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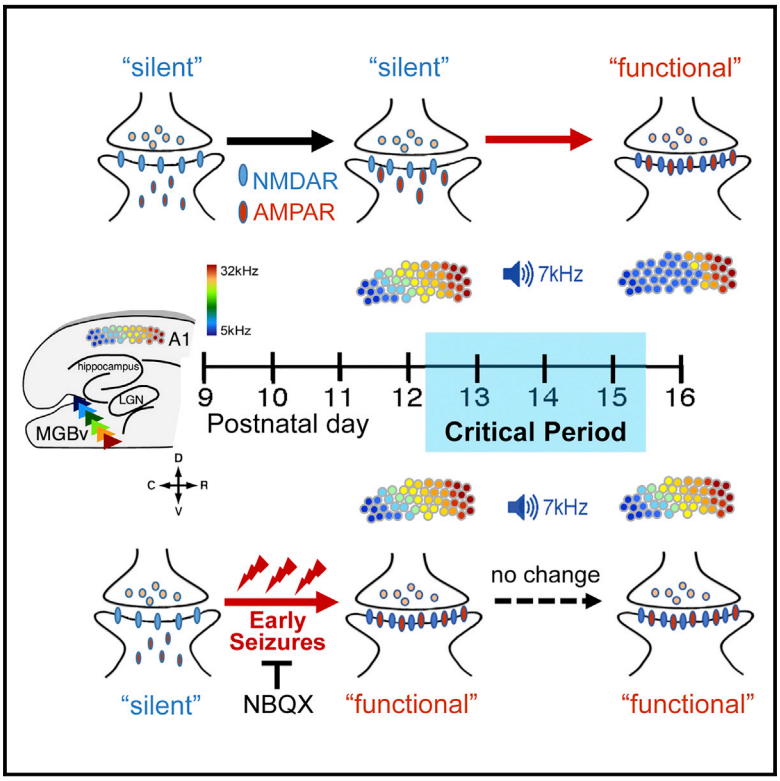
Early Seizures Prematurely Unsilence Auditory Synapses to Disrupt Thalamocortical Critical Period Plasticity

Graphical Abstract



Highlights

- Early-life seizures disrupt a critical period for tonotopic map plasticity in A1
- Maturational decrease in NMDA-only silent synapses characterizes this CP
- Seizures accelerate synapse unsilencing by AMPA receptor insertion
- An AMPAR antagonist prevents synapse unsilencing and rescues CP plasticity

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In Brief

Early-life seizures are often associated with intellectual disability and/or autism. Sun et al. show that seizures prematurely unsilence synapses to disrupt tonotopic plasticity in auditory cortex, revealing a mechanism for the relationship between seizures and later cognitive impairment.

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Early Seizures Prematurely Unsilence Auditory Synapses to Disrupt Thalamocortical Critical Period Plasticity

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SUMMARY

Heightened neural excitability in infancy and childhood results in increased susceptibility to seizures. Such early-life seizures are associated with language deficits and autism that can result from aberrant development of the auditory cortex. Here, we show that early-life seizures disrupt a critical period (CP) for tonotopic map plasticity in primary auditory cortex (A1). We show that this CP is characterized by a prevalence of "silent," NMDA-receptor (NMDAR)-only, glutamate receptor synapses in auditory cortex that become "unsilenced" due to activity-dependent AMPA receptor (AMPAR) insertion. Induction of seizures prior to this CP occludes tonotopic map plasticity by prematurely unsilencing NMDAR-only synapses. Further, brief treatment with the AMPAR antagonist NBQX following seizures, prior to the CP, prevents synapse unsilencing and permits subsequent A1 plasticity. These findings reveal that early-life seizures modify CP regulators and suggest that therapeutic targets for early post-seizure treatment can rescue CP plasticity.

INTRODUCTION

Up to 40% of children with autism and intellectual disability also suffer from epilepsy, and approximately 35% of children with infantile spasms develop long-term intellectual disabilities, including autism (Spence and Schneider, 2009; Tuchman, 2015). While there are multiple autism-linked genes that associate highly with epilepsy, these two disorders may also be co-acquired as a result of early-life brain injury and seizures (Froehlich-Santino et al., 2014; Jensen, 2011).

Children with intellectual disorders related to early-life epilepsy and autism often show impaired acoustic temporal and phonological processing that may contribute to later language deficits (Amaral et al., 2015; Matsuzaki et al., 2012; Oram Cardy et al., 2005). Such auditory encoding abnormalities in children with autism are linked to perturbed development of auditory cortex (Edgar et al., 2015). Mouse models of autism also show impaired auditory cortical development, particularly during the critical period (CP) for tonotopic plasticity (Engineer et al., 2014; Rotschafer and Razak, 2013; Kim et al., 2013).

Early postnatal development is characterized by a series of CPs for enhanced synaptic plasticity and learning, reflecting dynamic changes in excitatory and inhibitory circuits (Takesian and Hensch, 2013). An untoward result is a heightened susceptibility to seizures (Rakhade and Jensen, 2009) that can lead to long-term learning deficits, including autistic-like social behavior (Bernard and Benke, 2015; Lippman-Bell et al., 2013; Talos et al., 2012). The extreme levels of synaptic activity during early-life seizures may co-opt plasticity mechanisms (Rakhade et al., 2008) required for later experience-dependent CP plasticity modifications of cortical representations (Hensch, 2004). However, the manner in which early-life seizures may interfere with cortical development to impact plasticity during CPs, such as the reorganization of auditory cortical maps, remains largely unexplored. Understanding the precise synaptic changes following seizures may allow us to identify treatments to reverse the sequelae underlying long-term deficits in auditory processing and learning.

Experience-dependent plasticity during CPs has been well characterized in sensory areas (Hensch, 2004), such as primary visual or auditory cortex (A1). During a finite 3-day CP for tonotopic plasticity, passive tone exposure leads to an expanded representation of that tone by altering auditory thalamocortical connectivity (Barkat et al., 2011; de Villers-Sidani et al., 2007; Insanally et al., 2009). Here, we examined how tonotopic map plasticity in A1 may be influenced in a rodent model of early-life seizures to elucidate the common comorbidity of early-life epilepsy and auditory deficits (Amaral et al., 2015; Riva et al., 2007).

We show that a postnatal maturational switch from functionally "silent" thalamocortical synapses containing only NMDA receptors (NMDARs) to "unsilent" synapses containing both

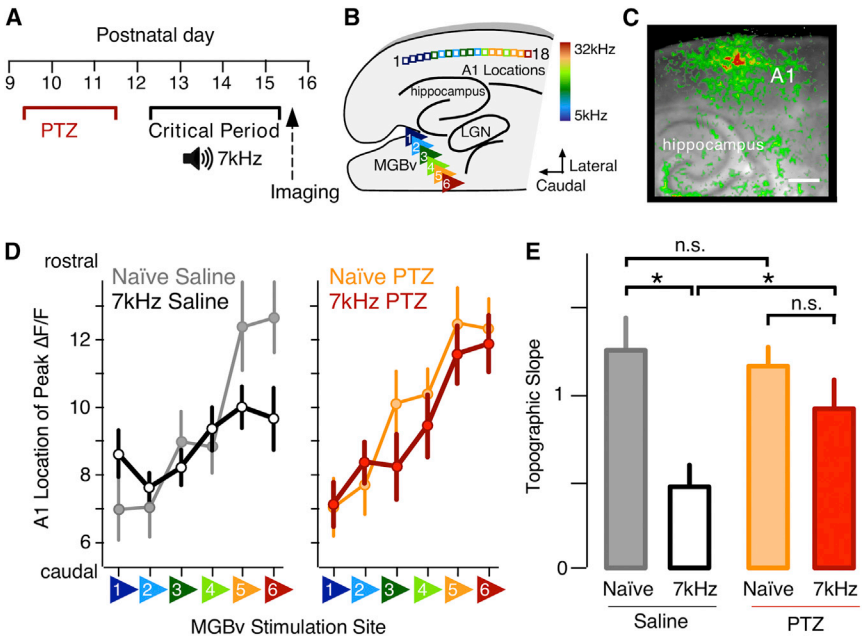

Figure 1. Early-Life Seizures Disrupt an Auditory Critical Period

(A) Experimental strategy to evaluate the effects of early-life seizures on auditory CP plasticity. P9–P11 mice were injected daily with PTZ (50 mg/kg, i.p.) or saline and then exposed to pulsed 7 kHz tones during a CP for tonotopic plasticity (P12–P15). Changes in thalamocortical connectivity were assessed *in vitro* at P15 using VSD imaging.

(B) Schematic of an auditory thalamocortical slice indicating six thalamic (MGBv) stimulus positions and 18 regions along L4 in A1. Electrical stimulation along the tonotopic axis of the MGBv produced a caudal-to-rostral shift in maximal VSD activity along the A1 tonotopic axis in naive mice. The color of each A1 region indicates the MGBv position that produced the maximal response across all naive mice. Inset shows approximate frequency representations of MGBv stimulus sites (Hackett et al., 2011).

(C) Sample A1 response (ΔF/F) to MGBv stimulation in a thalamocortical slice from a PTZ-treated mouse (P15). Scale bar, 500 μm.

(D) PTZ-induced seizures prevent the change of thalamocortical topography by 7 kHz tone exposure. The degree of orderly topography was calculated by the linear relationship between the location of peak response (ΔF/F) in A1 to MGBv stimuli. Average topographic curves are shown for naive saline-injected mice raised in a standard acoustic environment (black), 7 kHz exposed saline-injected mice (gray), naive mice with previous PTZ-induced seizures (orange), and 7 kHz exposed mice with previous PTZ-induced seizures (red).

(E) Comparison of topographic slopes shows a significant change following 7 kHz tone exposure in control mice injected with saline, but not in mice injected with PTZ (mean ± SEM; *$p < 0.05$, n.s. $p > 0.05$).

NMDARs and AMPA receptors (AMPARs) underlies CP tonotopic map plasticity in A1. Early-life seizures occlude this CP tonotopic plasticity by a premature unsilencing of both thalamocortical and intracortical synapses. Importantly, brief treatment with the AMPAR antagonist NBQX following seizures prevented this premature unsilencing of synapses and rescued CP plasticity, providing proof of principle for interventional therapies in early life.

RESULTS

Early-Life Seizures Disrupt Tonotopic Map Plasticity in A1 during an Auditory CP

The brief CP for A1 tonotopic map plasticity in mice begins on postnatal day (P) 12 and ends at P15 (Barkat et al., 2011). We induced generalized seizures prior to this CP onset with daily injections of pentylenetetrazol (PTZ; 50 mg/kg, intraperitoneally [i.p.]) from P9 to P11 (Rakhade et al., 2012). Following this period of acute seizures, we then exposed these mice to 7 kHz tones during the expected CP (P12–P15) (Figure 1A; Barkat et al., 2011).

The impact of seizures on tonotopic plasticity can be screened rapidly and reliably by mapping topography in peri-horizontal slices preserving the thalamocortical projection using voltage-sensitive dye (VSD) imaging (Barkat et al., 2011; Hackett et al., 2011). In slices from naive mice, electrical stimulation along the tonotopic axis of the auditory thalamus (the ventral division of the medial geniculate body; MGBv) produces a caudal-to-rostral shift in maximal VSD activity along the A1 tonotopic axis (Figures 1B and 1C). The degree of orderly topography was quantified as the linear relationship between the location of the maximal VSD response (ΔF/F) within cortical layer IV (L4) along the caudo-rostral axis of A1 and the MGBv stimulation site ("topographic slope"; Figure 1D).

Consistent with previous observations (Barkat et al., 2011), passive 7 kHz tone exposure during P12–P15 elicited large-scale shifts in the thalamocortical organization of A1 in saline-injected mice, reflected by a significant decrease of the topographic slope compared to saline-injected naive mice (Figures 1D and 1E; naive saline: 1.26 ± 0.18, n = 15 mice; tone-exposed saline: 0.46 ± 0.13, n = 22 mice; $p = 0.0008$, unpaired t test, two-tailed, equal variance). However, in littermate mice that had been injected with PTZ to induce seizures at P9–P11, this plasticity was significantly reduced (Figures 1D and 1E; topographic slope for tone-exposed saline versus tone-exposed PTZ = 0.92 ± 0.18, n = 16 mice; $p = 0.04$, unpaired t test, two-tailed, equal variance; for naive PTZ versus tone-exposed PTZ, $p = 0.25$, unpaired t test, two-tailed, unequal variance). To rule out an effect of seizures on thalamocortical topography under baseline acoustic conditions, naive mice were raised in a standard acoustic environment after PTZ-induced seizures. These mice showed similar thalamocortical topography to naive littermate controls injected with saline (no seizures), as evidenced by no significant difference in the slope of the topographic curves (Figures 1D and 1E; naive saline versus naive PTZ = 1.17 ± 0.11, n = 15 mice; unpaired t test, two-tailed, equal variance, $p = 0.66$). Taken together, these results suggest that seizure activity prior to the onset of the CP impairs subsequent tonotopic map plasticity in A1.

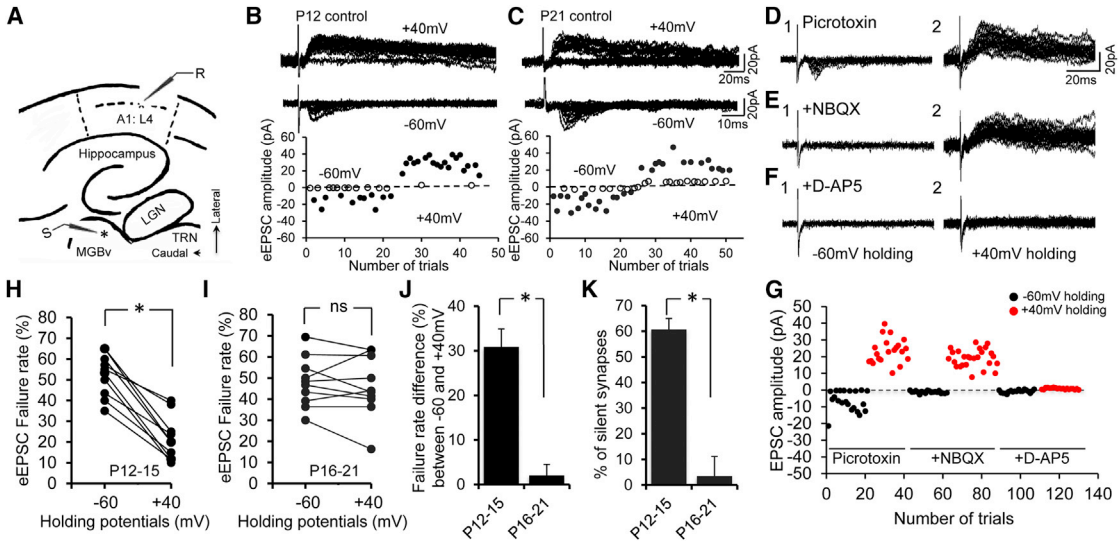

Figure 2. NMDAR-Only Silent Synapses in L4 Pyramidal Neurons in Naive Mouse A1 Decrease during Normal Development

(A) Schematic of stimulation and recording sites in thalamocortical slices.

(B and C) Representative minimal MGBv-evoked eEPSCs at +40 mV (upper traces) and −60 mV (lower traces) from P12 (B) and P21 (C) mice. Successes and failures of individual EPSCs are shown as filled and open circles, respectively.

(D–G) AMPARs mediate fast eEPSCs at −60 mV, and NMDARs mediate slow-decaying eEPSCs at +40 mV (n = 5) (D). Additional 20 μM NBQX in the ACSF abolished the eESPCs at −60 mV (E). 50 μM D-AP5 in the ACSF completely blocked the slow-decaying eEPSCs at +40 mV (F). Summary plot of individual eEPSC amplitudes during the time course of an experiment showing AMPAR- and NMDAR-mediated EPSCs at different holding potentials (G).

(H and I) Failure rates at −60 mV and +40 mV holding potentials from P12–P15 (H) and P16–P21 (I) mice.

(J) Summary of failure rate difference at −60 mV and +40 mV.

(K) Fraction of calculated silent synapses shows a significant decrease in L4 pyramidal neurons during normal development.

Error bars represent mean ± SEM. *$p < 0.05$, n.s. $p > 0.05$.

Maturation of Silent Synapses Corresponds to the CP for Tonotopy

To determine whether glutamatergic synapses are altered by early-life seizures, we first examined the normal maturational changes in NMDARs and AMPARs during the CP. A unique feature of the immature brain is that many excitatory synapses contain only NMDARs, which are functionally "silent" due to Mg^{2+} blockade at resting membrane potentials (Hanse et al., 2013; Kerchner and Nicoll, 2008).

The proportion of silent, NMDAR-only synapses to those unsilenced synapses also containing AMPARs can be determined by the difference of failure rates in thalamus-evoked excitatory postsynaptic currents (eEPSCs) at negative (−60 mV) and positive (+40 mV) membrane potentials (Figures 2A and 2D–2G). At −60 mV, EPSCs are mediated only by AMPARs, but at +40 mV, EPSCs are mediated by both AMPARs and NMDARs. To calculate the silent synapse ratio for each cell, we first set the baseline failure rate by initially adjusting the stimulus intensity to evoke synaptic responses with an ~50% failure rate at a holding potential of −60 mV, and then we raised the holding potential to +40 mV (Liao et al., 1995).

In thalamocortical slices from normal P12–P15 mice, L4 pyramidal neurons in A1 exhibited a failure rate change from 52.39 ± 3.85% at −60 mV to 21.54 ± 3.75% at +40 mV (stimulation intensity, 42.78 ± 7.69 μA; n = 10 cells, 6 slices from 6 mice; $p < 0.001$, paired t test; Figures 2B and 2H), yielding a difference of 30.85 ± 4.04%. These data suggest a prevalence of NMDAR-only, silent synapses. However, in normal mice at P16–P21, a significantly smaller change in failure rate (2.03 ± 2.48%; $p < 0.05$, unpaired t test, two-tailed, equal variance, versus that for P12–P15) was observed (47.90 ± 3.71% at −60 mV, and 45.87 ± 4.56% at +40 mV; stimulation intensity, 37.78 ± 6.75 μA; n = 10 cells, 5 slices from 5 mice; $p = 0.43$, paired t test; Figures 2C and 2I), suggesting that silent synapses dramatically decrease following the CP in normal mouse A1. The fraction of silent synapses showed a significant decline at P16–P21 (3.45 ± 7.70%; n = 10 cells, 5 slices from 5 mice) from the peak tonotopic plastic window (P12–P15, 57.23 ± 5.15%; n = 10 cells, 6 slices from 6 mice; $p < 0.001$, unpaired t test, two-tailed, equal variance; Figures 2J and 2K). These data reveal that, in normal mice, the availability of silent synapses is heightened during the auditory CP for A1 tonotopic map plasticity.

Premature Synapse Unsilencing Underlies the Disruption of Auditory CP Plasticity

Since there is overlap in the availability of NMDAR-only silent synapses in L4 and the previously delineated CP timing (Barkat et al., 2011), we asked whether premature unsilencing could account for seizure-induced disruption of tonotopic map plasticity in L4. We induced seizures in mice at P9–P11 with daily injections of PTZ and then measured excitatory synaptic function at P12–P15 using whole-cell patch-clamp recordings in L4 pyramidal cells. Indeed, following daily PTZ seizures, mice showed a significant increase in amplitude of AMPAR-mediated

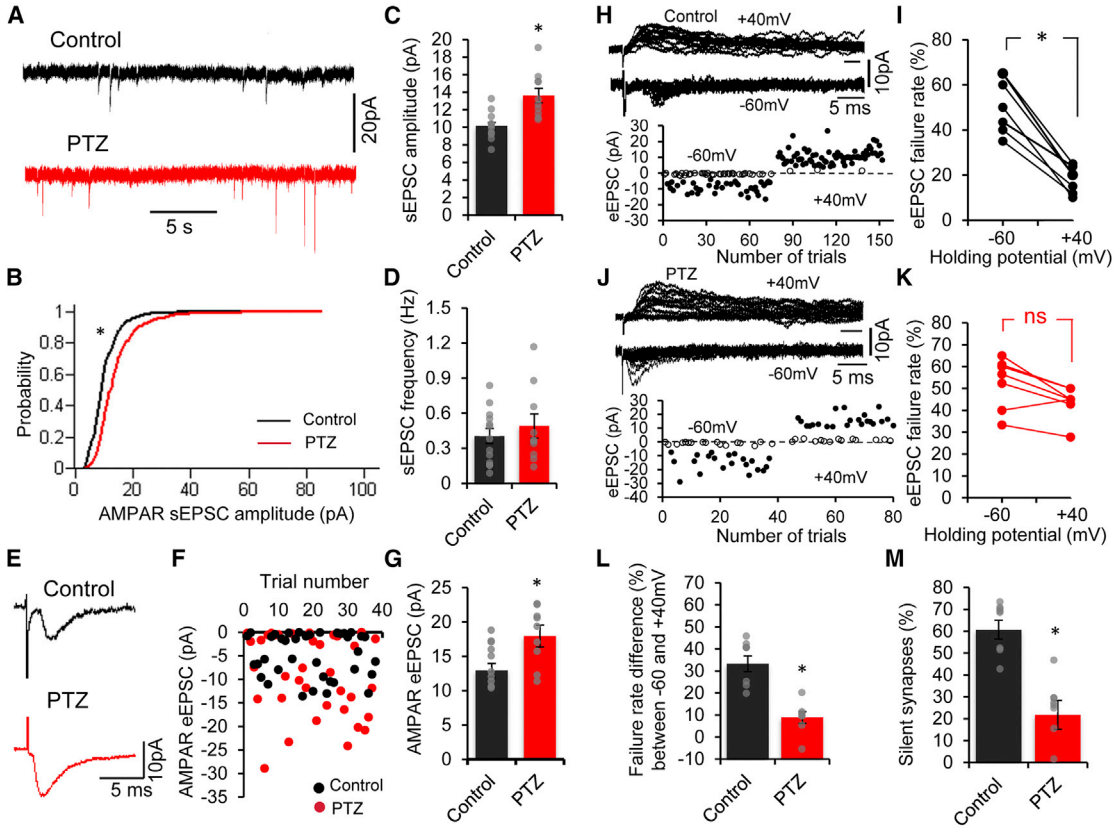

Figure 3. Early-Life Seizures Enhance AMPAR Function and Promote Thalamocortical Silent Synapse Loss in A1

(A) Sample AMPAR-mediated sEPSCs in L4 pyramidal neurons of A1 from a P12 mouse 24 hr post-PTZ seizures (red) and littermate controls (black).

(B) Cumulative distribution showing significantly larger sEPSC amplitudes from post-PTZ mice compared to littermate controls (*$p < 0.001$).

(C and D) PTZ seizures resulted in increased sEPSC amplitude (mean ± SEM; *$p < 0.05$) (C) but no alterations in frequency (D).

(E) Representative MGBv-evoked minimal eEPSCs in L4 pyramidal cells from each group.

(F and G) Shown in (F): typical peak amplitudes for 35 consecutive minimal eEPSC responses at −60 mV, showing that (G) minimal eEPSC amplitudes were increased after PTZ seizures (mean ± SEM; *$p < 0.05$).

(H) Representative eEPSC traces at +40 mV (upper traces) and −60 mV (lower traces) from a P12 control mouse. Successes and failures of individual EPSCs are shown as filled and open circles, respectively.

(I) Failure rates at −60 mV and +40 mV holding potentials for controls. *$p < 0.05$.

(J) Representative eEPSC traces at +40 mV (upper traces) and −60 mV (lower traces) from a P12 mouse at 24 hr post-PTZ.

(K) Failure rates at −60 mV and +40 mV holding potentials for post-PTZ mice. ns, not significant.

(L) Failure rate differences are significantly reduced after PTZ seizures (mean ± SEM; *$p < 0.05$).

(M) Fraction of calculated silent synapses shows a significant decrease in post-PTZ seizure mice compared to littermate controls (mean ± SEM; *$p < 0.05$).

spontaneous EPSCs (13.62 ± 0.82 pA; n = 10 cells, 6 slices from 6 mice; $p < 0.001$, unpaired t test, two-tailed, equal variance) as compared to controls (10.17 ± 0.40 pA; n = 13 cells, 8 slices from 8 mice; Figures 3A–3C), while these events occurred at comparable frequencies (0.49 ± 0.10 Hz and 0.41 ± 0.07 Hz, respectively; p = 0.48, unpaired t test, two-tailed, equal variance; Figures 3A, 3D). Consistent with the enhanced AMPAR-mediated sEPSCs, miniature AMPAR-mediated EPSCs also showed a significantly increased amplitude in slices from post-seizure mice (12.07 ± 0.34 pA; n = 10 cells, 5 slices from 5 mice) as compared with controls (10.52 ± 0.39 pA; n = 9 cells, 5 slices from 5 mice; p = 0.008, unpaired t test, two-tailed, equal variance; Figure S1).

To further investigate the contribution of postsynaptic receptors in the seizure-induced AMPAR functional enhancement, we measured minimally evoked AMPAR-mediated EPSCs by directly stimulating MGBv thalamocortical fibers at P12 (Chandrasekaran et al., 2007; Zhou et al., 2011). We found that minimal single-fiber stimulation successfully evoked larger AMPAR-mediated eEPSCs in post-seizure mice (17.97 ± 1.61 pA, n = 10 PTZ cells, 6 slices from 6 mice, versus 12.97 ± 1.01 pA, n = 10 control cells, 6 slices from 6 mice; p = 0.017, unpaired t test, two-tailed, equal variance; Figures 3E–3G). Thus, early-life seizures are associated with premature enhancement of baseline AMPAR function.

We next determined whether the AMPAR enhancement was associated with changes in the fraction of silent NMDAR-only synapses following seizures. As described earlier, age-matched P12–P13 controls were stimulated to produce a failure rate of 51.21 ± 4.35% at −60 mV and exhibited 17.94 ± 2.05% failures

at +40 mV (stimulation intensity, 43.50 ± 9.12 μA; n = 8 cells, 5 slices from 5 mice; Figures 3H and 3I), yielding a difference of 33.27 ± 3.62% (Figure 3L). However, in post-seizure mice, L4 pyramidal cells exhibited an attenuated change in failure rate at P12–P13 from an initial level of 52.58 ± 4.14% at −60 mV to 43.66 ± 2.65% at +40 mV (stimulation intensity, 39.25 ± 9.06 μA; n = 8 cells, 5 slices from 5 mice; Figures 3J and 3K), yielding a difference of 8.92 ± 2.66% that was significantly smaller than that observed in controls ($p = 0.004$, unpaired t test, two-tailed, equal variance) (Figure 3L). Further, a smaller fraction of silent synapses post-PTZ (21.77 ± 6.61% versus 60.71 ± 4.32% in controls; $p < 0.001$, unpaired t test, two-tailed, equal variance) suggests that early seizures unsilence NMDAR-only thalamocortical synapses (Figure 3M).

In addition to thalamic inputs, L4 pyramidal neurons in A1 also receive strong intracortical excitatory inputs from L6 (Kratz and Manis, 2015; Lee and Sherman, 2009). Following seizures, intracortical minimally evoked AMPAR-mediated EPSCs, elicited by stimulating intracortical L6 to L4 projection fibers, were larger compared with controls (18.68 ± 1.60 pA, n = 10 PTZ cells, 6 slices from 6 mice, versus 13.07 ± 0.92 pA, n = 9 control cells, 6 slices from 6 mice; $p = 0.005$, unpaired t test, two-tailed, equal variance; Figures S2A–S2D). Furthermore, age-matched controls exhibited a failure rate decrease from the initial 51.70 ± 2.66% at −60 mV to 22.18 ± 1.93% at +40 mV (stimulation intensity, 35.11 ± 6.32 μA; n = 9 cells, 6 slices from 6 mice; Figures S2E and S2G), yielding a difference of 29.52 ± 3.37% (Figure S2I). However, post-seizure mice exhibited a significantly smaller failure rate change from 48.90 ± 3.32% at −60 mV to 43.38 ± 2.40% at +40 mV at P12–P13 (stimulation intensity, 31.20 ± 6.61 μA; n = 10 cells, 6 slices from 6 mice; Figures S2F and S2H), yielding a difference of only 5.52 ± 2.10% that was significantly smaller than that observed in controls ($p < 0.001$, unpaired t test, two-tailed, equal variance; Figure S2I). This was confirmed by a smaller fraction of silent synapses post-PTZ (13.62 ± 5.55% versus 55.02 ± 4.80% in controls; unpaired t test, two-tailed, equal variance, $p < 0.001$; Figure S2J).

Taken together, our data reveal that early-life seizures prematurely diminish NMDAR-only silent synapses, regardless of input source. This seizure effect occludes the CP for tonotopic map plasticity in L4 of A1, which is normally associated with a conversion of silent NMDAR-only synapses to those containing both NMDARs and AMPARs.

AMPAR Antagonist Treatment Rescues Auditory CP Plasticity by Preventing Premature Synapse Unsilencing

Transient inactivation of AMPARs has been previously found to prevent acute and long-term seizure-induced changes in hippocampal neurons (Lippman-Bell et al., 2016; Rakhade et al., 2008; Zhou et al., 2011). Thus, we asked whether post-seizure treatment of the AMPAR antagonist NBQX restores proper CP plasticity by preventing the premature unsilencing of NMDAR-only synapses.

P9–P11 mouse pups were injected daily with PTZ (50 mg/kg) to induce seizures, followed 1 hr later by either saline or NBQX (20 mg/kg, i.p.). The pups were subsequently exposed to 7 kHz tones during the CP for tonotopic plasticity (P12–P15). The mice injected only with saline following seizures showed impaired tonotopic plasticity, consistent with our previous observation (Figure 4A; topographic slope, PTZ + saline = 1.34 ± 0.25, n = 9 mice). Importantly, plasticity was rescued in littermates injected with NBQX following seizures, as evidenced by a significant decrease in the slope of the topographic curves (Figure 4A; topographic slope, PTZ + saline versus PTZ + NBQX = 0.58 ± 0.18, n = 13 mice; $p = 0.03$, Mann-Whitney U test, two-tailed).

Consistent with the rescued plasticity, NBQX treatment reduced seizure-induced AMPAR enhancement and premature unsilencing of NMDAR-only synapses. NBQX prevented the increase in amplitude of AMPAR-mediated sEPSCs observed in L4 pyramidal cells at P12–P15 following PTZ (Figures 4B–4E) (percent change in sEPSC amplitude versus saline control, control: 100 ± 3.63%, n = 11 cells, 4 slices from 4 mice; PTZ + saline: 138.21 ± 8.41%, n = 14 cells, 5 slices from 5 mice; PTZ + NBQX: 99.49 ± 4.55%, n = 10 cells, 4 slices from 4 mice; $p = 0.0015$, one-way ANOVA followed by Bonferroni correction, PTZ + saline versus PTZ + NBQX). Furthermore, calculated fractions of silent synapses revealed that NBQX treatment reduced the premature unsilencing of thalamocortical synapses following PTZ-induced seizures (Figures 4F–4J) (control: 64.63 ± 3.66%, n = 8 cells, 4 slices from 4 mice; PTZ + saline: 17.87 ± 4.91%, n = 12 cells, 5 slices from 5 mice; PTZ + NBQX: 59.27 ± 5.53%, n = 10 cells, 4 slices from 4 mice; $p < 0.001$, one-way ANOVA followed by Bonferroni correction, PTZ + saline versus PTZ + NBQX). Thus, NBQX treatment may rescue tonotopic CP plasticity following seizures by preventing the accelerated maturation of A1 excitatory synapses.

DISCUSSION

Neonatal and childhood seizures are often comorbid with autism and intellectual disability (Spence and Schneider, 2009; Tuchman, 2015), yet it is unclear whether the seizures contribute to these developmental disorders. Early postnatal development is characterized by an enhanced excitatory neurotransmission that is necessary for activity-dependent synaptogenesis and synaptic plasticity (Ebert and Greenberg, 2013; Rakhade and Jensen, 2009) but can also lead to increased susceptibility to epileptic seizures (Jensen, 2009). Such seizures impair later synaptic plasticity, learning, and cognition (Bernard and Benke, 2015; Holmes and Ben-Ari, 2007). How seizures may affect intervening critical period windows of development remains unknown.

Neural circuit refinement through early life experience is important for normal sensory processing. A key feature of many maturing networks during early postnatal brain development is the gradual conversion of silent to active synapses (Hanse et al., 2013). Long-term potentiation (LTP), a form of synaptic plasticity, involves the trafficking of AMPARs into NMDAR-only synapses by calcium-dependent post-translational modifications of AMPAR subunits (Huganir and Nicoll, 2013). We had previously shown that seizure activity *in vitro* causes *de novo* unsilencing and strengthening of glutamatergic synapses in immature CA1 pyramidal neurons, occluding subsequent LTP (Rakhade and Jensen, 2009; Zhou et al., 2011). Here, we find that *in vivo* seizures induce a premature depletion of

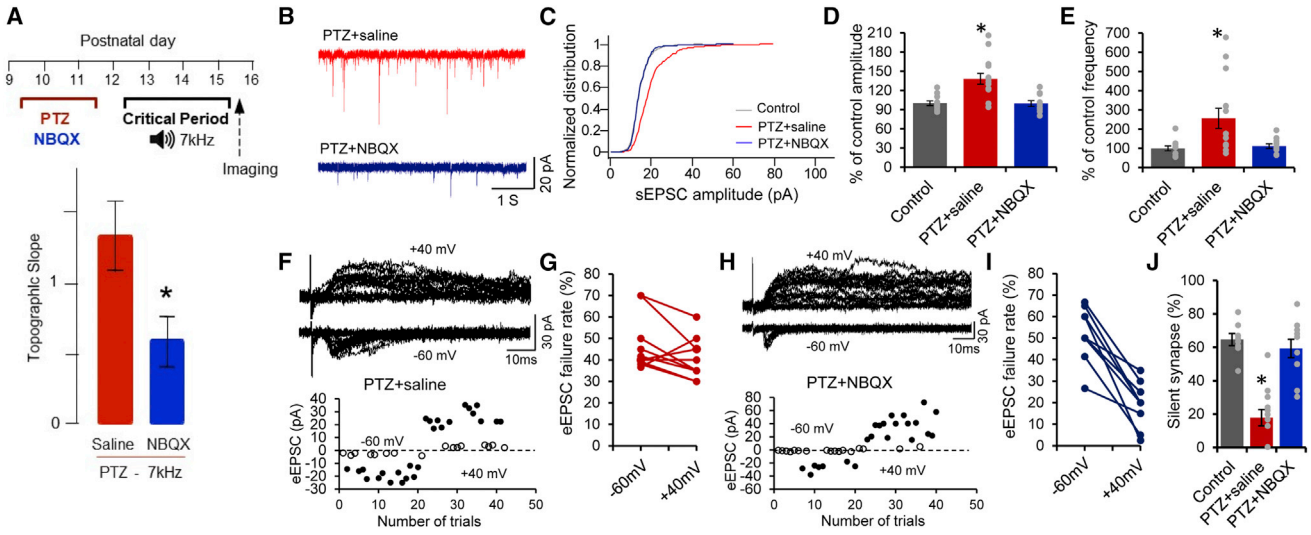

Figure 4. NBQX Rescues Auditory CP Plasticity following Early-Life Seizures by Preventing Premature Synapse Unsilencing

(A) Top: experimental strategy to evaluate the effects of NBQX treatment on seizure-induced impairment of auditory CP plasticity. P9–P11 mice were injected daily with PTZ (50 mg/kg, i.p.) followed by saline or NBQX (20 mg/kg, i.p.) at 1 hr post-seizures and then were exposed to pulsed 7 kHz tone pips during a CP for tonotopic plasticity (P12–P15). Tone-evoked changes in thalamocortical topography were assessed using *in vitro* VSD imaging at P15. Bottom: topographic slopes (*$p < 0.05$).

(B) Representative AMPAR-mediated sEPSCs in L4 pyramidal neurons of A1 from P12 mice 24 hr post-PTZ seizures treated with saline (red) or NBQX (blue).

(C) Cumulative distribution showing significantly smaller sEPSC amplitudes from NBQX-treated post-PTZ mice compared to saline-treated post-PTZ mice.

(D and E) Increased sEPSC amplitude (D) and frequency (E) induced by prior PTZ seizures were reversed by NBQX treatment (mean ± SEM; *$p < 0.05$).

(F) Sample eEPSC traces at +40 mV (upper traces) and −60 mV (lower traces) from a P12 mouse 24 hr post-PTZ seizures. Successes and failures of individual EPSCs are shown as filled and open circles, respectively.

(G) Failure rates at −60 mV and +40 mV for post-PTZ mice.

(H) Sample eEPSC traces at +40 mV (upper traces) and −60 mV (lower traces) from a P12 NBQX-treated post-PTZ mouse.

(I) Failure rates at −60 mV and +40 mV for NBQX-treated post-PTZ mice.

(J) The fraction of calculated silent synapses was significantly increased in post-PTZ mice treated with NBQX versus saline (mean ± SEM; *$p < 0.05$).

NMDAR-only silent synapses and occlude a CP for auditory circuit refinement, which may contribute to long-term deficits in auditory function.

PTZ may potentiate 2- to 4-Hz spike-wave discharges that are thought to reflect oscillations generated from dysfunctional thalamocortical circuits (Marescaux et al., 1984) but can also induce limbic seizures (Reddy et al., 2018). Importantly, altered activity of thalamocortical networks may also underlie limbic seizures and temporal lobe epilepsy (TLE) (Guye et al., 2006; He et al., 2017). Future studies will determine whether our findings generalize across various forms of epilepsy, including TLE, associated with neurodevelopmental disorders.

Importantly, we show that early intervention following seizures with the AMPAR antagonist NBQX preserves the silent synapse fraction and prevents the subsequent impairment of CP plasticity. A key feature of unsilencing is the insertion of AMPARs into NMDAR-only synapses. Neonatal seizures robustly increase PSD-95 expression (Rakhade et al., 2012), and PSD-95 promotes the functional maturation of AMPAR synapses in the visual cortex linked to the end of a CP for ocular dominance plasticity (Huang et al., 2015). Likewise, accelerated structural maturation of thalamo-recipient short stubby spines by genetic disruption of a synaptic cell-adhesion molecule shortens the CP in A1 (Barkat et al., 2011). The rapid strengthening of excitatory synapses following seizures may exacerbate the network hyperexcitability in response to subsequent epileptic triggers. Interventions are thus required to prevent this sequela. The half-life of NBQX is about 1 hr (Chizh et al., 1994), and we have shown there is no residual effect of prior NBQX treatment on AMPAR currents in controls at 24 hr after seizures (Rakhade et al., 2008). The NBQX treatment may act through both short- and longer-term mechanisms, preventing acute synaptic changes after a single seizure episode and protecting against AMPAR-driven hyperexcitability during subsequent seizures. Thus, our study identifies the AMPARs as one key therapeutic target to recover proper excitatory synapse development in A1 in order to curtail later impairments in auditory plasticity.

The rescue of an auditory CP by the AMPAR antagonist NBQX is proof-of-principle that plasticity is a dynamic target for therapeutic manipulation. Transient inactivation of AMPARs following early-life seizures may be a promising strategy to attenuate the premature unsilencing of cortical excitatory synapses, allowing for the expression of CP plasticity that underlies normal auditory and social development. Indeed, brief NBQX treatments prevent deficits in social behavior observed in juvenile rats following neonatal seizures (Lippman-Bell et al., 2013). Understanding how early-life manipulations of silent synapses impact developmental plasticity will provide important insight into the etiology and treatment of neurodevelopmental disorders.

EXPERIMENTAL PROCEDURES

Animals

Male and female C57BL/6J mice (The Jackson Laboratory) at P9–P25 were used for all experiments. All animal care protocols and procedures were approved by and in accordance with the guidelines of the Animal Care and Use Committee at the University of Pennsylvania (Philadelphia, PA), Boston Children's Hospital (Boston, MA), and Carleton University (Ottawa, ON). All efforts were made to minimize animal suffering and the number of animals used.

Early-Life Seizure Induction

Early-life seizures were induced by daily PTZ injections (50 mg/kg, i.p.) for 3 days from P9 to P11 prior to CP onset. PTZ acts mostly by blocking $GABA_A$ receptors via the t-butyl-bicyclophosphorothionate (TBPS) site (Olsen, 1981), while additional action of increasing sodium and calcium influx through Ca^{2+} channels has been reported (Papp et al., 1987). PTZ reliably causes spike and wave epileptic activity (Sierra-Paredes et al., 1989) and acute behavioral seizures in rodents (Rakhade et al., 2012). Only mice showing typical seizures, including repeated rearing, myoclonic jerks, forelimb clonus, or loss of posture and tonic-clonic movements were included for further examination. In some experiments, behavioral seizures after PTZ injection were videotaped and characterized. A single episode of seizures induced by one dose of PTZ (50 mg/kg, i.p.) showed a latency of 6.90 ± 1.10 min and an accumulated duration of 2.32 ± 0.45 min (n = 22). Age-matched littermates injected with the same amount of saline were used as controls. We found no significant differences in the body weights of the PTZ- versus saline-injected mouse pups when examined at P15 (saline, 7.12 ± 0.16 g, n = 37 mice; PTZ, 7.00 ± 0.15 g, n = 31 mice; p = 0.57).

In Vitro VSD Imaging

The mouse brain was quickly removed and sectioned peri-horizontally (600 μm, 15°) to preserve the MGBv and its projection to A1 (Cruikshank et al., 2002). Changes in thalamocortical topography were evaluated *in vitro* using VSD imaging as previously described (Barkat et al., 2011; Hackett et al., 2011). The acquisition and analysis of these experiments were performed blind to the drug condition of the mouse pups. Further details can be found in the Supplemental Experimental Procedures.

Whole-Cell Patch-Clamp Recording

Whole-cell voltage-clamp recordings were performed in L4 pyramidal neurons in A1 from P12–P21 mice, as previously described (Sun et al., 2013). Synaptic responses were evoked by stimulating the MGBv or L6. Stimulation intensity was adjusted to elicit detectable synaptic responses with about a 50% failure rate (Zhou et al., 2011). To examine the postsynaptic silent synapses, cells were first held at −60 mV and then stimulated with the same intensity at +40 mV. The failure responses of eEPSCs were visually verified blind to the drug condition of the mice. The fraction of silent synapses was calculated using the established formula: % silent synapses = $(1 - \ln(F_{-60})/\ln(F_{+40})) \times 100\%$. Further details can be found in the Supplemental Experimental Procedures.

Statistical Analyses

All data are expressed as mean ± SE. For two-group comparisons, statistical significance was assessed using the two-tailed Student's unpaired or paired t test for normally distributed data or the non-parametric two-tailed Mann-Whitney U test for data not distributed normally. Multi-group comparisons were performed using one-way ANOVA for normally distributed data or Kruskal-Wallis nonparametric tests. For comparison across groups with unequal variance, the unequal-variance t test using an unpooled SE was performed. The Shapiro-Wilk test was used to test for normality, and Levene's method was used to test for equal variance. Statistically significant differences were established at $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.04.108.

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AUTHOR CONTRIBUTIONS

H.S., A.E.T., J.J.L.-B., T.K.H., and F.E.J. designed the study. H.S., A.E.T., and T.T.W. conducted the experiments. H.S., A.E.T., T.K.H., and F.E.J. analyzed the data. H.S., A.E.T., J.J.L.-B., T.K.H., and F.E.J. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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