Inhibitory Inputs to Hippocampal Interneurons Are Reorganized in Lis1 Mutant Mice

Daniel L. Jones and Scott C. Baraban

Graduate Program in Neuroscience and Epilepsy Research Laboratory, Department of Neurological Surgery, University of California San Francisco, San Francisco, California

Submitted 5 May 2009; accepted in final form 8 June 2009

First published June 10, 2009; doi:10.1152/jn.00392.2009.

INTRODUCTION

Epilepsy, a neurological disorder defined by the presence of spontaneous, unprovoked seizures, is often associated with a brain malformation. These malformations can result from mutations in genes that encode molecular machinery required for proper brain development (Guerrini and Filippi 2005; Guerrini and Marini 2006). In humans, mutations in cytoskeleton-associated proteins such as LIS1, doublecortin, reelin, and ARX have been implicated in a condition known as lissencephaly, the hallmarks of which include a smooth cortical surface, disorganized cortical layering, enlarged ventricles, mental retardation, and severe epilepsy. Brain malformations in lissencephaly are consistent with known functions of these proteins, which include important roles in the process by which microtubules and microtubule-associated motor proteins regulate neuronal migration (Kato and Dobyns 2003; Tsai et al. 2005).

Although neuronal migration disorders (NMDs) are widely recognized as a pathology underlying epilepsy and cognitive dysfunction (Guerrini and Filippi 2005), how network function is disrupted when neurons migrate incorrectly is largely unknown. Previous studies of circuit changes associated with NMDs have primarily focused on neocortical malformations; characterizations of hippocampal malformations have largely been limited to p35-deficient mice and rats exposed to methyloxyazoxymethanol (MAM) in utero. Rats exposed to MAM develop nodular heterotopia in the CA1 region of hippocampus and exhibit disruptions in excitatory and inhibitory circuitry, including altered N-methyl-α-aspartate (NMDA) receptor function (Calcagnotto and Baraban 2005) and reduced γ-aminobutyric acid (GABA) uptake (Calcagnotto et al. 2002). p35 knock-out animals (p35−/− mice) also exhibit heterotopic pyramidal neurons in CA1/CA3 and granule cell dispersion in the dentate gyrus. Although abnormal field potential responses were observed in the dentate gyrus of p35−/− mice, detailed single-cell analysis of excitatory or inhibitory circuit function has not been reported (Wenzel et al. 2001). Similarly, studies of synaptic reorganization in cortical malformation models have primarily focused on inputs to principal glutamatergic neurons rather than GABAergic inhibitory interneurons. For instance, synaptic inputs to layer V cortical pyramidal neurons were extensively studied in a rat freeze-lesion model of polymicrogyria and these studies demonstrated that excitatory drive of pyramidal neurons located in malformed cortex is significantly enhanced before the appearance of epileptiform activity (Jacobs and Prince 2005; Zsombok and Jacobs 2007). Additionally, in the telencephalic internal structure heterotopia (tish) rat, a genetic cortical malformation model, cortical pyramidal neurons receive reduced inhibitory input at ages before seizures are observed (Trotter et al. 2006). Recently, mice lacking doublecortin and doublecortin-like kinase 2 were found to exhibit disrupted hippocampal lamination and spontaneous seizures, with significantly reduced GABAergic input to CA1 pyramidal neurons (Kerjan et al. 2009). In a similar model, an in utero small interfering RNA “double cortex” mouse, spontaneous currents and calcium responses were studied in cortical neurons overlying a heterotopia (Ackman et al. 2009). However, none of these studies examined synaptic inputs to interneurons and thus this remains an important topic of exploration for future experimental work in animal models of epilepsy and brain malformation.

To address these issues, we have been studying an animal model of lissencephaly—e.g., mice with one null allele of Lis1 (Lis1+/− mice). In these mice, the hippocampus is malformed, with pronounced disorganization of pyramidal cell layers,
dispersion of dentate granule cells, and enlarged ventricles (Hirotsune et al. 1998; Wang and Baraban 2007, 2008). The functional consequences of this disorganization include hippocampal hyperexcitability (Fleck et al. 2000), impairment in spatial learning and memory (Paylor et al. 1999), and spontaneous electrographic seizures (JSF Greenwood, RC Estrada, and SC Baraban, personal communication). We previously demonstrated that, in Lis1 (+/−) mice, hippocampal interneurons in area CA1 receive increased excitatory drive and provide enhanced inhibitory input to CA1 pyramidal neurons, although the intrinsic membrane properties of Lis1 (+/−) interneurons are within normal range. Amplitude and decay time of miniature inhibitory postsynaptic currents (mIPSCs) recorded from pyramidal neurons were normal, indicating that postsynaptic GABA_{A} receptor number and subunit composition are unchanged in CA1 pyramidal neurons (Jones and Baraban 2007). Here we continue our investigation of Lis1 (+/−) mice and examine the functional consequences of hippocampal circuit disorganization with an emphasis on inhibition of interneurons. We completed immunohistochemical studies, using interneuron-specific antibodies, and obtained in vitro patch-clamp recordings of spontaneous and miniature IPSCs onto hippocampal interneurons.

METHODS

Immunohistochemistry

Age-matched (P30) WT and Lis1 (+/−) mice were anesthetized and transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). Immediately following perfusion, the brain was removed, 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 them for 2 h in PBS with 0.1% Triton X-100, 10% goat serum, and 1% bovine serum albumin. We prepared immunohistochemical studies, using interneuron-specific antibodies, and obtained in vitro patch-clamp recordings of spontaneous and miniature IPSCs onto hippocampal interneurons.

Slice preparation

Mice were anesthetized and decapitated, after which the brain was quickly removed and placed 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_{2}PO_{4}, 0.5 CaCl_{2}, and 7 MgCl_{2}. After 2 min in hsACSF, the brain was blocked, glued to the stage of a Vibratome (VT1000; Leica, Bannockburn, IL), and cut into 300-μm horizontal brain slices containing hippocampus. To be consistent with previous studies of hippocampal circuits in Lis1 mutant mice, we chose to study synaptic function in horizontal slices (Fleck et al. 2000; Jones and Baraban 2007). Slices were then placed in a chamber containing ACSF (in mM: 124 NaCl, 3 KCl, 1.25 NaH_{2}PO_{4}, 2 MgSO_{4}, 7H_{2}O, 26 NaHCO_{3}, 10 dextrose, and 2 CaCl_{2}). After a 40-min incubation period at 35°C, slices were maintained at room temperature (6–8 h).

Electrophysiology

For recording, slices were placed in a submerged recording chamber (Warner Instruments, Hamden, CT) with oxygenated ACSF heated to 32°C and flowing at 2–4 ml/min. Interneurons were identified in area CA1 under infrared differential interference contrast (IR-DIC) optics. A micropipette puller (Sutter Instrument, Novato, CA) was used to pull patch pipettes of between 3 and 7 MΩ from 1.5-mm-OD borosilicate glass (World Precision Instruments, Sarasota, FL). Data were obtained using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA); data were digitized at 10 kHz and recorded using a Digidata 1320A and pClamp 8.2 software (Molecular Devices, Sunnyvale, CA). For all experiments, the internal recording solution (pH 7.20–7.25; 285–295 mOsm) contained (in mM): 40 K-glucanone, 100 KCl, 10 HEPES, 4 EGTA, 4 MgATP, 0.3 Na_{2}GTP, and 1.25 QX-314. For the current-clamp experiments described in Fig. 2, we recorded from postnatal day 15 (P15)–P22 mice because intrinsic membrane and firing properties allow cell types to be distinguished by this age (Holter et al. 2007; Massengill et al. 1997; Okaty et al. 2009). For all subsequent voltage-clamp and current-clamp experiments, mice were between P25 and P33.

After obtaining a stable voltage-clamp recording from a CA1 interneuron, the recording was switched to current-clamp mode and injected steps of hyperpolarizing and depolarizing current were used to determine the class of cell being recorded using published firing patterns and action potential (AP) properties (Butt et al. 2005; Flamme and Marin 2005). The recording was subsequently switched back to voltage-clamp mode (holding potential, −60 mV) for recording of sIPSCs or mIPSCs. Spontaneous IPSCs were pharmacologically isolated by perfusing the slice with ACSF containing 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] receptor blocker) and 50 μM t-2-amino-5-phosphonovaleric acid (t-APV, an NMDA receptor blocker; Sigma). Gabazine (Sigma) was added at the conclusion of the recording to confirm that DNQX- and t-APV–insensitive events were mediated by GABA_{A} receptors. Tetrodotoxin (TTX, 1 μM; Alomone Labs, Jerusalem, Israel) was added to isolate mIPSCs and each batch of TTX was tested to confirm its efficacy at blocking APs. 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). Whole cell access resistance and holding current were continuously monitored to confirm that recordings were stable.

IPSCs were analyzed using Mini Analysis Program 5.2.5 (Synaptoff, Decatur, GA), selecting each event by hand, and analyzing 300 events per cell. Histograms were constructed using the first 100 events per cell. For decay time histograms, decay time of individual events was defined as the time to 67% of peak amplitude. To calculate weighted decay times, an average IPSC was calculated for each cell, and its decay was fit to two exponentials using the following equation:

\[ t_{\text{decay}} = (\tau_{a1} + \tau_{a2})(a1 + a2) \]

(Sebe et al. 2003). All data are presented as means ± SE; we determined statistical significance of results using unpaired, two-tailed Student’s t-test, with \( P = 0.05 \) as the threshold for statistical significance. Where appropriate, the \( \chi^2 \) test was used to determine statistical significance.
RESULTS

Laminar distribution of interneuron subtypes

We examined interneuron distribution using the standard neurochemical markers calretinin (CR), parvalbumin (PV), and somatostatin (SOM). In hippocampus, at least three broad classes of interneurons are traditionally defined: 1) CR-positive, regular-spiking nonpyramidal (RSNP) cells; 2) PV-positive, fast-spiking (FS), basket-type cells, largely located in SO and stratum pyramidale (SP); 3) SOM-positive, burst-spiking (BST), oriens-lacunosum-moleculare interneurons (Flames and Marin 2005; Freund and Buzsáki 1996). First, we counted CR-, PV-, and SOM-positive somata in hippocampal area CA1 in coronal sections. To illustrate the overall distribution for interneuron subtypes in each section, we divided area CA1 into 400 \( \times \) 50-\( \mu \)m bins and counted the number of immunoreactive cell bodies in each bin. CR interneuron distribution in Lis1\(^{+/−}\) mice closely mirrored that of WT littermates (Fig. 1, A–D), with CR-positive somata distributed across all layers. Additionally, SOM-positive cells were mostly found in SO in WT and Lis1\(^{+/−}\) sections (Fig. 1, 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. 1, E–H). In sections from WT mice, nearly 50% of PV interneurons are located in SO, with the remaining cells divided evenly between SP and R/LM. In the Lis1\(^{+/−}\) hippocampus, about 65% of PV interneuron somas were located in R/LM, with only about 10% in SO (SO: \( P < 0.01 \); R/LM: \( P < 0.02 \)).

We previously reported that basic electrophysiological properties of Lis1\(^{+/−}\) and WT interneurons in CA1 were similar (Jones and Baraban 2007; Fig. 2, A–F). After sorting interneurons into subclasses according to firing properties, we found...
that the relative proportions of FS, RSNP, and BST cells in 
$Lis1^{+/−}$ animals ($n = 62$; Fig. 2G). Although other interneuron subtypes have been identified based on firing properties (Markram et al. 2004), we did not observe these firing patterns in our recordings. Here, RSNP was the predominant interneuron subtype encountered (71.0% in WT, 69.4% in $Lis1^{+/−}$; Fig. 2A and B). FS cells constituted about one fourth of the recorded interneurons (22.6% in WT, 24.5% in $Lis1^{+/−}$; Fig. 2C and D). BST cells were relatively infrequent (6.5% in WT, 6.1% in $Lis1^{+/−}$; Fig. 2E and F). However, when we sorted interneuron subtypes by anatomic location, we uncovered a shift in the
distribution of FS cells reminiscent of the shift observed in PV immunostaining studies. Although 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, χ² test; Fig. 2J). 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. 2H), in agreement with immunohistochemical data (Fig. 1).

GABAergic inputs to SO interneurons

Next, we explored functional consequences of interneuron disorganization by recording pharmacologically isolated sIPSCs on visually identified CA1 interneurons (n = 8 WT cells; n = 8 Lis1+/− cells) located in SO using bathing medium supplemented with glutamate receptor antagonists (20 μM DNQX and 50 μM d-APV; Fig. 3). For each cell, we confirmed interneuron identity in current-clamp mode (Fig. 3, A and B, bottom traces). To distinguish between regular-spiking interneurons and regular-spiking pyramidal neurons, we examined the fast afterhyperpolarization and spike width, both of which are significantly sharper in hippocampal interneurons (Lacaille and Williams 1990). We also distinguished between interneurons and pyramidal neurons based on morphology under IR-DIC optics, and their soma location outside the pyramidal cell body layer. Shown are sample voltage-clamp and current-clamp traces from RSNP interneurons in a WT (Fig. 3A) and Lis1 mutant (Fig. 3B) animal. Note that in all figure presentations traces shown are from the same interneuron. Interestingly, we found a greater than twofold increase in the frequency of sIPSCs onto SO interneurons in Lis1 mutant animals compared with that of controls (WT: 6.92 ± 1.48 Hz; Lis1+/−: 15.96 ± 2.67 Hz; P = 0.01; Fig. 3C). Mean sIPSC amplitude was unchanged (WT: 29.06 ± 3.05 pA; Lis1+/−: 33.02 ± 4.58 pA; P = 0.48; Fig. 3D), as was mean weighted decay time (WT: 3.95 ± 0.49 ms; Lis1+/−: 4.07 ± 0.46 ms; P = 0.85; Fig. 3E).

Next, we repeated these experiments in the presence of TTX, to isolate mIPSCs onto SO interneurons (Fig. 4; n = 11 WT cells; n = 10 Lis1+/− cells). Again, for each recorded cell, we also obtained a current-clamp recording to confirm interneuron identity (Fig. 4, A and B, bottom traces). Mean mIPSC frequency was not significantly different between WT and Lis1+/− littersates (WT: 6.26 ± 2.31 Hz; Lis1+/−: 8.42 ± 1.76 Hz; P = 0.47; Fig. 4C), suggesting that increased sIPSC frequency in mutant SO interneurons is due to AP-dependent release of GABA. We also observed no difference in mean weighted decay time (WT: 4.95 ± 0.81 ms; Lis1+/−: 4.96 ± 0.63 ms; P = 0.99; Fig. 4F). However, we found a significant decrease in mean mIPSC amplitude for Lis1+/− animals (WT: 30.58 ± 3.73 pA; Lis1+/−: 21.06 ± 1.67 pA; P = 0.04; Fig. 4D). Amplitude histograms constructed from mIPSC amplitude data demonstrated an increase in the frequency of small-amplitude events in SO interneurons from Lis1+/− animals (Fig. 4E). Interestingly, whereas WT sIPSC and mIPSC amplitude were similar, mIPSC amplitude was reduced by about 36% in mutant animals compared with sIPSC amplitude (Lis1+/− sIPSC: 33.02 ± 4.58 pA; Lis1+/− mIPSC: 21.06 ± 1.67 pA; P = 0.02). These data, along with sIPSC and mIPSC frequency results, are in agreement with our previous report that interneurons exhibit higher rates of spontaneous firing in Lis1 mutant mice, leading to increased AP-dependent GABA release onto pyramidal neurons and nearby SO interneurons.

GABAergic inputs to R/LM interneurons

Next, we recorded sIPSCs onto interneurons located in R/LM (Fig. 5; n = 14 WT cells; n = 13 Lis1+/− cells);
interneuron identity was confirmed in current-clamp mode for each cell (Fig. 5, A and B, bottom traces). We found no difference in mean sIPSC frequency (WT: 10.99 ± 2.87 Hz; Lis1+/−: 8.53 ± 2.22 Hz; P = 0.51; Fig. 5C) or amplitude (WT: 40.25 ± 4.12 pA; Lis1+/−: 43.53 ± 5.07 pA; P = 0.62; Fig. 5D). However, mean weighted decay time was significantly longer in R/LM interneurons from Lis1+/− animals (WT: 5.46 ± 0.36 ms; Lis1+/−: 7.74 ± 0.94 ms; P = 0.03; Fig. 5, E and F). A histogram of individual event decay times was consistent with this trend and showed more long-decay, presumably dendrite-innervating, events in cells from Lis1 mutants compared with those in WT littermate controls (Fig. 5G).

Finally, we recorded miniature IPSCs in voltage-clamp mode followed by current-clamp confirmation of interneuron identity (Fig. 6, A and B; n = 9 WT cells; n = 11 Lis1+/− cells). 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. 6C) and amplitude (WT: 29.52 ± 3.55 pA; Lis1+/−: 34.99 ± 5.96 pA; P = 0.47; Fig. 6D). In agreement with Shao and Dudek (2005) studies in a kainate model, we noted that mIPSC amplitudes were about 20–25% lower than sIPSC amplitudes in these experiments. Interestingly, mean mIPSC weighted decay time was significantly increased in Lis1 mutants (WT: 5.12 ± 0.51 ms; Lis1+/−: 7.37 ± 0.59 ms; P = 0.01; Fig. 6, E and F). A histogram of event decay times confirmed a rightward shift toward slower event decay (Fig. 6G).

**GABAergic inputs to FS and RSNP interneurons**

We considered the possibility that the differences we observed in inhibitory inputs to SO and R/LM interneurons might solely be a reflection of the different interneuron subtype composition in each layer. For instance, in WT SO, more of the recorded interneurons were FS cells; by contrast, in Lis1+/− SO, more interneurons were RSNP cells, due to the shift in FS cell organization that we report here. As a result, the differences that we have observed could simply be a result of comparing the FS-enriched population (WT SO) to the RSNP-enriched population (Lis1+/− SO). To address this possibility, we sorted sIPSC and mIPSC data according to interneuron subtype rather than hippocampal layer (Tables 1 and 2). Interestingly, we found that the frequency of inhibitory events on FS cells was almost doubled in Lis1 mutant mice compared with that on FS cells in WT mice, although this trend was significant only in mIPSCs (Table 1). We also found a non-significant trend toward larger sIPSC amplitudes in Lis1 mutant FS cells, as well as trends toward longer weighted decay times in IPSCs on FS cells (Table 1). When we examined inputs to RSNP interneurons, regardless of hippocampal layer (Table 2), we observed no differences in sIPSC or mIPSC frequency or amplitude. However, we found that sIPSC weighted decay time was significantly longer in RSNP cells, with a nonsignificant trend toward longer decay time in mIPSCs. These findings indicate that reorganizations of inhibitory inputs to interneurons occur not only according to laminar location, but also according to cell type.

**Discussion**

Here we report a significant reorganization of inhibitory circuits in the malformed hippocampus of a Lis1 mutant mouse. In Lis1+/− mice, the laminar location profile of PV-positive basket-type cells is disrupted and the inhibitory connectivity among interneurons is functionally altered. In patch-clamp recordings from Lis1+/− SO interneurons, we noted a significant increase in sIPSC frequency but a significant decrease in mIPSC amplitude. In recordings from Lis1+/− R/LM
interneurons, we uncovered a significant increase in the weighted decay time for sIPSCs and mIPSCs. Although anatomical reconstructions of interneuron axonal arbors were not performed, our electrophysiology findings are consistent with in vitro demonstrations by Golden and coworkers (McManus et al. 2004) that interneuron migration is slowed in \textit{Lis1} mutant mice, likely disrupting the synaptic integration of these cells.

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 these migration defects influence the laminar positioning of interneurons. After tangentially migrating from the ganglionic eminences to their final destinations in cortex and hippocampus, interneurons switch to a radial migration program to reach the proper layer (Hevner et al. 2004; Tanaka et al. 2003). Developing CA1 interneurons, including basket cells, appear to enter the developing hippocampus at the border between CA1/subiculum and the dentate gyrus, at which point they migrate radially through R/LM, SP, and finally, SO (Morozov et al. 2003, 2009). Fast-spiking interneurons might therefore populate R/LM in \textit{Lis1} mutant mice because the programs that shut down migration might turn on while cells are still migrating through R/LM on the way to SO. One factor that appears to be vital in allowing interneurons to settle in the proper laminar location is \textit{p35}, an activator of cyclin-dependent kinase 5 (Cdk5). Developing interneurons in mice lacking \textit{p35}, as in \textit{Lis1} mutant mice (McManus et al. 2004), exhibited slowed tangential migration (Rakic et al. 2009). Interneurons in \textit{p35} mutant mice, again showing similarities to \textit{Lis1} mutant mice, also populate neocortex in an altered laminar distribution (Rakic et al. 2009). In the hippocampus, dentate granule cell lamination is disrupted in \textit{p35} knock-out mice and this circuit disruption may contribute to hyperexcitability and spontaneous seizures (Patel et al. 2004; Wenzel et al.

![FIG. 5. sIPSC weighted decay time is increased in \textit{Lis1} R/LM interneurons. A, top trace: sIPSC recording from a WT R/LM interneuron. Bottom trace: current-clamp recording from the same interneuron. B, top trace: sIPSC recording from a \textit{Lis1} mutant R/LM interneuron. Bottom trace: current-clamp recording from the same interneuron. C: frequency of sIPSCs on R/LM interneurons not significantly different between WT and \textit{Lis1} mutant mice. D: amplitude of sIPSCs is unchanged in \textit{Lis1} R/LM interneurons. E: averaged sIPSC traces from representative WT (black) and mutant (gray) interneurons showing lengthened decay in \textit{Lis1} mutants. F: weighted decay time is significantly increased in \textit{Lis1} mutant R/LM interneurons (\textit{P} = 0.03, Student’s t-test). G: decay time histogram showing a trend toward longer decay events in \textit{Lis1} R/LM interneurons. Black bars: WT. Gray bars: \textit{Lis1}.](image-url)
2001). 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 any brain malformation and none has focused on interneurons in a malformed hippocampus.

**TABLE 1. sIPSC and mIPSC data recorded from fast-spiking interneurons**

<table>
<thead>
<tr>
<th></th>
<th>Frequency, Hz</th>
<th>Amplitude, pA</th>
<th>Weighted Decay Time, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. sIPSCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n = 4)</td>
<td>4.61 ± 1.00</td>
<td>26.25 ± 3.27</td>
<td>2.99 ± 0.27</td>
</tr>
<tr>
<td>Lis1&lt;sup&gt;+/−&lt;/sup&gt; (n = 7)</td>
<td>8.04 ± 2.33</td>
<td>41.20 ± 6.69</td>
<td>4.45 ± 0.79</td>
</tr>
<tr>
<td><strong>B. mIPSCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n = 7)</td>
<td>4.12 ± 1.21</td>
<td>30.58 ± 4.13</td>
<td>4.23 ± 0.52</td>
</tr>
<tr>
<td>Lis1&lt;sup&gt;+/−&lt;/sup&gt; (n = 6)</td>
<td>7.59 ± 1.07*</td>
<td>27.38 ± 3.39</td>
<td>4.91 ± 1.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P = 0.05.

**TABLE 2. sIPSC and mIPSC data recorded from regular-spiking interneurons**

<table>
<thead>
<tr>
<th></th>
<th>Frequency, Hz</th>
<th>Amplitude, pA</th>
<th>Weighted Decay Time, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. sIPSCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n = 15)</td>
<td>10.45 ± 2.50</td>
<td>36.25 ± 3.26</td>
<td>5.25 ± 0.36</td>
</tr>
<tr>
<td>Lis1&lt;sup&gt;+/−&lt;/sup&gt; (n = 14)</td>
<td>13.02 ± 2.45</td>
<td>38.69 ± 4.58</td>
<td>7.29 ± 0.92*</td>
</tr>
<tr>
<td><strong>B. mIPSCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n = 11)</td>
<td>8.68 ± 2.42</td>
<td>31.35 ± 3.77</td>
<td>5.47 ± 0.76</td>
</tr>
<tr>
<td>Lis1&lt;sup&gt;+/−&lt;/sup&gt; (n = 14)</td>
<td>7.58 ± 1.42</td>
<td>28.99 ± 5.14</td>
<td>6.94 ± 0.52</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05.

**FIG. 6.** mIPSC weighted decay time is increased in Lis1<sup>+/−</sup> R/LM interneurons. A, top trace: mIPSC recording from a WT R/LM interneuron. Bottom trace: current-clamp recording from the same interneuron. B, top trace: mIPSC recording from a Lis1<sup>+/−</sup> R/LM interneuron. Bottom trace: current-clamp recording from the same interneuron. C: frequency of mIPSCs on R/LM interneurons not significantly different between WT and Lis1<sup>+/−</sup> mutant mice. D: amplitude of mIPSCs is unchanged in Lis1<sup>+/−</sup> R/LM interneurons. E: averaged mIPSC traces from representative WT (black) and mutant (gray) interneurons showing lengthened decay in Lis1 mutants. F: weighted decay time is significantly increased in Lis1 mutant R/LM interneurons (P = 0.01, Student’s t-test). G: decay time histogram showing a shift toward longer decay events in Lis1<sup>+/−</sup> R/LM interneurons. Black bars: WT. Gray bars: Lis1<sup>+/−</sup>.

**GABAergic inputs to interneurons in epilepsy and brain malformations**

In the epileptic and malformed brain, significant reorganizations in synaptic transmission occur. These alterations include increased excitation and decreased inhibition of hippocampal and cortical principal neurons (Cobos et al. 2005;
neurons could enhance the synchronization of neural networks, principal neurons, resulting in disinhibition and hyperexcitability. RSNP cells in GABAergic input than do FS cells. The increased presence of animals, RSNP cells consistently receive a higher frequency of sIPSC frequency onto FS cells was nearly doubled in frequency by cell type, rather than by layer, we found that the reduction in mIPSC amplitude on Lis1+/− SO interneurons is not simply due to an increased presence of RSNP cells in this layer, but rather reflects a modification specific to all SO interneurons. In contrast with the presynaptic enhancement of inhibitory input to these interneurons, a reduction in mIPSC amplitude is likely to reflect a postsynaptic modification, such as 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 on dentate granule cells in kainate-treated rats, which the authors argue to be a compensatory response to decreased frequency of IPSCs on granule cells. In both examples, there appears to be an alteration in postsynaptic GABA_A receptor number in an attempt to counterbalance either an increase or a 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 weighted decay time of spontaneous and miniature GABAergic events is significantly longer in Lis1+/− R/LM interneurons. When we sorted decay time data by interneuron subtype, rather than by layer, we found that FS cells consistently showed trends toward faster weighted decay times than RSNP cells in both WT and mutant animals. We also found that IPSCs onto both FS and RSNP cells appeared to decay more slowly in Lis1 mutants compared with WT FS and RSNP cells. The increased decay time of Lis1+/− R/LM interneurons is not simply a result of recording more FS cells in mutant animals, then, for two reasons. First, both FS and RSNP cells appear to exhibit a lengthened decay time in mutant animals. Second, because IPSCs on FS cells decay more rapidly than IPSCs on RSNP cells in both WT and mutant mice, an increase in the proportion of FS cells in Lis1+/− R/LM would be expected to reduce, rather than lengthen, decay time.

A similar observation was reported for 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), suggesting that R/LM interneurons in Lis1 mutant mice express GABA_A receptors composed of subunits different 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), whereas 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 down-regulated and is largely replaced by α1 subunit expression (Heinen et al. 2002; Wenzel et al. 2000; Williams et al. 2007). Relevant to the studies described here, Morin and colleagues (1999), using electron microscopy, found layer-specific alterations in GABA synapses onto CA1 interneurons in kainic acid–injected rats. Specifically, inhibitory synapses on somata of lacunoumolecular interneurons 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. Despite this layer-specific reorganization of inhibitory input to interneurons, the properties of IPSCs onto CA1 interneurons are unchanged in kainate-treated rats—a discrepancy that remains unexplained (Morin et al. 1998). GABAergic inputs to certain hippocampal interneurons also undergo reorganization in humans with TLE. For example, anatomic studies demonstrate that calbindin (CB)-positive interneurons in CA1 receive an increased number of GABA synapses from both CB-positive and CB-negative interneurons (Wittner et al. 2002). This anatomic evidence, together with our electrophysiology studies, suggests that GABAergic inputs to interneurons undergo significant functional reorganization in many types of epilepsy.

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. Although loss of inhibition of principal neurons is commonly cited as an underlying cause of hyperexcitability and seizures, any understanding of the circuit rearrangements associated with epilepsy and malformations is necessarily incomplete without understanding inhibitory inputs to interneurons. Networks of interneurons are integral in controlling overall network excitability (Cossart et al. 2005). Here we found 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. When we analyzed sIPSC frequency by cell type, rather than by layer, we found that sIPSC frequency onto FS cells was nearly doubled in Lis1 mutant mice. We also found that, in both WT and mutant animals, RSNP cells consistently receive a higher frequency of GABAergic input than do FS cells. The increased presence of RSNP cells in Lis1+/− SO likely contributes, at least in part, to the increased sIPSC frequency onto the population of Lis1+/− SO interneurons. Increased inhibition of interneurons may contribute to an epileptic phenotype because 1) enhanced inhibitory input may reduce the output of SO interneurons onto principal neurons, resulting in disinhibition and hyperexcitability (Cohen et al. 2006), or 2) augmented inhibition of interneurons could enhance the synchronization of neural networks, which may lead to seizures (Cobb et al. 1995; Uhlhaas and Singer 2006; Wang and Buzsáki 1996).

Interestingly, we also find that mIPSCs on Lis1+/− SO interneurons are significantly reduced in amplitude. When we sorted mIPSC data by cell type, we found no evidence that subclass-specific alterations in mIPSC amplitude occur. Moreover, mIPSC amplitudes of FS and RSNP cells were comparable in WT and mutant animals. These data indicate that the reduction in mIPSC amplitude on Lis1+/− SO interneurons is not simply due to an increased presence of RSNP cells in this layer, but rather reflects a modification specific to all SO interneurons.
2004). Modifications in GABA$_A$ receptor subunits have been observed in TLE animal models (Poulter et al. 1999), with epileptic animals showing reduced $\alpha_1$ expression (Brooks-Kayal et al. 1998). Such modifications may also take place in the Lis1 mutant mouse hippocampus. The increased weighted decay time of sIPSCs and mIPSCs on Lis1$^{+/−}$/R/LM interneurons is consistent with the hypothesis that a developmental switch from $\alpha_2$/$\alpha_3$- to $\alpha_1$-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 (Lis1, 2006). Thus 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.

**Overall summary of inhibitory circuits in lissencephaly mice**

Our studies begin to provide a clear picture of the extensive inhibitory circuit reorganizations that take place in area CA1 of the Lis1 mutant hippocampus. Inhibitory interneurons within a disorganized region of hippocampus receive increased excitatory drive from CA3 pyramidal cells and fire more frequently than WT interneurons (Jones and Baraban 2007). These changes result in a significant enhancement of inhibitory input (e.g., sIPSCs) to CA1 pyramidal neurons and to nearby SO interneurons. In contrast, R/LM interneurons do not receive enhanced inhibition, although they may express an abnormal complement of GABA$_A$ receptor subunits. We also report a disruption of interneuron lamination. Together, these alterations may directly contribute to the clissencephalapy phenotypes, including seizures and cognitive deficits.

**ACKNOWLEDGMENTS**

We thank J. Sebe for helpful discussions and M. Dinday for genotyping and maintenance of the Lis1 mouse colony (founders generously donated by A. Wynshaw-Boris).

**GRANTS**

This work was supported by National Institute of Neurological Disorders and Stroke Grant R01 NS-40272 to S. C. Baraban.

**REFERENCES**


Goldstein PA, Elsen FP, Yang SW, Ferguson C, Homances GE, Harrison NL. Prolongation of hippocampal miniature inhibitory postsynaptic currents in mice lacking the GABA$_A$ receptor $\alpha_1$ subunit. J Neurophysiol 88: 3208–3217, 2002.


