

ALLN rescues an in vitro excitatory synaptic transmission deficit in *Lis1* mutant mice

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Sebe JY, Bershteyn M, Hirotsune S, Wynshaw-Boris A, Baraban SC. ALLN rescues an in vitro excitatory synaptic transmission deficit in *Lis1* mutant mice. *J Neurophysiol* 109: 429–436, 2013. First published October 24, 2012; doi:10.1152/jn.00431.2012.—*LIS1* gene mutations lead to a rare neurological disorder, classical lissencephaly, characterized by brain malformations, mental retardation, seizures, and premature death. Mice heterozygous for *Lis1* (*Lis1*^{+/-}) exhibit cortical malformations, defects in neuronal migration, increased glutamate-mediated synaptic transmission, and spontaneous electrographic seizures. Recent work demonstrated that in utero treatment of *Lis1*^{+/-} mutant dams with ALLN, a calpain inhibitor, partially rescues neuronal migration defects in the offspring. Given the challenges of in utero drug administration, we examined the therapeutic potential of ALLN on postnatal lissencephalic cells. Voltage- and current-clamp studies were performed with acute hippocampal slices obtained from *Lis1* mutant mice and age-matched littermate control mice. Specifically, we determined whether postnatal ALLN treatment can reverse excitatory synaptic transmission deficits, namely, an increase in spontaneous and miniature excitatory postsynaptic current (EPSC) frequency, on CA1 pyramidal neurons observed in tissue slices from *Lis1*^{+/-} mice. We found that acute application of ALLN restored spontaneous and miniature EPSC frequencies to wild-type levels without affecting inhibitory postsynaptic synaptic current. Furthermore, Western blot analysis of protein expression, including proteins involved in excitatory synaptic transmission, demonstrated that ALLN blocks the cleavage of the calpain substrate α I-spectrin but does not rescue *Lis1* protein levels in *Lis1*^{+/-} mutants.

epilepsy; lissencephaly; cortical malformation; ALLN; treatment

LISSENCEPHALY is a neurological disorder characterized by a smooth brain with regions of broad or no gyri, enlarged ventricles, and abnormal cortical lamination. Infants with lissencephaly are developmentally delayed, experience feeding problems, and are susceptible to pneumonia. Within the first year of life, children start exhibiting seizures, commonly infantile spasms, that can cause irreversible brain damage and additional cognitive impairment (Dobyns 2010). Children who suffer the most severe forms of this disorder (Dobyns et al. 1993), termed classical or type I lissencephaly, have a high mortality rate, with very few surviving into adulthood (Dobyns 2010). Although neuroanatomical defects in patients diagnosed with lissencephaly and other developmental disorders cannot be reversed, drugs that limit the cell damage and cognitive impairment normally associated with hyperexcitability (and seizures) may also reduce the prevalence of feeding problems

and episodes of pneumonia. One approach to discover effective medications is to elucidate the underlying cause of hyperexcitability in lissencephalic patients with a relevant animal model.

In humans, lissencephaly and associated developmental disorders are caused by mutations in a number of genes, e.g., *LIS1*, *DCX*, *RELN*, and *ARX* (Dobyns 2010; Hong et al. 2000; Kato and Dobyns 2003). Among these, *LIS1* haploinsufficiency is the most common genetic cause of classical lissencephaly and produces the most severe neuroanatomical, cognitive, and seizure defects (Dobyns 2010; Dobyns et al. 1993). A mouse model of classical lissencephaly that possesses a heterozygous mutation in the *Lis1* gene (*Lis1*^{+/-} mutants) (Hirotsune et al. 1998) has helped identify a critical *Lis1* role in brain development and the physiological consequences of *Lis1* protein deficiency. Similar to human patients, *Lis1*^{+/-} mice exhibit neuronal migration defects, enlarged ventricles, impaired learning, and spontaneous seizures (Fleck et al. 2000; Greenwood et al. 2009; Hirotsune et al. 1998; Paylor et al. 1999). *Lis1*-deficient neurons have been used to demonstrate a role for *Lis1* protein in a range of cell motility functions including anterograde and retrograde transport along microtubules (Yamada et al. 2008), cell division (Yingling et al. 2008), and neuronal positioning (Shu et al. 2004). For example, a reduction in *Lis1* gene expression results in neurons that migrate more slowly and have trouble locating correct lamina within the brain (Hirotsune et al. 1998). Anatomical defects characterized by a loose distribution of cells are evident in the *Lis1*^{+/-} mouse neocortex, olfactory bulb, and particularly the hippocampus (Greenwood et al. 2009; Hirotsune et al. 1998; Wang et al. 2007). The pyramidal cell layers of hippocampal CA1, CA2, and CA3 regions that are normally a continuous and tightly packed layer of cells are, in *Lis1*^{+/-} mutants, split into multiple, loosely organized cell layers. In addition, electrophysiological analysis (Greenwood et al. 2009) demonstrated that CA1 pyramidal cells within the loosely formed CA1 region of hippocampus receive an elevated level of excitatory glutamate-mediated synaptic input. One potential explanation for network hyperexcitability associated with an increased level of excitation is electron microscopy evidence suggesting a high density of glutamatergic synaptic vesicles in *Lis1*^{+/-} Schaffer collateral terminals (Greenwood et al. 2009).

A mouse model of lissencephaly with defects in neuronal migration and robust in vitro hyperexcitability also provides a useful tool with which to test new therapies. One family of therapeutic targets is the calpains, which are endogenous Ca²⁺-dependent cysteine proteases involved in normal brain function

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(Goll et al. 2003) that are activated at elevated levels in pathological conditions (Crocker et al. 2003; Saatman et al. 2010; Saito et al. 1993). Using *Lis1*^{+/-} mutants, Yamada et al. (2009) demonstrated that in utero administration of the calpain inhibitor ALLN partially rescues hippocampal and neocortical neuronal migration defects. Given these encouraging anatomical results, the primary aim of the present work is to determine whether ALLN can reverse the functional excitatory transmission defect exhibited by *Lis1*^{+/-} mutants. In contrast to the previous study that administered ALLN in utero and rescued neuroanatomical defects that occur at embryonic stages (Yamada et al. 2009), here we examined whether ALLN can reverse existing excitatory synaptic transmission defects in an already malformed postnatal brain.

METHODS

Slice preparation. Acute brain slices were prepared from P14–26 male or female wild-type (WT) or *Lis1*^{+/-} mice. All procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the University of California, San Francisco Institutional Animal Care and Use Committee. Mice were anesthetized with a ketamine-xylazine mixture and decapitated. The brain was rapidly removed, and 300- μ m slices were prepared with a vibratome (Leica VT1000S, Bannockburn, IL) in oxygenated high-sucrose artificial cerebrospinal fluid (ACSF; in mM: 150 sucrose, 50 NaCl, 25 NaHCO₃, 10 dextrose, 2.5 KCl, 1 Na₂HPO₄·H₂O, 0.5 CaCl₂, and 7 MgCl₂) at 4°C. During incubation (40 min at 35°C) and recording, slices were perfused with a carbogen-bubbled ACSF containing (in mM) 124 NaCl, 3 KCl, 1.25 NaH₂PO₄·H₂O, 2 MgSO₄·7H₂O, 26 NaHCO₃, 10 dextrose, and 2 CaCl₂. For recording, slices were maintained at 33–34°C with ACSF flowing at 9–10 ml/min. Immediately after slices were prepared, tissue was treated with ACSF containing either DMSO only or 10 μ M ALLN + DMSO. For electrophysiological experiments, slices were incubated for at least 3 h prior to recording and treatment continued throughout the recording.

Electrophysiology. All recordings were obtained from CA1 pyramidal cells that were visually identified with infrared-differential interference contrast (IR-DIC) optics on an Olympus BX-51WI microscope. Data were acquired with Clampex software (Molecular Devices, Sunnyvale, CA) at a gain of 5 and filtered at 5 and 10 kHz for voltage- and current-clamp recordings, respectively. For whole cell recordings of excitatory postsynaptic currents (EPSCs, spontaneous and miniature) and intrinsic membrane properties, patch electrodes (3 M Ω) were filled with (in mM) 140 K-gluconate, 1 NaCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, 5 EGTA, and 2 NaATP. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded with the following internal solution (in mM): 140 CsCl, 1 MgCl₂, 10 HEPES, 11 EGTA, 2 NaATP, and 0.5 Na₂GTP. To isolate AMPA receptor (AMPA)-mediated or GABAergic currents, slices were treated with 20 μ M bicuculline methiodide (BIC) or APV (25 μ M) and DNQX (20 μ M) (Sigma), respectively, and held at -60 mV. To isolate miniature (m)EPSCs, slices were perfused with BIC and TTX (1 μ M). To record intrinsic membrane properties, slices were bathed in ACSF only. For all whole cell recordings, the series resistance was measured after each recording and data were discarded if the resistance changed by >20% or if the series resistance was >20 M Ω . EPSCs and IPSCs were analyzed off-line with MiniAnalysis software. Two-way ANOVAs were used to compare means among the four treatment groups with SPSS 9.0 (SPSS).

Western blot. Hippocampal slices were prepared in the same way as for electrophysiological recordings and treated with vehicle DMSO only or 10 μ M ALLN for 4 h prior to protein extraction. For each sample, WT and *Lis1*^{+/-} slices were obtained from age-matched littermates. Cytosolic proteins were extracted with a Nuclear/Cytosol Fractionation Kit (BioVision) according to instructions accompanying

the kit. Protein concentrations were determined with a BCA Protein Assay Kit (Pierce), and equal amounts of protein (typically 10–15 μ g) were loaded into each well of a 4–20% TGX gradient gel and subjected to SDS-PAGE. Proteins were then transferred onto nitrocellulose membrane and subjected to Western blot coupled with ECL detection according to standard methods. The following primary antibodies were used: Lis1 (Santa Cruz sc-7577, goat 1:250), α -spectrin/ α -fodrin (Abcam ab75755, rabbit 1:1,000), actin (Sigma AC74, mouse 1:10,000), SNAP25 (Synaptic Systems 111 001, mouse 1:1,000), synaptotagmin (BD Bioscience, mouse 1:1,000), NMDA receptor (NMDAR) subunit 2A (NR2A; Chemicon AB10531, rabbit 1:1,000), NMDAR subunit 2A (NR2B; Invitrogen, 71-8600), GluR1 (Chemicon, AB1504, rabbit 1:1,000), reelin (Abcam ab18570, mouse 1:1,000). Representative Western blots from four independent experiments are shown in Fig. 5. Relative protein levels were measured with ImageJ software. Two-way ANOVAs were used to compare means among the four treatment groups with SPSS 9.0.

RESULTS

ALLN selectively rescues excitatory synaptic current deficit. To determine whether 1) calpain is endogenously active in postnatal WT or *Lis1*^{+/-} brain tissue and 2) an acute treatment protocol blocks calpain-mediated proteolysis, we treated horizontal slices including hippocampus and neocortex with either DMSO alone or DMSO + 10 μ M ALLN (4-h incubation). To assay for calpain activity, we analyzed the cleavage of a well-established calpain substrate, α II-spectrin, using Western blot analysis. Endogenous calpain normally cleaves the 240-kDa full-length (FL) α II-spectrin, producing a 145- to 150-kDa breakdown product (BDP) (Fig. 1A). Higher levels of BDP on Western blot reflect more calpain-mediated proteolysis. Figure 1B shows that relative levels of α II-spectrin FL to BDP are equivalent in tissue from WT versus *Lis1*^{+/-} mice; ALLN treatment effectively reduces BDP fragment levels in both genotypes. Given that ALLN inhibits endogenous calpain activity in WT and *Lis1*^{+/-} slices, we next examined whether ALLN can reverse the in vitro hyperexcitability associated with Lis1 deficiency. Using an acute hippocampal slice preparation and IR-DIC assisted patch clamp, we recorded spontaneous (s)EPSCs and mEPSCs in voltage-clamp mode from visually identified CA1 pyramidal WT or *Lis1*^{+/-} cells. Recordings were obtained at a holding potential of -60 mV; NMDARs are blocked at this potential, and EPSCs reflect AMPAR-mediated excitatory currents (Nowak et al. 1984) (Fig. 1B). As previously reported (Greenwood et al. 2009), *Lis1*^{+/-} CA1 pyramidal cells receive a nearly twofold higher frequency of sEPSCs relative to WT (WT: 2.0 \pm 0.3 Hz, n = 21; Lis1: 3.9 \pm 0.3 Hz, n = 21; 2-way ANOVA P < 0.05). After acute ALLN treatment (4 h), mean WT sEPSC frequency was statistically unchanged (1.2 \pm 0.2 Hz, n = 21), but mean *Lis1*^{+/-} frequency was restored to WT levels (2.0 \pm 0.2 Hz, n = 21; 2-way ANOVA P < 0.05; Fig. 1D). Mean sEPSC amplitudes and decays were unchanged across all treatment groups (Fig. 1, E and F).

Next, we recorded miniature, action potential-independent, EPSCs from WT or *Lis1*^{+/-} CA1 pyramidal cells in untreated or ALLN-treated slices (Fig. 2A). Again, we confirmed previous findings that *Lis1*^{+/-} cells receive a higher frequency of mEPSCs relative to WT (WT: 0.6 \pm 0.1 Hz, n = 13; Lis1: 1.5 \pm 0.2 Hz, n = 14; 2-way ANOVA P < 0.001). After ALLN treatment, mean WT mEPSC frequency was unchanged

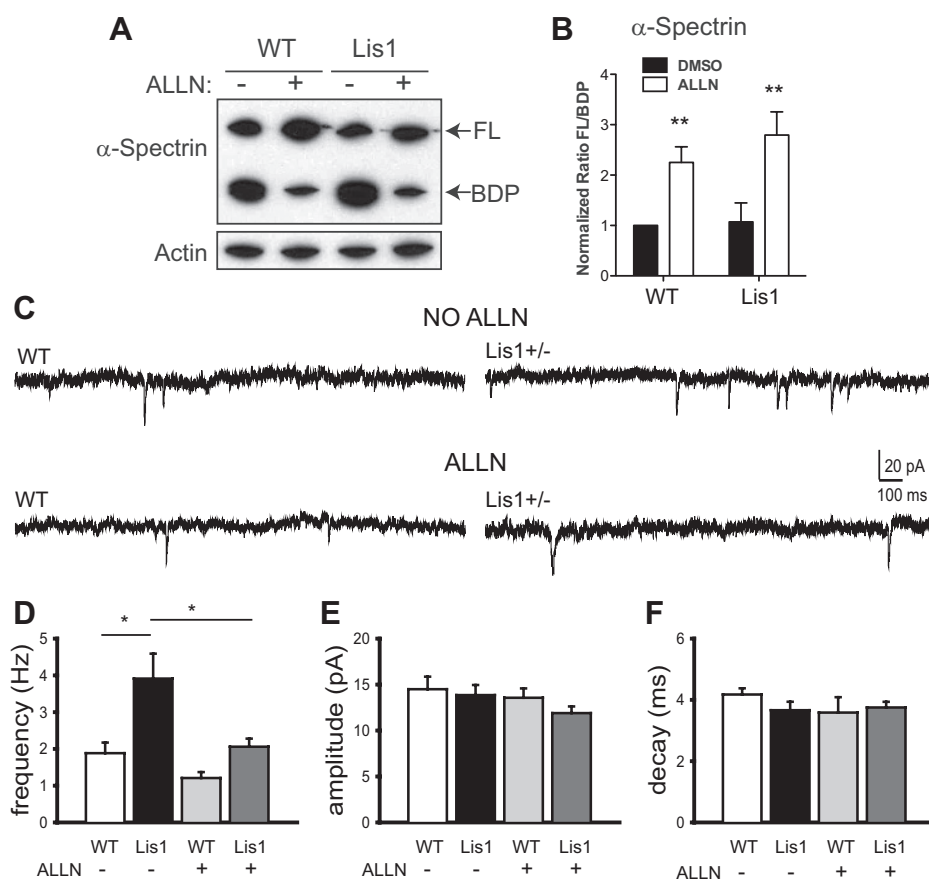


Fig. 1. ALLN incubation reduces the frequency of spontaneous excitatory inputs onto CA1 pyramidal cells. *A*: representative Western blot demonstrates that ALLN treatment inhibits cleavage of α -spectrin in both wild-type (WT) and *Lis1*^{+/-} brain slice lysates. FL, full-length protein; BDP, breakdown product. *B*: data are summarized as a ratio of FL vs. BDP. ***P* < 0.01, 2-way ANOVA; *n* = 4. *C*: representative raw traces of spontaneous excitatory postsynaptic currents (sEPSCs) in the absence of (*top*) and after incubation in (*bottom*) 10 mM ALLN. *D*: the frequency of sEPSCs was greater in *Lis1*^{+/-} mice relative to WT littermates. ALLN treatment reduced the frequency of sEPSCs recorded from *Lis1*^{+/-} cells to that of WT levels. *E* and *F*: amplitude (*E*) and decay (*F*) of sEPSCs recorded from CA1 pyramidal cells were not different between WT and *Lis1*^{+/-} mice. Furthermore, ALLN treatment did not affect either sEPSC amplitude or decay. **P* < 0.05, 2-way ANOVA; *n* = 21 cells for each condition. Means \pm SE.

(0.7 ± 0.1 Hz, *n* = 14) but mean *Lis1* frequency was restored to WT levels (0.9 ± 0.1 Hz, *n* = 12, 2-way ANOVA *P* < 0.05; Fig. 2*B*). Mean mEPSC amplitudes were unchanged across all treatment groups (Fig. 2*C*); mEPSCs recorded from *Lis1*^{+/-} DMSO- as well as ALLN-treated cells (Fig. 2*D*) decayed more rapidly than both groups of WT cells [no ALLN: WT = 5.7 ± 0.3 (*n* = 14), *Lis1* = 4.7 ± 0.3 (*n* = 13); ALLN: WT = 5.8 ± 0.4 (*n* = 13), *Lis1* = 4.7 ± 0.2 (*n* = 12); 2-way ANOVA].

Next, we examined sIPSCs under the same recording conditions (Fig. 3*A*). The baseline frequency of sIPSCs recorded

from CA1 pyramidal cells was not statistically different between WT and *Lis1*^{+/-} mice (no ALLN: WT = 19.3 ± 4.7 , *n* = 17; *Lis1* = 18.6 ± 4.5 , *n* = 17; Fig. 3*B*). These values were not affected by acute ALLN treatment (ALLN: WT = 19.0 ± 4.6 , *n* = 17; *Lis1* = 18.1 ± 4.4 , *n* = 17). Amplitude and decay times were not statistically different among all four groups (Fig. 3, *C* and *D*). Taken together, these data suggest that acute ALLN treatment selectively rescues excitatory synaptic transmission deficits associated with *Lis1* deficiency.

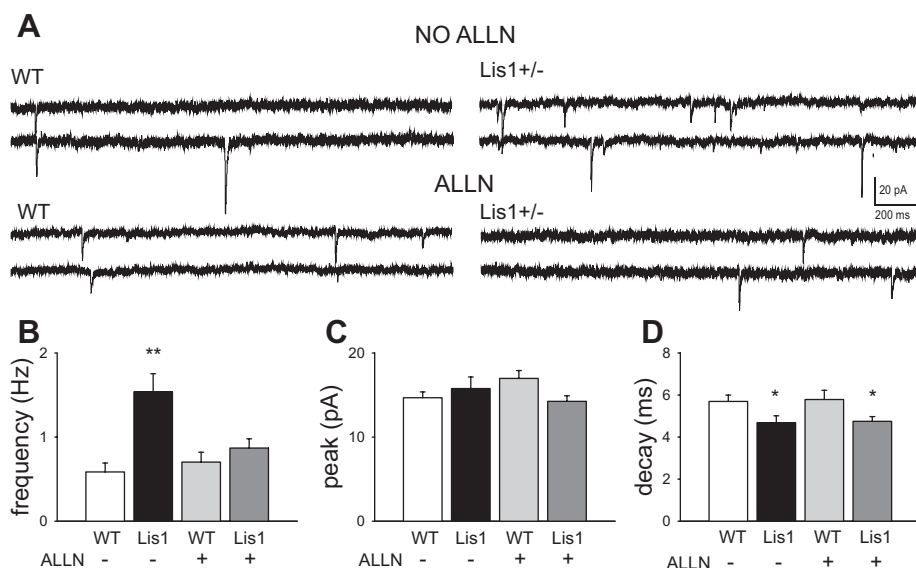


Fig. 2. ALLN incubation reduces the frequency of miniature excitatory inputs onto CA1 pyramidal cells. *A*: representative raw traces of miniature (m)EPSCs in the absence of (*top*) and after incubation in (*bottom*) 10 mM ALLN. *B*: the frequency of mEPSCs was greater in *Lis1*^{+/-} mice relative to WT littermates. ALLN treatment reduced the frequency of mEPSCs recorded from *Lis1*^{+/-} cells to that of WT levels. *C*: the mean amplitude of mEPSCs was indistinguishable among the 4 groups. *D*: mEPSCs recorded from both groups of *Lis1*^{+/-} cells decayed more rapidly than currents from untreated and treated WT cells. **P* < 0.05; ***P* < 0.01; 2-way ANOVA. Means \pm SE. No ALLN: WT (*n* = 14), *Lis1* (*n* = 13); ALLN: WT (*n* = 13), *Lis1* (*n* = 12).

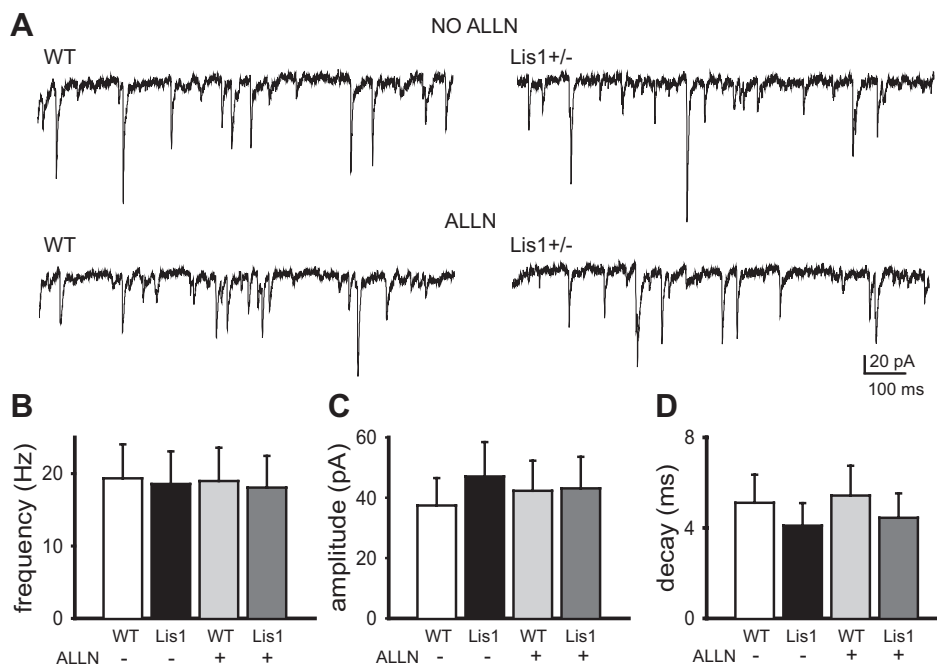


Fig. 3. ALLN incubation does not affect spontaneous inhibitory synaptic inputs onto CA1 pyramidal cells. *A*: representative raw traces of spontaneous inhibitory postsynaptic currents (sIPSCs) in the absence of (*top*) and after incubation in (*bottom*) ALLN. The frequency (*B*), amplitude (*C*), and decay (*D*) of sIPSCs recorded from CA1 pyramidal cells were not different between WT and *Lis1*^{+/-} mice (2-way ANOVA). Furthermore, ALLN treatment did not affect sIPSC amplitude or decay. *n* = 17 cells for each condition. Means \pm SE.

ALLN does not alter CA3 pyramidal cell excitability. To examine whether ALLN reduces the excitability of the presynaptic CA3 pyramidal cells that provide input to CA1 cells, we recorded intrinsic membrane properties from visually identified CA3 neurons in each of the four experimental groups. In current-clamp mode, we measured input resistance (R_{input}), resting membrane potential (V_{rest}), threshold potential ($V_{threshold}$), and firing frequency at twice the firing threshold. Representative recordings from untreated and ALLN-treated *Lis1*^{+/-} pyramidal cells are shown in Fig. 4, *A* and *B*. Consistent with previous recordings (Greenwood et al. 2009; Jones and Baraban 2007), none of these parameters was significantly different in WT versus *Lis1*^{+/-} cells before or after ALLN treatment (Fig. 4, *C–F*).

ALLN regulates cleavage and protein expression at excitatory terminals. Calpain regulates processing of a number of proteins at excitatory terminals, including glutamate receptors and vesicle fusion machinery. We therefore used Western blot analysis to quantify protein levels in tissue obtained from WT and *Lis1*^{+/-} slices that were incubated in vehicle DMSO or ALLN (10 μ M in DMSO) for 4 h. In contrast to ALLN treatment of mouse embryonic fibroblasts (MEFs) or dorsal root ganglia (DRG) neurons in vitro (Yamada et al. 2009), postnatal *Lis1*^{+/-} brain slices did not recover Lis1 protein levels after ALLN incubation (Fig. 5*A*). This may be due to the older age of the brain tissue and/or the acute nature of the in vitro ALLN treatment. ALLN treatment had no effect on the levels of actin, reelin, synaptotagmin, Gapdh, and FL NR2A (Fig. 5). These protein levels were also equivalent in WT versus *Lis1*^{+/-} lysates. Although FL SNAP-25 levels were unchanged regardless of genotype or treatment condition, ALLN blocked cleavage of SNAP-25. This latter effect is demonstrated by the absence and/or low level of the smaller SNAP-25 BDP after acute ALLN treatment (Fig. 5*A*). The ratio of FL to BDP is greater in both WT and *Lis1*^{+/-} lysates after ALLN treatment (SNAP-25 FL/BDP: WT DMSO = 1 ± 0 ; WT ALLN = 10 ± 2 ; Lis1 DMSO = 2 ± 1 ; Lis1 ALLN =

7 ± 0 ; *n* = 2; *P* < 0.01, 2-way ANOVA). AMPAR GluR1 and NMDAR NR2B subunit protein levels were equivalent for WT and *Lis1*^{+/-} lysates; however, ALLN increased the levels of these proteins in both genotypes. The increase in GluR1 protein following ALLN treatment is consistent with work by Yuen et al. (2007), yet Western blot analysis coupled with recordings of mEPSCs suggest that elevated AMPAR subunit levels do not necessarily correlate with increased synaptic glutamatergic transmission.

DISCUSSION

Here we demonstrated that ALLN treatment selectively rescues glutamatergic synaptic transmission defects in a disorganized hippocampus from a mouse model of lissencephaly. Furthermore, current-clamp recordings of CA3 pyramidal cells, which send inputs to CA1 cells, show no indication that lissencephalic neurons or ALLN treatment affects the firing properties of hippocampal cells. Western blot analysis of brain slice lysates suggests that ALLN does not rescue the excitability defect by acting on Lis1 or proteins involved in synaptic vesicle fusion that we examined. Together, our data suggest that the elevated excitatory transmission onto *Lis1*^{+/-} cells and its rescue by ALLN is mediated by an action potential-independent presynaptic mechanism that may be selective for glutamatergic synapses and could directly involve calcium-mediated packaging/release of neurotransmitter at the synaptic terminal.

ALLN-regulated physiological and pathological neural activity. The two main isoforms of calpain found in the brain, μ -calpain and m-calpain, are activated by micromolar and millimolar calcium concentrations, respectively, and serve similar physiological functions (Goll et al. 2003). Calpain-mediated proteolysis enables long-term potentiation (a synaptic mechanism thought to underlie learning and memory) by enhancing activation of mitogen-activated protein kinase (Shimizu et al. 2007; Zadrán et al. 2010) and mediates apoptosis

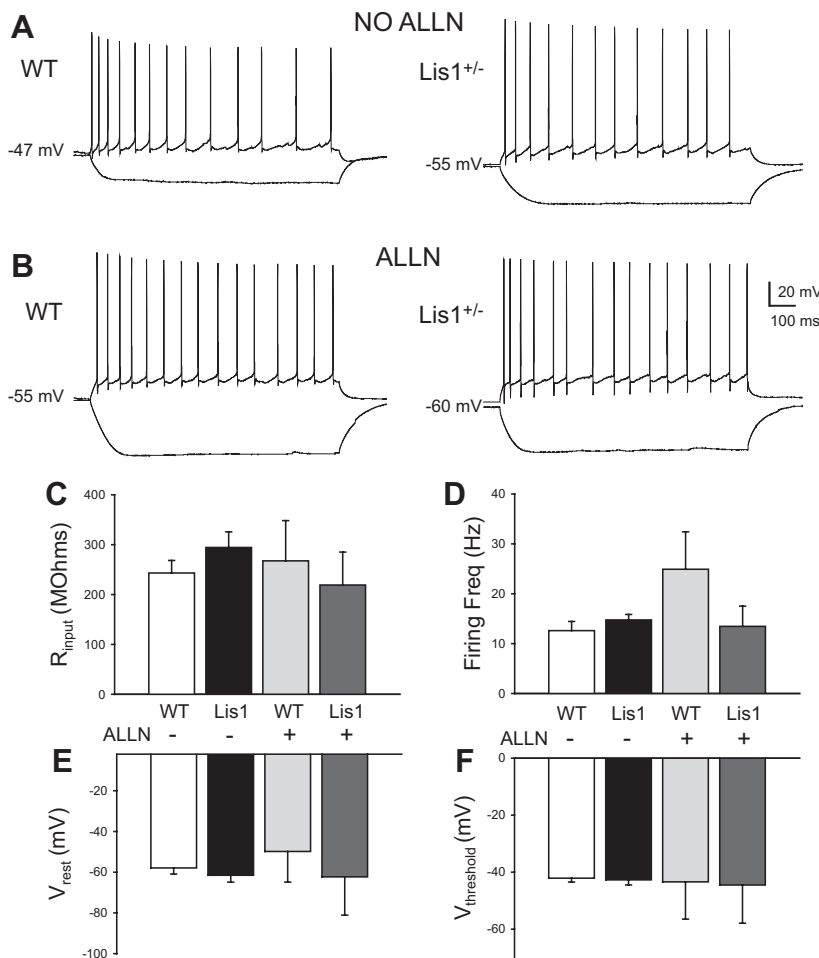


Fig. 4. ALLN does not affect the intrinsic membrane properties of CA3 pyramidal cells. *A* and *B*: representative current-clamp recordings of WT and Lis1 cells incubated in DMSO only (*A*) and in ALLN (*B*). All pairs of recordings show responses to -100 and 100 pA DC current steps. *C–F*: mean input resistances (R_{input} , *C*), firing frequencies (*D*), resting membrane potentials (V_{rest} , *E*), and threshold potentials ($V_{threshold}$, *F*) did not differ between genotypes or after ALLN treatment [no ALLN: WT ($n = 10$), Lis1 ($n = 10$); ALLN: WT ($n = 11$), Lis1 ($n = 10$); 2-way ANOVA].

(Raynaud and Marcilhac 2006), migration (Franco and Huttenlocher 2005), and cell cycle progression (Santella et al. 1998). Although extensive studies have examined the effect of calpain on both presynaptic and postsynaptic protein levels (Bi et al. 1994; Buddhala et al. 2012; Grumelli et al. 2008; Guttmann et al. 2001; Yuen et al. 2007), few studies have correlated protein levels with functional electrophysiological responses particularly with regard to pathological conditions. The few exceptions are studies of cultured cortical pyramidal cells that were treated with NMDA to model increased excitability and stimulate calpain activity (Yuen et al. 2007, 2008). Calpain increased both GluR1 protein levels and AMPAR currents elicited by exogenous glutamate application in these studies. In contrast, the present work examines synaptic glutamatergic currents and calpain activity in brain slices from a genetic mouse model of human lissencephaly. In this more intact circuit, we find that increased GluR1 levels following ALLN treatment do not correspond to increases in synaptic glutamatergic transmission. It is possible that ALLN treatment increases GluR1 subunits located extrasynaptically or in the cytosol and has a negligible effect on GluR1 subunits that compose functional, postsynaptically located AMPARs. Therefore, electrophysiological measures of physiological synaptic responses are useful in determining the functional relevance of protein analysis. In contrast to NMDA-treated cultured neurons and pathological human brain tissue (Crocker et al. 2003; Saito et al. 1993), calpain activity is apparently

equivalent in WT versus *Lis1*^{+/-} brain slices. In *Lis1*^{+/-} mice CA1 neurons receive enhanced glutamatergic transmission that likely contributes to the spontaneous seizures previously reported by our group (Greenwood et al. 2009). This suggests that substantial excitation and calcium overload are necessary to induce elevated calpain activity, but that abnormal calpain activity is not required for ALLN to reverse cellular hyperexcitability.

Factors affecting selective rescue of glutamate release. It is now well established that GABAergic (inhibitory) and glutamatergic (excitatory) synapses differ not only in their functional outputs but also in their expression of distinct pre- and postsynaptic proteins. Excitatory versus inhibitory synapses are equipped with different synapse-spanning neuroligin-neurexin complexes (Varoqueaux et al. 2004, 2006), components of the synaptic vesicle release machinery (Augustin et al. 1999; Varoqueaux et al. 2002; Verderio et al. 2004), and neurotransmitter receptor scaffolding proteins (Fritschy et al. 2008; Kim and Sheng 2004). In the present study, we found that glutamatergic and not GABAergic inputs onto CA1 pyramidal cells in *Lis1*^{+/-} mice are enhanced relative to WT. Furthermore, ALLN selectively rescued the frequency of both miniature and spontaneous excitatory events in *Lis1*^{+/-} cells to WT levels without changing parameters of spontaneous inhibitory events. Given that excitation is exclusively regulated in both cases, differences in the presynaptic release machinery of glutamatergic versus GABAergic terminals may underlie the observed

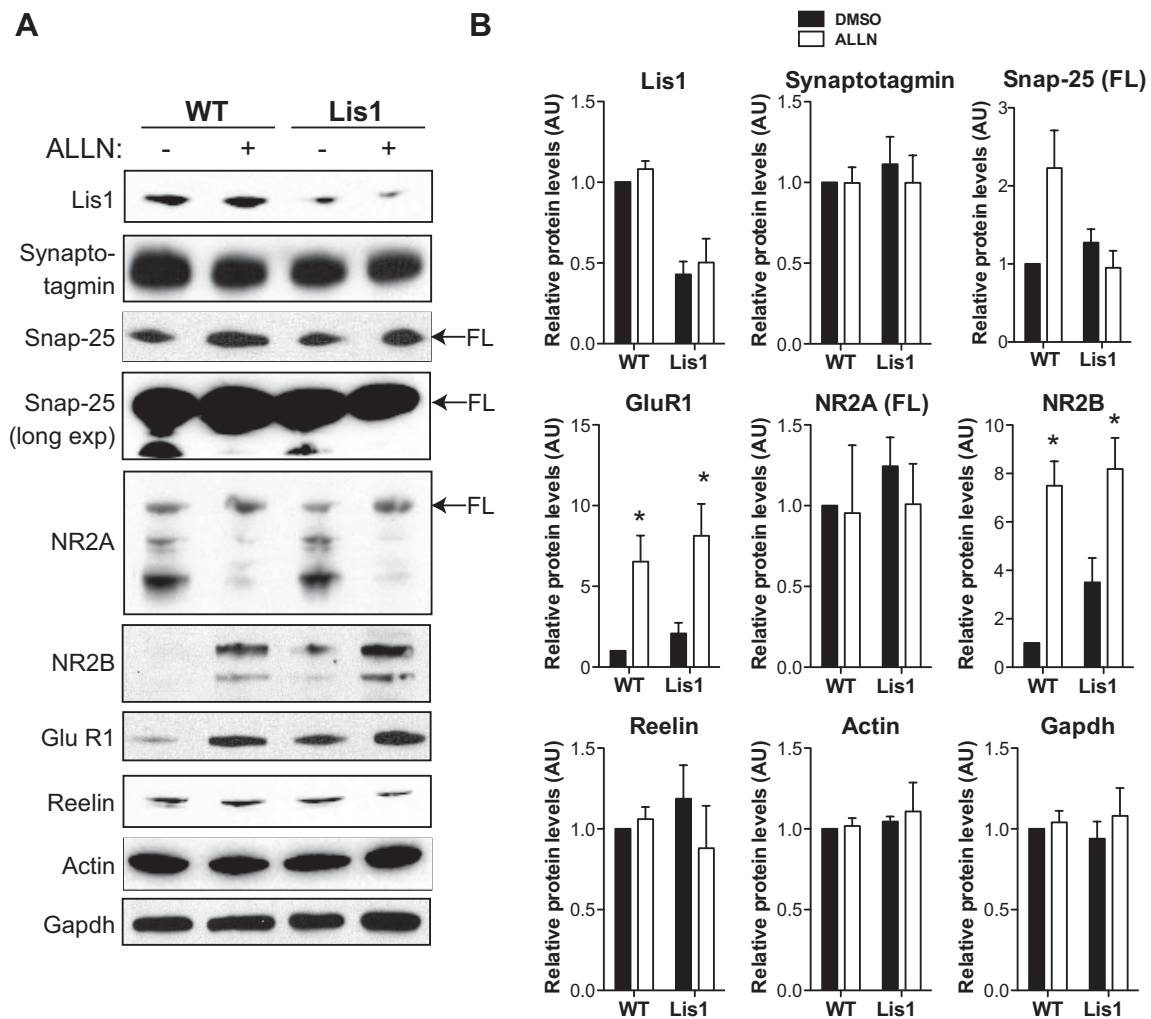


Fig. 5. Effect of ALLN treatment on the levels of proteins involved in synaptic transmission. *A*: representative Western blots showing the protein levels in WT vs. *Lis1* slices that were treated with DMSO only or ALLN + DMSO. FL, full-length protein. Loading control: actin and Gapdh. *B*: *Lis1* protein levels ($n = 4$) are lower in *Lis1*^{+/-} slices relative to WT regardless of ALLN treatment condition. Synaptotagmin ($n = 3$), reelin ($n = 2$), NR2A ($n = 3$), and FL SNAP-25 ($n = 5$) levels are unchanged across all 4 groups. In both genotypes, ALLN treatment increases the levels of GluR1 ($n = 5$) and NR2B ($n = 2$) while reducing the levels of SNAP-25 BDP ($n = 2$). * $P < 0.01$, 2-way ANOVA.

effects. GABAergic and a small fraction of glutamatergic terminals are characterized by their expression of a synaptic vesicle priming protein, Munc13-2. In contrast, Munc13-1 and SNAP-25, which regulate vesicle fusion, are restricted to glutamatergic terminals (Augustin et al. 1999; Varoquaux et al. 2002; Verderio et al. 2004). Reduced SNAP-25 levels are associated with increased glutamatergic synaptic vesicle number in reelin-deficient mutant mice, which like *Lis1*^{+/-} mice, exhibit hippocampal malformations and enhanced seizure susceptibility (Greenwood et al. 2009; Hellwig et al. 2011; Patrylo et al. 2006; Wang and Baraban 2008). Exogenous reelin treatment of *reeler* mutant slice cultures increased SNAP-25 levels and rescued synaptic vesicle number. Given the similarities in *Lis1*^{+/-} and *reeler* mice and the documented changes in SNAP-25 levels following calpain inhibition of cultured neurons (Ando et al. 2005), we also examined the levels of SNAP-25. We found that ALLN blocked cleavage of SNAP-25 in both WT and mutant slices; however, the levels of full-length SNAP-25 were not significantly changed across genotypes and treatment conditions. Further analysis of presynaptic proteins that are specific to glutamatergic terminals is nec-

essary to identify the therapeutic targets of calpain inhibition. Although the precise mechanism by which ALLN acts is yet to be elucidated, electrophysiological data suggest that calpain inhibition rescues glutamatergic transmission via a presynaptic mechanism that is unique to excitatory terminals.

A potential therapeutic approach for treating hyperexcitability. Calpain activation has been implicated in multiple neurological disorders including Alzheimer's, Parkinson's, and Huntington's diseases (Crocker et al. 2003; Saito et al. 1993). Activated calpain isoforms or calpain cleavage products are elevated in postmortem brain samples of Alzheimer's (Saito et al. 1993) and Parkinson's (Crocker et al. 2003) patients, respectively. Administration of a calpain inhibitor to a mouse model of Parkinson's disease was shown to rescue motor deficits to control levels (Crocker et al. 2003). Although numerous studies have examined the effect of calpain inhibition on AMPARs and NMDARs (Liu et al. 2008), and even on long-term potentiation and burst firing, this is the first study showing that calpain inhibition can rescue abnormal electrical defects in a model of epilepsy.

Classical lissencephaly disorders modeled by *Lis1*^{+/-} mice are rare; cortical malformations caused by defects in brain

development are associated with a substantial percentage (5–15%) of adult epilepsies (Brodtkorb et al. 1992; Hardiman et al. 1988; Hauser et al. 1993) and likely a larger fraction of pediatric cases. Therefore, a therapeutic drug candidate, such as ALLN, that reverses hyperexcitability associated with Lis1 deficiency may also be therapeutic in additional mutants that model neurodevelopmental disorders associated with epilepsy. Unfortunately, the current lack of a commercially available calpain inhibitor that can pass the blood-brain barrier significantly hinders preclinical in vivo studies aimed at testing the therapeutic efficacy of this approach in reversing behavioral deficits and seizures (the relatively low frequency of spontaneous seizures in this particular model may further complicate these potential studies). Nonetheless, our results represent a promising first step toward development of new treatments designed to reverse specific functional deficits associated with Lis1 deficiency.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.Y.S., M.B., A.W.-B., and S.C.B. conception and design of research; J.Y.S. and M.B. performed experiments; J.Y.S. and M.B. analyzed data; J.Y.S., M.B., and S.C.B. interpreted results of experiments; J.Y.S. and M.B. prepared figures; J.Y.S. drafted manuscript; J.Y.S., M.B., A.W.-B., and S.C.B. edited and revised manuscript; J.Y.S., M.B., S.H., A.W.-B., and S.C.B. approved final version of manuscript.

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