Astrocyte control of synaptic NMDA receptors contributes to the progressive development of temporal lobe epilepsy

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Astrocytes modulate neuronal activity, synaptic transmission, and behavior by releasing chemical transmitters in a process termed gliotransmission. Whether this process impacts epilepsy in vivo is not known. We show that genetic impairment of transmitter release from astrocytes by the expression of a glial dominant-negative SNARE domain in mice reduced epileptiform activity in situ, delayed seizure onset after pilocarpine-induced status epilepticus, and attenuated subsequent progressive increase in seizure frequency in vivo. The reduced seizure frequency was accompanied by attenuation of hippocampal damage and behavioral deficits. As the delay in seizure onset and the reduced seizure frequency were mimicked by intracerebroventricular delivery of the NMDA receptor (NMDAR) antagonist D-(-)-2-amino-5-phosphonopentanoate in WT littermates and because dominant-negative SNARE expression leads to a hypofunction of synaptic NMDARs, we conclude that astrocytes modulate epileptogenesis, recurrent spontaneous seizures, and pathophysiological consequences of epilepsy through a pathway involving NMDARs.

glia-to-neuron signaling | pilocarpine mouse | electroencephalography | hippocampus | interictal spikes

Temporal lobe epilepsy (TLE), one of the most common neurological disorders, which affects 1% of the population worldwide, is characterized by the occurrence of recurring unprovoked spontaneous seizures. Unfortunately, seizures in 30% of patients with TLE are not controlled by anticonvulsant agents, drugs that are directed to neuronal targets. In this case, patients may undergo resective epilepsy surgery to become seizure-free (1).

Emerging evidence suggests a critical role for the glial cells, astrocytes, in epilepsy (2, 3). Astrocytes play an important role in maintaining central nervous system function by releasing gliotransmitters, including glutamate, D-serine, and ATP (4). Whether gliotransmission contributes to epilepsy in vivo has not been established. It has recently been shown that gliotransmission regulates the trafficking and surface expression of the neuronal *N*-methyl-D-aspartate receptor (NMDAR) subunits 2A and 2B (5, 6). Because of the importance of NMDARs in epilepsy (7), we hypothesize that astrocytes modulate epileptic seizures by regulating these receptors. To test this hypothesis, we used astrocyte-specific molecular genetics in mice to inhibit transmitter release from astrocytes, along with the in vivo pilocarpine model of TLE, long-term continuous video EEG, and an in situ hippocampal slice model of epilepsy.

We show that genetic impairment of transmitter release from astrocytes by the expression of glial dominant-negative SNARE (dnSNARE) (8) delayed the onset of spontaneous recurrent seizures (SRSs) and attenuated the progressive increase in frequency of SRSs, hippocampal sclerosis, and the appearance of aberrant open-field behaviors. Similarly, chemically induced epileptiform activity in situ was attenuated by the expression of astrocytic dnSNARE. The ability of dnSNARE to reduce epileptiform activity in situ and seizure frequency in vivo was phenocopied by antagonism of the NMDAR with D-(-)-2-amino-5-phosphonopentanoate (D-AP5), indicating that astrocytic signals contribute to the progressive development of epilepsy through the modulation of neuronal NMDAR activity.

Results

Pilocarpine-Induced Status Epilepticus Is Comparable in WT and dnSNARE Mice. To determine contributions of astrocytes to epileptogenesis, we used the pilocarpine model of epilepsy in mice, a model known to be highly isomorphic with human TLE (9), and asked whether the expression of dnSNARE in astrocytes modulated the development of SRSs. Repeated low doses of pilocarpine (100 mg/kg) were injected i.p. until the onset of status epilepticus (SE; Fig. S1*A*). This ramping protocol has been shown to reduce mortality after SE (10). No differences in the behavioral seizure score were seen between dnSNARE animals and their WT littermates (Fig. S1 *A–C*). EEG recordings also showed that electrographic features of acute seizures and SE were similar between WT and dnSNARE mice (Fig. S1 *D–K*). These data indicate that astrocytic dnSNARE expression does not alter the acute responses to pilocarpine.

Astrocytic dnSNARE Expression Attenuates the Progressive Development of SRSs. To determine whether astrocytic dnSNARE expression impacts the development of epilepsy, we performed continuous video EEG recordings that commenced 5 d after SE and continued for as long as 150 d. The latency from SE to onset of the first SRS was significantly lengthened in dnSNARE mice compared with WT mice (latency to seizure onset in WT mice, 13.33 ± 0.55 d vs. 17.00 ± 1.34 d in dnSNARE mice; $n = 6$ per genotype; Student t test, $P < 0.05$, Fig. 1*A*). All mice that experienced SE subsequently exhibited interictal spikes, hallmarks of the epileptic brain (11), and developed epilepsy with more than

Significance

Temporal lobe epilepsy (TLE) is a chronic brain disorder characterized by the occurrence of spontaneous recurrent seizures. Much of our knowledge of epilepsy is based on how neurons contribute to this disorder. Here we provide a view in which glial cells (astrocytes) contribute to the progressive development of TLE. We have combined a model of epilepsy that more closely mimics the complex features of seizures in epileptic patients, with astrocyte-specific molecular genetics to identify how astrocytes modulate the progressive development of TLE, including seizure occurrence, brain damage, and behavioral deficits. We provide evidence of the glial regulation of neuronal NMDA receptors in this process. This study identifies astrocytes as a potential therapeutic target for the treatment of epilepsy.

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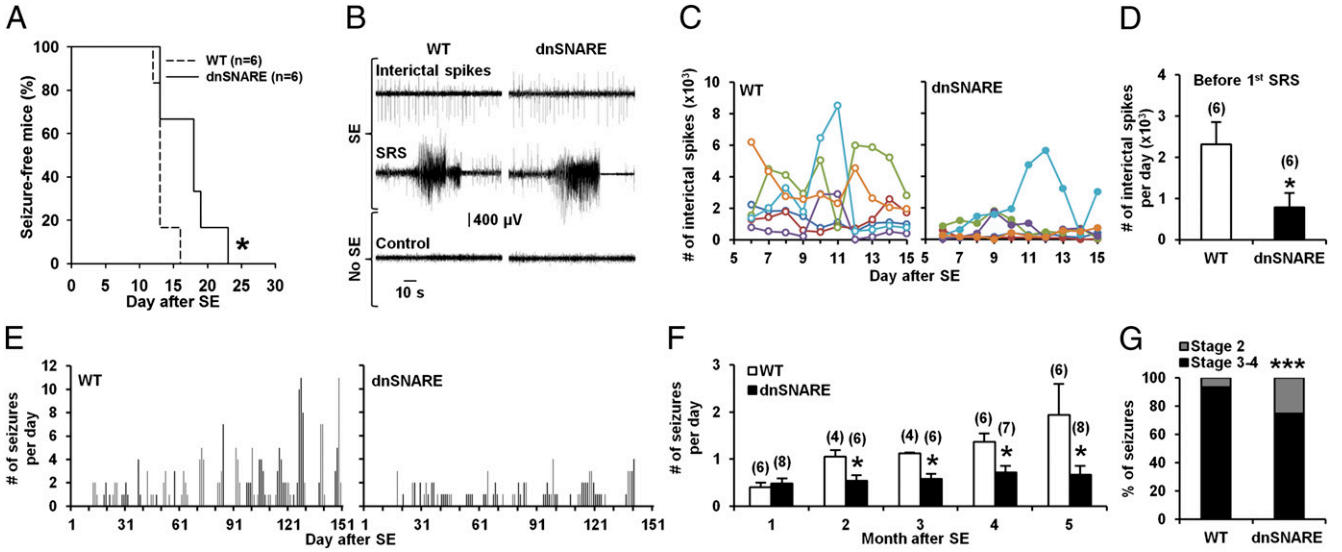

Fig. 1. Astrocytic dnSNARE expression delays and attenuates the progressive development of SRSs in the pilocarpine model of epilepsy. (*A*) Kaplan–Meier analysis of the time of SRS appearance after pilocarpine-induced SE showing a delayed seizure onset in dnSNARE mice compared with WT mice ($n = 6$ per genotype; log-rank test, $^*P < 0.05$). (*B*) Representative EEG recordings from SE and control no-SE WT and dnSNARE mice. In both genotypes, SE mice exhibited typical interictal spikes (*Top*) and SRSs (*Middle*) several days after pilocarpine treatment, whereas control no-SE mice (*Bottom*) were free of interictal spikes and seizure activity. (*C*) The individual total number of interictal spikes per day during the first 2 wk after SE was reduced in dnSNARE mice ($n = 6$, *Right*, closed circles) compared with WT mice ($n = 6$, *Left*, open circles). (*D*) The average number of interictal spikes after SE before seizure onset was smaller in dnSNARE mice compared with WT mice ($n = 6$ per genotype; Student *t* test, $^*P < 0.05$). (*E*) Representative plots of the seizure frequency over time showing seizure clusters in WT mice (*Left*) and dnSNARE mice (*Right*). Note fewer seizures per day after SE in dnSNARE animals compared with WT animals. (*F*) The average number of seizures per day for each 1-mo block was attenuated from 2 mo to 5 mo after SE in dnSNARE mice ($n = 6$–8) compared with WT mice ($n = 4$–6; ANOVA: genotype, $F = 14.893$, $P = 0.001$; time, $F = 5.410$, $P = 0.002$; genotype × time interaction, $F = 2.664$, $P = 0.048$; post hoc test, $^*P < 0.05$). (*G*) Seizures were less severe in dnSNARE mice (194 seizures from six animals) during the first 2 mo after SE compared with WT mice (168 seizures from four animals; χ^2 test, $^{***}P < 0.001$).

two SRSs (Fig. 1*B*). In both genotypes, each seizure was characterized by a typical increase in β-band power (13–30 Hz; Fig. S2). Control mice that had been injected with pilocarpine but did not enter SE displayed a normal EEG dominated by δ-band power (1–4 Hz; Fig. S2), with neither interictal spikes nor seizures (Fig. 1*B*). Additionally, the delayed seizure onset in dnSNARE mice was accompanied by a significant reduction in the number of interictal spikes (Fig. 1 *C* and *D*).

Continuous video EEG monitoring showed that SRSs occurred in clusters in both genotypes (Fig. 1*E*), a distinct feature of the pilocarpine model (12). During the first month after SE, there was no difference in initial seizure frequency between WT and dnSNARE mice (Fig. 1*F*). The frequency of SRSs increased progressively over time in WT mice, from 0.40 ± 0.10 seizures per day (1 mo after SE; $n = 6$) to 1.94 ± 0.65 seizures per day (5 mo after SE; $n = 6$; Mann–Whitney test, $P < 0.01$; Fig. 1 *E* and *F* and Fig. S3*A*), whereas it remained unchanged in dnSNARE mice (1 mo after SE, 0.48 ± 0.10 seizures per day vs. 0.66 ± 0.19 seizures per day at 5 mo after SE; $n = 8$ for both conditions; Student *t* test, $P > 0.05$; Fig. 1 *E* and *F* and Fig. S3*A*). The initial frequency of SRSs in WT mice at 1 mo after SE predicted the elevated frequency of SRSs 4 mo later (linear regression, $y = 6.96x - 0.31$, $r^2 = 0.7559$, $n = 5$; Fig. S3*B*) whereas, in dnSNARE mice, seizure frequency remained constant throughout this period (linear regression: $y = -0.03x + 0.68$, $r^2 = 0.0005$, $n = 8$; Fig. S3*B*). Whereas the severity of SRSs was reduced in dnSNARE mice compared with WT mice during the first 2 mo after SE (Fig. 1*G*), the duration of individual seizures remained similar over time (Fig. S3*C*). Additionally, the number of interictal spikes per day in WT mice increased progressively over time after SE (Fig. S3*D*), as described in another animal model of epilepsy (11). The average number of interictal spikes per day during the entire period of EEG recording was significantly reduced in dnSNARE mice compared with WT mice [WT mice, 9,614.88 ± 2,223.78 interictal spikes per day ($n = 5$), vs. 3,228.46 ± 629.44 interictal spikes per day in dnSNARE mice ($n = 7$); Mann–Whitney test, $P < 0.05$; Fig. S3*D*]. These results suggest that astrocytic dnSNARE expression prevents the progressive increase in seizure frequency and reduces interictal spiking during the chronic phase of epilepsy.

Astrocytic dnSNARE Expression Attenuates Behavioral Deficits During Epilepsy. In patients and animal models, TLE is associated with long-term behavioral abnormalities (13, 14). We then asked whether astrocytic dnSNARE expression would attenuate the development of these behavioral defects. To this end, we observed mice in the open field 8 mo after pilocarpine injections to examine gross locomotor activity and explorative habits. Control no-SE WT and dnSNARE mice exhibited the normal pattern of open field behavior that consisted of exploratory paths and stops, primarily in the corners of the arena (Fig. 2 *A* and *B*). SE increased the duration of time stopped in the open field in WT mice (Fig. 2 *A*, *C*, and *D*) but not in dnSNARE mice (Fig. 2 *B*, *C*, and *D*). In WT and dnSNARE mice, the cumulative distance and speed were not altered by SE (Fig. S4 *A* and *B*). Thus, astrocytic dnSNARE expression prevents the overall changes in the structure of the open-field behavior of epileptic mice.

Astrocytic dnSNARE Expression Reduces Neuronal Loss and Reactive Astrocytosis During Epilepsy. To evaluate the pathological correlates of seizure development in the pilocarpine model of TLE, we performed immunostaining in the hippocampus of mice 8 mo after pilocarpine treatment. To determine loss of neurons, we performed immunostaining against the neuronal marker NeuN (15). In WT and dnSNARE mice, SE caused striking neuronal loss in the CA1 and CA3 subfields (Fig. S5 *A* and *B*). SE also significantly reduced the number of dentate hilar neurons in WT mice but not in dnSNARE mice (Fig. 3*A*). We noted that, at 10 mo of age (8 mo after pilocarpine treatment), there was a small decline in dentate hilar neurons in dnSNARE mice compared with WT mice that did not exhibit SE (Fig. 3*A*). However, in control experiments at 2 mo of age, dnSNARE mice did not have

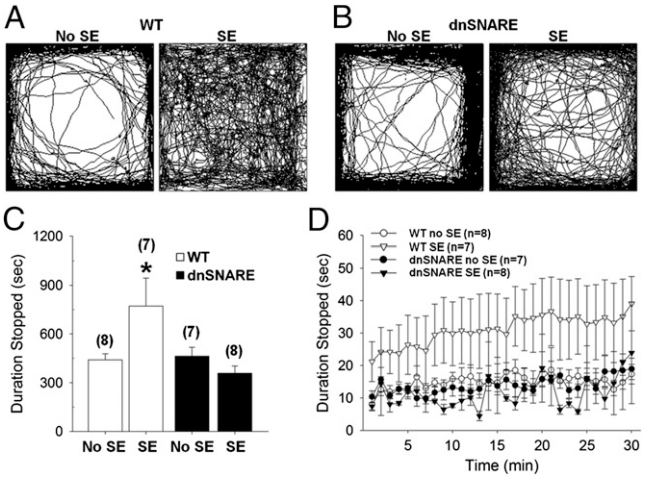

Fig. 2. Astrocytic dnSNARE expression attenuates behavioral consequences of epilepsy. (*A* and *B*) Representative 30 min of track plots of control no-SE and SE-experienced WT (*A*) and dnSNARE mice (*B*) placed in the open field 8 mo after pilocarpine treatment. (*C*) The average duration of time stopped over the 30-min test period was increased in SE WT mice but not in SE dnSNARE mice (WT no-SE, $n = 8$; WT SE, $n = 7$; dnSNARE no-SE, $n = 7$; dnSNARE SE, $n = 8$; ANOVA followed by post hoc test, $^*P < 0.05$, WT SE vs. WT no-SE). (*D*) Line graph shows duration of time stopped in 1-min bins over the 30-min test period.

fewer dentate hilar neurons compared with WT mice (WT mice, 500.81 ± 16.06 neurons per square millimeter, vs. 536.82 ± 37.21 neurons per square millimeter in dnSNARE mice; $n = 3$ animals for each genotype; Student t test, $P > 0.05$), suggesting that astrocytic dnSNARE expression does not impact the density of dentate hilar neurons at the age of onset of the study.

To assess reactive astrocytosis, we performed immunostaining against the glial marker GFAP (15) as well as vimentin (16). SE significantly increased GFAP and vimentin expression in the hippocampus of WT mice (Fig. 3*B* and Figs. S5 *C* and *D* and S6). In contrast, the expression of astrocytic dnSNARE prevented SE-induced reactive astrocytosis (Fig. 3*B* and Figs. S5 *C* and *D* and S6). These results show that astrocytic dnSNARE expression is neuroprotective in the dentate hilus and prevents reactive astrocytosis during epilepsy.

Astrocytic dnSNARE Expression Reduces Epileptiform Activity in Situ by Reducing NMDAR Activity. To understand the mechanism of astrocytic influence over SRSs, we used an acute slice model of epileptiform activity to ask whether glial control of neuronal NMDARs (5, 6) contributes to the modulation of SRSs. When hippocampal slices were perfused with an artificial cerebrospinal fluid (ACSF) containing 0 Mg^{2+} and picrotoxin (100 μM), extracellular recordings in the CA1 region showed typical epileptiform activity characterized by the occurrence of ictal-like events (duration > 2 s; low frequency < 1 event per minute; Fig. 4*A*). In agreement with results obtained on SRSs in vivo, slices from dnSNARE animals displayed a significant increase in the latency to onset of epileptiform activity (Fig. 4 *A* and *B*) and a significant decrease in the frequency of ictal-like events (Fig. 4 *A* and *C*). Duration of ictal-like events was not altered by the dnSNARE transgene [ictal-like event duration in WT mice, 24.82 ± 9.92 s ($n = 5$ slices from five animals), vs. 21.32 ± 6.72 s in dnSNARE mice ($n = 5$ slices from five animals); Student t test, $P > 0.05$; Fig. 4*A*].

Our previous studies have shown that astrocytic dnSNARE expression leads to a reduction in synaptic NMDA currents in the cortex (5, 6), which is expected to reduce neuronal excitability and could mediate the effects of astrocytic dnSNARE expression on epileptiform activity. We performed patch-clamp recordings from CA1 pyramidal neurons and determined that astrocytic dnSNARE expression increased the AMPA/NMDA ratio at the hippocampal synapses (Fig. 4*D*). To determine whether this was caused by a postsynaptic modulation of NMDARs, we recorded mixed AMPA and NMDA miniature excitatory postsynaptic currents (EPSCs; mEPSCs) in ACSF containing 1 μM TTX and 0 Mg^{2+} to enhance the NMDA component of mEPSCs (Fig. S7) as described by Deng et al. (6). By using the NMDAR antagonist D-AP5 (50 μM), we found that the amplitude of mixed mEPSCs 20 ms following the peak was a reliable indicator of the NMDA current contribution (Fig. 4*E*). Astrocytic dnSNARE expression significantly decreased the amplitude of synaptic NMDA current by 35% (Fig. 4 *E* and *F*) without changing the amplitude of synaptic AMPA current (Fig. 4 *E* and *G*).

To determine whether the reduced epileptiform activity observed in dnSNARE slices could be mimicked by reducing NMDAR activity, we perfused WT slices with 1 μM D-AP5, a concentration that reduced the synaptic NMDA current by 32% (Fig. S8). Application of 1 μM D-AP5 in WT slices significantly increased the latency to onset of epileptiform activity (Fig. 4 *A* and *B*) and significantly decreased the frequency of ictal-like events (Fig. 4 *A* and *C*), mimicking the in situ and in vivo dnSNARE phenotype. In contrast, astrocytic dnSNARE expression occluded the effects of D-AP5 (Fig. 4 *A*–*C*). Thus, these results indicate that the reduced epileptiform activity seen in dnSNARE slices is a consequence of astrocytic dnSNARE-dependent hypofunction of NMDARs.

Before asking whether in vivo treatment of WT mice with D-AP5 would phenocopy dnSNARE mice, we determined whether the astrocytic dnSNARE-dependent hypofunction of NMDARs was conserved during epilepsy. The AMPA/NMDA current ratio

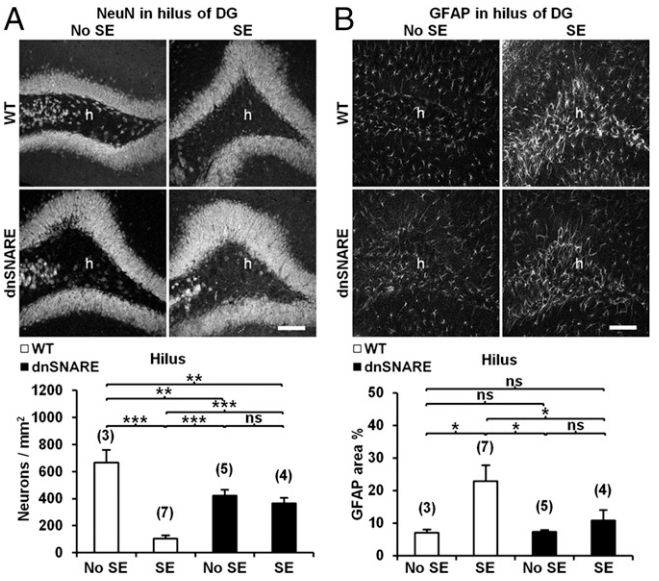

Fig. 3. Astrocytic dnSNARE expression attenuates hippocampal sclerosis during epilepsy. (*A*) Representative hippocampal sections from control no-SE and SE-experienced WT and dnSNARE mice stained with an antibody directed against the neuron-specific epitope NeuN 8 mo after pilocarpine treatment. Striking neuronal cell loss was observed in the hilus (h) of the dentate gyrus (DG) in SE WT mice but not in SE dnSNARE mice. Average neuronal density in the hilus of the DG is presented under the corresponding sections (WT no-SE, $n = 3$ animals; WT SE, $n = 7$ animals; dnSNARE no-SE, $n = 5$ animals; dnSNARE SE, $n = 4$ animals; ANOVA followed by post hoc test, $^{**}P < 0.01$ and $^{***}P < 0.001$; ns, nonsignificant). (*B*) Same hippocampal sections as in *A* but stained with an antibody directed against the astrocyte-specific epitope GFAP. GFAP expression was increased in the hilus (h) of the DG in SE WT mice but not in SE dnSNARE mice. Average GFAP area in the hilus of the DG is presented under the corresponding sections (ANOVA followed by post hoc test, $^*P < 0.05$; ns, nonsignificant). (Scale bars: 100 μm.)

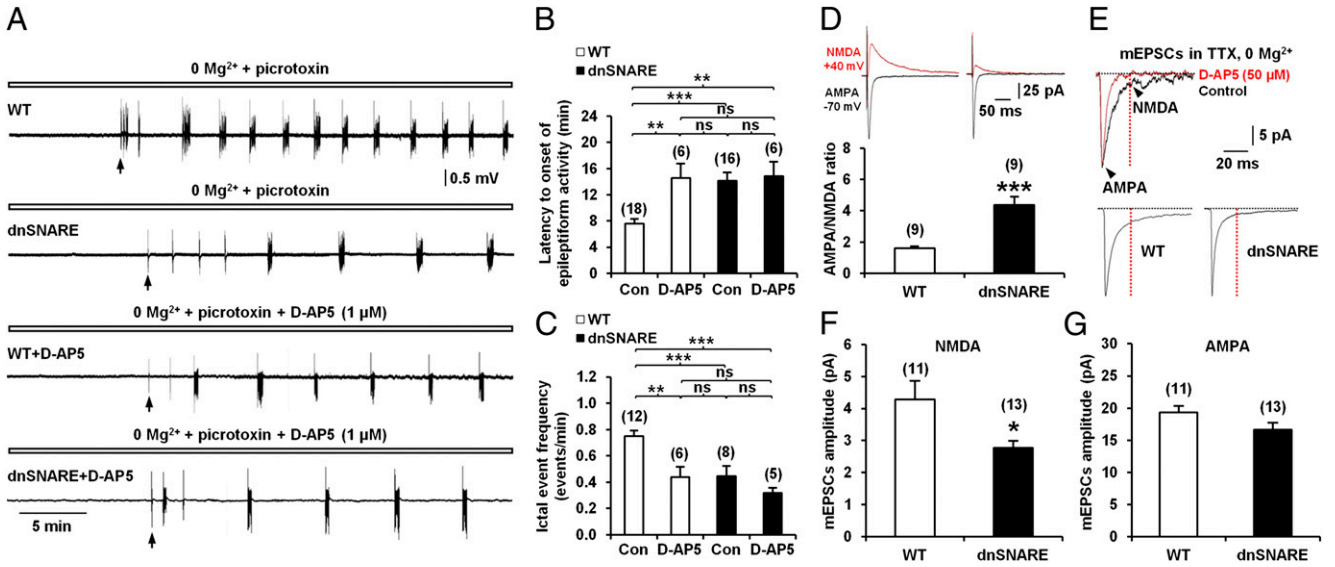

Fig. 4. Astrocytic dnSNARE expression reduces chemically induced epileptiform activity in hippocampal slices by reducing synaptic NMDAR activity. (*A*) Representative extracellular recordings showing the initiation of epileptiform activity in proconvulsant ACSF (0 Mg^{2+}, 100 μM picrotoxin) in CA1 region of WT and dnSNARE mice in the absence (two upper traces) or presence of the NMDAR antagonist D-AP5 (two lower traces). Slices from dnSNARE mice showed a delayed epileptiform activity onset (arrow) and a reduced ictal-like event frequency. Pretreatment with 1 μM D-AP5 delayed epileptiform activity onset and reduced ictal-like event frequency in WT slices but not in dnSNARE slices. (*B*) Average latency to onset of epileptiform activity in different conditions [WT slices (control) not pretreated with D-AP5, $n = 18$ slices from nine animals; WT slices pretreated with D-AP5, $n = 6$ slices from three animals; dnSNARE slices (control) not pretreated with D-AP5, $n = 16$ slices from eight animals; dnSNARE slices pretreated with D-AP5, $n = 6$ slices from three animals; ANOVA followed by post hoc test, $^{**}P < 0.01$ and $^{***}P < 0.001$; ns, nonsignificant]. (*C*) Average of ictal-like event frequency in different conditions [WT slices (control) not pretreated with D-AP5, $n = 12$ slices from six animals; WT slices pretreated with D-AP5, $n = 6$ slices from five animals; dnSNARE slices (control) not pretreated with D-AP5, $n = 8$ slices from three animals; dnSNARE slices pretreated with D-AP5, $n = 5$ slices from three animals; ANOVA followed by post hoc test, $^{**}P < 0.01$ and $^{***}P < 0.001$; ns, nonsignificant). (*D*) Whole-cell recordings of evoked AMPA- and NMDA-EPSCs in CA1 pyramidal cells showing that AMPA/NMDA ratio was increased in dnSNARE mice ($n = 9$ cells from six animals) compared with WT mice ($n = 9$ cells from seven animals; Mann–Whitney test, $^{***}P < 0.001$). (*E, Upper*) D-AP5 (50 μM) blunted the slow NMDAR component of the mEPSC recorded in CA1 pyramidal cells from WT slices in 1 μM TTX and 0 Mg^{2+} without affecting the AMPA current. (*E, Lower*) Average traces of mEPSCs in WT ($n = 4$ cells from four animals; *Lower Left*) and dnSNARE mice ($n = 4$ cells from three animals; *Lower Right*) showing that astrocytic dnSNARE expression reduced the NMDAR component of the mixed mEPSCs without affecting the AMPA current. (*F*) Average amplitude of the slow NMDAR component of the mEPSCs taken 20 ms after the peak of the AMPA current in WT ($n = 11$ cells from nine animals) and dnSNARE mice ($n = 13$ cells from five animals; Mann–Whitney test, $^{*}P < 0.05$). (*G*) Average amplitude of the AMPA peak component of the mEPSCs in WT ($n = 11$ cells from nine animals) and dnSNARE mice ($n = 13$ cells from five animals; Student *t* test, $P > 0.05$).

measured in CA1 pyramidal neurons 5 mo after pilocarpine treatment was significantly increased in dnSNARE mice compared with WT mice regardless of whether they had previously exhibited SE (Fig. 5 *A* and *B*). Pyramidal neurons from mice with SE were filled with biocytin for post hoc cell type identification (Fig. 5*B*). Additionally, the dnSNARE transgene did not alter the frequency and amplitude of AMPA receptor (AMPAR)-mediated spontaneous EPSCs (sEPSCs) in mice with or without SE (Fig. 5 *C*–*F*), indicating that the increased AMPA/NMDA current ratio was not caused by a modulation of AMPARs but rather by a change in NMDAR current. Thus, these data demonstrate that the reduced NMDAR current seen in dnSNARE mice was maintained throughout the period of SRSs.

In Vivo Antagonism of NMDARs Attenuates the Progressive Development of SRSs. We then asked whether in vivo antagonism of NMDAR activity would lead to a long-term reduction in SRSs. To this end, we used osmotic minipump to intracerebroventricularly (i.c.v.) introduce the NMDAR antagonist D-AP5 (or ACSF vehicle) in WT and dnSNARE mice 5 d after SE for 2 wk while continuously recording video EEG for 2 mo (Fig. 6*A*). Treatment with D-AP5 significantly delayed the onset of the first SRS (seizure onset in WT mice treated with ACSF, 8.20 ± 0.96 d, vs. 16.50 ± 2.39 d in WT mice treated with D-AP5; $n = 5$ animals per condition; Student *t* test, $P < 0.05$; Fig. 6*B*). Treatment with D-AP5 also significantly reduced the frequency of SRSs, and this was correlated with an increase in the interseizure interval duration (Fig. 6*C*). Animals treated with D-AP5 exhibited fewer stage 3 to 4 seizures (Fig. 6*D*), although the duration of individual seizures was not altered by prior D-AP5 treatment (seizure duration in WT mice treated with ACSF, 32.49 ± 1.28 s, vs. 34.80 ± 2.60 s in WT mice treated with D-AP5; $n = 5$ animals per condition; Student *t* test, $P > 0.05$). In contrast, astrocytic expression of dnSNARE occluded the action of D-AP5 (Fig. 6 *E*–*G*). Thus, these results demonstrate that chronic in vivo antagonism of NMDARs after SE delays seizure onset, reduces seizure severity, and attenuates the progressive increase in seizure frequency, mimicking the dnSNARE phenotype.

Discussion

In summary, we have used genetic, electrophysiological, behavioral, and histological tools to elucidate the role of astrocytes in epilepsy. Our studies demonstrate a major role for astrocytes in the progressive development of SRSs after SE. Genetic impairment of the SNARE complex in astrocytes leads to a hypofunction of postsynaptic NMDARs and ameliorates the development of epilepsy that includes progressive increase in seizure frequency, hippocampal sclerosis, and behavioral abnormalities.

By using the pilocarpine model of TLE along with continuous video EEG, we demonstrate that inhibition of transmitter release from astrocytes lengthens the latent phase of epilepsy, reduces seizure severity, and attenuates the progressive increase in seizure and interictal spike frequency during the chronic phase of epilepsy. As defined by previous studies that showed the importance of chronic video EEG recordings in studies of epilepsy (12, 17, 18), sustained monitoring was required to reveal the epilepsy phenotype in dnSNARE mice. The progressive nature of epilepsy and the seizure clustering presented here have been

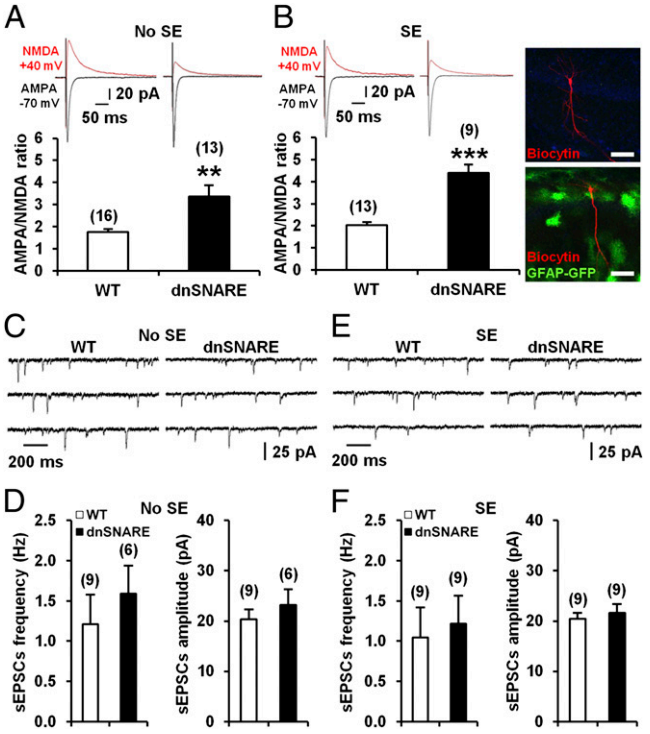

Fig. 5. The astrocytic dnSNARE-dependent hypofunction of synaptic NMDARs is conserved during epilepsy. (*A*) The AMPA/NMDA ratio in CA1 pyramidal cells 5 mo after pilocarpine treatment was increased in control no-SE dnSNARE mice ($n = 13$ cells from four animals) compared with control no-SE WT mice ($n = 16$ cells from six animals; Mann–Whitney test, $^{**}P < 0.01$). (*B*) The increased AMPA/NMDA ratio was conserved in SE dnSNARE mice ($n = 9$ cells from four animals) compared with SE WT mice ($n = 13$ cells from five animals; Student *t* test, $^{***}P < 0.001$) 5 mo after pilocarpine treatment. (*Right*) Images show CA1 pyramidal neurons filled with biocytin (red) in SE WT (*Upper*) and SE dnSNARE mice (*Lower*) during AMPA/NMDA ratio measurements. The presence of GFP (*Lower*) confirms that the dnSNARE transgene was expressed in astrocytes (green) of dnSNARE animals (8). (Scale bars: 100 μm.) (*C*) Representative continuous recording of CA1 pyramidal cells held at −70 mV depicting spontaneous EPSCs (sEPSCs) in the presence of 4 mM Mg^{2+} and 100 μM picrotoxin in control no-SE WT (*Left*) and dnSNARE mice (*Right*) 5 mo after pilocarpine treatment. (*D*) Frequency and amplitude of sEPSCs were similar between control no-SE WT ($n = 9$ cells from four animals) and dnSNARE mice ($n = 6$ cells from four animals; Student *t* test, $P > 0.05$). (*E*) Same experiments as in *C* but in WT (*Left*) and dnSNARE mice (*Right*) that entered SE. (*F*) Frequency and amplitude of sEPSCs were the same in WT ($n = 9$ cells from four animals) and dnSNARE mice that experienced SE ($n = 9$ cells from four animals; Student *t* test, $P > 0.05$).

described in epileptic patients (19, 20) and various animal models of epilepsy (12, 17, 18). It is surprising that SE was not affected in dnSNARE mice given that recent in situ studies suggest that astrocytic calcium signals could contribute to the initiation of seizure during SE through a glutamate-mediated mechanism (21). However, this mechanism operates at the seizure focus by lowering the epileptic threshold and becomes dispensable in the presence of saturating stimuli (21). It is likely that our pilocarpine treatment is a saturating stimulus that masks this threshold-modulating mechanism.

In accordance with our previous studies showing that dnSNARE expression in astrocytes leads to a reduction in the surface expression of synaptic NMDARs in the cortex (5, 6), we identified a reduced activity of NMDARs at the CA1 hippocampal synapses that persisted during epilepsy. Importantly, chronic i.c.v. administration of D-AP5 following SE in WT animals mimicked the dnSNARE phenotype by increasing the latency to seizure onset, reducing seizure severity and attenuating the progressive increase in seizure frequency over time. Equally important, these seizure-suppressing effects of D-AP5 were occluded following astrocytic dnSNARE expression, confirming that there was a smaller contribution of NMDARs to epilepsy in dnSNARE animals. These results are further supported by the fact that the regulation of NMDAR trafficking directly influences epileptiform discharges in situ (22) and seizure susceptibility in vivo (23). Interestingly, we found that treatment with D-AP5 during the latent period of epileptogenesis was sufficient to phenocopy the long-term effects of astrocytic dnSNARE expression, suggesting that there is a temporal window during which the reduced activity of NMDARs leads to long-term consequences on epilepsy.

We have previously identified a possible mechanism by which astrocytes control neuronal NMDAR activity (6). We have shown that astrocytic dnSNARE expression leads to a reduction in synaptically accessible adenosine acting on adenosine A1 receptors

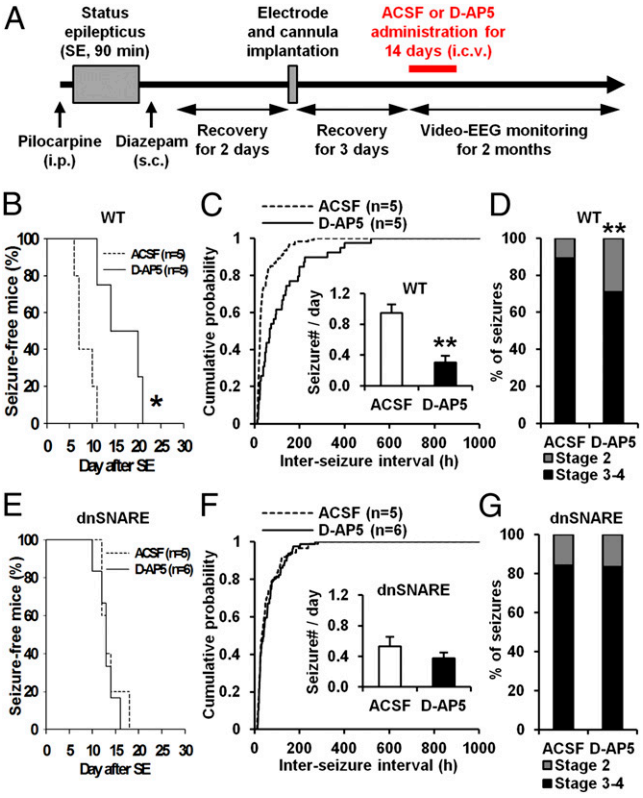

Fig. 6. Pharmacological blockade of NMDARs during the latent phase of epilepsy attenuates seizure development in WT mice but not in dnSNARE mice. (*A*) Schematic diagram of the pilocarpine model of epilepsy and D-AP5 treatment in vivo. ACSF vehicle or D-AP5 were administered for 14 d (i.c.v.) in WT and dnSNARE mice 5 d after pilocarpine-induced SE. (*B*) Kaplan–Meier analysis of the time of seizure appearance after SE in WT mice shows that D-AP5 treatment delayed seizure onset compared with ACSF vehicle treatment ($n = 5$ per group; log-rank test, $^{*}P < 0.05$). (*C*) Cumulative distribution plot of interseizure intervals and average number of seizures per day (*Inset*) in WT mice showing that D-AP5 treatment reduced seizure frequency compared with ACSF vehicle treatment ($n = 5$ per group; Kolmogorov–Smirnov test, $P < 0.001$; *Inset*, Mann–Whitney test, $^{**}P < 0.01$). (*D*) Seizure severity in WT mice was reduced by D-AP5 treatment ($n = 84$ seizures from five animals) compared with ACSF vehicle treatment ($n = 206$ seizures from five animals; χ^2 test, $^{**}P < 0.01$). (*E*) D-AP5 treatment in dnSNARE mice ($n = 6$) did not affect the latency to seizure onset compared with ACSF vehicle treatment ($n = 5$; log-rank test, $P > 0.05$). (*F*) No change in seizure frequency was observed in dnSNARE mice treated with D-AP5 ($n = 6$) compared with ACSF vehicle treatment ($n = 5$; Kolmogorov–Smirnov test, $P > 0.05$; *Inset*, Student *t* test, $P > 0.05$). (*G*) Seizure severity in dnSNARE mice was not affected by D-AP5 treatment ($n = 164$ seizures from six animals) compared with ACSF vehicle treatment ($n = 124$ seizures from five animals; χ^2 test, $P > 0.05$).

(8, 24, 25), which in turn causes a delayed reduction in the surface expression of postsynaptic NMDARs (6). Whether astrocytes use this mechanism to modulate NMDAR activity during epileptic conditions will be investigated in future studies.

Several studies have raised the potential for a causal role for astrocytic glutamate in epilepsy (21, 26), although Fellin et al. (27) describe a different view. It is important to note that these studies were mainly performed in situ and used pharmacological approaches that do not allow the selective manipulation of astrocytic signals. Additionally, the NMDAR coagonist D-serine, released from astrocytes through a SNARE-dependent mechanism (28), could also contribute to our epilepsy phenotype. However, the glutamate and D-serine levels measured with microelectrode biosensors in hippocampal slices were not affected by astrocytic dnSNARE expression (Fig. S9). Conclusions must be drawn carefully because biosensors measure average signals and they may make such measurements in a different spatial domain than local to the tripartite synapse. However, changes in glutamate and D-serine believed to arise from astrocytes have been previously measured by using biosensors, suggesting that this approach has the appropriate spatiotemporal sensitivity (29, 30). Consequently, our inability to measure dnSNARE-dependent changes in glutamate and D-serine with biosensors makes it unlikely that the nonprogressive development of epilepsy discovered in dnSNARE animals was a consequence of an impaired release of glutamate and/or D-serine from astrocytes.

In agreement with studies showing that experimental and human TLE are associated with behavioral abnormalities (13, 14), we observed that exploratory behavior of epileptic WT mice was strongly affected in the open field test. Epileptic WT mice presented a striking increase in duration of stops between subsequent locomotor excursions. Interestingly, the dnSNARE transgene strongly attenuated these abnormal behavioral defects. It is important to note that, because of the striking impact of epilepsy in WT mice on open-field behavior, it was not possible to assess other cognitive functions of these mice because impaired locomotor activity in the open field is a significant experimental confound for other tasks (31). Thus, by comparing WT and dnSNARE mice, we established a link between the degree of behavioral impairment and the severity of hippocampal sclerosis during epilepsy.

In conclusion, by combining a model of epilepsy that more closely mimics the complex features of seizures in epileptic patients with astrocyte-specific molecular genetics, we demonstrate that astrocytes modulate the progressive development of epilepsy in vivo including seizure occurrence, brain damage, and behavioral deficits. We also provide strong evidence of the importance of NMDARs in this process.

Materials and Methods

Animals. The generation of dnSNARE mice has been previously reported (8). Details are given in *SI Materials and Methods*.

Pilocarpine-Induced Epilepsy. Male mice (2 mo old) were subjected to pilocarpine treatment to induce SE. Protocols for surgical implantation of cortical EEG electrodes, video EEG monitoring, and scoring of seizure behavior are detailed in *SI Materials and Methods*.

Behavioral Study. Detailed protocol for the open-field test is given in *SI Materials and Methods*.

Immunofluorescence Staining. After behavioral study, mice were anesthetized and perfused with paraformaldehyde. Coronal brain sections (40-μm thickness) were immunostained, examined, and analyzed as described in *SI Materials and Methods*.

Electrophysiology. Acute coronal cortical–hippocampal slices (310–400 μm) were prepared, and extracellular recordings, whole-cell recordings, and data analysis were performed as described in *SI Materials and Methods*.

In Vivo Pharmacology. Two days after SE, mice were implanted with cortical EEG electrodes and brain cannulas. Osmotic minipumps were filled with D-AP5 (5 mM) or vehicle (ACSF), externalized, and connected to the brain cannula. Details are given in *SI Materials and Methods*.

Statistical Analysis. Statistical analysis is detailed in *SI Materials and Methods*. Significance was set at $P < 0.05$. Data are shown as mean ± SEM.

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