Maladaptive myelination promotes generalized epilepsy progression

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Activity-dependent myelination can fine-tune neural network dynamics. Conversely, aberrant neuronal activity, as occurs in disorders of recurrent seizures (epilepsy), could promote maladaptive myelination, contributing to pathogenesis. In this study, we tested the hypothesis that activity-dependent myelination resulting from absence seizures, which manifest as frequent behavioral arrests with generalized electroencephalography (EEG) spike-wave discharges, promote thalamocortical network hypersynchrony and contribute to epilepsy progression. We found increased oligodendrogenesis and myelination specifically within the seizure network in two models of generalized epilepsy with absence seizures (Wag/Rij rats and Scn8a<sup>+</sup>/<sup>-mut</sup> mice), evident only after epilepsy onset. Aberrant myelination was prevented by pharmacological seizure inhibition in Wag/Rij rats. Blocking activity-dependent myelination decreased seizure burden over time and reduced ictal synchrony as assessed by EEG coherence. These findings indicate that activity-dependent myelination driven by absence seizures contributes to epilepsy progression; maladaptive myelination may be pathogenic in some forms of epilepsy and other neurological diseases.

Neuronal activity can modulate myelin structure during development1–3 and throughout life4–10 by promoting proliferation of oligodendrocyte progenitor cells (OPCs), generation of new myelinating oligodendrocytes and changes to myelin structure11–13. Remodeling of myelin by existing oligodendrocytes can also occur in response to neuronal activity14–16. This plasticity of myelin and oligodendroglial cells has been best demonstrated to date in cortical and callosal axons4–6,11. Activity-regulated myelination is adaptive in the healthy brain, where it is hypothesized to increase neural network synchrony12–14, and myelin plasticity contributes to cognitive functions, including attention, learning and memory11,14,15,17.

The effects of myelin plasticity on network function in the healthy brain raise the question of how activity-regulated myelination may modulate network function in disease states characterized by abnormal patterns of neuronal activity, such as epilepsy. Diffusion-based imaging has demonstrated abnormal white matter microstructure in various forms of epilepsy in humans and rodent models18–24; however, definitive conclusions cannot be drawn about underlyin myelin and axonal structure in the absence of gold-standard ultrastructural histology, nor is it known how altered white matter structure may contribute to epilepsy pathophysiology.

Absence seizures occur in multiple forms of human generalized epilepsy and are associated with behavioral arrest and generalized, but frontally predominant, spike-wave discharges25,26. Absence seizures originate in abnormal oscillations between the thalamus and cortex27 and propagate along myelinated tracts, including the anterior portions of the corpus callosum28. In humans and rodents, absence seizures are brief but very frequent, occurring hundreds of times per day29,30. Thus, generalized epilepsy with absence seizures presents an ideal paradigm to examine the relationship between activity-regulated myelination and seizure pathophysiology.

Genetic rodent models of generalized epilepsy with absence seizures exhibit defined periods of seizure onset followed by seizure progression, in which absence seizures increase in daily frequency over time29,30. This pattern of developmental seizure onset with progression is similar to the natural history of untreated, medically refractory and/or progressive forms of generalized epilepsy in children12,29. Blockade of seizures throughout the period of seizure progression in one model of absence epilepsy—Wag/Rij rats—prevents or delays seizure onset31, indicating that aberrant neuronal activity induces pathological network changes that contribute to subsequent progression in seizure burden. Although mechanisms of absence seizure onset and progression are incompletely understood, a well-documented feature is excessive synchrony (coordinated firing of groups of neurons) in the thalamocortical network12,13,15. Given the proposed effect of activity-regulated myelination on network synchrony12–14, we hypothesized that abnormally increased myelination within the seizure network, induced by absence seizures, might contribute to seizure progression.

Results

Increased seizure network myelination after epilepsy onset. To test the putative relationship between absence seizures and myelination, we used a well-established model of absence seizures—Wag/Rij rats29. Wag/Rij is an inbred rat strain that develops spontaneous, stereotyped absence seizures characterized by brief behavioral arrest, similar to absence seizures in humans31. The EEG correlate of these episodes in Wag/Rij rats is ~4–8 Hz, generalized, frontally predominant spike-wave discharges that are maximal over the somatosensory cortices31. Absence seizures arise from neurons connecting the thalamus and the cortex25,26. In rodents, absence seizures are particularly prominent in relays between the ventral nuclear complex of the thalamus and somatosensory cortex,
driven by complex circuitry involving interneurons of the reticular thalamic nucleus27,28. Seizures propagate throughout the brain via myelinated tracts, including the internal capsule (interconnects the thalamus and cortex) and the corpus callosum, a commissural tract that is required for seizure generalization24 (Fig. 1a). In Wag/Rij rats, infrequent seizures spontaneously begin around 2 months of age and steadily increase in daily frequency until the rate plateaus at 20–30 seizures per hour at about 6 months of age26. A closely related rat strain from which Wag/Rij is derived, Wistar, does not typically develop absence seizures during this time frame and, therefore, is used as a control for Wag/Rij rats26.

To investigate whether absence seizures cause aberrant activity-regulated myelination within the seizure network, we began by assessing the proliferation of OPCs together with the number of mature oligodendrocytes in the mid-region (body) of the corpus callosum, focusing specifically on the area interconnecting the somatosensory cortices that is involved in the absence seizure network (Allen Brain Atlas, http://atlas.brain-map.org/). Given anatomical differences between Wag/Rij and Wistar (control) rats (Extended Data Fig. 1a), we used unbiased stereological methods to assess total cell numbers (Fig. 1b–c) as well as volume of the corpus callosum (Extended Data Fig. 1b) and oligodendrocyte density (Extended Data Fig. 1c). Before seizure onset, at 1.5 months of age, control rats and Wag/Rij (seizure) rats exhibit equivalent OPC proliferation in the corpus callosum. However, at 6 months of age, when seizures are well-established, Wag/Rij rats exhibit a 69% increase in dividing OPCs relative to age-matched control rats (cells co-expressing Ki67, PDGFRα and Olig2; 6,647 ± 585 dividing OPCs in control versus 11,259 ± 615 dividing OPCs in Wag/Rij; Fig. 1b–c). Compared to age-matched control rats, Wag/Rij rats also exhibit a 56% increase in Olig2, CC1-expressing mature oligodendrocytes at 6 months of age, indicative of increased oligodendrogenesis (mean ± s.e.m.: 373,697 ± 42,161 mature oligodendrocytes in control versus 581,490 ± 60,898 in Wag/Rij; Fig. 1d–e and Extended Data Fig. 1c). Both the total number and density of oligodendrocytes were increased, indicating that these differences were not a passive consequence of differences in callosal volume. In contrast, Wag/Rij and control rats exhibit similar numbers of oligodendrocytes at 1.5 months of age, before seizure onset (Fig. 1d–e). Given these findings indicating that oligodendrogenesis increases in parallel with seizure progression, we next investigated whether myelin structure is also altered. We used transmission electron microscopy to visualize cross-sections of myelinated axons in the mid-sagittal plane of the body of the corpus callosum (Fig. 1f), where oligodendrogenesis was assessed. We measured myelin sheath thickness relative to axon diameter, g-ratio4,6,11,15, in 1.5-month-old and 6-month-old Wag/Rij rats and Wistar controls. We found an increase in mean myelin sheath thickness (decreased g-ratio) in 6-month-old Wag/Rij rats compared to controls (control: 0.7368 ± 0.0036, Wag/Rij: 0.6862 ± 0.0099; Fig. 1h,i). This difference in myelin sheath thickness is similar in magnitude to functionally relevant changes in previous studies4,6,11 and is meaningful particularly considering that the dynamic range of central nervous system g-ratios is typically ~0.6–0.84,6,11,15.

Differences in g-ratios were not observed before seizure onset at 1.5 months (Fig. 1g,i) and are not attributable to strain differences in axon diameter or total axon number either before (1.5 months) or after (6 months) onset of seizures (Extended Data Figs. 2a and 3a). There was no difference in the percentage of axons that were myelinated in Wag/Rij and control rats at either 1.5 months or 6 months (Extended Data Fig. 3b); changes in myelin sheath thickness observed at 6 months were not restricted to a particular range of axon diameters (Extended Data Fig. 4a,b).

Absence seizures in rodents are most prominent in the somatosensory cortices11. We reasoned that, if abnormally increased myelination is caused by seizure activity, these changes would be specific to the seizure-affected regions. Therefore, we assessed myelin in the posterior corpus callosum (splenium), connecting cortical regions where seizure activity is less prominent in humans and rodents4,6,11,15. The seizure-associated myelin difference observed in the body of the corpus callosum was not found in the splenium, as shown in Extended Data Fig. 5. Taken together, these data demonstrate increased oligodendrogenesis and abnormally increased myelination in a temporal and anatomical pattern that parallels seizure activity.

Seizures are necessary for aberrant callosal myelination. We reasoned that absence seizures likely induce aberrant activity-regulated myelination. To confirm that seizures are required for the observed increase in callosal myelination, we treated Wag/Rij and control rats with the anti-seizure drug ethosuximide (ETX) at ~300 mg/kg/day, a dose known to prevent or reduce seizures in Wag/Rij rats26. This dosing led to a mean plasma concentration of 101.3 ± 10.33 μg ml⁻¹ (mean ± s.e.m., n = 20 rats), without signs of toxicity and similar to therapeutically levels in humans, typically between 40 μg ml⁻¹ and 100 μg ml⁻¹ (https://pubchem.ncbi.nlm.nih.gov/compound/Ethosuximide). Treatment was initiated at 1.5 months of age, before seizure onset. After 5 months of treatment, EEG at 6.5 months of age revealed frequent absence seizures of 5–15 per 0.5 second duration in vehicle-treated Wag/Rij rats (Fig. 2a,b) as previously described26. ETX robustly decreased or prevented seizures, as expected (Fig. 2b). We examined callosal myelination in control and Wag/Rij rats after vehicle or ETX administration at 7 months of age. Similarly to the findings described in Fig. 1, myelin sheath thickness was increased in vehicle-treated 7-month-old Wag/Rij rats compared to controls. However, seizure blockade with ETX treatment normalized myelin sheath thickness (g-ratio) in Wag/Rij rats (Fig. 2c–e). ETX did not...
influence g-ratio in control rats (Fig. 2e), nor did it affect axonal diameter in any group (Extended Data Fig. 2b).

Together, these findings indicate that absence seizures increase myelination specifically within the seizure-affected network and suggest a mechanism of aberrantly increased activity-dependent myelination that could be deleterious (maladaptive), contributing to epilepsy pathogenesis. To further test this hypothesis, we sought to evaluate seizure-related myelin changes in a second model of absence seizures.

**Increased myelination in a second model of generalized epilepsy.**

We next quantified oligodendrogenesis and myelin structure in a second model of generalized epilepsy. Increased myelination in a second model of generalized epilepsy.
Fig. 2 | Seizures are necessary for aberrant callosal myelination. a, Representative spike-wave discharge seizure from a 6.5-month-old VEH-treated Wag/Rij rat (upper panel); spectral analysis demonstrating that the predominant seizure frequency is ~8 Hz (lower panel). b, Mean seizures per hour for each rat. Control-VEH, n = 8 rats; Control-ETX, n = 9 rats; Wag/Rij-VEH, n = 7 rats; Wag/Rij-ETX, n = 7 rats. Kruskal–Wallis analysis revealed significant variance in seizure burden (seizures per hour) between groups (Kruskal–Wallis statistic, 25.14, P < 0.0001). Dunn’s post hoc testing: Control-VEH versus Wag/Rij-VEH, P < 0.0001, Control-ETX versus Wag/Rij-VEH, P < 0.0001, Wag/Rij-VEH versus Wag/Rij-ETX, P = 0.0099. c, Representative transmission electron micrographs from the mid-sagittal body of the corpus callosum of 7-month-old rats. Scale bars, 2 μm. d, Scatter plots of g-ratios in 7-month-old VEH-treated or ETX-treated Wag/Rij rats. Each dot represents the g-ratio of one axon. Mean g-ratios for each 7-month-old Wag/Rij rat and control rat from measurements shown in d. Control-VEH, n = 3 rats; Control-ETX, n = 3 rats; Wag/Rij-VEH, n = 3 rats; Wag/Rij-ETX, n = 3 rats. One-way ANOVA revealed significant variance in group g-ratios F(3, 16) = 11.36, P = 0.0021. Tukey testing with corrections for multiple comparisons revealed decreased g-ratio (increased myelin thickness) in Wag/Rij-VEH rats with seizures compared to control rats (Control-VEH versus Wag/Rij-VEH, P = 0.0015, and Control-ETX versus Wag/Rij-VEH, P = 0.0028). This increase in myelin sheath thickness was prevented with seizure blockade by ETX (Wag/Rij-VEH versus Wag/Rij-ETX, P = 0.0038), which normalized g-ratios (Control-VEH versus Wag/Rij-ETX, P = 0.5841, and Control-ETX versus Wag/Rij-ETX, P = 0.9952). ETX treatment did not alter g-ratios in control rats (Control-VEH versus Control-ETX, P = 0.4492). Each dot represents the mean for one rat (b, e) shown with group means ± s.e.m.; control rats are represented with black dots, and Wag/Rij rats are represented with red dots. *P < 0.05, **P < 0.01, ***P < 0.001, NS, non-significant (P > 0.05). VEH, vehicle; ETX, ethosuximide.
in the corpus callosum of Scn8a<sup>−/−</sup> mice compared to Scn8a<sup>+/+</sup> mice (Extended Data Fig. 6b). There was no difference in callosal OPC or oligodendrocyte cell death, assessed with TUNEL staining (Extended Data Fig. 6c,d). Because neuro-inflammation related to microglial and astrocyte reactivity can affect activity-dependent myelination<sup>11,28</sup>, we quantified microglia density and activation state and assessed astrocyte density and hypertrophy. We found a small increase in callosal microglial cell density in P45 Scn8a<sup>−/−</sup> mice relative to Scn8a<sup>+/+</sup> mice, whereas microglial reactivity (assessed with CD68 immunostaining) was equivalent between P45 Scn8a<sup>−/−</sup> and Scn8a<sup>+/+</sup> mice (Extended Data Fig. 7a–c). Astrocytes did not exhibit hypertrophy or increased cell density (Extended Data Fig. 7d–g). Taken together, these findings are not suggestive of a pronounced state of microglial or astrocyte reactivity in P45 Scn8a<sup>−/−</sup> mice.

Transmission electron microscopy revealed that myelin sheath thickness was increased in association with established seizures at P45 in Scn8a<sup>−/−</sup> mice relative to Scn8a<sup>+/+</sup> littermate controls (Scn8a<sup>+/+</sup>) g-ratio: 0.75 ± 0.0016 and Scn8a<sup>−/−</sup> g-ratio: 0.71 ± 0.008; Fig. 3e–h). Before seizure onset at P21, g-ratios were equivalent in Scn8a<sup>+/+</sup> and littermate control mice (Fig. 3f,h). Mean myelinated axon diameter was equivalent at P21 and at P45 in Scn8a<sup>−/−</sup> mice relative to Scn8a<sup>+/+</sup> littermate controls, indicating that altered axon size does not contribute to g-ratio differences (Extended Data Fig. 2c). We also found an increase in the percent of axons that were myelinated at P45 in Scn8a<sup>−/−</sup> mice compared to Scn8a<sup>+/+</sup> mice, whereas total axon diameter was equivalent (Fig. 3) and Extended Data Fig. 6e). Similar to Wag/Rij rats, we did not observe that increased myelin sheath thickness in Scn8a<sup>−/−</sup> mice was restricted to any particular axon size (Extended Data Fig. 4c,d).

Taken together, findings in Wag/Rij rat and Scn8a<sup>−/−</sup> mouse models demonstrate that absence seizures induce increased OPC proliferation and increased oligodendrocyte generation together with abnormally increased myelination within the affected thalamocortical seizure network.

We next sought to determine the functional effect of seizure-associated myelination and tested the hypothesis that aberrantly increased myelination contributes to disease pathogenesis.

**Myelin plasticity promotes generalized epilepsy progression.** In the healthy brain, activity-dependent myelination is thought to promote coordination between regions within distributed neuronal networks, a process that supports multiple forms of learning<sup>31–33</sup>. We hypothesized that absence seizure-associated, abnormally increased myelination might contribute to thalamocortical network hypersynchrony<sup>34,35</sup>, increasing disease severity. To assess the functional effect of myelin plasticity in absence seizure progression, we sought to block activity-dependent myelination.

Activity-dependent secretion of brain-derived neurotrophic factor (BDNF), and its subsequent signaling through the TrkB receptor on OPCs, is required for activity-dependent myelination of corticocortical projection neurons<sup>36</sup>.Conditional deletion of TrkB from OPCs prevents activity-dependent oligodendrogenesis and myelination in the corpus callosum but does not alter homeostatic oligodendrogenesis nor lead to myelin loss<sup>37</sup>.

To enable blockade of activity-dependent myelination in Scn8a<sup>−/−</sup> mice, we bred Scn8a<sup>−/−</sup> and Scn8a<sup>+/−</sup> littermates with floxed TrkB<sup>Cre</sup> in the presence or absence of Cre, inducibly expressed under the PDGFRα promoter (Scn8a<sup>−/−</sup>/TrkB<sup>fl/fl</sup>, PDGFRα<sup>Cre</sup>-ER). Induction of Cre in this model with tamoxifen leads to TrkB deletion in about 80% of OPCs<sup>11</sup>; leak of Cre expression is not found in neurons<sup>29</sup>. Our cross yielded four littermate groups of mice: (1) Scn8a<sup>−/−</sup>;TrkB<sup>fl/fl</sup> (referred to as Scn8a<sup>−/−</sup>+, wild-type mice with intact activity-dependent myelination); (2) Scn8a<sup>−/−</sup>;TrkB<sup>fl/fl</sup>;PDGFRα<sup>Cre-ER</sup> (referred to as Scn8a<sup>−/−</sup>+, OPC conditional knockout (CKO), wild-type mice with impaired activity-dependent myelination); (3) Scn8a<sup>−/−</sup>;TrkB<sup>fl/fl</sup> (referred to as Scn8a<sup>−/−</sup>−, mice with absence seizures and intact activity-dependent myelination); and (4) Scn8α<sup>−/−</sup>;TrkB<sup>fl/fl</sup>;PDGFRα<sup>Cre-ER</sup> (Scn8α<sup>−/−</sup>−, OPC CKO, mice with absence seizures and impaired activity-dependent myelination). All mice were treated with tamoxifen to ensure that any differences between genotype groups do not reflect differences in tamoxifen treatment. After tamoxifen treatment, mice were implanted for EEG to monitor seizures.

The original Scn8a<sup>−/−</sup> mouse line is on a C3HeB/FeJ background, whereas Scn8α<sup>−/−</sup>;TrkB<sup>fl/fl</sup> mice have a mixed C3HeB/FeJ and C57BL6 background. Background strain can influence the age of seizure onset and progression<sup>37</sup>. Therefore, we determined the timeline of epileptogenesis in Scn8α<sup>−/−</sup> mice with this mixed background. In Scn8α<sup>−/−</sup> mice (mixed background) with intact activity-dependent myelination, 4–8 Hz absence seizures begin around P90. Seizures then increase steadily and occur 20–30 times per hour, on average, by 6 months of age (Extended Data Fig. 8).

We next confirmed that deletion of the TrkB receptor from OPCs prevents the myelination response to seizures. As expected, deletion of the TrkB receptor from OPCs in Scn8α<sup>−/−</sup>;TrkB<sup>fl/fl</sup>;PDGFRα<sup>Cre</sup> mice (Scn8α<sup>−/−</sup>−, OPC CKO) prevented the aberrantly increased myelination (decreased g-ratio) observed in Scn8α<sup>−/−</sup> mice (Fig. 4a–c). Differences in g-ratios were not related to changes in axonal diameter (Extended Data Fig. 2d).

Having elucidated the timeline of seizure progression and confirmed that TrkB deletion from OPCs prevents aberrant myelination in association with seizures, we next examined the number of seizures per hour in Scn8α<sup>−/−</sup> mice lacking TrkB expression in OPCs (Scn8α<sup>−/−</sup>−, OPC CKO). We found that seizure burden was strikingly reduced in Scn8α<sup>−/−</sup> OPC CKO mice with impaired activity-dependent myelination. Scn8α<sup>−/−</sup>− mice with intact activity-regulated myelination exhibit a marked increase in the number of seizures per hour over time (Fig. 4d–f). In contrast,
Myelin plasticity promotes ictal synchrony. Mechanisms of seizure genesis are thought to originate in intrinsic neuronal properties, independent from myelination\(^n\),\(^3\), raising the question of how changes in myelination might contribute to seizure burden. We hypothesized that aberrant myelination might promote seizure progression by further facilitating ictal synchrony once the thalamocortical network has already become prone to seizures. To test this idea, we assessed inter-hemispheric somatosensory cortical ictal coherence with EEG in 6-month-old \(\text{Scn8a}^{+/+}\) and \(\text{Scn8a}^{-/+}\) OPC cKO mice, focusing on theta frequency activity (4–8 Hz). We found that ictal EEG coherence between the somatosensory cortices was substantially diminished in \(\text{Scn8a}^{-/+}\) OPC cKO mice that lack activity-dependent myelination, compared to \(\text{Scn8a}^{+/+}\) mice (Fig. 4g–i). By contrast, there was no difference in ictal coherence in the bilateral visual cortices (Extended Data Fig. 10), where seizure activity is minimal and where myelin changes were not observed in Wag/Rij rats (Extended Data Fig. 5).

Blockade of myelin plasticity abrogates epilepsy progression. To further assess the role of activity-dependent myelination in seizure progression, and to assess the potential for targeting maladaptive myelination in the treatment of absence seizures, we next pharmacologically blocked activity-dependent myelination in \(\text{Scn8a}^{-/+}\) mice on the C3HHeB/FJ background (with seizure onset at P21), to assess the effect on seizure burden. Trichostatin A (TSA) is a histone deacetylase (HDAC) inhibitor that is known to prevent activity-regulated myelination exhibit substantially fewer seizures (4-month time point: \(\text{Scn8a}^{-/+}\) mice, \(n=6\) mice, \(P=0.0042\)). The mean duration of individual seizures was substantially diminished in \(\text{Scn8a}^{-/+}\) OPC cKO mice, focusing on theta frequency activity (4–8 Hz). We found that ictal EEG coherence between the somatosensory cortices was substantially diminished in \(\text{Scn8a}^{-/+}\) OPC cKO mice that lack activity-dependent myelination, compared to \(\text{Scn8a}^{+/+}\) mice (Fig. 4g–i). By contrast, there was no difference in ictal coherence in the bilateral visual cortices (Extended Data Fig. 10), where seizure activity is minimal and where myelin changes were not observed in Wag/Rij rats (Extended Data Fig. 5).

Fig. 4 | Activity-dependent myelination contributes to generalized epilepsy progression. a, Representative transmission electron micrographs from corpus callosum body of 6-month-old mice. Scale bar, 2 \(\mu\text{m}\). b, g-ratios from 6-month-old \(\text{Scn8a}^{-/+}\) and \(\text{Scn8a}^{+/+}\) OPC cKO mice. Each dot represents the g-ratio for one axon. c, Mean g-ratios for each mouse. \(\text{Scn8a}^{+/+}\), \(n=4\) mice; \(\text{Scn8a}^{-/+}\) OPC cKO, \(n=4\) mice; \(\text{Scn8a}^{+/+}\) OPC cKO, \(n=4\) mice; \(\text{Scn8a}^{-/+}\) OPC cKO, \(n=4\) mice. One-way ANOVA, \(F_{1,11}=8.753\), \(P=0.0024\). Post hoc Tukey’s test: \(\text{Scn8a}^{-/+}\) versus \(\text{Scn8a}^{+/+}\), \(P=0.0085\); \(\text{Scn8a}^{-/+}\) versus \(\text{Scn8a}^{+/+}\), \(P=0.0042\). \(\text{Scn8a}^{-/+}\) OPC cKO versus \(\text{Scn8a}^{+/+}\) versus \(\text{Scn8a}^{-/+}\), \(P=0.99\). \(\text{Scn8a}^{-/+}\) OPC cKO versus \(\text{Scn8a}^{+/+}\) OPC cKO, \(P=0.99\). \(\text{Scn8a}^{-/+}\) OPC cKO, \(P=0.0066\). \(\text{Scn8a}^{+/+}\) OPC cKO, \(P=0.0075\). d, Representative seizure in a \(\text{Scn8a}^{-/+}\) mouse. e, Continuous EEG recordings showing decreased incidence of seizures (arrowheads) in \(\text{Scn8a}^{-/+}\) OPC cKO mice at 6 months. f, Mean seizures per hour for each mouse. 3 months: \(\text{Scn8a}^{+/+}\), \(n=3\) mice; \(\text{Scn8a}^{-/+}\) OPC cKO, \(n=8\) mice; \(\text{Scn8a}^{+/+}\) OPC cKO, \(n=7\) mice. One-way ANOVA: \(F_{1,11}=5.814\), \(P=0.0063\). Post hoc Tukey’s test: \(\text{Scn8a}^{-/+}\) versus \(\text{Scn8a}^{+/+}\), \(P=0.045\). \(\text{Scn8a}^{-/+}\) OPC cKO versus \(\text{Scn8a}^{+/+}\) OPC cKO, \(P=0.6\). 4 months: \(\text{Scn8a}^{+/+}\), \(n=5\) mice; \(\text{Scn8a}^{-/+}\) OPC cKO, \(n=6\) mice; \(\text{Scn8a}^{+/+}\) OPC cKO, \(n=4\) mice. One-way ANOVA: \(F_{1,11}=23.05\), \(P=0.0001\). Tukey’s test: \(\text{Scn8a}^{-/+}\) versus \(\text{Scn8a}^{+/+}\), \(P<0.0001\). g, Schematic of recording electrodes over somatosensory cortices, created with BioRender. h, Representative seizure from a 6-month-old \(\text{Scn8a}^{-/+}\) mouse with coherence plot. i, Ictal theta band coherence. \(\text{Scn8a}^{-/+}\), \(n=6\) mice, \(\text{Scn8a}^{+/+}\) OPC cKO, \(n=7\) mice. Two-tailed t-test: \(P=0.047\). Each dot represents the mean for one animal (c, f, i), with group means ± s.e.m. \(\text{Scn8a}^{-/+}\), black dots; \(\text{Scn8a}^{+/+}\) OPC cKO, gray dots; \(\text{Scn8a}^{-/+}\), red dots; \(\text{Scn8a}^{+/+}\) OPC cKO, blue dots. * \(P<0.05\), ** \(P<0.01\), *** \(P<0.001\), NS, non-significant (\(P>0.05\)).
models, such that early blockade of absence seizures and/or their downstream effects mitigates morbidity\textsuperscript{29,40}. Thus, improved understanding of mechanisms underlying seizure progression could enable discovery of disease-modifying and/or curative treatments for generalized and other forms of epilepsy.

The findings presented here indicate that maladaptive myelination is a consequence of seizures and/or related changes in neural activity and does not precede the onset of recurrent seizures in the models that we studied, underscoring the primary role of neurons in epilepsy onset\textsuperscript{16,32}. Our findings contrast with, but do not exclude,
the possibility that, in some cases, developmental myelin differences preceding seizures, including within the corpus callosum, could also influence seizure severity. Both Wag/Rij rats and Scn8a/mut mice exhibit increased myelin sheath thickness (decreased g-ratio), whereas Scn8a/mut mice also exhibit an increase in myelinated axons, indicative of de novo myelination. These differences may be related to species and/or age of onset of seizures, which is ~2 months in Wag/Rij rats and ~P21 in Scn8a/mut mice. The observed increases in callosal myelin sheath thickness were subtle, but this magnitude of change could substantially modulate network function given the dynamic range of g-ratios. Small changes within this range can meaningfully modulate neuronal network function and behavior. Whether the change in myelinated axon number reflects de novo myelination of previously unmyelinated axons or discontinuously myelinated axon segments, and whether thicker myelin sheaths reflect newly generated internodes or activity-regulated remodeling by existing oligodendrocytes, remain to be determined. Furthermore, it will be important to determine whether myelin internode length changes in association with seizures using models amenable to such measurements. The effects of seizure-associated myelination on inhibitory interneurons and other neuronal subtypes remain to be explored.

The observed myelination-dependent increase in icat somatosensory EEG coherence suggests that aberrantly increased myelination within the thalamocortical network may increase the potential for highly synchronous activity that underlies absence seizures, thus enabling more frequent transition of the network to the seizure state. Activity-regulated myelination may also contribute to epilepsy by influencing spike timing-dependent synaptic plasticity, temporal dynamics involving interneuron function and neuronal excitability, and/or by serving as a compensatory mechanism that provides metabolic support to enable rapid firing during seizures.
Notably, HDAC inhibition has been shown to promote synaptic plasticity⁶⁶; therefore, the observed effects of TSA treatment are unlikely to be explained by impaired synaptic plasticity. HDAC inhibition has also been shown to improve the course of absence epilepsy when initiated before seizure onset in Wag/Rij rats⁶⁶, although the link to oligodendrogenesis has not been previously appreciated.

Our studies also suggest a link between BDNF signaling in OPCs and aberrant myelination that promotes seizures. The role of BDNF signaling in epilepsy is complex (Supplementary Note 1). An important consideration for our study is that BDNF to TrkB signaling was prevented specifically in OPCs (in Scn8a<sup>−/−</sup> OPC cKO mice). Thus, we did not determine the effect of BDNF signaling blockade across all cell types (such as neurons) on seizures, nor do we conclude that BDNF antagonism would be a useful therapeutic for the treatment of absence seizures. Future work should explore whether additional molecular pathways that link neurons, oligodendrogenesis and myelination are involved in epilepsy (Supplementary Note 1).

The broader implications of these findings in rodent models to generalized epilepsy in humans remain to be fully elucidated, and several open questions remain for future study (Supplementary Note 2). Given the many mechanisms occurring in different human forms of epilepsy (including differences in age at onset, seizure location, seizure severity, etiology and associated neuro-inflammation), it is likely that the extent and role of myelin plasticity also varies between different types of epilepsy.

Mounting evidence suggests that a range of aberrant patterns of myelination may predispose brain networks toward seizures, through multiple mechanisms. Our findings in models of generalized epilepsy indicate that activity-regulated myelination, previously described in the setting of neural network adaptation related to learning, can also reinforce deleterious patterns of neural activity. Therefore, myelin plasticity may become maladaptive in some contexts. More myelin is not necessarily better, and seizure-related plasticity that increases myelination of axons beyond a normal optimum could disrupt the normal function, for example by interfering with precise oscillatory synchrony between brain regions that supports cognition⁶²–⁶⁴. This raises a key question about how oligodendrocyte lineage cells may sense and integrate circuit-level information to fine-tune circuit dynamics in an optimal manner in health. Further study of maladaptive myelination in disease contexts may elucidate novel strategies to treat neurological diseases, such as epilepsy, while also providing greater insight into mechanisms of myelin plasticity that promote function in the healthy brain.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-022-01052-2.

Received: 10 May 2021; Accepted: 14 March 2022; Published online: 2 May 2022

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Methods

Rodent colony maintenance. All experiments were conducted in accordance with protocols approved by the Stanford University Institutional Animal Care and Use Committee (protocols 27215, 12363 and 33969). Mice or rats were group or single housed to five mice or two rats per cage according to standard guidelines, with ad libitum access to food and water on a 12-hour light/dark cycle. In rooms where mice and rats were housed, the ambient temperature was 70 ± 2 °F, and the relative humidity was 30–70%. No animals were manipulated other than for the experiment—there is no history of drug exposures, surgeries or behavioral testing for the animals used other than that reported for the given experimental group. Mice and rats were healthy and tolerated all experimental manipulations well.

The ages of all mice and rats used in specific studies are indicated in the figures and throughout the text. In brief, in studies using Wag/Rij and control rats, 1.5-month-old animals were used to assess endpoints before seizure onset, and 6–7-month-old animals were used to assess endpoints after seizures are well-established. In studies using Scn8a+/− mice and wild-type littermates, P21 mice were used to assess endpoints before seizure onset, whereas P45 mice were used to assess endpoints after seizures are well-established. In studies in which Scn8a+/− and other mouse lines were bred (Fig. 1), because seizure onset is delayed, later time points (3–6 months) were used to study seizure progression, as described in detail in the text and methods below.

Both males and females (rats and mice) were used in equal numbers whenever possible. The exact numbers of male and female rats and mice are listed in the source files. There was no discernable effect of male or female sex upon any of the endpoints, aside from body weight. For most experiments, individual animals used came from at least 2–3 distinct litters.

Wag/Rij rats. Wistar (control) and Wag/Rij rats were purchased from Charles River Laboratories (Wistar: cat. no. 005; Wag/Rij, Charles Rivers Italy: strain code 638). A colony of Wag/Rij mice has subsequently been maintained in the laboratory of J.R.H. at Stanford50.

Scn8a+/− and Scn8a+/− mice were treated with the HDAC inhibitor TSA or vehicle on a previously published dosing schedule shown to prevent activity-dependent myelination in mice1. Male and female littermates were randomly assigned to either vehicle or TSA treatment with the following conditions: 30% PEG300 (Selleck Chemicals, S6704) and 2% Tween 80 (Sigma-Aldrich, P21754) in sterile-filtered DMSO (Tocris, 3176) just before each administration. Then, 10 mg/kg of TSA or vehicle was administered each day by intraperitoneal injection between P28 (starting after the first EEG recording on the same date) and P45. Mice were weighed every 1–2 days, and their health was monitored throughout the study. We did not observe any deleterious effects of TSA treatment.

EEG. Mice and rats were stereotactically implanted with wires (A-M Systems, 78570) to screws (J.H. Morris, F850CE125) over the left and right somatosensory cortices as well as a reference wire over cerebellum, and implants were secured with dental cement (Metabond, S399, S371 and S398; also Jet Set4 Liquid, Long Dental, 380226). The following stereotactic coordinates were used, relative to bregma: primary somatosensory cortex (SIbF), AP −1.3 mm and lateral 3.3 mm; primary visual cortex (V1), AP −3.3 mm, lateral 2.5 mm. Implanted wires were integrated into custom-made Mill-Max headpieces (Digi-Key Electronics, ED90267–ND) that could be connected to a head stage, consisting of a digitizer and amplifier board (Intan Technologies, C3334). Awake and freely behaving animals were tethered to an acquisition board (Open Ephys) with lightweight SPI interface cables (Intan Technologies, C3206). Continuous real-time EEG was recorded with Open Ephys software (https://open-ephys.org, version 0.14.7.1. Data were sampled at 2 kHz and bandpass filtered between 1 Hz and 300 Hz. All animals underwent 3–4 hours of continuous EEG recording between the hours of 10:00 and 18:00. Longitudinal data were collected whenever possible (for example, when not precluded by EEG implant loss or failure).

Immunohistochemistry. Rats were given a lethal dose of Fatal Plus (sodium pentobarbital, Vortech Pharmaceuticals, NDC: 0298–9373–68) and transcardially perfused with 40–80 ml of ice-cold 0.1 M PBS, followed by 40–80 ml of 4% paraformaldehyde (PFA) in PBS before brain extraction and tissue processing. Mice were given a lethal dose of Fatal Plus or 2.5% Avertin (2,2,2-tribromoethanol, Sigma–Aldrich, T4402) and transcardially perfused with 10–20 ml of ice-cold PBS, followed by 10–20 ml of 4% PFA in PBS. In TSA studies, brains were bisected in the sagittal plane at the inter-hemispheric fissure. Brains or hemispheres were post-fixed in 4% PFA overnight at 4 °C before transfer to 30% sucrose for cryoprotection for several days. Cryoprotected brain samples were embedded in Tissue-Tek OCT compound (Sakura, 4583) and sectioned in the coronal plane at 50 μm using a sliding microtome (Microm HM450, Thermo Fisher Scientific). TUNEL assays were performed using the Click-IT Plus TUNEL Assay Kit from Thermo Fisher Scientific (C10617). For immunohistochemistry, sectioned coronal sections were rinsed three times in TBS and incubated in blocking solution (3% normal donkey serum, Jackson Immunoresearch, AB2337258; 0.3% Triton X–100 in TBS) at room temperature for 30 minutes. Rabbit anti-Olig2 (1:400, Millipore, AB9610), goat anti-PDGFRα (1:200, R&D Systems, AF3141), mouse anti-APC (CC1, 1:50, Calbiochem, OP89) and rat anti-iKita (1:200, Life Technologies, 14-5698-82) were diluted in staining solution (1% normal donkey serum, 0.3% Triton X–100 in TBS) and incubated with sections at room temperature for 2.5 days; when staining for CC1, sections were incubated at 4 °C for 10 days. In other studies, sections were incubated with rabbit anti-SOX9 (1:500, Abcam, Ab185966), mouse anti-α-GEAP (1:200, Thermo Fisher Scientific, 14-9882–92), rabbit anti-iBa1 (1:1,000, Wako, 019-17941) and/or rabbit anti-CD68 (1:200, Abcam, AB53444) overnight at 4 °C. Sections were then rinsed three times in TBS and incubated in secondary antibody solution containing Alexa Fluor 488 donkey anti-rabbit IgG (1:500, Jackson Immunoresearch, 711-545-152), Alexa Fluor 647 donkey anti-goat IgG (1:500, Jackson Immunoresearch, A21417), Alexa Fluor 594 donkey anti-mouse IgG (1:500, Jackson Immunoresearch, 715-585-150), Alexa Fluor 647 donkey anti-mouse IgG (1:500, Jackson Immunoresearch, 715-605–150), Alexa Fluor 594 donkey anti-rat IgG (1:500, Jackson Immunoresearch, 715-585–150) and/or Hoechst 33342 (1:2,000, Life Technologies, H3459) in staining solution at 4 °C for 12 hours or overnight. Sections were rinsed three times in TBS and mounted with ProLong Gold Mounting Medium (Life Technologies, P36930).

ETX treatment. Wistar (control) and Wag/Rij rats were treated with ETX or vehicle on a previously published dosing schedule shown to prevent or and/or substantially reduce seizures. Male and female littersmates were randomly assigned to either vehicle or ETX treatment. Pharmaceutical-grade ETX solution (Akorn Pharmaceuticals, 230 mg/ml), NDC: 61748-024-16) was added to drinking water in tubes that were shielded from light at a concentration of 2.5 mg/ml, leading to approximate dosing of 300 mg/kg/day. The solution was changed at least every 7 days, and the health, weight and drinking of animals were monitored regularly throughout the study. Before perfusion, 1–2 ml of blood was taken. The samples were centrifuged at 2,000g, 4 °C, for 5 minutes, and plasma was collected for the measurement of ETX concentration using liquid chromatography–mass spectroscopy, performed at the Stanford BioADD center (http://med.stanford.edu/bioad.html).

TSA treatment. Scn8a+/− and Scn8a+/− mice were treated with the HDAC inhibitor TSA or vehicle on a previously published dosing schedule shown to prevent activity-dependent myelination in mice1. The polymerase chain reaction (PCR) protocol was as follows: F: ATGTCGCCCTGGCTGAAGTG; TrkB + Cre TTA · GC. The master mix of REDEX reagent, primers as indicated below, water and extracted brain tissue was adjusted to a final volume of 25 μl and incubated at 94 °C for 5 minutes, followed by 30 seconds at 94 °C, 30 seconds at 65 °C for generating a 1.4% agarose gel, and a band size of 450 base pairs (bp) indicated the presence of the floxed allele. For detection of the Cre, the following primers (IDT) were used: Cre Up: GTA GAT TCC TAT GCA AAC TCC AGC; Cre Down: GCT AAA CAT GCT TCA TCG TCG C; PCR protocol was 94 °C for 5 minutes × 1; then 94 °C for 1 minute, 65 °C for 1.5 minutes and 72 °C for 1.5 minutes, all × 40 cycles; then 72 °C for 7 minutes × 1; then held at 12 °C until used in gel electrophoresis. The PCR products were run on a 1.8% agarose gel, and a band size of 450 base pairs (bp) indicated the presence of the floxed allele. For detection of Cre, the following primers (IDT) were used: Cre Up: GTT CTG CCA TGT TGA AAC TCC AGC; Cre Down: GCT AAA CAT GCT TCA TCG TCG C; PCR protocol was 94 °C for 5 minutes × 1; 94 °C × 15 seconds, 65 °C × 30 seconds × 30 cycles; then 72 °C × 1 minute × 30 cycles; then 94 °C × 15 seconds, 55 °C × 30 seconds, 72 °C × 1 minute × 2 cycles; then 72 °C × 1 minute × 7 cycles; then held at 4 °C until used in gel electrophoresis. The PCR products were run on a 1.8% agarose gel indicated an animal positive for the Cre gene. Automated genotyping for the Scn8a ‘med’ allele was done using tail samples via Transnetyx (www.transnetyx.com).
Confocal microscopy. Representative images shown in Figs. 1 and 3 were taken on a Zeiss LSM800 scanning confocal microscope at ×63 magnification, using ZEN software (version 2.1). Z-stack images for counting microglia, astrocytes and TUNEL cells were taken on a Zeiss LSM700 scanning confocal microscope at ×20, and CD68⁺ microglia were imaged at ×40 magnification. For measurement of glial fibrillary acidic protein (GFAP) area, images were taken in the corpus callosum using a Zeiss LSM700 confocal microscope at ×40 magnification, using ZEN software (version 2.3). Fluorescent signal was enhanced uniformly in the representative merged images to enable visualization of all fluorophores and their co-localization.

Electron microscopy. Rats were given a lethal dose of Fatal Plus and transcardially perfused with 40–80 ml of ice-cold 0.1 M PBS, followed by 40–80 ml of Karnovsky fixative, consisting of 4% PFA (Electron Microscopy Services (EMS), 15700) and 2% glutaraldehyde (EMS, 16000) in 0.1 M sodium cacodylate buffer (EMS, 12330) in PBS before brain extraction and tissue processing. Mice were given a lethal dose of Fatal Plus or Avertin and transcardially perfused with 10–20 ml of ice-cold PBS followed by 10–20 ml of Karnovsky fixative. Samples were post-fixed in Karnovsky fixative for at least 2 weeks. A ~1 mm² block of tissue was dissected from the midline sagittal corpus callosum body, at the rostrocaudal location overlying the dentate gyrus and the hippocampal fornix, enabling cross-sectional views of callosal projection axons. Another ~1 mm² block was taken from the corpus callosum splenium. The dissected block was processed for transmission electron microscopy as described previously. In brief, tissue was post-fixed in 1% osmium tetroxide (EMS, 19100) for 1 hour at room temperature, washed three times in ultra-filtered water, en bloc stained for 2 hours at room temperature before dehydration in gradient ethanols and then rinsed in 100% ethanol twice, followed by acetonitrile (Thermo Fisher Scientific, A21-1). Samples were then embedded in 1:1 EMbed-812 (EMS, 14120):acetonitrile, followed by EMbed-812 for 2 hours and then placed into TAAB capsules filled with fresh resin before incubation in a 65 °C oven overnight. Next, 75–90 nm sections from this block were mounted on formvar/carbon-coated slot grids and contrast stained for 30 seconds in 3.5% uranyl acetate in 50% acetone (EMS, 10015), followed by 0.2% lead citrate (EMS, 0378) for 30 seconds. Samples were imaged with a JEOL JEM-1400 transmission electron microscope at 120 kV, and images were collected with a Gatan Orius digital camera.

Quantification of TUNEL, microglia and astrocytes. Fiji software (https://imagej.net/software/fiji, version 2.1.0) was used to quantify cell number per callosal volume (TUNEL, microglia and astrocyte counts). ImageJ software (version 2.0) was used to quantify the area occupied by GFAP in volume (TUNEL, microglia and astrocyte counts). ImageJ software (version 2.0) was used to quantify the area occupied by GFAP in volume (TUNEL, microglia and astrocyte counts). ImageJ software (version 2.0) was used to quantify the area occupied by GFAP in volume (TUNEL, microglia and astrocyte counts). ImageJ software (version 2.0) was used to quantify the area occupied by GFAP in volume (TUNEL, microglia and astrocyte counts).

Unbiased stereology. OPCs and oligodendrocytes were visualized with an MRF Zeiss Axiocam light microscope. Cell numbers were determined through unbiased stereology using Stereo Investigator software (MBF Bioscience, versions 13.1.3.6 and 13.2.0.9). A representative area was defined within each ROI and for each genotype, with a minimum of 80 regions counted per genotype. The percentage of myelinated axons was calculated as 100 × (myelinated / (myelinated + unmyelinated) axons). Total (myelinated + unmyelinated) axon number was determined by quantifying the average number of axons per ×4000 electron micrograph. Rat g-ratios: In experiments comparing myelination in 1.5- and 6-month-old rats, for each rat, 195–264 axons were measured from 8–18 electron micrographs (Fig. 1). In studies involving conditional knockout of TrkB from OPCs in Scn8a+/- mice and for those studies involving conditional knockout of TrkB from OPCs in Wag/Rij rats, similarly to other investigators29,55. Seizures were associated with behavioral arrest, and the corresponding EEG demonstrated predominantly 4–8 Hz frequency spike-wave morphology, amplitude ~1.5–2 times that of the background and duration of more than 1 sec.56,57 Coherence was calculated using an adapted version of MATLAB coherence software (https://www.mathworks.com/help/wavelet/ref/wcoherence.html). The custom software used for quantitative analysis of EEG is available on GitHub: https://github.com/huguenardlab/EEG.

EEG analysis. EEG data acquired with Open Ephys software were displayed and seizures from 3–4 hours of EEG recording were visually identified, marked and subsequently used by a blinded reviewer using custom MATLAB code. For many rats, the axonal diameter in its short axis divided by the diameter of the entire fiber in the same axis (axonal diameter / axonal diameter + myelin sheath), using ImageJ software (https://imagej.nih.gov/ij/) to measure the g-ratio. Myelinated and unmyelinated axons were quantified from ×4000 transmission electron micrographs. The percentage of myelinated axons was calculated as 100 × (myelinated / (myelinated + unmyelinated) axons). Total (myelinated + unmyelinated) axon number was determined by quantifying the average number of axons per ×4000 electron micrograph.

Data Fig. 2b); mice were treated with TSA (Fig. 5, and Extended Data Fig. 9b); for those studies involving conditional knockout of TrkB from OPCs in Scn8a+/- mice and Wag/Rij rats, similarly to other investigators29,55. Seizures were associated with behavioral arrest, and the corresponding EEG demonstrated predominantly 4–8 Hz frequency spike-wave morphology, amplitude ~1.5–2 times that of the background and duration of more than 1 sec.56,57 Coherence was calculated using an adapted version of MATLAB coherence software (https://www.mathworks.com/help/wavelet/ref/wcoherence.html). The custom software used for quantitative analysis of EEG is available on GitHub: https://github.com/huguenardlab/EEG.

Statistical analysis. All data collection and analysis were performed by experimenters blinded to animal identity and experimental condition. Full details of statistical analyses can be found in the figure legends. For all studies, n refers to the number of mice or rats included in each experimental group, and, unless indicated otherwise (for example, g-ratio scatter plots), each data point in a graph represents the mean from one mouse or one rat. For all studies, n = 3 or more mice or rats per group, with the exact n specified in the figure legends. Sample sizes were based on the variance of data in pilot experiments and were generally estimated by power calculations that determined the number of animals needed for 80% power to detect a 20–30% difference between genotypes. GraphPad Prism software (versions 8 and 9) was used to perform statistical analyses. Statistical significance was defined as P < 0.05 throughout. Rarely, data were excluded for the following pre-established reasons: (1) samples incurred damage during histological processing that precluded accurate analysis; (2) EEG recordings were not of sufficient quality to enable accurate interpretation (for example, one channel not working or presence of a major artifact); and (3) statistical outliers, defined as data points >2 standard deviations from the group mean. The Shapiro–Wilk test was used to determine whether data were normally distributed; parametric tests were used where indicated. Non-parametric tests were used for non-parametric datasets. One-way ANOVA followed by Sidak’s post hoc test, correcting for multiple comparisons, was used to compare two groups at specific ages (for example, Figs. 1 and 3 and Extended Data Figs. 1a,b, 2ac, 3, 6ae and 9). When two experimental groups within one time point were compared (Extended Data Figs. 1c, 5c, 6bd, 7 and 10), a two-tailed t-test was used. For experiments in which rats were treated with ETX (Fig. 2 and Extended Data Fig. 2b), mice were treated with TSA (Fig. 5, and Extended Data Fig. 9b); and for those studies involving conditional knockout of TrkB from OPCs in Scn8a+/- mice and Wag/Rij rats, similarly to other investigators29,55. Seizures were associated with behavioral arrest, and the corresponding EEG demonstrated predominantly 4–8 Hz frequency spike-wave morphology, amplitude ~1.5–2 times that of the background and duration of more than 1 sec.56,57 Coherence was calculated using an adapted version of MATLAB coherence software (https://www.mathworks.com/help/wavelet/ref/wcoherence.html). The custom software used for quantitative analysis of EEG is available on GitHub: https://github.com/huguenardlab/EEG.
For experiments in which rats were treated with ETX, or in which mice were treated with TSA, rats or mice within each litter were randomized to treatment groups.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
Raw data are available in the source data files for Figs. 1–5 and Extended Data Figs. 1–10. Source data are provided with this paper.

**Code availability**
Custom MATLAB code used for EEG analyses is available at https://github.com/huguenardlab/EEG.

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**Acknowledgements**
The authors gratefully acknowledge support from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health (NIH) (R01NS092597 to M.M, K12NS08482 and K08NS119800 to J.K.K. and R01NS034774 and R01NS117150 to J.R.H.); the NIH Director's Pioneer Award (DP1NS111132 to M.M.); the Robert J. and Helen C. Kleberg Foundation (to M.M.); the Stanford Maternal and Child Health Research Institute (to M.M. and J.K.K.); the Bio-X Institute (to M.M. and J.K.K.); Cancer Research UK (to M.M.); the American Epilepsy Society (to J.K.K.); the CURE Epilepsy Foundation (to J.K.K) and the Child Neurology Foundation (to J.K.K.).

The authors thank M. Fogerson, J. Sorokin, A. Reese and C. Makinson for their guidance on performing and analyzing rodent EEG. The authors also thank S. Chinn at Stanford Children’s Health for assistance with ETX experiments and A. Geraghty for guidance on techniques related to OPC cKO mice and confocal microscopy.

**Author contributions**
J.K.K. performed experiments and analyzed quantitative microscopy and electrophysiological data. C.S., E.F., L.T.T., D.F., A.B., T.S., H.X. and S.T. performed experiments and assisted with data analysis. L.T.T., A.B., K.V. and S.T. assisted with animal husbandry and drug administration. L.N. performed electron microscopy. J.K.K., M.M. and J.R.H. conceived of the project. J.K.K. and M.M. wrote the manuscript. J.R.H., H.X., A.B., E.F., L.T.T., D.F., T.S. and S.T. edited the manuscript. M.M. and J.R.H. supervised all aspects of the work.

**Competing interests**
M.M. is on the Scientific Advisory Board of Cygnal Therapeutics. The authors declare no other competing interests.

**Additional information**
Extended data is available for this paper at https://doi.org/10.1038/s41593-022-01052-2.

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41593-022-01052-2.

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**Peer review information** *Nature Neuroscience* thanks Klaus-Armin Nave, Robert Wykes and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | Callosal oligodendroglial cell density increases with absence seizures in Wag/Rij rats. (a) Control and Wag/Rij rat body weights. 1.5-month timepoint: control = 7 rats; Wag/Rij = 5 rats. 6-month timepoint: control = 5 rats, Wag/Rij = 5 rats. One-way ANOVA: F(3, 18) = 39.83, p < 0.0001. Sidak’s post-hoc testing: Wag/Rij vs. Wistar at 1.5-months, p = 0.0032; at 6-months, p < 0.0001. (b) Callosal volumes for each rat. 1.5-month timepoint: control = 7 rats; Wag/Rij = 5 rats. 6-month timepoint: control = 5 rats, Wag/Rij = 5 rats. One-way ANOVA: F(3, 18) = 18.28, p < 0.0001. Sidak’s post-hoc testing: Wag/Rij vs Wistar at 6-months: p = 0.0022, at 1.5-months: p = 0.9910. (c) Oligodendrocyte densities for each rat. Control = 3 rats, Wag/Rij = 4 rats. Two-tailed t-test, Wag/Rij vs control: p = 0.0025. Each dot represents the mean for one rat, shown with group means ± SEM; control rats are represented with black dots and Wag/Rij rats are represented with red dots. *p < 0.05, **p < 0.01, ***p < 0.001, n.s. = nonsignificant (p > 0.05).
Extended Data Fig. 2 | Myelinated axon diameters do not contribute to g-ratio differences in Wag/Rij rats and Scn8a\(^{+/+}\) mice. (a) Mean diameters of myelinated axons for each rat. 1.5-month timepoint, n=4 control (black dots), 3 Wag/Rij (red dots); 6-month timepoint, n=4 control, 4 Wag/Rij rats. One-way ANOVA: F(3,11)=1.065, p=0.4033. (b) Myelinated axon diameter in the body of the corpus callosum at 7-months of age in control (black dots) and Wag/Rij (red dots) rats treated with vehicle (VEH) or ethosuximide (ETX). (Control-VEH, n=4 rats; Control-ETX, n=3 rats; Wag/Rij-VEH, n=3 rats; Wag/Rij-ETX, n=3 rats). One-way ANOVA: F(3,9)=0.9890, p=0.44. (c) Mean myelinated axon diameters for Scn8a\(^{+/+}\) (black dots) and Scn8a\(^{+/+}\)mut (red dots) mice. P21, Scn8a\(^{+/+}\) n=4 mice; Scn8a\(^{+/+}\)mut n=3 mice. P45, Scn8a\(^{+/+}\) n=4 mice; Scn8a\(^{+/+}\)mut n=4 mice. One-way ANOVA: F(3,11)=2.822, p=0.088. (d) Myelinated axon diameters in the body of the corpus callosum in 6-month-old Scn8a\(^{+/+}\) (black dots, n=4 mice), Scn8a\(^{+/+}\)OPC cKO (gray dots, n=4 mice), Scn8a\(^{+/+}\)mut (red dots, n=4 mice) and Scn8a\(^{+/+}\)mut OPC cKO mice (blue dots, n=4 mice). One-way ANOVA: F(3,12)=1.324, p=0.31. Each dot represents the mean for one rat or mouse, shown with group means ± SEM. *p<0.05, **p<0.01, ***p<0.001, n.s.=nonsignificant (p>0.05).
Extended Data Fig. 3 | Percent axonal myelination and total axon number are similar in control and Wag/Rij rats. (a) Total number of axons per electron micrograph (field), for each rat (1.5-month timepoint: control=3 rats; Wag/Rij=3 rats. 6-month timepoint: control=3 rats, Wag/Rij=3 rats). One-way ANOVA: F(3, 8)=1.302, p=0.3388. (b) Percent of total callosal axons (myelinated + unmyelinated) that are myelinated in control and Wag/Rij rats. 1.5-month timepoint: control=3 rats; Wag/Rij=3 rats. 6-month timepoint: control=3 rats, Wag/Rij=3 rats. One-way ANOVA: F(3, 8)=3.337, p=0.0768. Sidak’s post-hoc test, percent myelination in Wag/Rij vs control rats at 1.5-months (p=0.8764), and at 6-months (p=0.9283). Each dot represents the mean for one rat, shown with group means ± SEM; control rats are represented with black dots and Wag/Rij rats are represented with red dots. *p<0.05, **p<0.01, ***p<0.001, n.s.=nonsignificant (p>0.05).
**Extended Data Fig. 4 | Increased myelin sheath thickness across axon diameters in Wag/Rij rats and Scn8a\textsuperscript{+/+} mice.** (a) Relative frequency of myelinated axons across axon diameters in 6-month old Wag/Rij (red dots) and control rats (black dots). N=4 control rats and 4 Wag/Rij rats. One-way ANOVA: F(7,24)=3.566, p = 0.009. Post-hoc Sidak’s testing, control vs. Wag/Rij: 201-400 nm, p=0.88; 401-600 nm, p=0.99; 601-800 nm, p=0.99; >801 nm, p=0.27. (b) g-ratios in axon populations sorted by diameter. N=4 control rats and 4 Wag/Rij rats. One-way ANOVA: F(7,24)=36.79, p<0.0001. Sidak’s testing, control vs. Wag/Rij: 201-400 nm, p=0.0186; 401-600 nm, p=0.0194; 601-800 nm, p=0.0759; >801 nm, p=0.0095. (c) Relative frequency of myelinated axons across axon diameters in P45 Scn8a\textsuperscript{+/+} mice (black dots) compared to Scn8a\textsuperscript{+/-} mice (red dots). N=4 Scn8a\textsuperscript{+/-} and 4 Scn8a\textsuperscript{+/+} mice. One-way ANOVA: F(7,24)=8.067, p<0.0001. Post-hoc analysis with Sidak’s testing, Scn8a\textsuperscript{+/-} vs. Scn8a\textsuperscript{+/+} mice: 201-400 nm, p=0.9971; 401-600 nm, p=0.9988; 601-800 nm, p=0.9990; >801 nm, p=0.9954. (d) g-ratios from Scn8a\textsuperscript{+/-} and Scn8a\textsuperscript{+/+} mice, sorted by axon diameter (N=4 Scn8a\textsuperscript{+/-} and 4 Scn8a\textsuperscript{+/+} mice). One-way ANOVA: F(7,24)=126.2, p<0.0001. Sidak’s test, Scn8a\textsuperscript{+/-} vs. Scn8a\textsuperscript{+/+} g-ratios: 201-400 nm, p=0.0509; 401-600 nm, p=0.0003; 601-800 nm, p=0.0059; >801 nm, p=0.0013. Each dot represents the mean for one rat or mouse, shown with group means ± SEM. *p<0.05, **p<0.01, ***p<0.001, n.s.=nonsignificant (p>0.05).
Extended Data Fig. 5 | Increased myelination is specific to the absence seizure network. (a) Representative transmission electron micrographs showing similar appearing myelinated axons in the splenium of a 6-month-old control rat (upper panel) and a Wag/Rij rat (lower panel). Scale bar=2 μm. (b) Scatterplot of g-ratios showing overlap between control and Wag/Rij rats’ g-ratios. Each data point represents one axon’s g-ratio, with control g-ratios in black and Wag/Rij g-ratios in red. n=3 control, 3 Wag/Rij rats. (c) Each dot represents the mean g-ratio for one rat. n=3 control, 3 Wag/Rij rats. Data are shown with group means ± SEM and were analyzed with a two-tailed t-test (n.s., non-significant, p=0.27).
**Extended Data Fig. 6** | Increased callosal oligodendroglial cell density and equivalent callosal axon density in Scn8a<sup>−/−</sup> mice. (a) Callosal volumes for Scn8a<sup>+/+</sup> and Scn8a<sup>−/−</sup> mice. P21: Scn8a<sup>+/+;</sup> Scn8a<sup>−/−</sup> = 4 mice; Scn8a<sup>+/+;</sup> Scn8a<sup>−/−</sup> = 4 mice. P45: Scn8a<sup>+/+;</sup> Scn8a<sup>−/−</sup> = 8 mice, Scn8a<sup>+/+;</sup> Scn8a<sup>−/−</sup> = 8 mice. One-way ANOVA: F(3, 20) = 8.443; p = 0.0008. Sidak’s post-hoc test, Scn8a<sup>+/+;</sup> Scn8a<sup>−/−</sup> vs Scn8a<sup>+/+;</sup> Scn8a<sup>−/−</sup> at P45, p = 0.0027. At P21, p = 0.94. (b) Oligodendrocytes in the body of the corpus callosum in P45 mice, normalized to callosal volume (Scn8a<sup>+/+;</sup> n = 8 mice; Scn8a<sup>−/−;</sup> n = 6 mice). Two-tailed t-test, p = 0.017. Apoptotic OPCs (c) and apoptotic mature oligodendrocytes (d) in P45 Scn8a<sup>+/+;</sup> and Scn8a<sup>−/−;</sup> were labeled with TUNEL staining. (c) Scn8a<sup>+/+;</sup> n = 4 mice, Scn8a<sup>−/−;</sup> n = 4 mice, two-tailed t-test: p = 0.3471. (d) Scn8a<sup>+/+;</sup> n = 4 mice, Scn8a<sup>−/−;</sup> n = 4 mice, two-tailed t-test: p = 0.3625. (e) Total number of callosal axons (myelinated and unmyelinated) per electron micrograph (field) in Scn8a<sup>+/+;</sup> and Scn8a<sup>−/−;</sup> mice. P21: Scn8a<sup>+/+;</sup> Scn8a<sup>−/−;</sup> = 3 mice; Scn8a<sup>−/−;</sup> = 3 mice. P45: Scn8a<sup>+/+;</sup> Scn8a<sup>−/−;</sup> = 4 mice; Scn8a<sup>−/−;</sup> = 4 mice. One-way ANOVA revealed no significant variance in total axon number among the four groups, F(3, 10) = 4.047, p = 0.04. Sidak’s post-hoc test, Scn8a<sup>+/+;</sup> Scn8a<sup>−/−;</sup> vs Scn8a<sup>+/+;</sup> at P45, p = 0.37. At P21, p = 0.13. Scn8a<sup>+/+;</sup> mice are indicated by black dots and Scn8a<sup>−/−;</sup> mice are indicated by red dots. Data are shown with group means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, n.s. = nonsignificant (p > 0.05).
Extended Data Fig. 7 | See next page for caption.
Increased callosal microglial density in Scn8a<sup>+/+<sup> mice. (a) Representative photomicrographs of callosal microglia co-labeled for Iba1 (green), CD68 (red) and nuclear Hoescht staining (blue), from P45 Scn8a<sup>+/+<sup> and Scn8a<sup>+/<sup>mut mice. Scale bars=20 μm. (b) Density of Iba1-expressing microglia for each mouse. Scn8a<sup>+/+<sup> n=4 mice; Scn8a<sup>+/<sup>mut n=4 mice. Data were analyzed with two-tailed t-test, p=0.0035. (c) The percent of Iba1+ microglia co-expressing CD68 appears to be relatively high in C3H/FeJ mice, but was not significantly different in Scn8a<sup>+/+<sup> (n=4 mice) and Scn8a<sup>+/<sup>mut (n=4 mice); two-tailed t-test, p=0.0806. (d) Representative photomicrographs of callosal astrocytes co-labeled for Sox9 (green) and GFAP (red), from P45 Scn8a<sup>+/+<sup> and Scn8a<sup>+/<sup>mut mice. Scale bars=20 μm. (e) Total astrocyte density (GFAP and Sox9- and Sox9-only expressing astrocytes). Scn8a<sup>+/+<sup>, n=3 mice and Scn8a<sup>+/<sup>mut, n=3 mice; two-tailed t-test, p=0.6390. (f) The percentage of GFAP positive astrocytes was equivalent (Scn8a<sup>+/+<sup>, n=3 mice and Scn8a<sup>+/<sup>mut, n=3 mice; two-tailed t-test, p=0.2286). (g) Astrocyte size as assessed by % area occupied by GFAP staining was equivalent between Scn8a<sup>+/+<sup> and Scn8a<sup>+/<sup>mut (Scn8a<sup>+/+<sup>, n=4 mice and Scn8a<sup>+/<sup>mut, n=4 mice; two-tailed t-test, p=0.4844). Each dot represents the mean for one mouse; group means ± SEM are shown. Scn8a<sup>+/+<sup> mice are shown in black and Scn8a<sup>+/<sup>mut are shown in red. *p<0.05, **p<0.01, ***p<0.001, n.s.=nonsignificant (p>0.05).
Extended Data Fig. 8 | Timing and kinetics of seizure onset and epilepsy progression in Scn8a<sup>+/__</sup>mut mice on original (C3HeB/FeJ) and new (C3HeB/FeJ/C57/BL6) genetic backgrounds. To genetically block activity-dependent myelination in Scn8a<sup>+/__</sup>mut mice (originally on a C3HeB/FeJ background), we generated Scn8a<sup>+/__</sup>mut;TrkB<sup>fl/fl;PDGFRα::Cre-ER</sup> mice (on a C3HeB/FeJ and C57/BL6 mixed genetic background). In Scn8a<sup>+/__</sup>mut mice with the new mixed C3HeB/FeJ and C57/BL6 genetic background and intact activity-dependent myelination (Scn8a<sup>+/__</sup>mut, new background), seizure onset and progression occurred at later timepoints (solid red line, P45-P180, 1.5- to 6-months, P45, n = 4 mice, P90, n = 6 mice, P120, n = 6 mice, P180, n = 5 mice). In the original line (Scn8a<sup>+/__</sup>mut, original background), seizures begin at ~P21 and increase until P35-P45 (dashed red line, n = 4 mice per timepoint, data from Makinson et al, Neuron 2017). Graph displays group means with SEM.
Extended Data Fig. 9 | Activity-dependent myelination does not impact seizure duration. (a) Mean seizure duration in Scn8a+/mut (red dots) and Scn8a+/mut OPC cKO mice (blue dots). 3-month-old timepoint: Scn8a+/mut n=7 mice, Scn8a+/mut OPC cKO, n=3 mice. 4-month-old timepoint: Scn8a+/mut, n=6 mice, Scn8a+/mut OPC cKO, n=4 mice. 6-month timepoint: Scn8a+/mut, n=5 mice, Scn8a+/mut OPC cKO, n=4 mice. One-way ANOVA, F(5, 23)=0.7687, p=0.58. (b) Mean seizure duration in Scn8a+/mut treated with vehicle (VEH, red dots) or trichostatin A (TSA, blue dots). P28 timepoint: Scn8a+/mut-VEH n=6 mice, Scn8a+/mut-TSA n=5 mice. P45 timepoint: Scn8a+/mut-VEH n=6 mice, Scn8a+/mut-TSA n=5 mice. Data were analyzed with one-way ANOVA revealing significant variation in seizure duration across groups, F(3,18)=8.062, p=0.0013. However, Sidak’s test comparing seizure duration between groups within each timepoint revealed no significant differences (P28, p=0.57; P45, p=0.90). Each data point represents mean seizure duration for one mouse; data are shown with group means ± SEM. n.s., nonsignificant (p>0.05).
Extended Data Fig. 10 | Blockade of activity-dependent myelination does not alter ictal coherence in visual cortices. In contrast to ictal coherence in the somatosensory cortices (Fig. 4i), ictal theta band coherence when recorded from visual cortices is similar in 6-month-old Scn8a<sup>+/mut</sup> OPC cKO mice compared to Scn8a<sup>+/mut</sup> mice. Scn8a<sup>+/mut</sup> (red dots), n=6 mice, Scn8a<sup>+/mut</sup> OPC cKO (blue dots), n=6 mice. Each dot represents the mean ictal theta coherence from visual cortices for one mouse, shown with group means ± SEM. Two-tailed t-test, p=0.5142. n.s., nonsignificant (p>0.05).
### Reporting Summary

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### Software and code

**Policy information about availability of computer code**

**Data collection**

Representative images and images used to quantify TUNEL positive OPCs, TUNEL positive oligodendrocytes, microglia and astrocytes were collected with a Zeiss confocal microscope, LSM800 or LSM700 model, using Zen 2.1 or 2.3 software, as indicated in the Methods. Fluorescent images used to quantify cells with unbiased stereology were collected with Stereo Investigator software (MBF Bioscience, versions 2017-2020). Continuous real-time EEG was recorded with Open Ephys software (https://open-ephys.org, version 0.4.4.1).

**Data analysis**

Cell counts were performed by unbiased stereology using Stereo Investigator software (MBF Bioscience, versions 2017-2020). ImageJ software (https://imagej.nih.gov/ij/, versions 1.53a - 2.0) and Fiji software (imagej.net/software/fiji/, version 2.1.0) were used to quantify g-ratios from transmission electron micrographs and cell counts where indicated in the Methods. Unmyelinated axons and myelinated axons were counted from transmission electron micrographs. Seizures from EEG recordings and EEG coherence were quantified using custom Matlab software, version R2019B, available at: https://github.com/huguenardlab/EEG. GraphPad Prism software (GraphPad Software, versions 8 and 9) was used to perform statistical analyses.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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Data Availability: Raw data is available in the source data files for Figures 1-5 and Extended Data Figures 1-10.
Code availability: Custom Matlab code used for EEG analyses is available at: https://github.com/huguenardlab/EEG

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | For all studies, n ≥ 3 or more mice / rats per group, with the exact n specified in figure legends. Sample sizes were based on the variance of data in pilot experiments, and were generally estimated by power calculations which determined the number of animals “n” needed for 80% power to detect a 20-30% difference between genotypes, at the p<0.05 significance level. |
| Data exclusions | Rarely, data were excluded for the following pre-established reasons: (1) samples incurred damage during histological processing that precluded accurate analysis (2) EEG recordings were of insufficient quality to enable accurate interpretation (e.g. one channel not working or significant artifact) and (3) statistical outliers, defined as data points > 2 standard deviations from the group mean. |
| Replication | To ensure reproducibility, individual animals utilized came from > or = 2 distinct litters for the majority of experiments, and data were collected in separate, sequential experiments. For all experiments included in this study, data were successfully replicated. |
| Randomization | In experiments involving drug or vehicle treatment groups, treatment was assigned to individual animals randomly and in a method ensuring equal numbers of male and female animals assigned to each treatment group. |
| Blinding | All data collection and analyses were performed by experimenters blinded to subject experimental condition. |

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| Materials & experimental systems | Methods |
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| ☒ ☒ | ☒ | ChIP-seq |
| ☒ | ☒ | Flow cytometry |
| ☒ | ☒ | MRI-based neuroimaging |

Antibodies

Antibodies used

Primary antibodies: Rabbit anti-Olig2 (1:400, Millipore AB9610, lots 3172075, 2987464 and 3045562), Goat anti-PDGFRα (1:200, R&D Systems AF1062, lots HMQ0218081, HMQ0216021), mouse anti-APC (CC1,1:50, Calbiochem, OP80, lots 3031622, 2834781), or Rat anti-Ki67 (1:200, Life Technologies, 14-5698-82, lots 2056928, 2002315, 4328926), Rabbit anti- SOX9 (1:500, Abcam AB185966. lot GR3241181 - 12), Mouse anti-GFAP (1:200, Thermo Scientific 14-9892-82, lot 2358413), Rabbit anti-Iba1 (1:1000, Wako 019-19741, lot LEK0542), and/or Rat anti-CD68 (1:200, Abcam AB53444, Lot GR3384340-2).

Secondary antibodies: Alexa 488 donkey anti-rabbit IgG (1:500, Jackson Immuno Research, 711-545-152, lots 141848, 127725, 127498), Alexa 647 donkey anti-goat IgG (1:500, Jackson Immuno Research, A21447, lot 1977345), Alexa 594 donkey anti-mouse IgG
Validation

Millipore Rabbit anti-Olig2, R&D Systems Goat anti-PDGFRα and Calbiochem mouse anti-APC(CC1) as listed above have each been validated and used in rodent brain tissue in multiple previous publications (e.g. Gibson and Monje, Science 2014 PMID 24727982; Geraghty and Monje, Neuron 2019 PMID 31122677). Throughout the manuscript, representative photomicrographs are shown which clearly resemble those of prior publications (Figures 1, 3; for comparison see publications PMID 31018125, 24727982). In brain tissue from mice and rats used in this study, cells which co-expressed Olig2 and PDGFRα expressed in a characteristic ramified pattern around the nuclear Olig2 stain were considered to be oligodendrocyte precursor cells (similar to Dang et al, Cell Reports 2019, PMID 31018125, shown in Figures 1 and 3). To identify dividing OPCs, we performed immunostaining with Life Technologies Rat anti-Ki67 and counted Ki67-expressing OPCs. This Ki67 antibody has been used to label dividing cells in the mouse brain (for example, see Arimura et al, PMID 30422377) and clearly co-localized with the nuclear stain Olig2 in mice and rats (Figures 1 and 3). Mature oligodendrocytes co-expressed CC1 (peri-nuclear) and Olig2 (nuclear) as previously demonstrated in Gibson and Monje, Science 2014 PMID 24727982. Abcam anti-Sox9 and Thermo Fisher anti-GFAP have been previously used to label astrocytes in vivo in mice, in published work (PMIDs 31433295, 32381088) and cells co-labeled with Sox9 and GFAP exhibited morphology consistent with astrocytes as shown in representative photomicrographs in the manuscript. Wako anti-Iba1 and Abcam Rat anti-CD68 have been used by our laboratory to study microglial reactivity in previously published work: Gibson and Monje, Cell 2019, PMID 30528430.

In addition, antibodies were validated by the company from which each antibody was purchased:
- Anti-Olig2: https://www.emdmillipore.com/US/en/product/Anti-Olig-2-Antibody,MM_NF-AB9610; confirmed reactivity in mice and rats
- Anti-PDGFRα: https://www.rndsystems.com/products/mouse-pdgf-ralpha-antibody_af1062; confirmed reactivity in mice; reactivity in rats confirmed as above
- Anti-Ki67: https://www.thermofisher.com/antibody/product/Ki-67-Antibody-Clone-SolA15-Monoclonal/14-5698-82; confirmed reactivity in mice and rats
- Anti-CC1: https://www.emdmillipore.com/US/en/product/Anti-APC-Ab-7-Mouse-mAb-CC-1,EMD_BIO-OP80; confirmed reactivity in mice and rats
- Anti-Sox9: https://www.abcam.com/sox9-antibody-epr14335-78-ab185966.html; confirmed reactivity in mice and rats
- Anti-GFAP: https://www.thermofisher.com/antibody/product/GFAP-Antibody-Clone-GA5-Monoclonal/14-9892-82, confirmed reactivity in mice and rats
- Anti-Iba1: https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-1974.html, confirmed reactivity in mice and rats
- Anti-CD68: https://www.abcam.com/cd68-antibody-fa-11-ab53444.html, confirmed reactivity in mice

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

All experiments were conducted in accordance with protocols approved by the Stanford University Institutional Animal Care and Use Committee (IACUC; protocols 27215, 12363 and 33969). Mice or rats were group or single housed (up to 5 mice or 2 rats per cage) according to standard guidelines with ad libitum access to food and water in a 12 hour light/dark cycle. In rooms where mice and rats were housed, the ambient temperature was 70 +/- 2 degrees F and the relative humidity was 30-70%. The strains and ages of all mice and rats used in specific studies are indicated in the figures and throughout the text. Male and female mice were used in all studies. Briefly, in studies using Wag/Rij and control (Wistar) rats, 1.5-month-old rats were used to assess endpoints prior to seizure onset and 6-7-month-old rats were used to assess endpoints after seizures are well established. In studies using Scn8a+/mut mice and wild-type littersmates on a C3HeB/FeJ background strain, post-natal day (P)21 mice were used to assess endpoints prior to seizure onset, P28 mice were used to assess timepoints during seizure progression and P45 mice were used to assess endpoints after seizures are well established. In studies in which Scn8a+/mut mice were bred onto a mixed C3HeB/FeJ and C57/BL6 background (Figure 4), because seizure onset is delayed, later time points (3 to 6 months) were used to study seizure progression, as described in detail in the text.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected in the field.

Ethics oversight

All experiments were conducted in accordance with protocols approved by the Stanford University Institutional Animal Care and Use Committee (IACUC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.