosis by ~1 month of age in mice lacking *Dlx1*, and these interneurons target their inhibitory output to pyramidal cell

dendrites. Curiously, IPSC recordings of pyramidal cells showed a loss of soma-targeted inhibition as well (Cobos et al. 2005). We demonstrated that surviving interneurons receive reduced excitatory drive, helping to explain this puzzling finding. However, many interesting questions remain with regard to the function of *Dlx1* in interneurons.

First, although virtually all CR and SOM interneurons appear to express *Dlx1*, only about ¼ of these cells undergo apoptosis in mice lacking *Dlx1*. Clearly, *Dlx1* expression is not absolutely required for survival of these interneurons, because most SOM and CR interneurons survive in the absence of *Dlx1* (Cobos et al. 2005). Because interneurons that express these neurochemical markers do not constitute uniform subclasses of cells (Markram et al. 2004), it is possible that *Dlx1* is indeed required for survival of certain types of interneurons, and that classifying these cells using SOM or CR is too broad. Further fine-grained analysis of *Dlx1* expression may clarify whether the interneurons that undergo apoptosis are a uniform subclass as opposed to a diverse cross-section of the different types of SOM and CR interneurons.

Second, *Dlx1* expression is low or absent in parvalbumin-positive basket cells, which provide powerful inhibitory input to perisomatic regions of pyramidal neurons (Markram et al. 2004). Much of this inhibition is lost in $Dlx1^{-/-}$ mice, suggesting that, even at extremely low levels of expression, *Dlx1* may be important in the function of these interneurons. By crossing *Dlx1* knockout mice with mice expressing GFP under a basket cell-specific promoter, an in-depth physiological analysis of basket cell function could be undertaken, including paired recordings to assess the functionality of basket cell-pyramidal cell synapses in $Dlx1^{-/-}$ mice. It is also likely that surviving CR and SOM interneurons exhibit functional deficiencies that have yet to be uncovered; for instance,

the reduction in AMPA receptor number that we observed could be a result of loss of *Dlx1* signaling. Targeted recordings of these neurons could illuminate potentially novel roles for *Dlx1* in the function of adult interneurons.

Third, while the loss of inhibition and putative compensatory reduction in excitatory drive are absent at P9-12 but present by one month of age in mice lacking *Dlx1*, their temporal relationship remains unknown. These experiments should be repeated at time points between the second and fourth postnatal week. It is also possible that these events are unrelated. Transplantation of interneuron progenitors into mice lacking *Dlx1* could help to address this question. If AMPA receptor removal persists in >P30 *Dlx1*$^{-/-}$ mice that have received MGE grafts, it would suggest that AMPA receptors are removed as a result of *Dlx1* loss, rather than as a compensatory response to inhibition loss.

Finally, mice lacking *Dlx1* should be used as a tool to test the principles that underlie neural progenitor cell grafts. Because *Dlx1*$^{-/-}$ mice are missing certain subtypes of interneurons, the ability of MGE or CGE grafts to replace these interneurons could be tested. Additionally, because MGE and CGE grafts each generate distinct populations of interneurons, their success at reducing seizures and restoring inhibition could be compared in *Dlx1*$^{-/-}$ mice. Finally, because loss of interneurons clearly precedes the development of seizures in mice lacking *Dlx1*, progenitor cells could be grafted at various time points (e.g., before apoptosis and epilepsy, after apoptosis but before epilepsy, and after apoptosis and epilepsy) in order to clarify the temporal requirements for success of progenitor cell transplants. These data will be vital as cell transplantation moves toward the treatment of humans, because previous tests of graft efficacy have only examined

animals receiving grafts well before the onset of epilepsy. The main promise of MGE grafts lies with their potential ability to reverse seizures, not to prevent them, because patients do not seek medical treatment until after they begin having seizures.

Concluding remarks

When we began these studies, little was known about interneurons in the malformed hippocampus, and almost no information existed regarding connectivity between interneurons in epilepsy or malformation models. In addition, researchers were just beginning to examine models of genetic epilepsies, as more "epilepsy genes" continue to be identified. We have been able to shed a great deal of light on these issues, undertaking in-depth analyses of inhibitory interneurons – their intrinsic properties, their inputs, and their outputs – in two genetic models of epilepsy. We have also been able to contribute significantly to the small but growing body of work regarding neural progenitor cell transplantation as a potential treatment for epilepsy, highlighting some of the benefits that MGE grafts might have as compared to traditional pharmacotherapies.

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Appendix 1: Materials and Methods

Experimental protocols

Immunohistochemistry of interneurons in Lis1 mutant mice

We anesthetized and transcardially perfused age-matched WT and *Lis1*$^{+/-}$ mice with 4% paraformaldehyde in PBS. Immediately following perfusion, we removed the brain, postfixed overnight in 4% paraformaldehyde, and cryoprotected in 30% sucrose in PBS for 2 days at 4°C. We cut 40 μm coronal sections and blocked sections for two hours in PBS with 0.1% Triton X-100, 10% goat serum, and 1% bovine serum albumin. Primary antibodies, including mouse anti-calretinin (1:000, Chemicon, Temecula, CA), mouse anti-parvalbumin (1:2000, Sigma, St. Louis, MO), and rabbit anti-somatostatin (1:500, Chemicon), were added overnight at 4°C. Sections were then incubated at room temperature for two hours in biotinylated goat anti-mouse IgG or goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA), and then for two hours in ABC complex (Vector Laboratories). We processed sections with diaminobenzidine (Sigma, St. Louis, MO) before mounting, dehydrating with ethanol, and coverslipping.

Cell counts of interneuron subtypes in Lis1 mutant mice

We counted parvalbumin, somatostatin, and calretinin-immunoreactive cell bodies in 40 μm sections from three wild-type and three age-matched *Lis1*$^{+/-}$ mice (four sections per mouse). We used NIH Image software to divide area CA1 into bins of 400 μm by 50 μm, and counted immunoreactive somata in each bin, in order to ascertain the overall distribution of each interneuron subtype in CA1.

Transplantation of MGE cells

We followed the transplantation protocol described by Alvarez-Dolado et al. (2006). Interneuron precursors are generated in the ventricular and subventricular zones of the MGE. These regions were dissected out from E13.5 *GPF*$^{+/+}$ embryos, and the cells were mechanically dissociated by passing them through a pipette tip 10-20 times. We washed MGE cells in 1 mL of L-15 media containing DNase, and centrifuged cells for 2 minutes at 800 *g* in order to obtain an MGE cell pellet. After resuspension in L-15 media at high concentrations (approximately 200,000 cells/μL), the cell suspension was front-filled into a 50 μm diameter glass pipette and was injected into 2-3 day old mice, which were anesthetized by exposure to low temperature (4 °C) on an aluminum plate. Each mouse received an injection of 500,000 cells into deep layers of cortex.

Slice preparation

In order to obtain acute brain slices for electrophysiology, we anesthetized and decapitated mice, removed the brain, and placed it into oxygenated, ice-cold, high sucrose artificial cerebrospinal fluid (hsACSF), containing (in mM): 150 sucrose, 50 NaCl, 25 $NaHCO_3$, 10 dextrose, 2.5 KCl, 1 NaH_2PO_4-H_2O, 0.5 $CaCl_2$, 7 $MgCl_2$. After 2 minutes in the hsACSF, we blocked the brain and glued it to the stage of a Vibratome (Leica VTS1000, Bannockburn, IL), and we cut 300 μm brain slices in oxygenated hsACSF at 4°C. Afterwards, slices were placed in a chamber containing ACSF (in mM, 124 NaCl, 3 KCl, 1.25 NaH_2PO_4-H_2O, 2 $MgSO_4$-$7H_2O$, 26 $NaHCO_3$, 10 dextrose, and 2 $CaCl_2$). After a 40 minute incubation at 35°C, we transferred the slice chamber to room temperature for the remainder of the day.

Electrophysiology

For recording, we placed slices in a recording chamber with oxygenated ACSF flowing at 2-4 mL/min, and we identified interneurons and pyramidal cells under infrared differential intereference contrast (IR-DIC) optics. A micropipette puller (Sutter Instrument Company, Novato, CA) was used to pull patch pipettes of between 3-7 MΩ from 1.5mm outer diameter borosilicate glass (World Precision Instruments, Sarasota, FL). We obtained all data using an Axopatch 1D amplifier; data were digitized at 10kHz and recorded using a Digidata 1320A and pClamp 8.2 software (Molecular Devices, Sunnyvale, CA).

Inhibition of interneurons in Lis1 mutant mice

For all experiments, internal recording solution (pH 7.20-7.25, 285-295 mOsm) contained, in mM, 40 KGluconate, 100 KCl, 2 NaCl, 10 HEPES, 4 EGTA, 4 MgATP, 0.3 Na_2GTP, 1.25 QX-314. After obtaining a stable voltage-clamp recording from a CA1 interneuron at 32 °C, we switched to current clamp mode and injected steps of hyperpolarizing and depolarizing current in order to determine the class of cell being recorded, based on firing patterns and action potential properties (Butt et al. 2005); we subsequently switched back into voltage clamp mode, holding the cell at -60 mV, in order to commence recording of sIPSCs or mIPSCs. We isolated sIPSCs by perfusing the slice with ACSF containing 20 μM DNQX, an AMPA receptor blocker, and 50 μM *D*-APV, an NMDA receptor blocker (Sigma), and confirmed that each batch of DNQX and *D*-APV was functional by perfusing cells with 100 μM Gabazine (Sigma), confirming that

DNQX and *D*-APV insensitive events were mediated by $GABA_A$ receptors. TTX (Alomone Labs, Jerusalem, Israel) was added to isolate mIPSCs, and we tested each batch of TTX to confirm its efficacy at blocking action potentials. All recordings were obtained at 32 °C. Voltage clamp recordings were low-pass filtered at 1 kHz, and band-pass filtered at 60 Hz (Hum Bug, AutoMate Scientific, Berkeley, CA). We monitored whole cell access resistance and holding current to confirm that recordings were stable.

Inhibition of pyramidal neurons in Lis1 mutant mice

We recorded sIPSCs from CA1 pyramidal neurons held at –60 mV at room temperature in the presence of 3 mM kynurenic acid (Tocris, Ellisville, MO) to block glutamatergic transmission; 100 μM gabazine (SR-95531; Sigma) was added at the end of some experiments (n=25) to confirm that all synaptic events recorded in the presence of kynurenic acid were GABAergic. To isolate mIPSCs from sIPSCs, we added 1 μM TTX (Alomone Labs, Jerusalem, Israel), and tested each batch of TTX to confirm its efficacy at blocking action potentials.For tonic inhibition experiments, we followed the protocol described by Maguire et al. (2005). Briefly, we obtained stable sIPSC recordings as previously described, with 0.005 mM GABA included in the ACSF. After a stable baseline in 3 mM kynurenic acid was established (>5 minutes), we perfused the slice with 100 μM gabazine and continued recording until a stable baseline was re-established and all synaptic events were eliminated.

For evoked IPSC (eIPSC) studies, we obtained voltage clamp recordings of sIPSCs as previously described. A monopolar stimulating electrode was placed in stratum radiatum, 50-100 μm from the pyramidal cell being recorded. Stimulus

parameters were controlled using pClamp software, an A310 Accupulser (World Precision Instruments), and an A360 Stimulus Isolator (World Precision Instruments). 100 μs pulses were delivered at low frequency (0.05 Hz). We obtained a plot of stimulus intensity versus response size for each cell, and performed paired-pulse experiments at a stimulus strength that reliably elicited a response of approximately 100 pA for the first pulse. Interpulse intervals tested in these experiments were 40, 100, 400, 500, and 700 ms. At the end of some experiments, 5 μM bicuculline (Sigma) was added to ensure that eIPSCs were mediated by $GABA_A$ receptors (n=3).

Excitatory drive and spontaneous firing of interneurons in Lis1 mutant mice

For cell-attached recordings of spontaneous firing in interneurons, we used a slightly modified ACSF containing 5 mM KCl and obtained recordings at 32°C. We recorded spontaneous firing in voltage-clamp mode at –60 mV before switching to current-clamp mode and rupturing the patch to obtain a whole-cell current clamp recording, so that interneuron sub-type could be identified. Internal pipette solution (pH 7.20-7.25, 285-295 mOsm) for spontaneous firing and current-clamp recordings contained, in mM, 120 K-Gluconate, 10 KCl, 1 $MgCl_2$, 0.025 $CaCl_2$, 0.2 EGTA, 2 Na_2ATP, 0.2 Na_2GTP, 10 HEPES. We injected 1000 ms depolarizing and hyperpolarizing current steps to measure membrane and firing properties of each interneuron recorded; passive membrane properties were examined occasionally throughout the experiment to ensure the health of the cell and the integrity of the seal.

Recordings of spontaneous excitatory postsynaptic currents (sEPSCs) on interneurons were obtained at 32°C in the presence of 5 μM bicuculline, in accordance

with standard recording protocols (Perez et al. 2006). We used the previously described current-clamp internal patch pipette solution, and recorded events at a holding potential of –60 mV. Immediately after obtaining a whole-cell recording, we switched to current-clamp mode and injected current steps in order to identify the interneuron sub-type (cells with firing properties reminiscent of CA1 pyramidal neurons were discarded), and then switched back into voltage-clamp mode to record sEPSCs. At the end of some experiments, 3 mM kynurenic acid was added to confirm that sEPSCs were successfully isolated (n=3). Whole-cell access resistance and holding currents were monitored throughout all recordings; cells for which these values changed by more than 25% were excluded from analysis.

Excitatory drive and intrinsic properties of interneurons in Dlx1 knockout mice

All recordings were obtained at 32°C, using the previously described current clamp and EPSC internal patch pipette solution. EPSCs were recorded using the same protocol as in *Lis1* mutant mice, and current clamp recordings were obtained and analyzed as with *Lis1* mutant mice.

Inhibition of host interneurons and intrinsic properties of MGE cells in grafted mice

In cortical slices obtained from grafted and media-injected controls, sIPSCs and current clamp recordings were obtained as with *Lis1* mutant mice.

Data analysis

All voltage clamp recordings were low-pass filtered at 1 kHz, and band-pass filtered at 60 Hz (Hum Bug, AutoMate Scientific, Berkeley, CA). We analyzed all synaptic events using Mini Analysis Program 5.2.5 (Synaptosoft, Decatur, GA), selecting each event by hand, and analyzing 300 events per cell. Histograms were constructed using the first 100 events per cell. Decay time was defined as the time to 67% of peak amplitude. To calculate weighted decay time constants, an average IPSC was calculated for each cell, and its decay was fit to two exponentials. All data are presented as mean ± SEM, and we determined statistical significance of results using unpaired, two-tailed Student's *t*-tests, with p=0.05 as the threshold for statistical significance.

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