Myelination synchronizes cortical oscillations by consolidating parvalbumin-mediated phasic inhibition

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Abstract

Parvalbumin-positive (PV⁺) γ -aminobutyric acid (GABA) interneurons are critically involved in producing rapid network oscillations and cortical microcircuit computations but the significance of PV⁺ axon myelination to the temporal features of inhibition remains elusive. Here using toxic and genetic mouse models of demyelination and dysmyelination, respectively, we find that loss of compact myelin reduces PV⁺ interneuron presynaptic terminals, increases failures and the weak phasic inhibition of pyramidal neurons abolishes optogenetically driven gamma oscillations *in vivo*. Strikingly, during behaviors of quiet wakefulness selectively theta rhythms are amplified and accompanied by highly synchronized interictal epileptic discharges. In support of a causal role of impaired PV-mediated inhibition, optogenetic activation of myelin-deficient PV⁺ interneurons attenuated the power of slow theta rhythms and limited interictal spike occurrence. Thus, myelination of PV axons is required to consolidate fast inhibition of pyramidal neurons and enable behavioral state-dependent modulation of local circuit synchronization.

Introduction

GABAergic interneurons play fundamental roles in controlling rhythmic activity patterns and the computational features of cortical circuits. Nearly half of the interneuron population in the neocortex is parvalbumin-positive (PV^+) and comprised mostly of the basket-cell (BC) type (Hu et al., 2014; Tremblay et al., 2016). PV^+ BCs are strongly and reciprocally connected with pyramidal neurons (PNs) and other interneurons, producing temporally precise and fast inhibition (Bartos et al., 2002; Gonchar and Burkhalter, 1997; Tamás et al., 1997). The computational operations of PV^+ BCs, increasing gain control, sharpness of orientation selectively and feature selection in the sensory cortex (Atallah et al., 2012; Cardin et al., 2009; Lee et al., 2013; Yang et al., 2017; Zucca et al., 2017) are mediated by a range of unique molecular and cellular specializations. Their extensive axon collaterals targeting hundreds of PNs, are anatomically arranged around the soma and dendrites, electrotonically close to the axonal output site and the unique calcium (Ca^{2+}) sensors in PV^+ BCs terminals, synaptotagmin 2 (Syt2), are tightly coupled to Ca^{2+} channels mediating fast and synchronized release kinetics (Chen et al., 2017; Sommeijer and Levelt, 2012), powerfully shunting excitatory inputs and increasing the temporal precision of spike output (Hu et al., 2014; Somogyi et al., 1983; Tamás et al., 1997; Thomson et al., 1996).

Recent findings have shown that the proximal axons of PV^+ interneurons are covered by myelin sheaths (Micheva et al., 2016; Peters and Proskauer, 1980; Somogyi et al., 1983; Stedehouder et al., 2017; Tamás et al., 1997; Yang et al., 2020). How interneuron myelination defines cortical inhibitory interneuron functions remains, however, still poorly understood. Myelination of axons provides critical support for long-range signaling by reducing the local capacitance which results

in rapid saltatory conduction and by maintaining the axonal metabolic integrity (Cohen et al., 2020; Nave and Werner, 2014). For PV⁺ BCs, however, the average path length between the axon initial segment (AIS) and release sites involved in local circuit inhibition is typically less than ~200 μm (Micheva et al., 2021; Schmidt et al., 2017; Tamás et al., 1997) and theoretical and experimental studies indicate the acceleration by myelin may play only a limited role in tuning inhibition (Micheva et al., 2021, 2016). Another notable long-standing hypothesis is that myelination of PV⁺ axons may be critical for the security and synchronous invasion of presynaptic terminals (Somogyi et al., 1983). In support of a role in reliability, in Purkinje cell axons of the long Evans shaker (*les*) rat, which carries a deletion of *Mbp*, spike propagation shows failures and presynaptic terminals are disrupted (Barron et al., 2018). Interestingly, in a genetic model in which oligodendrocyte precursor cells lack the $\gamma 2$ GABA_A receptor subunit, fast-spiking interneuron axons in the neocortex are aberrantly myelinated and feedforward inhibition is impaired (Benamer et al., 2020). At the network level, PV⁺ BC-mediated feedback and feedforward inhibition is critical to produce local synchronization between PNs and interneuron at the gamma (γ) frequency (30 – 80 Hz) which is a key rhythm binding information from cell assemblies, allowing synaptic plasticity and higher cognitive processing of sensory information (Buzsáki, 2006; Cardin et al., 2009; Hu et al., 2014; Sohal et al., 2009; Veit et al., 2017). Here, we determined whether PV⁺ BC driven neocortical rhythms require myelination by using de- and dysmyelination models, studying the cellular properties of genetically labelled PV⁺ BCs and examining the functional role of myelin by longitudinally examining the frequency spectrum of cortical oscillations.

Results

Behavioral state-dependent increase in theta power and interictal epileptiform discharges.

We investigated *in vivo* cortical rhythms by recording local field potential (LFP) in layer 5 (L5) together with surface electrocorticogram (ECoG) signals from both primary somatosensory (S1) and visual (V1) areas. Freely moving mice (C57BL/6, 7–9 weeks at the start of the experiment) were recorded in their home cage every second week (18–24 hours/week) across an 8-week cuprizone treatment, inducing toxic loss of oligodendrocytes in white- and gray matter areas (Clarner et al., 2012; Hamada and Kole, 2015; Kipp et al., 2009). Remarkably, after 6 weeks of cuprizone feeding we detected high-voltage spike discharges (~5 times the baseline voltage and ~50–300 ms in duration, **Figure 1a-c**). These brief spike episodes on the ECoG and LFP (**Figure 1c**) occurred bilaterally and near synchronously in S1 and V1, resembling the interictal epileptiform discharges (also termed interictal spikes) that are a hallmark of epilepsy (Cohen et al., 2002; Dubey et al., 2018; Hoffmann et al., 2008; Tóth et al., 2017). Automated detection of interictal spikes in the raw ECoG–LFP signal was performed with a machine-based learning classifier (see **Figure 1–figure supplement 1a** and **Methods**), revealing a progressively increasing number of interictal spikes, from ~5/hour at 4 weeks up to ~70/hour at 8 weeks of cuprizone treatment (**Figure 1d**). Interestingly, interictal spikes were highly dependent on vigilance state and present exclusively during quiet wakefulness (30 out of 30 randomly selected LFP segments from awake or quiet wakefulness, Chi-square test $P < 0.0001$, $n = 6$ cuprizone mice), with no other discernible association to specific behaviors (**Figure 1–figure supplement 1b** and **video 1**). Whether the pathological cortical oscillations were specific to certain frequency bands, including gamma (γ , 30–80 Hz), was examined by plotting the power spectrum density of the LFP in S1 during periods of quiet wakefulness or active movement (**Figure 1e**). During quiet wakefulness LFP power in cuprizone-treated mice was selectively amplified in the theta frequency band (θ , 4–12 Hz, Šidák’s multiple comparisons test, $P = 0.0013$, **Figure 1e-f**, and **Figure 1–figure supplement 1c**). In contrast, during active states when mice were moving and

exploring no differences were observed in the power spectrum, in none of the frequency bands (Šidák's multiple comparisons test, $P > 0.166$, **Figure 1e, f, Source data 1**). Finally, to more firmly establish whether interictal epileptiform discharges result from the lack of myelin we analyzed ECoG signals in the dysmyelinated shiverer mice (Mbp^{Shi}) lacking compact myelin due to a truncating mutation in *Mbp* (Readhead et al., 1987). Shiverer mice suffer progressively increasing number of epileptic seizures beginning at approximately 8 weeks of age (Chernoff, 1981; Readhead et al., 1987). ECoG recordings at 8 weeks showed that in addition to ictal discharges, interictal spikes were detected with a rate of $\sim 1/\text{minute}$, comparable to cuprizone-treated mice (**Figure 1g,h** and **Figure 1-figure supplement 2**). Although the waveform of interictal spikes in shiverer was substantially longer in duration ($\sim 100\text{--}500$ ms), comparison of the power across the four frequency bands around interictal spikes was similar (Two-way ANOVA $P = 0.7875$, $n = 6$ mice for both groups, **Figure 1-figure supplement 2**)

Loss of myelin impairs fast PV⁺ BC-mediated inhibition

Increased power of sensory-driven slow oscillations and epileptiform activity in the neocortex of normally myelinated brains is also observed when PV⁺ interneurons are optogenetically silenced (Brill et al., 2016; Veit et al., 2017; Yang et al., 2017). To investigate how myelin loss affects the PV⁺ interneuron morphological and functional properties we crossed the *Pvalb*^{Cre} mouse line, having Cre recombinase targeted to *Pvalb* expressing cells, with a tdTomato fluorescence (Ai14) Cre reporter line (hereafter called PV-Cre; Ai14 mice). The cytoplasmic fluorescence allowed quantification of PV⁺ cell bodies and their processes in the primary somatosensory cortex (**Figure 2a, b** and **Figure 2-figure supplement 1a**) and immunofluorescent labelling with myelin basic protein (MBP) revealed substantial myelination of large-diameter PV⁺ axons (mean $\pm$ SEM, $80.15 \pm 9.95\%$ along 83 mm of PV⁺ axons analyzed, $n = 3$ slices from 2 mice, z -stack

with a volume of $7.66 \times 10^5 \mu\text{m}^3$, (**Figure 2b** and **Figure 2–figure supplement 1a**). Electron microscopy (EM) immunogold-labeled tdTomato showed that PV^+ axons possessed multi-lamellar compact myelin sheaths (on average, 6.33 ± 0.80 myelin lamella) with 10.8 ± 0.76 nm distance between the major dense lines and a mean g-ratio (axon diameter/fiber diameter) of 0.74 ± 0.01 ($n = 6$ sheaths, **Figure 2c**). PV-Cre; Ai14 mice fed with 0.2% cuprizone for 6 weeks showed strongly reduced MBP in S1 and PV^+ axons were largely devoid of myelin (**Figure 2a-b** and **Figure 2–figure supplement 1b**) while the total number of PV^+ cell bodies across cortical layers remained constant (control density, 326 ± 14 cells mm^{-2} vs. cuprizone density, 290 ± 48 cells mm^{-2} , $n = 6$ sections from $N = 6$ animals/group, Mann-Whitney test $P = 0.1649$, **Figure 2d**). Further single cell analysis was performed on biocytin-filled PV-Cre⁺ interneurons which were re-sectioned and stained for MBP to identify the location of myelin and the axon morphology (**Figure 2e** and **Figure 2–figure supplement 1c, d**). Myelin was present on multiple proximal axonal segments of all control BCs (4/4 fully reconstructed axons, on average $2.8 \pm 1.2\%$ myelination). In contrast, none of the BCs from cuprizone treated mice showed myelinated segments (0/6 axons). Furthermore, the total number of axon segments (~ 80 per axon, Mann-Whitney test, $P = 0.3032$, **Figure 2f**) as well as the total path length were unaffected by cuprizone treatment (on average ~ 4.5 mm in both groups, range 2.0–9.5 mm, Mann-Whitney test, $P = 0.9871$, **Figure 2g**, **Figure 2–figure supplement 1c-f**).

To examine whether myelin loss changes the intrinsic excitability of PV^+ BCs, we made whole-cell recordings in slices from PV-Cre; Ai14 mice (**Figure 2h**). Recording of steady-state firing properties by injecting increasing steps of currents injections revealed an increase in the rheobase current (~ 90 pA, **Figure 2i-j**) and a ~ 50 Hz reduced firing frequency during low amplitude current injections (two-way ANOVA, treatment $P = 0.0441$, Šidák's multiple comparison post

hoc test at 200 pA; $P = 0.0382$, 250 pA; $P = 0.0058$, 300 pA; $P = 0.0085$) without a change in the
 maximum instantaneous firing rate (two-way ANOVA, Šidák's multiple comparison post hoc
 test, $P = 0.92$, data not shown). However, neither the AP half-width (control, $290 \pm 10 \mu\text{s}$, $n = 34$
 cells from 12 mice versus cuprizone, $295 \pm 10 \mu\text{s}$, $n = 15$ cells from 7 mice, two-tailed Mann-
 Whitney U test $P = 0.7113$) nor AP amplitude was affected by cuprizone treatment (control
 $78.12 \pm 1.66 \text{ mV}$, $n = 34$ cells from 12 mice versus cuprizone, $80.13 \pm 2.48 \text{ mV}$, $n = 15$ cells
 from 7 mice, $P = 0.4358$). In contrast, the resting membrane potential (V_{RMP}) of PV^+ interneurons
 was on average $\sim 4 \text{ mV}$ significantly more hyperpolarized (**Figure 2k**) without a change in the
 apparent input resistance (control, $133.3 \pm 8.55 \text{ M}\Omega$, $n = 42$ cells from 21 mice versus cuprizone
 $125 \pm 8.96 \text{ M}\Omega$, 27 cells out of 13 mice, $P = 0.5952$). In addition to the hyperpolarization in
 V_{RMP} , demyelinated PV^+ interneurons also had a $\sim 3 \text{ mV}$ more hyperpolarized AP voltage
 threshold (control, $-40.51 \pm 0.97 \text{ mV}$, $n = 34$ cells from 12 mice), cuprizone -43.65 ± 1.29 ($n =$
 15 cells from 7 mice, Mann-Whitney test $P = 0.0269$). Taken together, the results indicate that
 cuprizone treatment completely demyelinates proximal branches of PV^+ interneuron axons, and
 while not affecting axon morphology, causes a net decrease in the intrinsic PV^+ interneuron
 excitability.

Is myelin required for PV^+ BC-mediated inhibition? Single PV^+ BCs typically make 5 to
 15 synapses with a PN in a range of $< 200 \mu\text{m}$, forming highly reliable, fast and synchronized
 release sites (Micheva et al., 2021; Packer and Yuste, 2011; Tamás et al., 1997; Thomson et al.,
 1996). Intercellular variations in both myelin distribution and aberrant myelin patterns have been
 associated with conduction velocity changes (Benamer et al., 2020; Micheva et al., 2021). To
 examine the role of myelin on inhibitory transmission more directly, we made paired recordings
 of PV^+ BCs and L5 PNs with and without myelination, in control or cuprizone-treated PV-Cre;

172 Ai14 mice, respectively (**Figure 3**). We evoked APs in PV⁺ BCs while recording unitary
173 inhibitory post-synaptic currents (uIPSCs) under conditions of physiological Ca²⁺/Mg²⁺ (2.0/1.0
174 mM in $n = 78$ pairs, **Figure 3a, b**). Concordant with optogenetic mapping of PV⁺ inputs onto L5
175 PNs in mouse S1 (Packer and Yuste, 2011), in control slices the probability of a given PV⁺ cell
176 being connected to a nearby PN was high (~0.48, **Figure 3c**). In contrast, the connection
177 probability was significantly lower in cuprizone-treated mice (~0.23, $P = 0.0182$, **Figure 3b, c**).
178 In 13 stable connected pairs, we examined unitary IPSC properties including failure rate and
179 amplitude, as well as rise- and decay time, using automated fits of the uIPSCs ($n > 80$ trials per
180 connection, **Figure 3d**). Cuprizone treatment led to a significant increase in the number of
181 failures (from 0.05 to 0.26, **Figure 3e**) and a ~2.5-fold reduction in the average uIPSC peak
182 amplitude (**Figure 3f**). To obtain an estimate of propagation speed, we determined on successful
183 trials the latency between the AP peak and uIPSCs at 10% peak amplitude (**Figure 3d**).
184 Interestingly, both the mean latency as well as the trial-to-trial latency variability remained
185 unchanged (average ~800 μ s; Mann-Whitney test $P > 0.999$; SD in cuprizone 319 ± 65 μ s, $n = 7$
186 pairs, SD in control, 276 ± 38 μ s, $n = 5$ pairs, $P > 0.60$, **Figure 3g**).

187 To further examine the properties of GABA release in demyelinated PV-BCs we
188 recorded uIPSCs during a train of five APs at 100 Hz (averaging > 50 trials, **Figure 3h**).
189 Consistent with the temporary facilitation in IPSCs of adult Purkinje cells (Turecek et al., 2016),
190 uIPSC recordings in control PV BCs showed that paired-pulse ratios were on the second spike
191 facilitated by 20% (uIPSC₂/uIPSC₁ 1.20 ± 0.060) and gradually depressed on the subsequent
192 spikes (spikes 3 to 5). In contrast, in cuprizone-treated mice uIPSC were depressed during the
193 second and subsequent pulses (Two-way RM ANOVA pulse $\times$ treatment effect $P < 0.021$,
194 Šidák's multiple comparison tests for uIPSC₂/uIPSC₁ 0.89 ± 0.041 , $P = 0.0339$, **Figure 3h**).

The uIPSC failures and impairment of temporary facilitation may reflect failure of AP propagation along demyelinated PV axons, changes in the GABA release probability or a lower number of active release sites (< 5 , Refs. (Tamás et al., 1997; Thomson et al., 1996). To further examine the properties of inhibition at L5 PNs we recorded miniature inhibitory postsynaptic currents (mIPSCs). In line with the uIPSC findings the results showed that mIPSC were significantly reduced in peak amplitude (from ~ 20 to ~ 7 pA, $P = 0.002$) without a change in frequency (**Figure 3-figure supplement 1c**). Furthermore, using PV immunofluorescence staining with biocytin filled L5 PNs the number of PV⁺ puncta was 40% reduced both at the soma and the primary apical dendrite, correlating with the overall reduction in immunofluorescent signals in cuprizone treatment (**Figure 3-figure supplement 1g-i**). Interestingly, in contrast to the loss of perisomatic PV⁺ BC puncta, putative PV⁺ chandelier cell inputs, identified by co-staining with the AIS marker β IV-spectrin, were preserved (~ 8 puncta/AIS, Mann-Whitney test $P = 0.96$, **Figure 3-figure supplement 2**). Furthermore, staining for Syt2, a Ca²⁺ sensor protein selective for PV⁺ presynaptic terminals (Sommeijer and Levelt, 2012; Xu et al., 2007) confirmed a $\sim 35\%$ reduction (**Figure 3-figure supplement 3a-b**). Together with loss of uIPSC amplitudes (**Figure 3f**) these data suggest that cuprizone-induced demyelination causes a loss of presynaptic PV⁺ terminal sites. Interestingly, Syt2⁺ puncta analysis in the dysmyelinated shiverer mouse line also showed a reduced number of Syt2⁺ puncta at L5 PN somata and a reduced frequency of mIPSCs ($P = 0.019$, **Figure 3-figure supplement 3c-g**), indicating that compact myelin is not only required for maintaining PV⁺ interneuron inputs but also for PV⁺ BC presynaptic terminal development.

Cuprizone treatment did not affect PV⁺ axon length (**Figure 2-figure supplement 1f**) suggesting that the density of presynaptic terminals should be reduced. To test this idea, we performed Syt2 immunolabeling of individually biocytin-filled PV⁺ BCs (**Figure 3i**). Consistent

with the hypothesis, cuprizone treatment significantly reduced the density of Syt2⁺ puncta by 2-fold (cuprizone, ~1 Syt2⁺ puncta per 10 μ m vs. 1 Syt2⁺ puncta per 5 μ m in control, Mann-Whitney test $P < 0.0001$, **Figure 3j-k**). Interestingly, recordings of miniature EPSCs from PV⁺ interneurons of control and cuprizone-treated mice showed no changes in peak amplitude nor frequency (**Figure 3–figure supplement 4**), in keeping with the preservation of excitatory inputs onto L5 PN following cuprizone-induced demyelination (Hamada and Kole, 2015) and suggesting that myelin loss has a larger impact inhibitory synapse stabilization and maintenance.

Thus, myelin loss reduces the number of presynaptic sites, causing an increase of GABA release failures and a frequency-dependent depression ultimately limiting the fast component of BC to PN inhibitory transmission.

PV⁺ activation rescues interictal spikes and theta oscillations, but not the loss of gamma

To understand next how myelin deficits and loss of fast PV inhibition of PNs impacts network dynamics, we used AAV1-mediated delivery of Cre-dependent channelrhodopsin-2 (ChR2) into L5 of PV-Cre; Ai14 mice (**Figure 4a**). The ChR2 transduction rate was comparable between control and cuprizone mice (~70%, **Figure 4b, c**). In acute slices, we voltage-clamped L5 PNs and optogenetically evoked IPSC (oIPSC) with full-field blue light illumination (**Figure 4d**). Consistent with S1 L5 pyramidal neurons receiving converging input from >100 PV⁺ interneurons (Packer and Yuste, 2011), control oIPSCs rapidly facilitated to a peak amplitude of ~700 pA followed by rapid synaptic depression (**Figure 4f, g**). In slices from cuprizone mice, however, the oIPSC peak amplitude was significantly reduced (~2-fold) while neither the steady-state amplitude during vesicle replenishment nor the total charge transfer reached a significant difference (Control, -99.58 ± 28.5 pC vs. cuprizone, -54.3 ± 20.57 pC, $P = 0.236$, $n = 9$ control and $n = 8$ cuprizone neurons, **Figure 4f, h**).

Impaired phasic PV⁺ interneuron-mediated inhibition predicts a disrupted γ rhythm. Experimental and computational studies show that in most cortical areas γ rhythms are strongly shaped by electrically and synaptically coupled PV⁺ interneurons, which by temporally synchronizing firing rates, synaptic inhibitory time constants ($\cong 9$ ms) and the recurrent excitatory feedback from PNs, give rise to network resonance in the 30 to 80 Hz bandwidth (Bartos et al., 2002; Cardin et al., 2009; Sohal et al., 2009; Traub et al., 1997; Wang and Buzsáki, 1996). To test the cellular and circuit properties of the γ rhythm we examined the extent of evoked γ modulation by leveraging optogenetic activation of PV⁺ interneurons with AAV1-hChR2-YFP, and introducing a laser fiber into L5 and recording the LFP (**Figure 5a**). Evoking brief pulses of blue light (1 ms at a low γ frequency of 30 Hz) showed that local circuit currents were modulated and highly phase-locked in slices from control mice (bandpass filter 25 and 40 Hz, **Figure 5b, c** and **Figure 5-figure supplement 1**). In striking contrast, no modulation or entrainment was observed in cuprizone-treated mice, neither when using high laser power (up to 6.5 mW, **Figure 5b-e, Figure 5-figure supplement 1**).

Could the diminished PV⁺ BC activity cause the emergence of θ rhythm and interictal spikes during quiet behavioral states of wakefulness? To test the direct contribution of PV⁺ BCs we activated ChR2 for 1 s duration pulses in PV-Cre; Ai14 mice to generate tonic GABA release (**Figure 6a**). In cuprizone-treated mice we found that optically driving PV⁺ interneurons normalized the LFP power in the θ band to control levels, without affecting δ , β , and γ rhythms (two-way ANOVA Treatment $\times$ Light $P = 0.0124$, Šidák's multiple comparison tests in cuprizone, light on vs. off; for δ , $P = 0.9975$; θ , $P = 0.0076$; β , $P = 0.9481$; γ , $P = 0.9998$, **Figure 6a-c and Source data 1**). Furthermore, activation of blue light significantly reduced the

frequency of interictal epileptic discharge frequency ($P = 0.0089$, **Figure 6d-e**, **Figure 6–video 1**). The normalization of cortical rhythms by elevating sustained PV⁺ mediated activity suggests that GABA_A receptors are insufficiently activated in the demyelinated cortex. Finally, to directly examine the role of GABA_A receptors agonism in dampening global interictal spikes we administered a non-sedative dose of diazepam (2 mg/kg i.p.), an allosteric modulator of post-synaptic GABA_A receptors, in cuprizone-treated mice (8-weeks treatment). The results showed that diazepam significantly suppressed the interictal epileptiform discharges in cuprizone mice, indicating a prominent role of GABA in the deficits of circuit excitability (**Figure 6f** and **Figure 6–figure supplement 1**).

Discussion

In this study we identified that the cellular microarchitecture of myelination of PV⁺ BCs is required for stimulus-induced fast gamma frequencies, limiting the power of slow cortical oscillations and interictal spikes during quiet wakefulness. Interictal discharges identified as spikes on the EEG are an important diagnostic criterium in epilepsy and reflect hypersynchronized burst firing of pyramidal neurons and interneurons (Cohen et al., 2002; Tóth et al., 2017; Zhou et al., 2007). The brief episodic and generalized nature of EEG spikes we recorded in both demyelinated and dysmyelinated cortex (~50 to 500 ms in duration and ~1/minute) resemble interictal spikes reported in epilepsy models (Kleen et al., 2010; Zhou et al., 2007) and are concordant with recordings in the hippocampus of cuprizone-treated mice by Hoffmann et al. (Hoffmann et al., 2008). Here we fundamentally extend the insights into interictal spikes by showing their spatiotemporal synchronization across cortical areas and hemispheres and a selective manifestation during the vigilance state of quiet wakefulness. During brain states of quiescence, for example when whiskers are not moving, whole-cell *in vivo*

recordings in the barrel cortex reveals low frequency (<10 Hz) highly synchronized membrane potential fluctuations of PNs and interneurons, during which fast-spiking PV⁺ interneurons are dominating action potential firing (Gentet et al., 2010; Poulet and Petersen, 2008). Consistent with the state-dependent increased activity of cortical interneuron firing we found a selective amplification of the LFP theta power during quiet wakefulness, which may be explained by the reduced intrinsic excitability of PV⁺ BCs and deficiency of fast inhibitory transmission in the demyelinated cortex (**Figures 2, 3**). In support of this conjecture, optogenetic inhibition of PV⁺ interneurons in the normally myelinated cortex is sufficient to increase PN firing rates, elevating the power of slow oscillations and triggering epileptiform activity (Brill et al., 2016; Veit et al., 2017; Yang et al., 2017). While our optogenetic activation of demyelinated PC⁺ BCs normalized the power in the theta bandwidth, these interneurons are not critical for generating theta (Cardin et al., 2009; Sohal et al., 2009). Physiological theta oscillations are strongly driven by the long-range corticothalamic circuitry and cortical PN firing during non-rapid eye movement (NREM), also called thalamocortical spindles (Steriade, 1997). Interestingly, recordings in epilepsy patients showed that interictal discharges occur frequently during NREM sleep stages coupled with the spindle activity (Dahal et al., 2019; Ujma et al., 2017). Whether spindle activity or sleep stages are affected in cuprizone-treated mice is not known but the present results warrant investigation of the contribution of the corticothalamic loop to the generation of interictal discharges.

A major limitation of the experimental toolbox available to experimentally study myelination is the lack of axon- or cell-type selectivity. Whether amplified delta- and abolished gamma-frequency oscillations are the consequence of PV⁺ axon demyelination, the loss of excitatory axon myelination or the combination thereof, remains to be further examined when more refined genetic or molecular methods become available to interrogate oligodendroglial

myelination of specific cell types. In the absence of such strategies, however, our *in vivo* experiments optogenetically driving selectively PV⁺ interneurons or activating GABA_A receptors (**Figure 5**) uncovers important converging evidence for a role of interneurons in the amplification and synchronization of slow oscillations and epileptic discharges. Interestingly, while tonically driving action potential firing in myelin-deficient PV⁺ interneurons with optogenetic activation was able to rescue theta hypersynchrony, using a 30 Hz stimulation regime failed to entrain the LFP in the low-gamma frequency. This may suggest that for gamma, precisely timed spike generation of PV⁺ BCs alone is insufficient and requires synchronized inhibitory synaptic transmission, which is selectively reduced in demyelinated PV⁺ BCs. Alternatively, a specific circuit connectivity or presynaptic GABA release dynamics may be lost in cuprizone-treated mice. In future studies, the role of myelination could be further examined by exploring whether remyelination restores the ability of PV⁺ interneurons to modulate gamma oscillations.

Myelination of PV⁺ axons determine synapse assembly and maintenance

The requirement of myelination of PV⁺ BCs to generate gamma rhythms is surprising in view of its sparse distribution in patches of ~25 µm across < 5% of the total axon length (Micheva et al., 2021, 2016; Stedehouder et al., 2019) (**Figure 2** and **Figure 2-figure supplement 1**). The sparseness of interneuron myelination previously raised the question whether myelin speeds conduction velocity in these axon types (Micheva et al., 2016; Stedehouder et al., 2019; Stedehouder and Kushner, 2017). In a genetic mouse model with aberrant myelin patterns along fast-spiking interneuron axons the inferred conduction velocity was reduced (Benamer et al., 2020). In contrast, we found that the average uIPSC latency (~800 µs) in completely demyelinated axons was normal and well within range of previous paired recordings between

myelinated PV⁺ BC and PNs (700–900 μ s, (Miles, 1990; Rossignol et al., 2013)). Assuming a typical axonal path length of ~200 μ m between the AIS and presynaptic terminals connected with a PN, combined with a ~250 μ s delay for transmitter release, the calculated conduction velocity would be 0.4 m s⁻¹, consistent with optically recorded velocities in these axons (~0.5 m s⁻¹, ref. (Casale et al., 2015)). Our paired recordings, made near physiological temperature (34 – 36 °C), may have had a limited resolution to detect temporal differences and are not excluding changes in the order of microseconds. To study submillisecond changes it may be necessary to employ simultaneous somatic and axonal whole-cell recording (Hu and Jonas, 2014) and/or high-resolution anatomical analysis of myelin along the axon path, which recently showed a small albeit a positive correlation between percentage of myelination and conduction velocity (Micheva et al., 2021). Furthermore, conduction velocity tuning by myelination of GABAergic axons may become more readily apparent for long-range projections. Another constraint of the present study is the lack of information on the nodes of Ranvier along demyelinated PV⁺ BC axons. Aberrant interneuron myelin development, causing myelination of branch points impairs the formation of nodes of Ranvier (Benamer et al., 2020). Reorganization of nodal voltage-gated ion channel clustering also occurs with the loss of myelin or oligodendroglial secreting factors causing deficits in action potential propagation (Freeman et al., 2015; Lubetzki et al., 2020). How PV⁺ BC interneuron myelin loss changes the nodal ion channel distribution remains to be examined.

Converging evidence from the two distinct models (shiverer and cuprizone) showed that interneuron myelination critically determines PV⁺ release site number, dynamics and connection probability (**Figure 3** and **figure 3-figure supplement 3**), concordant with the observed synapse loss in Purkinje axons of the *les* rat (Barron et al., 2018). The molecular mechanisms how compact myelination of proximal axonal segments establishes and maintains GABAergic

terminals in the higher-order distal axon collaterals are not known and remains to be further investigated. The PV⁺ interneuron myelin sheath contains high levels of non-compact 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) protein (Micheva et al., 2018, 2016), which is part of the inner cytoplasmic inner mesaxon (Edgar et al., 2009). One possible mechanism may be that in the absence of inner cytoplasmic loops of oligodendroglial myelin, interneuron axons lack sufficient trophic support (Fünfschilling et al., 2012). In support of this idea, amyloid precursor protein, a marker of disrupted axonal transport, has been observed in early phases of cuprizone treatment and in multiple sclerosis (MS) (Berg et al., 2017; Lindner et al., 2009; Sorbara et al., 2014). Another possibility is pruning of GABAergic synaptic terminals by microglia (Chen et al., 2014; Favuzzi et al., 2021; Ramaglia et al., 2021). Microglia become increasingly activated during sub-demyelinating stages within the first week of cuprizone treatment (Caprariello et al., 2018; Skripuletz et al., 2013), and in aged *Mbp*^{+/-} mice (Poggi et al., 2016). In future studies it needs to be examined whether attenuation of microglia activation could protect against PV⁺ synapse loss and interictal epileptiform discharges.

Implications for cognitive impairments in gray matter diseases

The identification of a cellular mechanism for interictal spikes may shed light to the role of PV⁺ axon myelination in cognitive impairments in MS (Benedict et al., 2020) and possibly other neurological disorders. In preclinical models of epilepsy and epilepsy patients interictal spikes have been closely linked to disruptions of the normal physiological oscillatory dynamics such as ripples required to encode and retrieve memories (Cohen et al., 2002; Henin et al., 2021; Kleen et al., 2013, 2010). Interictal epileptic discharges are also a prominent hallmark in other cognitive diseases, including Alzheimer (Lam et al., 2017). Notably, reduced gray matter myelination and oligodendroglia disruption are reported in multiple epilepsy models and recently

in Alzheimer (Chen et al., 2021; Drenthen et al., 2020). Therefore, the cellular and circuit functions controlled by PV⁺ interneurons may represent a common mechanism for memory impairments in neurological disease encompassing myelin pathology. In support of this idea, neuropathological studies in MS show a specific loss of PV⁺ interneuron synapses in both cortex and hippocampus (Ramaglia et al., 2021; Zoupi et al., 2021). In MS patients increased connectivity and synchronization in delta and theta band rhythms during resting state or task-related behavior have been reported (Schoonheim et al., 2013; Tewarie et al., 2014) and low GABA levels in sensorimotor and hippocampal areas are correlated with impairments of information processing speed and memory (Cawley et al., 2015; Gao et al., 2018). Taken together with the present work, promoting PV⁺ interneuron myelination, and thereby strengthening fast inhibition, may provide important new therapeutic avenues to improve cognition.

Methods

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
strain, strain background (<i>Mus musculus</i> male/female)	C57BL6	Janvier Labs	Cat# 2670020, RRID:MGI:2670020	
strain, strain background (<i>Mus musculus</i> male/female)	B6;129P2- <i>Pvalb</i> ^{tm1(cre)Arbr} /J	Jackson laboratories	Stock No: 008069 RRID:IMSR_JAX:008069	

strain, strain background (<i>Mus musculus</i> male/female)	B6;129S6- <i>Gt(ROSA)26Sor^{tm14(CAG-tdTomato)Hze}/J</i>	Jackson laboratories	Stock No: 007908 RRID:IMSR_JAX:007908	
strain, strain background (<i>Mus musculus</i> male/female)	C3Fe.SWV- <i>Mbp^{shi}/J</i>	Jackson laboratories	Stock No: 001428 RRID:IMSR_JAX:001428	
Transfected construct (<i>Mus musculus</i>)	pAAV-EF1a-double-floxed-hChR2(H134)-EYFP-WPRE-HGHpA	Addgene.org	#20298 RRID:Addgene_20298	
antibody	Rabbit monoclonal, anti-MBP	Santa Cruz	Cat# sc-13564, RRID:AB_675707	(1:300)
antibody	Mouse monoclonal, anti-PV	Swant	Cat#: 235 RRID:AB_10000343	(1:1000)
antibody	Rabbit polyclonal, anti-syt2	Synaptic Systems	Cat# 105 223, RRID:AB_10894084	(1:500)
antibody	Rabbit polyclonal, anti-βIV-spectrin	M. Ransband (BCM)	N/A	(1:200)
peptide, recombinant protein	Alexa 488 -streptavidin	Thermofisher	Cat#: S32354 RRID:AB_2315383	(1:500)
peptide, recombinant protein	Alexa 633- streptavidin	Thermofisher	Cat#: S21375 RRID:AB_2313500	(1:500)
chemical compound,	Bis(cyclohexanone)oxaldi-hydrazone	Merck	Cat#: C9012	

drug				
chemical compound, drug	Diazepam	Centrafarm Nederland B.V	Cat#: RVG56691	
chemical compound, drug	Biocytin	Sigma	Cat#: B4261	
chemical compound, drug	D-AP5	HelloBio	Cat#: 0225	
chemical compound, drug	Gabazine	Sigma	Cat#: S106	
chemical compound, drug	CNQX	HelloBio	Cat#: HB0205	
chemical compound, drug	Tetrodotoxin (TTX) citrate	Tocris	Cat#: 1069	
software, algorithm	Neurolucida 360 Neurolucida Explorer	MBF Bioscience	V2018.02 RRID:SCR_01775	
software, algorithm	Synaptic puncta quantification	FIJI (ImageJ)	V2.0, V.2.0.0-rc-65/1.5w RRID:SCR_002285	
software, algorithm	Neuroarchiver tool	Open source Instruments Dubey et al., 2018	LWDAQ_8.5.29	
software, algorithm	Igor pro 8	WaveMetrics	V8.04 RRID:SCR_000325	

software, algorithm	Axograph	Axograph	RRID:SCR_014284	
software, algorithm	GraphPad	Prism 8 and 9	RRID:SCR_002798	
software, algorithm	SP8 X (DM6000 CFS)	Leica application Suite	AF v3.2.1.9702 RRID:SCR_013673	
other	Electrode (90% Pt, 10% Ir)	Science Products	Cat#: 101R-5T	
other	Optical fiber	Thorlabs	Cat#: FP200URT	
other	Ceramic ferrule	Thorlabs	Cat#: CFLC230-10	
other	Fiber coupled blue laser	Shanghai Laser & Optics Co.	Cat#: 473 nm, DPSS Laser with fiber coupled (T3)	
other	Cyclops LED driver	Open-ephys.org	Cat#: Cyclops LED driver 3.7	
other	Patch-clamp amplifier	Dagan Corporates	BVC-700A	
other	Patch-clamp amplifier	Molecular device	Axon Axopatch 200B	
other	<i>In-vivo</i> multichannel systems (Portable-ME-systems) amplifier	Multi channels systems	ME16-FAO-uPA	

402

403

404

Animals

We crossed *Pvalb*^{Cre} mice (B6;129P2-*Pvalb*^{tm1(cre)Arbr}/J, Stock No: 008069, Jackson laboratories, RRID:IMSR_JAX:008069) with the Ai14 Cre reporter line B6;129S6-*Gt(ROSA)26Sor*^{tm14(CAG-tdTomato)Hze}/J (Stock No: 007908, Jackson laboratories, RRID:IMSR_JAX:007908). For other experiments we used C57BL/6 mice (Janvier Labs, Saint-Berthevin Cedex, France, RRID:MGI:2670020). Shiverer mice were obtained from Jackson (C3Fe.SWV-*Mbp*^{shi}/J Stock No: 001428, RRID:IMSR_JAX:001428) and backcrossed with C57BL/6 mice for >10 generations. All mice were kept on a 12:12 h light-dark cycle (lights on at 07 am, lights off at 19 pm) with ad libitum food and water. For cuprizone treatment either PV-Cre; Ai14 or C57BL/6 male or female mice, from 7 to 9 weeks of age, were fed *ad libitum* with normal chow food (control group) or were provided 0.2% (w/w) cuprizone (Bis(cyclohexanone)oxaldihydrazone, C9012, Merck) added either to grinded powder food or to freshly prepared food pellets (cuprizone group). Cuprizone-containing food was freshly prepared during every 2nd or 3rd day for the entire duration of the treatment (6–9 weeks). The average maximum weight loss during cuprizone feeding was ~11% (*n* = 31). All animal experiments were done in compliance with the European Communities Council Directive 2010/63/EU effective from 1 January 2013. The experimental design and ethics were evaluated and approved by the national committee of animal experiments (CCD, application number AVD 80100 2017 2426). The animal experimental protocols were designed to minimize suffering and approved and monitored by the animal welfare body (IvD, protocol numbers; NIN17.21.04, NIN18.21.02, NIN18.21.05, NIN19.21.04 and, NIN20.21.02) of the Royal Netherlands Academy of Arts and Science (KNAW).

In vitro electrophysiology

Mice were briefly anaesthetized with 3% isoflurane and decapitated or received a terminal dose of pentobarbital sodium (5 mg kg⁻¹) and were transcardially perfused with ice-cold artificial CSF (aCSF) of the composition (in mM): 125 NaCl, 3 KCl, 25 glucose, 25 NaHCO₃, 1.25 Na₂H₂PO₄, 1 CaCl₂, 6 MgCl₂, 1 kynurenic acid, saturated with 95% O₂ and 5% CO₂, pH 7.4. After decapitation, the brain was quickly removed from the skull and parasagittal sections (300 or 400 μm) containing the S1 cut in ice-cold aCSF (as above) using a vibratome (1200S, Leica Microsystems). After a recovery period for 30 min at 35 °C brain slices were stored at room temperature. For patch-clamp recordings, slices were transferred to an upright microscope (BX51WI, Olympus Nederland) equipped with oblique illumination optics (WI-OBCLD; numerical aperture, 0.8). The microscope bath was perfused with oxygenated (95% O₂, 5% CO₂) aCSF consisting of the following (in mM): 125 NaCl, 3 KCl, 25 D-glucose, 25 NaHCO₃, 1.25 Na₂H₂PO₄, 2 CaCl₂, and 1 MgCl₂. L5 pyramidal neurons were identified by their typical large triangular shape in the infragranular layers and in slices from PV-Cre; Ai14 mice the PV⁺ interneurons expressing tdTomato were identified using X-Cite series 120Q (Excelitas) with a bandpass filter (excitation maximum 554 nm, emission maximum 581 nm). Somatic whole-cell current-clamp recordings were made with a bridge current clamp amplifier (BVC-700A, Dagan Corporation, US) using patch pipettes (4–6 MΩ) filled with a solution containing (in mM): 130 K-gluconate, 10 KCl, 4 Mg-ATP, 0.3 Na₂-GTP, 10 HEPES, and 10 Na₂-phosphocreatine, pH 7.4, adjusted with KOH, 280 mOsmol/kg, to which 10 mg mL⁻¹ biocytin was added. Voltage was analog low-pass filtered at 10 kHz (Bessel) and digitally sampled at 50–100 kHz using an analog-to-digital converter (ITC-18, HEKA Electronic) and data acquisition software Axograph X (v.1.7.2, Axograph Scientific, RRID:SCR_014284). The access resistance was typically < 20 MΩ and fully compensated for bridge balance and pipette capacitance. All reported membrane potentials were corrected for experimentally determined junction potential of –14 mV. Analysis

for the electrophysiological properties includes PV interneuron recordings from cells in normal ACSF and in the presence of CNQX and d-AP5 with high chloride intracellular solution (see below).

mIPSC and mEPC recordings

Whole-cell voltage-clamp recordings were made with an Axopatch 200B amplifier (Molecular Devices). Patch pipettes with a tip resistance of 3–5 M Ω were pulled from thin wall borosilicate glass. During recording, a holding potential of -74 mV was used. Both the slow- and fast pipette capacitance compensation were applied, and series-resistance compensated to ~ 80 – 90% . Patch pipettes were filled with high chloride solution containing (in mM): 70 K-gluconate, 70 KCl, 0.5 EGTA, 10 HEPES, 4 MgATP, 4 K-phosphocreatine, 0.4 GTP, pH 7.3 adjusted with KOH, 285 mOsmol kg $^{-1}$ and IPSCs isolated by the presence of the glutamate receptor blockers 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μ M), d-2-Amino-5-phosphonovaleric acid (d-AP5, 50 μ M) and the sodium (Na $^{+}$) channel blocker tetrodotoxin (TTX, 1 μ M Tocris). Individual traces (5 sec duration) were filtered with a high-pass filter of 0.2 Hz and decimated in Axograph software (RRID:SCR_014284). Chart recordings of mIPSCs were analyzed with a representative 30 ms IPSC template, using the automatic event detection tool of Axograph. Detected events were aligned and averaged for further analysis of inter-event intervals (frequency) and peak amplitude. For mEPSC recordings from PV $^{+}$ interneurons we filled patch pipettes with a solution containing (in mM): 130 K-gluconate, 10 KCl, 4 Mg-ATP, 0.3 Na $_2$ -GTP, 10 HEPES, and 10 Na $_2$ -phosphocreatine, pH 7.4, adjusted with KOH, 280 mOsmol/kg and both gabazine (4 μ M) and TTX (1 μ M) were added to the bath solution. The mEPSCs were analyzed using events detection tool in Axograph. The recorded signals were bandpass filter (0.1 Hz to 1 kHz) and

recordings analyzed with a representative 30 ms EPSC template, after which selected EPSCs aligned and averaged for further analysis of inter-event intervals (frequency) and peak amplitude.

uIPSC recording and analysis

PV⁺ interneurons (visually identified in PV-Cre; Ai14 mice based on tdTomato fluorescence expression) were targeted for whole-cell current-clamp recording within a radius of 50 μ m from the edge of the L5 soma recorded in voltage-clamp configuration. APs in PV⁺ interneurons were evoked with a brief current injection (1–3 ms duration) and uIPSCs recorded in the L5 PN from a holding potential of –74 mV. Only responses with $2 \times$ S.D. of baseline noise were considered being connected. Both fast and slow capacitances were fully compensated, series-resistance compensation was applied to ~80-90% and the current and voltage traces acquired at 50 kHz. For stable recordings with > 50 uIPSCs the episodes were temporally aligned to the AP and the uIPSCs were fit with a multiexponential function in Igor Pro. The curve fitting detected the baseline, uIPSC onset, rise time, peak amplitude and decay time and was manually monitored. Fits were either accepted or rejected (e.g. when artefacts were present) and the number uIPSC failures were noted for each recording.

In vitro optogenetics

50 nL of AAV1 particles (titer 1×10^{12} cfu mL⁻¹) produced from pAAV-EF1a-double-floxed-hChR2(H134)-EYFP-WPRE-HGHpA (Addgene.org #20298, RRID:Addgene_20298) was injected into L5 of S1 (co-ordinates from bregma; AP-0.15 mm ML-0.30 mm and DL-0.75 mm) of 6–9 weeks old PV-Cre; Ai14 mice. About 7 days after the injection, a subset of mice was placed on 0.2% cuprizone diet for 8 to 9 weeks. PV⁺ interneurons expressing hChR2 were identified using td-tom and YFP co-expression. Whole-cell voltage-clamp recordings were made

from L5 PNs and optically induced inhibitory postsynaptic currents (oIPSCs) were evoked with a X-cite 120Q, fluorescent lamp using filter BA460-510 (Olympus) in the presence of CNQX (50 μ M) and dAP5 (20 μ M) in the bath solution. The oIPSCs were evoked by illumination of large field with 5 light pulses of each 1 ms and 100 ms apart. Peak amplitude and area under curve (charge) of oIPSC was quantified using Axograph. Only the first pulse was used for the quantification.

In-vivo electrophysiology and automated event detection

Chronic ECoG and LFP recordings were performed using in-house made electrodes of platinum-iridium wire (101R-5T, 90% Pt, 10% Ir, complete diameter of 200 μ m with 127 μ m metal diameter, Science Products). The perfluoroalkoxy alkanes (PFA) coated wire platinum-iridium wire was only exposed at the tip to record the local field potential (LFP). For placement of the recording electrode, animals were anesthetized with isoflurane (3%, flow rate 0.8 L/min with maintenance 1.5–1.8%, flow rate 0.6 L/min). A 1 cm midline sagittal incision was made starting above the interaural line and extending along the neck to create a pocket for subcutaneous placement of the transmitter along the dorsal flank of the animal. The recording electrodes in each hemi-sphere (stereotaxic coordinates relative to bregma: S1; –0.15 mm anterior and $\pm$ 0.30 mm lateral; for LFP; ventral 0.75 mm, V1; 0.40 mm anterior and $\pm$ 0.30 mm lateral; for LFP; ventral 0.75 mm) and ground electrode (6 mm posterior and 1 mm lateral) were implanted subdurally through small holes drilled in the skull, held in place with stainless steel screws (A2-70, Jeveka) and subsequently sealed with dental cement. Mice were provided with Metachem analgesic (0.1 mg per kg) after surgery and allowed to recover for 4–7 days before recordings. To obtain multiple hours recordings of ECoG-LFP at multiple weeks, mice remained in their home cage during an overnight recording session. ECoG–LFP data were collected using a

ME2100-system (Multi channel Systems); ECoG-LFP data were acquired at a sampling rate of 2 kHz using the multi-channel experimenter software (Multi channel systems). An additional 0.1–200 Hz digital band-pass filter was applied before data analysis. Large noise signals, due to excessive locomotion or grooming, were manually removed from the data. The ECoG and LFP recordings were processed offline with the Neuroarchiver tool (Open Source Instruments, http://www.opensourceinstruments.com/Electronics/A3018/Seizure_Detection.html). To detect interictal spikes an event detection library was built as described previously (Dubey et al., 2018). During the initial learning phase of the library the observer, if needed, overruled the identity of each new event by the algorithm, until automated detection reached a false positive rate < 1%. Subsequently, the ECoG-LFP data were detected by using a single library across all ECoG-LFP recordings. For determining the interictal rate, only S1 LFP signals were used for quantification.

In-vivo optogenetics with simultaneous ECoG-LFP recordings

50 nl of AAV1 particles (titer 1×10^{12} cfu ml⁻¹) produced from pAAV-EF1a-double-floxed-hChR2(H134)-EYFP-WPRE-HGHpA (Addgene #20298, RRID:Addgene_20298) was injected unilaterally into the L5 of S1 (coordinates from bregma; AP-0.15 mm ML-0.30 mm and DL-0.75 mm) of 6–9 weeks old PV-Cre; Ai14 mice. ECoG-LFP electrode (stereotaxic coordinates relative to bregma: –0.15 mm anterior and $\pm$ 0.30 mm lateral; for LFP; ventral 0.75 mm) and ground electrode (6 mm posterior and 1 mm lateral) were implanted through small holes drilled in the skull, held in place with stainless steel screws (A2-70, Jeveka). Through the drilled hole, a polished multimode optical fiber (FP200URT, Thorlabs) held in ceramic ferrule (CFLC230-10, Thorlabs) was driven into the layer 5 and ~50 μ m above virus injection site. Once optical fiber and electrode were correctly placed, the drilled hole subsequently sealed with dental cement. A blue fiber-coupled laser (473 nm, DPSS Laser T3, Shanghai Laser & Optics Co.) was used to

activate the ChR2. Cyclops LED Driver (Open ephys) together with customized program was used to design the on and off state of the laser. The driving signal from LED driver was also recorded at one of the empty channels in multi-channel systems. This signal was used to estimate the blue light on or off condition. For gamma entrainment in S1, 40 pulses of blue light were flashed with 1 ms on and 28 ms off pulse.

To inhibit interictal spikes, 300 pulses of blue light were flashed with 1 sec on and 100 ms off by manual activation of light pulses when periods of high interictal spikes were observed (> 10 interictals/min). Aged-matched control mice were stimulated during the resting phase of the EEG, which was estimated using online EMG signal and video observation. For interictal counts, 5 min LFP signals were used from before light stimulation, during, and post light stimulation. Interictal were detected using event detection library. For analysis of the cortical rhythms, epochs were extracted using 2 second window at the start and after 180 pulses of blue light. Epoch containing interictal were not included in the analysis.

For pharmacology experiment, continuous LFP recordings of >10 -12 hours duration from the circadian quiet phase (from 19:00 to 09:00) of 6 cuprizone mice (7 weeks treatment) and 3 control mice were used for the analysis. To activate GABA_A receptors in cuprizone-treated mice, we used diazepam (Centrafarm Nederland B.V) prepared in a 10% solution of (2-Hydroxypropyl)- β -cyclo-dextrin (Sigma-Aldrich). A non-sedative dose of 2 mg kg^{-1} diazepam was injected intraperitoneally, and data was acquired for a period of 10 hours, starting 15 min after injection of drug in control and cuprizone mice. The automated event detection library (**Figure 1-supplement 1**) was used to determine the event frequency before and after diazepam injection.

In-vivo power spectrum analysis

Power spectral density (PSD) analysis was done using multi-taper PSD toolbox from Igor Pro 8.0 (RRID:SCR_000325). The absence of high voltage activity in the EMG electrode was classified as quiet wakefulness (**Figure 1–figure supplement 1** and **Figure 1–video 1**). For PSD analysis during interictal activity, a 2 sec window was used to extract LFP signal epochs. Epochs from control animals were selected comparing the EMG activity with cuprizone EMG activity. The interictal activity itself was excluded from the analysis. Selected LFP epochs were band pass filtered between different frequency bands; delta, δ , (0.5-3 Hz), theta, θ , (4-12 Hz), beta, β , (12.5-25 Hz) and gamma, γ , (30-80 Hz). Multi-taper PSD function (Igor Pro 8.0) was applied to the filtered the data to plot the power distribution within each frequency band. Areas under the curve was measured for each frequency band to compare power density between the control and cuprizone groups.

Immunohistochemistry

L5 PNs were filled with 10 mg ml⁻¹ biocytin during whole-cell patch clamp recording for at least 30 minutes. Slices were fixed for 30 min with 4% paraformaldehyde (PFA) and stored in 0.1 M phosphate buffered saline (PBS; pH 7.4) at 4 °C. Fixed 400 μ m slices were embedded in 20% gelatin (Sigma-Aldrich) and then sectioned with a Vibratome (VT1000 S, Leica Microsystems) at 80 μ m. Sections were pre-incubated with blocking 0.1M PBS containing 5% normal goat serum (NGS), 5% bovine-serum albumin (BSA; Sigma-Aldrich) and 0.3% Triton-X (Sigma) during 2 hours at 4 °C to make the membrane permeable. For biocytin-labelled cells, streptavidin

biotin-binding protein (Streptavidin Alexa 488, 1:500, Invitrogen, RRID:AB_2315383) was diluted in 5% BSA with 5% NGS and 0.3% Triton-X overnight at 4 °C. Sections including biocytin-filled cells were incubated again overnight at 4 °C with primary antibody rabbit anti-βIV-spectrin (1:200; gift from M.N. Rasband, Baylor College of Medicine), mouse anti-myelin basic protein (MBP) (1:250; Covance), mouse anti-PV (1:1000; Swant, RRID:AB_10000343) rabbit anti-syt2 (1:500, Synaptic Systems, RRID:AB_108 94084) in PBS blocking solution containing 5% BSA with 5% NGS and 0.3% Triton-X. Secondary antibody were used to visualize the immunoreactions: Alexa 488-conjugated goat anti-rabbit (1:500; Invitrogen), Alexa 488 goat anti-mouse (1: 500; Sanbio), Alexa 488 goat anti- guinea pig, Alexa 555 goat anti-mouse (1:500; Invitrogen), Alexa 555 goat anti-rabbit (1:500; Invitrogen), Alexa 633 goat anti-guinea pig (1:500; Invitrogen), Alexa 633 goat anti-mouse (1:500; Invitrogen) and Alexa 633 goat anti-rabbit (1:500; Invitrogen). Finally, sections were mounted on glass slides and cover slipped with Vectashield H1000 fluorescent mounting medium (Vector Laboratories, Peterborough, UK) and sealed.

Confocal imaging

A confocal laser-scanning microscope SP8 X (DM6000 CFS; acquisition software, Leica Application Suite AF v3.2.1.9702, RRID:SCR_013673) with a 63× oil-immersion objective (1.3 NA) and with 1× digital zoom was used to collect images of the labelled L5 neurons and the above-mentioned proteins. Alexa fluorescence was imaged using corresponding excitation wavelengths at 15 units of intensity and a z-step of 0.3 μm. Image analysis was performed with

Fiji (ImageJ) graphic software (v.2.0.0-rc-65/1.5w, National Institutes of Health, RRID:SCR_002285).

Synaptic puncta counting and image analysis

The intensity of PV⁺ or Syt2 immunostaining was measured with a z-axis profile, calculating the mean RGB value for each z-plane. When quantifying the axosomatic puncta, the soma was defined to extend into the apical dendrite maximally ~4 µm and a boundary was drawn around the maximum edges (ROI). For counting apical dendritic puncta, a 200 µm length of apical dendrite was selected as ROI. Linear immunofluorescent signals from βIV-spectrin were identified as AIS and used as ROI. For all analyses, the RGB images were separated into single color channels using the color deconvolution plugin in Image J. The single-color channel containing boutons signals was subjected to thresholding and particle filter of 0.5 µm. The threshold was saved and applied to all images in the same staining group. The boutons were selected by scanning through the 3D projection of ROI with 0.35 µm z-steps. Trained experimenters identified the boutons either by colocalization of the ROI and PV/Syt2 or direct contact of the two. The boutons were characterized as round spots with a minimal radius of 0.5 µm ranging to almost 2 µm. Three experimenters blinded to the identity of the experiment group independently replicated the results. All image analysis was done in Fiji (ImageJ) graphic software (v.2.0.0-rc-65/1.5w, National Institutes of Health, RRID:SCR_002285).

PV⁺ axon reconstruction and quantification

For immunolabeling of biocytin-filled PV⁺ interneuron, 400 µm electrophysiology slices were incubated overnight at 4 °C in PFA. Slices were rinsed with PBS followed by staining using streptavidin 488 (1:300, Jackson) diluted in PBS containing 0.4% Triton-X and 2% normal horse

serum (NHS; Gibco) overnight at 4 °C. Confocal images of 400 µm thick slices were taken (see Methods, Confocal Imaging) and immediately after, thoroughly rinsed with 0.1M PB and 30% sucrose at 4 °C overnight. Next, slices were sectioned into 40-µm thick and preserved in 0.1 M PB before staining. Sections were pre-incubated in PBS blocking buffer containing 0.5% Triton-X and 10% NHS during one hour at room temperature. Sections were stained with primary mouse anti-MBP (1:300, Santa Cruz, RRID:AB_675707), rat anti-syt2 (RRID:AB_108 94084) in 0.4% Triton-X and 2% NHS with PBS solution for 72 h. Alexa 488-conjugated secondary antibodies (1:300, Invitrogen) were added in PBS containing 0.4% Triton-X and 2% NHS, posterior to washing steps with PBS. Then, sections were mounted on slides and cover slipped with Vectashield H1000 fluorescent mounting medium, sealed and imaged. Biocytin-labelled PV⁺ neurons were imaged using upright Zeiss LSM 700 microscope (Carl Zeiss) with 10× and 63× oil-immersion objectives (0.45 NA and 1.4 NA, respectively) and 1× digital zoom with step size of 0.5 µm. Alexa 488 and Alexa 647 were imaged using 488 and 639 excitation wavelengths, respectively. The 10× image was taken to determine the exact location of biocytin-filled cells. Subsequently, axonal images were taken at 63× magnification. Axons were analyzed as described previously (Stedehouder et al., 2019) and identified by their thin diameter, smoothness, obtuse branching processes and occasionally by the presence of the axon bleb. Images were opened in Neurolucida 360 software (v2018.02, MBF Bioscience, RRID:SCR_001775) for reconstruction using the interactive user-guided trace with the Directional Kernels method. Axon and myelinated segments were analyzed using Neurolucida Explorer (MBF Bioscience, RRID:SCR_001775). Axonal segments were accepted as myelinated when at least one MBP-positive segment co-localized with streptavidin across the internode length.

Statistics

All statistical tests were performed using Prism 8 or 9 (GraphPad Software, LLC, San Diego, CA, RRID:SCR_014284). For comparisons of two independent groups, we used two-tailed Mann-Whitney U tests. For multiple group comparisons, data were initially assessed for normality and subsequently we either used ordinary one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons or two-way ANOVA with repeated measures followed by Šidák's multiple comparisons tests to correct for multiple comparisons. The level of significance was set to 0.05 for rejecting the null hypothesis. A detailed overview of the statistical analyses performed in this study together with the numbers used for figures and statistical testing is provided in **Source Data 1**.

Data availability

The raw ECoG and EMG data of **Figure 1d**, for the 8-weeks period, is accessible via Dryad (doi:10.5061/dryad.pk0p2ngpk) and available during the review process via this link: <https://datadryad.org/stash/share/0HSJ4h6Zbd-vMa5W8PBYQ8qwrT6fxU85srjcpigLhqk>

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Figure Legends

Figure 1. Loss of compact myelin causes interictal spikes and behavioral state-dependent amplification of theta rhythms

(A) Schematic of the ECoG and LFP recordings in freely moving mice. Electrodes were placed right (S_R) and left (S_L) in the primary somatosensory cortex, and a left LFP electrode (S_{L-LFP}) into L5. A similar array of electrodes was positioned in the primary visual cortex (V_R , V_L and V_{L-LFP}). One electrode was placed around neck muscle recording electromyography (EMG) and one used as reference (Ref). (B) Interictal spikes (*) appear from 4 weeks cuprizone and onwards. Example raw LFP traces (S_{L-LFP}). (C) Representative interictal spike example showing spatiotemporal synchronization of the spike across cortical areas and hemispheres. Higher magnification of interictal spikes (red, ~50 to 300 ms duration) overlaid with the average (black). (D) Cuprizone treatment caused a progressively increasing frequency of interictal spikes (Mixed-effects two-way ANOVA. Time $\times$ treatment interaction $P < 0.0001$, Šidák's multiple comparison

tests, cupri vs con; 4 weeks $P = 0.121$, 6 weeks $*P = 0.0406$ and 8 weeks $**P = 0.0054$. (E) Power spectral content during two different brain states, awake and moving (am, dotted lines) and quiet wakefulness (qw, solid lines) in control (black) and cuprizone (red). Red arrow marks amplified theta band power (θ) during quiet wakefulness in cuprizone mice (Cupr_qw). (F) Cuprizone amplifies selectively θ power during quiet wakefulness (Two-way ANOVA treatment $P < 0.0001$, Šidák's multiple comparisons cupr vs con; for δ , $P = 0.8118$; θ , $P = 0.0013$; β , $P = 0.8568$ and for γ , $P = 0.9292$) but not during moving (Two-way ANOVA treatment $P = 0.0575$). (G) *Left*, Schematic of ECoG and LFP recordings from $Mbp^{+/+}$ and Mbp^{Shi} mice with example trace showing interictal spikes (*). *Right*, overlaid individual interictal spikes in Mbp^{Shi} mice (red) combined with the population average (black). (H) Bar plot of interictal rate in Mbp^{Shi} mice. Two-tailed Mann-Whitney test $**P = 0.0095$. Data show mean $\pm$ SEM with gray lines (D) or open circles (H) individual mice.

Figure 2. Demyelination preserves PV⁺ interneuron number and morphology but reduces excitability. (a) *Left*, confocal z-projected overview image of S1 in a PV-Cre; Ai14 mouse (tdTomato⁺, red) overlaid with myelin basic protein (MBP, cyan). *Right*, overview image showing loss of MBP after 6 weeks cuprizone. (b) myelinated PV⁺ axons in control (*left*) and PV⁺ axons demyelination with cuprizone treatment (*Right*). (c) EM of transverse cut tdTomato⁺ immunogold-labelled axons (false colored red). *Right*, higher magnification of immunogold particles and ultrastructure of the PV interneuron myelin sheath. (d) PV⁺ interneuron number across the cortical lamina was not affected by cuprizone treatment (Two-way ANOVA, treatment effect $P = 0.6240$). (e) *Top*, example of a high-resolution 3D reconstruction of a biocytin-labelled PV axon (red) labelled with MBP (cyan) of a control mouse, showing the first

~6 axonal branch orders. *Bottom*, control axonogram showing axon branch order and myelinated segments (cyan). **(f-g)** Total axon branch number and length are not changed by demyelination (Mann-Whitney tests $P = 0.3032$ and $P = 0.8822$, respectively). n.s., not significant. **(h)** *Left*, brightfield/fluorescence overlay showing patch-clamp recording from a tdTomato⁺ interneuron. *Right*, example PV⁺ interneuron APs from control (dotted line) and cuprizone treated mice (continuous lines). **(i)** Steady-state sub- and supra-threshold voltage responses during 700 ms current injections. Firing rates near threshold reduced and the **(j)** rheobase current significantly increased in cuprizone (*** $P = 0.0003$). **(k)** PV BCs showed a ~4 mV hyperpolarized resting membrane potential (** $P = 0.0036$). Data show mean $\pm$ SEM and open circles individual cells.

Figure 3. Demyelination decreases connectivity, reliability and rapid facilitation of PV⁺ unitary IPSCs

(a) Immunofluorescence image of a connected control PV⁺ BC (red) and L5 PN (white). **(b)** Example traces of ten single trial uIPSC traces (gray) overlaid with mean average (>60 trials, black). Inset, uIPSCs abolished by gabazine (GABA_A blocker, 4 μ M). **(c)** Cuprizone-treated mice show significantly lower connection probability between PV⁺ BC and L5-PN (Chi-square test * $P = 0.0182$, $n = 78$ pairs). **(d)** Example fits (blue) of uIPSCs for rise- and decay time, amplitude, failure rate, amplitude and latency analyses (δ , AP to 10% uIPSC peak amplitude). **(e)** Cuprizone increased failures by 5-fold and the average peak amplitude by ~2.5-fold (Mann-Whitney test ** $P = 0.012$) and **(f)** reduces the mean amplitude (Mann-Whitney test * $P = 0.0256$). **(g)** uIPSCs latency remained unchanged (Mann-Whitney test n.s., $P > 0.999$). **(h)** Cuprizone impairs short-term facilitation. Dotted line indicates expected amplitude for uIPSC₂ (scaled from uIPSC₁). **(i)** *Left*, confocal z-projected image of a control PV⁺ axon (red)

immunostained with Syt2 (green). *Right*, surface rendered 3D-image of the same axon. **(j)** Example sections of 3D reconstructions. **(k)** Cuprizone increased the Syt2⁺ puncta spacing by ~2-fold (Mann-Whitney test **** $P < 0.0001$, $n = 12$). Data shown as mean $\pm$ SEM and open circles individual axons or pairs. n.s., not significant.

Figure 4. Demyelination impairs phasic PV⁺ BC inhibition of L5 PNs

(a) Immunofluorescent image of AAV1-hChR2-YFP expression (green) injected into L5 of S1. **(b)** Confocal images of separate fluorescent channels showing td-Tomato⁺ cell bodies and neurites (red, top), the localization of AAV1-hChR2-YFP (green, middle) and the merge image (bottom). The majority of tdTomato⁺ cells were YFP⁺ (white arrows). **(c)** Average transfection rate of AAV1-hChR2-YFP in the L5 (>70%) is comparable in control and cuprizone conditions (Mann-Whitney test $P = 0.889$, $n = 5$). **(d)** schematic showing full-field blue light optogenetically-evoked postsynaptic inhibitory currents (oIPSCs) in L5 PNs. **(e)** Example trace of a whole-cell current-clamp recording from a PV⁺ interneurons (bottom red) compared to a separate whole-cell voltage-clamp recordings from a L5 PN. A 1 sec blue light field illumination (blue bar) produces sustained firing in *PV-Cre AAV1-ChR2* interneurons. **(f)** Single trial oIPSCs (gray) from different experiments (1 sec duration pulses) overlaid with the average oIPSC (black) revealing a lower peak amplitude in cuprizone (red arrow). **(g)** Population data revealed a ~2-fold reduction in oIPSCs peak amplitude (Mann-Whitney test $P = 0.0172$). **(h)** Steady-state oIPSCs amplitude did not reach significance, n.s., Mann-Whitney test $P = 0.0789$.

Figure 5. Demyelination impairs optogenetically evoked γ entrainment

(a) Schematic for chronic LFP recordings and *in vivo* optogenetic stimulation in freely moving PV-Cre; Ai14 mice. (b) Time frequency plot showing low gamma frequency (γ) entrainment (40 blue light pulses of 1-ms duration at 30 Hz) in control but not in cuprizone mice. (c) raw LFP (*top*) and band pass filtered trace (25–40 Hz, *bottom*) from control and cuprizone during low- γ entrainment. (i) Population data of γ power with increasing laser power reveals impaired γ in cuprizone-treated mice. (d) Myelin deficient mice low- γ band entrainment to optogenetic stimuli (two-way ANOVA followed by Šidák's multiple comparisons cuprizone vs control, 0 mW, $P > 0.999$; 1.5 mW, $P = 0.979$; 4.5 mW, $*P = 0.0221$, 6.5 mW, $**P = 0.0046$). (e) Data are shown as mean $\pm$ SEM with gray lines individual cells, gray lines individual mice. n.s., not significant.

Figure 6. Optogenetic activation of myelin deficient PV⁺ interneurons rescues theta rhythm and interictal epileptiform discharges

(a) Schematic drawing for chronic LFP and optogenetic stimulation in freely moving mice. A 1 sec blue light pulse with 100 ms off periods activated PV⁺ interneurons. Blue light was switched on during high interictal activity (>10 spikes/min). (b) power spectral content collected from 2 sec epoch windows in control (black) and cuprizone (red) before (left) and during 3 min (right) optogenetic activation of PV⁺ interneurons. *Insets*, raw LFP signals (*top*) and θ content (*bottom*) in control (black) and cuprizone (red) condition. (c) Population data showing optogenetic activation of PV⁺ cells attenuated the amplified θ frequency in cuprizone mice to control levels (Šidák's multiple comparisons test in cuprizone, light on vs light off for θ , $**P = 0.0076$). (d) Example time frequency plot (*top*) and raw LFP traces (*below*) showing suppression of interictal spikes during light on conditions. See also **Figure 6–video 1**. (e) Population data of transient optogenetic suppression of the interictal activity in 8 weeks-cuprizone treated mice (One-way

ANOVA $P = 0.0165$, Tukey's multiple comparison tests; off vs on, $**P = 0.0089$; on vs off, $P = 0.0539$; off versus off, $P = 0.928$). (f) 2 mg/kg i.p injection of diazepam in cuprizone-treated mice reduces interictal rate for at least 10 hours (two-tailed Student's t test $*P = 0.024$). Data show mean $\pm$ SEM and grey lines individual mice.

Figure supplement legends

Figure 1—figure supplement 1. State-dependent interictal activity in cuprizone mice and automated interictal event detection library

(a) Automated event detection library used for interictal classification. Three-dimensional projection metric space, showing coherence, spikiness and signal power (a.u. = arbitrary units), with colors corresponding to interictal (red) and baseline/normal (black) events. The event library was constructed by an operator who classified events as “normal” or interictal events. The blue domain represents the population of interictal events from Mbp^{Shi} mice. (b) Example of a 30 s recording from multiple electrodes from 6 weeks-cuprizone treated mouse. *Left*, traces during the awake state, note the high voltage EMG activity. *Right*, same mouse during quiet wakefulness with low EMG activity. Interictal discharge indicated with red asterisk (*). (c) Example traces showing raw LFP signals from S1 (top) and bandpass filtered traces (bottom) at different brain states in control (black) and cuprizone (red) for delta (δ , 0.5-3.5 Hz), theta (θ , 4-12 Hz), beta (β , 12.5-25 Hz) and gamma (γ , 30-80 Hz).

Figure 1–figure supplement 2. Ictal, interictal activity and power spectrum in Mbp^{Shi} mice

(a) Schematic drawing showing ECoG and LFP recordings from S1 of $Mbp^{+/+}$ and Mbp^{Shi} mice. Example LFP trace showing pre-ictal and ictal discharge from 8 weeks old Mbp^{Shi} mouse (top). Bottom, higher temporal resolution of the top LFP trace showing ECoG activity with interictals (1) and ictal discharge (2 and 3). (b-c) Comparable power spectral content during interictal activity in cuprizone (red) and Mbp^{Shi} (blue). (Two-way ANOVA myelin models $P < 0.0001$, Šidák's multiple comparisons cuprizone vs Mbp^{Shi} ; for δ , $P = 0.9952$; θ , $P > 0.9999$; β , $P > 0.9999$ and for γ , $P = 0.9346$). Data show mean $\pm$ SEM.

Figure 2–figure supplement 1. Cuprizone treatment causes loss of PV^+ axon myelination but preserves axon length and complexity

(a) *Left*, confocal image of control staining in L5 of S1 showing a PV^+ interneuron. *Right*, higher magnification of the image in *right*, illustrating the trajectory of a myelinated PV^+ interneuron axon (yellow arrows). Note that also PV^+ axon swellings are frequently myelinated. (b) A confocal z -stack image examples of the L5 region in control (left) and cuprizone (right) treated mice. (c) Axonograms of a control and (d) a cuprizone axon. Myelinated segments indicated with cyan. (e, f) Segment number and length per branch order. Both number and length of axon segments were not significantly affected by cuprizone-induced demyelination (Two-way ANOVA Treatment $\times$ Branch order interaction $P = 0.8028$ and $P = 0.6236$, respectively). Data show mean $\pm$ SEM.

Figure 3—figure supplement 1. Cuprizone decreases miniature IPSCs and somatodendritic PV puncta numbers

(a) *Left*, Example traces of mIPSCs at the soma of L5 pyramidal neurons in the presence of CNQX, d-AP5 and TTX in control (top) and demyelinated conditions (bottom). (b, c) Population data showing a ~3-fold mIPSC peak amplitude but not frequency reduction (Mann-Whitney test $**P = 0.0025$; n.s., $P = 0.728$). (d) Example images of maximum z-projection of a biocytin filled L5 PNs (white) overlaid with PV immunofluorescence (red). Dotted lines indicate the soma borders with number indicating counted PV⁺ puncta. (e, f) Population analysis reveals a significant loss in the number of PV⁺ puncta at the L5 soma (Mann-Whitney test $*P = 0.0035$) and the primary proximal apical dendrite ($< 200 \mu\text{m}$, $*P = 0.0044$). (g, h) Example confocal z-stack images of L5 in control (left) and cuprizone treated mice (right) reveals a global significant reduction in PV immunofluorescence intensity. (i) Regression plot reveals the mean PV immunofluorescence intensity correlates with the number of PV⁺ boutons on large NeuN⁺ pyramidal neurons cell bodies ($r^2 = 0.755$). NeuN⁺ immunofluorescent signals are not shown. The soma diameters of pyramidal neurons were unchanged (cuprizone $18.23 \pm 0.99 \mu\text{m}$, $n = 6$ vs. $18.37 \pm 0.68 \mu\text{m}$, Mann-Whitney U test $P = 0.954$, $n = 9$).

Figure 3—figure supplement 2. Putative PV⁺ chandelier inputs at the AIS are unaffected by cuprizone-induced demyelination.

(a) A confocal z-projection of a biocytin filled (cyan) L5 pyramidal neuron overlaid with βIV spectrin (green) and parvalbumin (PV). (b) The number of putative chandelier PV⁺ boutons were

preserved in cuprizone-treated mice (Mann-Whitney test $P = 0.96$). Data are shown as mean $\pm$ SEM with open circles individual neurons. n.s., not significant.

Figure 3—figure supplement 3. Demyelination and dysmyelination reduces miniature IPSCs and perisomatic Syt2⁺ puncta

(a) Maximum z -projection of biocytin-filled L5 PN (white) overlaid with Syt2⁺ immunofluorescence (green). Numbers indicate the Syt2⁺ puncta counted at the soma. (b) Population analysis reveals a significant Syt2⁺ puncta loss (two-tailed Mann-Whitney U test $*P = 0.0216$). (c) Maximum z -projection of a biocytin filled L5 soma (white) overlaid with Syt2⁺ immunofluorescence (green) from $Mbp^{+/+}$ and Mbp^{Shi} mice. (d) Population analysis shows a significant loss of Syt2⁺ puncta in the Mbp^{Shi} mice ($**P = 0.0013$). (e) Example traces of mIPSCs of L5 PNs in the presence of CNQX, d-AP5 and TTX in $Mbp^{+/+}$ (top) and Mbp^{Shi} mice (bottom). (f, g) mIPSCs peak amplitude was unaffected but frequency is reduced in Mbp^{Shi} (n.s. $P = 0.797$ and $*P = 0.019$, respectively). Data are shown as mean $\pm$ SEM with open circles individual neurons. n.s., not significant.

Figure 4—figure supplement 1. Demyelination does not affect excitatory drive of PV⁺ BCs

(a) *Left*, schematic of whole-cell voltage-clamp recording for mEPSCs in identified parvalbumin interneurons in the PV-Cre; Ai14 mouse line. mEPSCs were recorded in control mice and mice with 6-weeks cuprizone feeding. (b, c) Population data of mEPSC recordings revealed no difference in amplitude (Mann-Whitney test n.s. $P = 0.240$) nor mEPSC frequency (Mann-

Whitney test, n.s. $P = 0.937$). Data are shown as mean $\pm$ SEM with open circles individual neurons. n.s., not significant.

Figure 5—figure supplement 1. Myelin loss abolishes optogenetically evoked entrainment of γ rhythm

(a) Raw LFP and low gamma (γ) 25-40 Hz band-pass filtered trace during 30 Hz blue light stimulation in control (black) and cuprizone (red) mice. Blue light shifted the phase or extended the γ cycle in control mice. (b) Averaged power spectral density content of low- γ entrainment during *light on* (blue lines) or *off* (black lines). (c) 30 Hz blue light stimulation (blue lines) showed a lack of γ band entrainment in cortex of demyelinated mice (closed triangles) in comparison to the control mice (closed squares). Two-way ANOVA frequency $\times$ treatment $P = 0.0002$, Šidák's multiple comparison tests; γ , **** $P < 0.0001$; all other bands $P > 0.910$. Data show mean $\pm$ SEM.

Figure 6—figure supplement 1. GABA_A receptor agonism suppresses interictal epileptiform discharge frequency

(a) The GABA_A receptor agonist, diazepam, was injected i.p. at 7 weeks of cuprizone treatment, and LFP recordings performed 10 hours post diazepam injection (b) Example time frequency plot before (*left*) and after diazepam injection (*right*) showing suppression of interictal epileptiform discharges in cuprizone-treated mice.

Figure 1—video 1. Brain-state dependent interictal spikes

Example video of ECoG, LFP and behavioral recordings in a 6-weeks cuprizone treated mouse. Raw signals show ECoG from right and left primary somatosensory cortex (S_R , S_L , respectively) and LFP from the left L5 region (S_{L-LFP}). Similar configuration for primary visual cortex (V_R , V_L and V_{L-LFP}). An additional electrode was connected to the neck muscle recording electromyography (EMG). Note the high EMG activity during awake and moving states. Interictal epileptiform discharges was automatically detected (red asterisks) and only occur during quiet wakefulness.

Figure 6–video 1. Optogenetic activation of myelin-deficient PV^+ interneurons attenuates interictal spikes

Example video of ECoG, LFP and behavioral recordings in a 7-weeks cuprizone treated mouse. Raw signals show ECoG from right primary somatosensory cortex (S_R) and LFP from the layer 5 region (S_{R-LFP}). Interictal epileptiform discharges (red asterisks) were suppressed during laser-induced optogenetic activation of PV^+ interneurons in layer 5.

Figure 1

a **b** **c**

a **b** **c**

Figure 3

a PV Biocytin β IV spectrin

b

Figure 4

Figure 5

a

b

c

a **b**