Myelinating satellite oligodendrocytes are integrated in a glial syncytium constraining neuronal high-frequency activity

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Satellite oligodendrocytes (s-OLs) are closely apposed to the soma of neocortical layer 5 pyramidal neurons but their properties and functional roles remain unresolved. Here we show that s-OLs form compact myelin and action potentials of the host neuron evoke precisely timed Ba²⁺-sensitive K⁺ inward rectifying (Kir) currents in the s-OL. Unexpectedly, the glial K⁺ inward current does not require oligodendrocytic Kir4.1. Action potential-evoked Kir currents are in part mediated by gap-junction coupling with neighbouring OLs and astrocytes that form a syncytium around the pyramidal cell body. Computational modelling predicts that glial Kir constrains the perisomatic [K⁺]o increase most importantly during high-frequency action potentials. Consistent with these predictions neurons with s-OLs showed a reduced probability for action potential burst firing during [K⁺]o elevations. These data suggest that s-OLs are integrated into a glial syncytium for the millisecond rapid K⁺ uptake limiting activity-dependent [K⁺]o increase in the perisomatic neuron domain.
Neuron–glia interactions are critical for diverse functions of the central nervous system. Recent work shows that activity of glia modulates long-term synaptic potentiation or depression, controls rhythmic network activity, and contributes to neuronal dysfunction. A tight organization of the glial syncytium around neurons has previously been observed to contribute to regulation of neuronal activity, suggesting essential functional roles of local glia arrangements. In the grey matter, all glia cell types, including astrocytes and microglia, can be found in a satellite position around neurons but oligodendrocytes (OLs) are most frequently observed in the glial syncytium around neurons has previously been observed. These OLs are referred to as perineuronal or satellite OLs (s-OLs). The present view of s-OLs is that they are primarily non-myelinating, in contrast to the main function of OLs as myelin-producing cells. However, s-OLs can provide metabolic support for neurons, protect neurons against apoptosis or remyelinate axons following demyelinating injuries. Since s-OLs are often located at the base of the soma, close to the action potential (AP) initiation site and the axon initial segment (AIS), we have hypothesized that their privileged position enables s-OLs to influence AP firing.

To investigate the functional and anatomical properties of s-OLs, we combine simultaneous whole-cell patch-clamp recordings and live-confocal imaging with post hoc immunofluorescence and electron microscopy of neuron–s-OL pairs in acute neocortical slices from adult mice. We find that s-OLs myelinate surrounding axons and exhibit time-locked Ba2+- and carbamol-sensitive inward currents in response to APs generated in the host neurons. Unexpectedly, the AP-evoked inwardly rectifying K⁺ (Kir) currents are not mediated by somatically expressed OL-specific Kir4.1 channels. Instead, the AP-evoked currents reflect junctional coupling to nearby astrocytes forming together with s-OLs a perisomatic glial syncytium. Moreover, during repetitive high-frequency AP firing of pyramidal neurons, s-OL coupled syncytia constrain the neuronal AP generation during periods of fast accumulation of K⁺. These results suggest that s-OLs within a glial syncytium perform multiple functional and anatomical roles, spatially buffering K⁺ and myelinating axons within the perisomatic domain.

Results

Distribution of s-OLs around neocortical layer 5 neurons.

To study s-OLs, we used a transgenic mouse line that expressed enhanced cyan fluorescent protein (ECFP) under control of a PLP promoter (PLP-ECFP, see Experimental Procedures; Fig. 1a) and focused on the neocortical layer 5 region. Electron microscopy identification of ECFP expressing s-OLs (Fig. 1b) revealed a characteristic very close apposition to neuronal cell membranes over long stretches with an interstitial space of 12.3 ± 1.8 nm (n = 4 cells). A close apposition was also seen in confocal analyses of neuron–s-OL pairs in which s-OLs were often found to fit in concavely shaped membrane regions of layer 5 neuron cell bodies and primary basal dendrites (Fig. 1c; Supplementary Movie 1). Estimates of the opposing membrane area yielded an average of 83 ± 8 μm² (range: 60–110 μm², n = 6). The initial description of s-OLs noted that these cells often locate to the base of the soma. To examine whether s-OLs have a preferred location, we combined simultaneous whole-cell patch-clamp recordings and confocal imaging of ECFP expressing s-OLs in the transgenic PLP-ECFP mouse. Scale bar, 150 μm. (b) Left: s-OL (red pseudo-colour) in a PLP-ECFP mouse neocortex exhibits positive immuno-gold labelling in the cell body. The host neuron is pseudo-labelled in light green and nuclei are labelled with N. Scale bar, 3 μm. Right: higher magnification image of the box indicated in the left image, which shows a distinct and confined location of gold particle-labelled ECFP in the cell body (black dots). Scale bar, 200 nm. (c) Confocal image of labelled OLs in a PLP-ECFP mouse and two biocytin-filled L5 neurons and corresponding orthogonal views. The s-OL cell body is in direct contact with the neuronal soma. Processes of OLs are not visible because laser intensity was reduced to a minimum. Scale bar, 10 μm. Also, see Supplementary Movie 1. (d) Heatmap illustrating the location probability of 145 s-OLs in relation to the soma of an example L5 neuron (grey). s-OLs were positioned at multiple locations around the soma, but the highest percentages were found around the base of neurons close to the AIS and the basal dendrites. See also Supplementary Fig. 1.

Identified layer 5 s-OLs myelinate axons.

For electrophysiological recording and single-cell fluorescence characterization, we targeted putative s-OLs based on their position at the soma of medium- to large-sized layer 5 pyramidal neurons (Fig. 2a,b). s-OLs were recorded either individually (total n = 70) or during s-OL–neuron paired recordings (total n = 71 pairs). While various glia cell types can be in contact with neurons, OLs are most frequently in a satellite position. In line, satellite glia cells of wild-type (WT) animals were in some cases astrocytes or OL precursor cells (combined ~20%; Supplementary Fig. 1d). Quantification of the soma area from 16 neuron–s-OL pairs revealed that the soma size of s-OLs significantly and positively correlated with the host neuron cell body dimensions (Fig. 2c). Live-confocal scans showed that all s-OLs had internode-like
structures oriented in vertical, horizontal and various diagonal directions, and resembled myelin based on the transmitted light images (Fig. 2b; Supplementary Movie 2; Supplementary Fig. 2a). s-OLs had on average 32 ± 2 internodal-like branches with a mean length of 43.4 ± 1.3 μm (n = 32; Fig. 2d).

Myelination by s-OLs is unexpected as s-OLs are referred to as non-myelinating in the literature. To further examine whether axon wrapping by s-OLs forms compact myelin, we pursued two independent approaches. First, biocytin- and Alexa 594-filled s-OLs were positively post hoc labelled for myelin basic protein (MBP; n = 4; Fig. 2e). Second, to identify single s-OL internode–axon assemblies at the ultrastructural level, we filled s-OLs (n = 5 cells) with horseradish peroxidase. Electron microscopy processing (Supplementary Fig. 2b) revealed that s-OLs made on average ~4 myelin wraps (range: 3–7, n = 12; Fig. 2f) around axons with diameters ranging between 0.2 and 1.1 μm (Fig. 2g; Supplementary Fig. 2c). The average g-ratio was 0.70 ± 0.02 (n = 45 axons, n = 5 s-OLs; Fig. 2g). Notably, we found that s-OLs never myelinated the first internode of their host neuron. This is most likely explained by the length of the first internode of the layer 5 axon extending up to ~110 μm from the soma edge, which is beyond the maximum expansion of the s-OL internodes (~80 μm from the centre of the s-OL cell body, n = 40). These data highlight that all s-OLs myelinate axons of varying small diameter with short internodal processes, classifying them as type 1 OLs.15.

s-OLs sense APs at high temporal resolution. Whole-cell recordings revealed that s-OLs have a resting membrane potential of on average ~–86 mV, a very low input resistance (Rm) of ~13 MΩ and a linear current–voltage relationship (Supplementary Table 1; Supplementary Fig. 3a–c). Given the close proximity to layer 5 somata and AIS, we tested in dual-whole-cell recordings (Fig. 3a) whether s-OLs respond to APs. In response to a single AP, s-OLs displayed a small and transient inward current (12.5 ± 3 pA, n = 23) temporally aligned with the capacitive charge during the AP depolarization (Fig. 3b). The capacitive transient was followed by an inward current with amplitude of on average ~2.2 ± 0.3 pA (n = 7; Fig. 3c). Interestingly, the onset of this inward current aligned with the beginning of the repolarization of the AP and continued for several hundreds of milliseconds (Fig. 3d). Subthreshold current injections in neurons did not lead to detectable inward currents (n = 6; Supplementary Fig. 3d). Since layer 5 neurons can generate APs at frequencies up to ~300 Hz (ref. 19), we assessed the impact of a high-frequency train of APs and the response of s-OLs (Fig. 3e). Five APs with a frequency of 100 Hz resulted in an inward current of ~15.9 ± 1.8 pA in amplitude, cumulating with each AP and decaying double exponentially (τ1 = 39 ± 4 ms, τ2 = 235 ± 24 ms, τweighted = 74 ± 9 ms, n = 22). Due to the low input resistance in current-clamp configuration, five APs depolarized s-OLs by only 0.5 ± 0.1 mV (n = 5; Fig. 3e). The charge transferred per AP remained constant between 1 and

Figure 2 | Experimental targeting of s-OLs reveals compact myelin and wrapping of surrounding axons. (a) Illustration of the experimental approach. s-OL (red) and layer 5 neuron (black) targeted with patch-clamp pipettes in mouse primary somatosensory neocortex. (b) Left: Bright-field image of a neuron-s-OL pair. Patch pipettes are visible. Middle: right: Confocal maximum z-projected image of a neuron–s-OL pair after intracellular loading with Alexa 488 (neuron) and Alexa 594 (s-OL) dyes. The white box is displayed at higher magnification on the right. White arrows indicate one internode. All scale bars, 10 μm. See Supplementary Movie 2. (c) Correlation of soma size between neurons and s-OLs as estimated from two separate data sets. Closed circles display data from maximum z-projected live-confocal scans and open triangles are measurements from bright-field images. Both data sets were fit by linear regression (confocal scans: n = 76, correlation coefficient r² = 0.33, P = 0.02; bright field: n = 24, r² = 0.29, P = 0.007). (d) Distribution histogram of s-OL internode length measured from confocal live image z-stacks (n = 32 cells). The data were fit by a Gaussian function (red line, y = 24.36 e^(-0.5((x-39.30)/15.93)²)). Data presented are mean ± s.e.m. (e) A s-OL was filled with biocytin and Alexa 594 during recordings and subsequently post hoc labelled for myelin basic protein (MBP). Internodal structures are positively co-labelled by MBP and biocytin (red and white arrowheads). Scale bar, 10 μm. (f) Left: electron microscopy image of an identified horseradish peroxidase (HRP)-filled transversal cut s-OL process shows multiple layers of myelin wrapped around an axon. Gold particles are visible as black dots in the outer tongue and all myelin layers, suggesting a direct cytoplasmic connection with the cell body. Right: a high magnification image of the same s-OL process shows seven layers of myelin wraps (range: 3–7, n = 5 cells) with horseradish peroxidase. Electron microscopy processing (Supplementary Fig. 2b) revealed that s-OLs made on average ~4 myelin wraps (range: 3–7, n = 12; Fig. 2f) around axons with diameters ranging between 0.2 and 1.1 μm (Fig. 2g; Supplementary Fig. 2c). The average g-ratio was 0.70 ± 0.02 (n = 45 axons, n = 5 s-OLs; Fig. 2g). Notably, we found that s-OLs never myelinated the first internode of their host neuron. This is most likely explained by the length of the first internode of the layer 5 axon extending up to ~110 μm from the soma edge, which is beyond the maximum expansion of the s-OL internodes (~80 μm from the centre of the s-OL cell body, n = 40). These data highlight that all s-OLs myelinate axons of varying small diameter with short internodal processes, classifying them as type 1 OLs.15.
Figure 3 | Action potentials evoke time-locked inward currents in s-OLs. (a) Confocal z-projected image of dual-whole-cell recording from LS neuron–s-OL pair in the somatosensory neocortex. Scale bar, 20 μm. (b) The dV/dt of the AP aligns with the fast transient in the s-OL, reflecting the capacitive charge of the s-OL membrane during the rising phase of the AP. In current clamp, the s-OL membrane voltage change is slow (dV/dt) compared with the neuronal AP. Note the dV/dt is from a different recording and filtered for display. The other traces are from the same cell as in c and d. Scale bars, (top to bottom) □ pA, 1Vs, 30 mV (middle), 100Vs and 0.1 ms. (c) AP repolarization temporally aligns with a rapid inward current in the s-OL. Average of 35 trials; scale bars, 5 pA, 30 mV, 50 ms and 0.7 nA. Capacitive current transient clipped for clarity. (d) The single AP evoked a rapid inward current in the s-OL (red trace, top) that decayed slowly (held at –91 mV; black fit, t1 = 78 ms, t2 = 451 ms). Traces displayed are the average of 25 trials. Scale bars, 2 pA, 30 mV, 50 ms and 1 nA. (e) Five APs evoked at 100 Hz potentiated the inward current amplitude in the s-OL (clamped to –88 mV, upper trace) that decayed slowly (held at –91 mV; black fit, t1 = 78 ms, t2 = 451 ms). Traces displayed are the average of 25 trials. Scale bars, 10 pA, 1Vs, 30 mV and 0.7 nA. Capacitive current transient clipped for clarity. (f) Illustration of the targeted activation of layer 5 neurons by ChR2 while simultaneously recording from s-OL–neuron pairs. (g) Single light-evoked neuronal AP led to a capacitive transient (clipped) in the s-OL, followed by an inward current that activated during AP repolarization. (h) The synchronised network activation of layer 5 neurons by ChR2 led to a large AP evoked inward current in a simultaneous recorded single s-OL. Scale bars, 20 pA (top), and 30 mV and 0.2 s (bottom).

200 Hz (Kruskal–Wallis test, P = 0.5, n = 35; Supplementary Fig. 3e) and was on average 0.42 ± 0.05 pC per AP. Varying the holding potential of s-OLs to positive voltages diminished, but did not abolish, the inward currents (Supplementary Fig. 3f). We next assessed the impact of a synchronously activated network of layer 5 neurons by photostimulating layer 5 neurons that expressed channelrhodopsin 2 (ChR2, ref. 20; Fig. 3f). Single APs of the host neuron were well discernible and similar to the response of electrically evoked inward currents (Fig. 3g). During long light pulses, inward currents had an average amplitude of –92 ± 19 pA with a total charge of –136 ± 36 pC that decayed double exponentially (t_weighted = 377 ± 113 ms, n = 6; Fig. 3h). These results show that during in vivo-like synchronized activity of layer 5 pyramidal neurons, a substantial inward current is generated in s-OLs.

AP-evoked currents are glial specific and Ba^{2+} sensitive. We next asked whether these inward currents are specific for s-OLs. Paired recordings of pyramidal neurons and astrocytes in a satellite position on average 2 ± 1 μm away (n = 5, Mann–Whitney test, P = 0.18, compared to s-OLs (n = 22)) revealed inward currents of comparable amplitude (–23.26 ± 7 pA, n = 5, Mann–Whitney test, P = 0.4) and decay kinetics (t_weighted = 64 ± 9 ms, n = 5, Mann–Whitney, P = 0.62, Fig. 4a,b). In contrast, AP-evoked inward currents were absent in interneurons that were in a satellite position to pyramidal neurons (–0.94 ± 0.3 pA, n = 4, Fig. 4a,b).

OLs that were not in a satellite position to the recorded neuron (>10 μm membrane separation) had significantly smaller inward currents in response to APs (–2.1 ± 1.1 pA, n = 8, Mann–Whitney, P = 0.0002, Fig. 4b) and were not detectable beyond an intercellular distance of 50 μm (n = 4).

To identify the source of the inward current, we first applied blockers against ionotrophic glutamate receptors, known to be expressed in adult OLs and OPCs21,22. However, the AP-evoked inward currents were unaffected by the NMDA (N-methyl-d-aspartate) receptor blocker D-AP5 (50 μM) or 20 μM of the kainate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor blocker CNQX (ratio (ctrl/blocker) of 1.1 ± 0.1 and 0.9 ± 0.1, respectively, Wilcoxon signed-rank test, P = 0.99, n = 3; P = 0.65, n = 2; Fig. 4c). Similarly, blockers against GABA_A (γ-aminobutyric acid; 5 μM gabazine) and GABA_B (50 μM CGP-35348) receptors also did not affect the inward currents (0.96- and 1.04-fold change, respectively, Wilcoxon signed-rank test, P = 0.65, n = 3; P = 0.18, n = 2; Fig. 4c). Adult rat OLs express inwardly rectifying potassium (K^+) channels at the cell body that are sensitive to barium (Ba^{2+})23. Following 100 μM Ba^{2+} application, the maximum amplitude of the AP-evoked inward current was reduced by 80.7 ± 2.6% (Wilcoxon signed-rank test, P = 0.043, n = 5, Fig. 4c) and the decay time constant of the remaining current was significantly slowed (control: t_weighted = 58 ± 8 ms, Ba^{2+}: t_weighted = 505 ± 148 ms, n = 5, Wilcoxon signed-rank test, P = 0.04).
Kir4.1 channels are not mediating inward K\(^+\) currents. On the basis of our experiments (Figs 3 and 4) and a report of Ba\(^{2+}\) -sensitive inward currents in OPGs and adult OLs\(^{29,31}\), we hypothesized that the potassium channel Kir4.1 is responsible for the observed inward current after AP firing. To test this hypothesis, we utilized a floxed Kir4.1 mouse\(^{26}\) in which we specifically removed Kir4.1 in adult OLs. After conditionally knocking out Kir4.1 (Kir4.1\(^{-/-}\)) the deletion was confirmed by the absence of immuno-gold labelling at electron-dense OL cell bodies (Fig. 5a,b; Supplementary Fig. 4)\(^9\).

In WT, gold particles were found in cell bodies but never in myelin sheaths, which is in agreement with previous published data\(^{27,28}\). To examine the consequence of Kir4.1 loss in OLs, we assessed the Ba\(^{2+}\) -sensitive current in voltage clamp of Kir4.1\(^{-/-}\) and WT s-OLs (Fig. 5c). The Ba\(^{2+}\) -sensitive conductance as estimated from steady-state I–V curves was significantly smaller in Kir4.1\(^{-/-}\) mice (4.1 ± 2 nS, n = 5) compared with WT s-OLs (13.1 ± 1.9 nS, n = 6, Mann–Whitney test, P = 0.017; Fig. 5c,d; Supplementary Fig. 5a–c). Moreover, consistent with the sensitivity of Kir4.1 to intracellular spermine\(^{29}\), the Ba\(^{2+}\) -sensitive current of wild-type s-OLs was modulated and reduced in outward rectification when spermine was added intracellularly (Supplementary Fig. 5d).

Next, we assessed in paired recordings from neurons and s-OLs the consequence of Kir4.1 knockout on K\(^+\) inward currents evoked by APs (Fig. 5e,f). Unexpectedly, an inward current was recorded in all pairs without a difference in their maximum amplitude (unpaired t-test, P = 0.81), charge (Mann–Whitney test, P = 0.6) or the weighted decay time constant (unpaired t-test, P = 0.09) compared with WT (WT: n = 16; Kir4.1\(^{-/-}\), n = 9; Fig. 5g, WT data set same as Fig. 3). To further analyse the origin of the AP-mediated current in Kir4.1\(^{-/-}\) s-OLs, we applied Ba\(^{2+}\). Similar to WT s-OLs 100 µM Ba\(^{2+}\) reduced the AP-evoked current in Kir4.1\(^{-/-}\) s-OLs by 86 ± 1 % (n = 4 Mann-Whitney test, P = 0.011). In line with these data, s-OLs from Kir4.1\(^{-/-}\) animals appeared indistinguishable in their resting membrane properties compared with WT s-OLs (Supplementary Table 1). Thus, despite successful deletion of Kir4.1 in OLs and a reduction of Ba\(^{2+}\) -sensitive current, the AP-dependent inward current and resting membrane properties are maintained. These data either suggest that other members of the Kir family or gap–junction coupled astrocytes contribute to the observed inward current.

s-OLs connect to the astrocytic syncytium. Panglial dye coupling has been reported in acute grey and white matter slices\(^{30–32}\), electrical coupling between OLs and astrocytes has been shown to occur in explant cultures\(^{33}\) and gap–junctions are implicated in K\(^+\) buffering\(^{34}\). We therefore tested reciprocal electrical gap–junction coupling between the pairs of s-OLs and between s-OLs and neighbouring astrocytes (Fig. 6a,b). The average coupling ratio between s-OLs and astrocytes (n = 12) was 3 ± 1% and between OL–OL pairs (n = 3) 0.6 ± 0.1% (Mann–Whitney test, P = 0.0044; Fig. 6c) when recorded from similar distances (Mann–Whitney test, P = 0.82). Interestingly, the coupling was distance dependent with a strong attenuation at distances >20 µm (Fig. 6c). In addition, astrocytes were dye coupled with 9 ± 1 other astrocytes within a domain (n = 20). To test whether electrical coupling between s-OLs and astrocytes is mediated by gap–junctions, we bath applied 100 µM carbeneoxolone (CBX). After wash in the voltage coupling between s-OLs and astrocytes was reduced by 67 ± 6% (Wilcoxon signed-rank test, P = 0.03, n = 6; Fig. 6d). Finally, we tested the impact of CBX on the AP-evoked inward current in s-OLs. CBX caused a reduction in s-OL current amplitude by 58.1 ± 4.4% (Wilcoxon signed-rank test, P = 0.043 n = 5; Fig. 6e).

Figure 4 | Inward currents are glial specific and Ba\(^{2+}\) sensitive. (a) Left: Z-projected confocal images of paired neuron-astrocyte and neuron–interneuron recordings. Scale bars, 20 µm. Right: traces from paired recordings show that astrocytes in satellite position show similar inward currents as s-OLs, but interneurons exhibit no inward currents; capacitive transients are still visible. The electrophysiology traces do not correspond to the image shown on the left. Scale bars, 10 pA (top and middle) and 30 mV, and 50 ms and 1 nA (bottom). (b) Summary data of the charge for five APs for s-OLs (n = 22), oligodendrocytes not in satellite position (n = 8), astrocytes (n = 5) and interneurons (n = 4) reveal that only glia in close proximity to the firing neuron exhibit a substantial inward current. Mann–Whitney test, P = 0.0004 and P = 0.129. Data are mean ± s.e.m. (c) Left: extracellular application of 100 µM Ba\(^{2+}\) (orange) blocks the AP-induced inward current in s-OLs. Scale bars, 20 pA, 40 mV and 20 ms. (d) Population data of blocking experiments. Neither glutamatergic (50 µM D-AP5, n = 3; 20 µM CNQX, n = 2) nor GABAergic receptor blockers (5 µM gabazine, n = 3; 50 µM CGP-35348, n = 2) affected the inward current (for all Wilcoxon signed-rank test, P > 0.18). In contrast, 100 µM Ba\(^{2+}\) led to an ~80% reduction of the s-OL inward current (Wilcoxon signed-rank test, n = 5, P = 0.043). Data shown are mean ± s.e.m.

On the basis of previous recordings of voltage-gated K\(^+\) currents in somatic outside-out patches from rat L5 neurons\(^{34}\) the K\(^+\) charge per AP was estimated to be 5.3 eC µm\(^{-2}\). The L5 soma width (14.4 ± 0.3 µm, n = 34) accounted for a somatic surface area of ~707 µm\(^2\) (assuming a ball) and a single AP would thus generate a K\(^+\) charge of 3.7 pC. An individual s-OL, generating 0.42 eC charge per AP, thereby takes up ~11% of the somatically extruded K\(^+\) ions. Assuming a cleft dimension (δ) of 20 nm (or 0.02 µm) between the neuron and the s-OL (Fig. 1b; Supplementary Movie 1), the numerical estimation of the K\(^+\) charge within the extracellular volume would be equivalent to 2.7 mM per AP (see Methods). Taken together, these data show that Ba\(^{2+}\) -sensitive inward currents are specific for glia cells in direct contact or close proximity to the firing neuron, suggesting that they play a role in the activity-dependent K\(^+\) uptake.
Figure 5 | Oligodendrocytic Kir4.1 does not contribute to AP-evoked inward currents in s-OLs. (a,b) Electron microscopy images of WT and Kir4.1−/− tissue immuno-gold labelled with an antibody against Kir4.1. In the WT tissue, gold particles are present (white arrowheads) at the soma that were absent in the Kir4.1−/− OLs. Scale bars, 1μm. (c) Ba2+-sensitive currents from WT (black) and Kir4.1−/− (grey) s-OLs evoked by the displayed voltage steps. Scale bars, 0.5nA and 0.1s. (d) Summary of the experiments in c shown in an I–V curve. Kir4.1−/− s-OLs have a reduced Ba2+-sensitive conductance. Data are mean ± s.e.m. (e) Combined bright-field and tdTomato fluorescence example image of a Kir4.1−/− s-OL-neuron pair in layer 5. Scale bar, 10μm. (f) Recording of the Kir4.1−/− s-OL-neuron pair shown in e overlaid with a recording from a WT experiment and the inward current evoked by five APs. s-OL currents were normalized to the maximum amplitude. Scale bars, 30mV, 50ms and 1nA. (g) Summary data of the maximum amplitude, charge and weighted decay time constant reveal no differences of the action potential-evoked inward current properties between wild-type and Kir4.1−/− s-OLs. Data are mean ± s.e.m.

without changing decay kinetics (control: τweighted = 76 ± 18 ms, CBX: τweighted = 136 ± 46 ms, n = 5; Wilcoxon signed-rank test, P = 0.14). These results show that astrocytes and s-OLs in the vicinity of neuronal cell bodies are coupled by gap–junctions likely forming a syncytium to efficiently taking up K+ from the extracellular perisomatic region.

Experimental and modelling predictions of potassium uptake.

To examine how changes in [K+]o relate to the s-OL inward currents, we applied K+ (1–30 mM) to the soma of s-OLs with pipettes closely positioned near the cell body (7.2 ± 0.4 μm, n = 21; Fig. 7a) and a holding potential of −84 mV. A [K+]o application of 1 mM led to small transient outward currents (peak amplitude of 48.6 ± 13.7 pA, n = 5). However, when [K+]o was higher than the control [K+]o of 3 mM, inward currents increased up to a peak amplitude of on average −514 ± 106 pA (n = 9; Fig. 7b) at 30 mM [K+]o. By fitting these data with a linear regression (R2 = 0.99), we determined that the [K+]o evoked peak currents reversed polarity at 3.2 mM. On the basis of the total s-OL surface area of ~400 μm2 and an apposition area of ~80 μm2 to the host neuron, we assumed that five times smaller current is generated at the s-OL soma during APs. In the adjusted linear regression (y = −3.8x + 12.3), we substituted our peak amplitude current from five APs. Using the experimentally recorded range from −5 to −30 pA (Fig. 3e), we calculated a range of concentrations from 4.51 to 11.0 mM [K+]o. The average increase was up to 7.2 mM [K+]o for five APs equivalent to ~1 mM [K+]o rise per single AP. To evaluate the contribution of astrocytes, we applied CBX while puffing 30 mM [K+]o. The inward current reduced amplitude and charge by 52 ± 8% (n = 2; Fig. 7c) indicating that gap–junction coupling to astrocytes may contribute for ~50% of the inward current. These data suggest that the observed inward current is reflecting a participation of s-OLs in buffering [K+]o.

Next, we simulated the spatial K+ charge profile by a single AP in a published spatially accurate computational model of a L5 pyramidal neuron (Supplementary Fig. 6a). The model revealed that the AIS, soma and proximal dendrites are the main membrane sites contributing to the K+ efflux during the initiation and back propagation of a single AP strongly overlapping with the spatial arrangement of s-OLs. Given the critical role of the nearby AIS in determining the AP threshold in the axosomatic region35, we aimed to test whether local K+ buffering by the perisomatic syncytium influences neuronal excitability. Since Ba2+ is not specific for glial Kir, this excludes its use to distinguish the impact of glial and neuronal Kir. As an alternative, we first explored the impact of glial Kir-mediated K+ buffering on neuronal excitability using a published computational model with simplified neuronal morphology36,37. Varying the intercellular distance between neuron and glia in this model between 10 and 2,000 nm showed that AP-evoked [K+]o increased between 8 and 0.1 mM, respectively (Fig. 7d). As expected, the AP-evoked [K+]o increase was highly dependent on the extracellular volume; logarithmically reducing the intercellular distance between the neuron and glia (δ) increased
Evidence for modulation of neuronal excitability by s-OLs. To experimentally test whether the perisomatic syncytium around pyramidal neurons impacts neuronal firing, we took advantage of the fact that s-OLs are only found at $\sim$20% of L5 neurons (Supplementary Fig. 1b). Bright-field and fluorescence inspection in the PLP-ECFP mouse in combination with post hoc labelling of s-OLs confirmed their presence or absence (Fig. 8a; Supplementary Fig. 7a). Assessment of the local three-dimensional arrangement of OLs around these two neuron populations confirmed differences in proximity (with s-OL, $n = 13$; without s-OL, $n = 8$; Kolmogorov–Smirnoff test $P = 0.04$; Fig. 8b). Comparative analysis of the intrinsic properties of L5 neurons with and without s-OL showed that the resting membrane potential of neurons lacking a s-OL ($–76.4 \pm 0.5$ mV, $n = 17$) was on average 2.6 mV more depolarized ($–79 \pm 0.4$, $n = 51$, unpaired t-test, $P = 0.002$). Furthermore, the firing properties evoked by a steady depolarizing current step revealed that, while the large majority of neurons with s-OLs were regular firing (86%, 45 out of 51), L5 neurons without s-OL showed less regular firing (47%, 9 out of 19, Fisher’s test, $P = 0.0007$) and instead were more burst firing (53%, 10 out of 19). Firing behaviour of L5 neurons may be influenced by several factors including composition of intrinsic conductances, the dendritic morphology, as well as the axonal projection targets. To isolate the role of extrinsic factors, we grouped L5 neurons according to their firing patterns (Fig. 8c). Analysis of single L5 neurons without s-OL showed no difference in amplitude half-width, afterdepolarisation or the AP frequency. In contrast, for low frequencies, the neuronal AP firing showed no differences between high or low Kir (Fig. 7g). With higher current injections ($> 50$ pA), APs were generated at higher frequencies ($> 170$ Hz) and reduction of Kir in the glial compartment led to more APs (Fig. 7f,g; Supplementary Fig. 6c). Indeed, when the extracellular volume was increased ($\delta > 250$ nm), the AP frequency reduced and glial Kir played no role in the initiation of APs (Fig. 7h).

Taken together, the modulating effects suggest that placing glia close to the soma membrane causes two opposite changes. On the one hand, a narrower extracellular space increases neuronal excitability by amplifying activity-dependent $[K^+]_o$. On the other hand, the glia-specific K$^+$ buffering limits $[K^+]_o$ and thereby prevents excessive AP generation. Moreover, glia-specific K$^+$ buffering was in particular noticeable when APs occur at high frequency.

**Figure 6** | s-OLs are gap-junction coupled to other s-OLs and astrocytes. (a) Confocal z-projected image of a s-OL and astrocyte. Scale bar, 30 μm. (b) Gap-junction coupling between s-OL and astrocytes was estimated by bidirectional current injections into s-OLs or adjacent astrocytes and simultaneous recordings of the response in the corresponding cell. Traces are averages of 10 trials from pair in a. Open circle indicate the measurement region at the end of the pulse. Scale bars, (inject) 5 mV, (record) 200 μV and 50 ms, respectively. (c) Summary plot of the coupling ratio in the steady state as a function of the distance between recorded s-OL/astrocyte ($n = 12$) and s-OL/s-OL ($n = 4$) pairs. The open red circle indicates the experiment shown in a and b. Data were fitted by single exponential equations: s-OL/astrocyte: $y = 165.2 \times e^{(–0.27 \times x)} + 1.46$ (dotted line); s-OL/s-OL: $y = 10.76 \times e^{(–0.249 \times x)} + 0.435$ (black line). (d) Left: the gap-junction blocker carbenoxolone (CBX) reduced the cross gap-junctional current between s-OLs and astrocytes compared with control conditions. Scale bars, 0.1 mV and 50 ms. Right: summary data showing the inhibition of the gap-junction-mediated current ($n = 6$, Wilcoxon signed-rank test, $P = 0.03$). Data are mean ± s.e.m. (e) Left: CBX reduced the AP-mediated inward current between neuron–s-OL pairs by 60%. Scale bars, 20 pA, 40 mV and 20 ms. Right: summary plot showing the reduction of the inward current in s-OLs in the presence of CBX in response to five APs ($n = 5$). Data are mean ± s.e.m. in interstitial $[K^+]_o$ up to a peak concentration of nearly $\sim 10$ mM, and depolarized $E_k$ by an additional $\sim 5$ mV (Fig. 7f). As a result, the APs were associated with a reduced afterhyperpolarization, facilitating the generation of additional APs (Fig. 7f). In contrast, for low frequencies, the neuronal AP firing showed no differences between high or low Kir (Fig. 7g). With higher current injections ($> 50$ pA), APs were generated at higher frequencies ($> 170$ Hz) and reduction of Kir in the glial compartment led to more APs (Fig. 7f,g; Supplementary Fig. 6c). Indeed, when the extracellular volume was increased ($\delta > 250$ nm), the AP frequency reduced and glial Kir played no role in the initiation of APs (Fig. 7h).

Finally, the computational model predicted that glial Kir becomes critical when $[K^+]_o$ reaches higher concentrations, for
example, during repetitive neuronal firing (Fig. 7g). Increased $[K^+]_o$ can then in turn facilitate the generation of additional APs due to depolarization of $E_K$. To assess the impact of potassium buffering, we recorded from neurons with or w/o s-OL in baseline (3 mM) and elevated (8 mM) $[K^+]_o$ conditions (Fig. 8e). In 8 mM $[K^+]_o$ neurons in both the groups showed a strongly depolarized resting membrane potential (with s-OL: 13.5 ± 1.2 mV, without s-OL 14.1 ± 1.3 mV, n = 7 cells per group, unpaired t-test P = 0.7) and consequently the F-I curve showed a rheobase shift to lower thresholds (Supplementary Fig. 7c). In elevated $[K^+]_o$ neurons from both the groups generated high-frequency APs (Fig. 8e), which were more numerous in neurons w/o s-OL during current injections of 200 and 250 pA (two-way ANOVA, P = 0.0001, n = 7 cells per group; Fig. 8f,g). Subsequently, on the basis of these results, we calculated the burst probability for the 200 pA current step (Fig. 8h). Consistent with the model predictions of a large role of $[K^+]_o$ during bursts, the interaction analysis showed that the burst probability was more strongly increased in neurons w/o s-OL, further supporting our observation that the presence of s-OLs contributes to limit AP generation (repeated measures ANOVA, interaction P = 0.016; Fig. 8h). In summary, these data suggest that the presence of s-OLs can be interpreted as a proxy for local differences in arrangement of the glial syncytium, which reduces the AP-dependent $[K^+]_o$ elevations and acts to limit the positive feedback on neuronal excitability.

**Discussion**

Satellite oligodendrocytes are ubiquitously found throughout the cortex and hippocampus in rodents and humans. The presence of s-OLs can be interpreted as a proxy for local differences in arrangement of the glial syncytium, which reduces the AP-dependent $[K^+]_o$ elevations and acts to limit the positive feedback on neuronal excitability.
Interaction significance was assessed with a two-way ANOVA-repeated measures. Differences in the average firing rate were observed at 250 pA (two-way ANOVA, probability for neurons with and without s-OL in 3 and 8 mM [K+]o to 8 mM neurons depolarized and fired more APs with the same current injection. Arrowheads indicate cluster APs. Scale bar, 20 μm and 5 ms. Bottom right: summary data of the average APs per cluster for neurons with and without s-OL. Scale bars, 30 mV and 0.1 s. Top right: high temporal resolution of the first high-frequency AP cluster generated by L5 neurons with or without s-OL. Scale bar, 20 μV and 25 ms. (e) Summary plot of the sum of cluster APs for neurons with and without s-OL indicate that the largest difference is observed for current steps between 200 and 300 pA (two-way ANOVA, P = 0.0001, n = 7 cells per group). Data are mean ± s.e.m. (f) Summary F-I plot of neurons with and without s-OL that generated high-frequency cluster APs. Differences in the average firing rate were observed at 250 pA (two-way ANOVA, P = 0.0038). Data are mean ± s.e.m. See also Supplementary Fig. 7b.

While classified in the literature as non-myelinating similar to OPCs10,12,14,15, we present morphological, ultrastructural and molecular evidence that s-OLs abundantly myelinate axons indistinguishable from type I OLs (Fig. 2) reaffirming their original classification by del Rio Hortega13.

What is the functional role of the close apposition of the outer membranes of OLs and neurons? Previous work suggested that s-OLs may provide metabolic support for neurons39, protect neurons against apoptosis17 or remyelinate axons following injury12. Specific arrangements between neurons and glia cells in satellite position, such as the cerebellar Bergmann glia, dynamically regulate extracellular K+30, thereby ensuring firing bistability of the Purkinje cell7,41. The perisomatic domain of pyramidal layer 5 neurons generates substantial outward K+ current when APs back propagate from the axon into the soma and dendritic tree, and activate fast voltage-gated K+ channels26. It is well established that buffering of [K+]o and water in the brain is a complex process controlled by passive diffusion, K+ uptake through Na+/K+ pumps and Kir channels as well as spatial K+ redistribution via electric coupling of glial cells34,42–45. AP-evoked K+ release into the extracellular space may be most efficiently controlled when glial syncytia are organized perisomatically and in close contact with the neuronal membrane. Consistent with these features we found that AP-evoked inward currents in s-OLs were highly sensitive to Ba2+ a Kir channel blocker and partially gap–junction mediated. Importantly, these currents were not different from perisomatic astrocytes that are widely implicated in K+ homeostasis6,34,41,44). Based on these results it may be postulated that s-OLs participate in regulation of extracellular K+.

Given the electrical coupling of s-OLs to an astrocyte syncytium what is the role of a satellite OL? The conditional OL-specific knockout of Kir4.1 was characterized by impaired Ba2+-sensitive currents strongly supporting the finding that Kir4.1 is at the membrane in mature OLs (Fig. 4)28,46. Unexpectedly, OL Kir4.1 was not required for AP-evoked inward current and setting the resting membrane properties. These results contrast with deletion or mutation of Kir4.1 in astrocytes causing impaired K+ siphoning, myelin defects and epilepsy26,47–49. There are several possibilities that can account for the lack of detectable impact on K+ uptake in Kir4.1−/− s-OLs. First, since mature OLs are connected to the astrocyte...
The electrical coupling maintains the isopotentiality of a synaptical form ensuring a constant driving force for K⁺ uptake. Indeed, the disruption of OL gap-junctions Cx32 and Cx47 have been implicated in deficits of potassium and water siphoning, and myelination defects. Given the dominant role of electrical coupling Kir4.1 an activity of single-s-OL may thus be masked by the synaptical. Second, the lack of impact of OL-specific Kir4.1 knockout on the inward current may reflect a differential molecular composition of Kir expression in OLs and astrocytes. OLs express homotrimers of Kir4.1/kir5.1 characterized by an approximately fourfold larger single-channel conductance compared with Kir4.1 homotrimers at 28,53,54. Third, it cannot be excluded that OL-specific uptake of K⁺ is complemented by Kit2.1, although this channel is expressed at lower levels compared with Kir4.1 (refs 28,55). Furthermore, a dominant role of astrocytic Kir4.1 in a OL–astrocyte syncytium is supported by the Kit4.1 knockout studies showing not only deficits in potassium buffering and glutamate uptake but also white matter vacuolization 28,56,57, which could be a consequence of elevated [K⁺]o, leading to myelin degeneration. In line, inward currents of white matter OLs are predominantly carried by K⁺ (ref. 56) supporting an important role for OL physiology. On the basis of these and our present findings, further experiments are required to examine the properties of perisomatic astrocytes and OLs in local AP-evoked K⁺ uptake using the specific conditional astrocytic knockout of Kir4.1 or directly assessing [K⁺]o, by potassium-sensitive dye imaging. The Nerst equation predicts that depolarization of EK reduces the driving force for K⁺, which causes membrane depolarization and increased neuronal excitability. Our computational simulations demonstrated that due to the fast temporal dynamics of Kir-mediated uptake, conducting logistically with K⁺o (ref 29,57), EK is mostly influenced during high-frequency APs (Fig. 7). In good agreement with these predictions, the presence of s-OLs was associated with neuronal excitability changes selectively during high-frequency burst firing and as a function of the baseline K⁺ o. These findings may suggest that s-OLs have a role in anatomically organizing the perisomatic glial synactium. This hypothesis remains to be addressed in future studies using the conditional knockout of Kir4.1 in astrocytes or directly visualizing the entire perisomatic glial synactium. Our results predict that perisomatic synactea increase the diversity of populations of pyramidal neurons in the neocortex. Since high-frequency burst firing of neocortical pyramidal neurons occurs in vivo during active whisking behaviours and during coincidence detection, perisomatic synactea potentially may contribute to modalities of sensory integration. In summary, the present work shows that satellite cells are typical type I OLs and an integral component of an electrically coupled glial synactium-mediating K⁺ buffering in the perisomatic domain. The results add to an emerging view that the current Kir4.1 expression was specific for OLs. For breeding, a feature which is shared with the endfeet (refs 29,57), a feature which is shared with the endfeet.
Analysis of electrophysiological data. Analyses of electrophysiology recordings were performed with Axograph X. Rs was determined by fitting the linear range of voltage responses to current injections (± 50 pA for L5 neurons and up to ± 400 pA for OLs) around the resting membrane potential. The resting membrane potential was determined from traces with zero-current injection or alternatively read from the built-in display after breaking into whole-cell mode (Axograph 2008). Resting conductance (G) was calculated from voltage-clamp recordings as G = ΔV/V, where V was a 10 mV hyperpolarizing voltage step from −70 mV and ΔV the respective current difference. AP parameters were determined from single-evoked APs or steady-current injection as indicated. The threshold was determined when the slope of the voltage reached > 50 V/s (ref 35.61). Single APs were only analysed when they initiated with a delay after the end of the current pulse. Gap-junction coupling of cells was tested by injecting current in one cell and recording the voltage response in both cells; current injections to assess coupling were performed reciprocally. For the analysis, current responses of 10 trials were averaged and the voltage was measured at the end of each current injection step for each cell. The coupling ratio (V_foating cell / V_injected cell) was calculated for each current step and averaged for all positive and negative current injections. Subsequently, an average coupling ratio was calculated for each cell pair. The slopes of the F-I curves were estimated from linear fits to either all available data points (regular firing neurons) or to the first data points that were linear (burst-firing repetitive APs at high frequencies19). Neurons with a short burst of two or more APs were defined as high-frequency firing neurons and neurons with > 10 Hz interspike frequencies as regular firing neurons.

Photoactivation of layer 5 neurons. For photoactivation of the ChR2 variant ChETA in layer 5 neurons, we used a blue light-emitting diode (LED; peak at 470 nm, Thorlabs Inc., Newton NJ, USA) mounted into the light path of the microscope. For photostimulation purposes, the LED output power was directly measured in the optical path (~1.8 mw) with an optical power metre (Newport, Irvine, CA, USA). With this LED intensity, a short light pulse of 3 ms was sufficient to evoke single APs in layer 5 neurons. To control the area of illumination, a field stop was coupled into the excitation light path.

Live imaging. During electrophysiological recordings, live fluorescence z-stacks were obtained with an upright microscope equipped with a confocal scanning unit (Olympus BX61/FV1000, Olympus) and laser lines for 488 and 594 nm (multi-line argon laser (Showa Optronics, Tokyo, Japan); helium–neon laser (Melles Griot, Carlsbad, CA, USA)). Images were acquired with either × 40 numerical aperture (NA) 0.8, × 60 NA 1.0 or × 60 NA 1.1 Olympus water immersion objectives in z-steps sizes of 1 μm (Fluoview software, Olympus).

Immunohistochemistry and image analysis. For post hoc labelling of intracellular dye-filled cells slices were fixed in 4% paraformaldehyde (PFA) for 10–20 min before further processing. We used antibodies against MPB (rabbit polyclonal anti-MPB, #AB980, Millipore or monoclonal mouse anti-MBP, #SMI-99P, Covance both 1:250), GAP43 (ab65536, 1:1500, Abcam or 75–132, 1:100, Neuronab), Kir4.1 (rabbit polyclonal or APC-035, 1:300, Alomone, Israel), NeuN (polyclonal, guinea-pig, 1:1000, #AB909, Millipore) and NG2 (polyclonal rabbit, #AB5320, 1:250, Millipore). Corresponding secondary antibodies were conjugated with Alexa, 488, 555, 594 or 633 (1:500, all Life Technologies) and chosen as suitable. Immunohistochemical labelling was performed as described below. Kir4.1 immunofluorescence labelling was performed on tissue that was fixed for 20 min in 4% PFA and subsequently processed identical as described below. For quantifying the distribution of OLs in neocortical layer 5, we immersion-fixed the brains of three mice (C57BL/6, ~75 days) in 4% PFA for 4 h and cut slices of 40-μm thickness on a vibratome (VT1000S, Leica Microsystems, Germany). Sections were labelled with a polyclonal MBP antibody (AB980) for which some isoforms are known to be present in the cytoplasmic domain of OLs42 and a NeuN antibody (ABN90), a pan-neuronal marker, in the presence of 1% Triton-X 100 at 4 °C overnight after blocking for 2 h at room temperature in the same solution without primary antibodies. Secondary antibodies were incubated for 2 h at room temperature and then washed in PBS, three times. Sections were mounted onto slides coated with a solution of 1% gelatin and 2% DABCO, and then gold anti-rabbit-Alexa555 (Life Technologies). Subsequently, the sections were coveredslipped with Vectashield with 4,6-diamido-2-phenylindole (DAPI, Vector Labs). For each animal, eight ROIs from four randomly selected slices in the layer 5 of the somatosensory neocortex were selected and 40 μm z-stacks were scanned as described above. The Z-projected, thresholded and normalized images were subsequently separately using the plug-in “Analyze Particles” (from Image J) to detect the number of neurons and nuclei that were identified by DAPI. NeuN and DAPI images were z-projected, thresholded and normalized. Finally, the number of neurons and nuclei that were identified by DAPI was counted using the “Analyze Particles” tool of the plugin “3D VESSEL FAMILY TOOL” (from Adobe) with a diameter of 5 μm.

Electron microscopy. To obtain electron microscopy images from single s-OLs, we first identified s-OLs in acute slices of PLP-ECFP mice and filled them with 10 μM Alexa 488–labeled cholera toxin B subunit (Sigma-Aldrich) in the whole-cell recording condition. After pipette removal slices containing a single OL were fixed for ~17 min in freshly prepared 5% glutaraldehyde in Na-cacodylate (0.1 M, pH 7.4), and then washed in Na-cacodylate buffer and cryoprotected in 25% sucrose. After saturation slices were embedded in an aluminum boat, frozen on dry ice and then cut at 60 μm with a freezing microtome. Sections of 75–100 nm were collected in a Tris–HCl buffered diaminobenidine solution containing 0.03% H2O2. The diaminobenidine reaction product was subsequently intensified by a gold-substituted silver permanganate method55. Sections were then rinsed in sodium cacodylate buffer (0.1 M, pH 7.4) and post fixed for 20 min in 1% OsO4 supplemented with 1% ferricyanide in cacodylate buffer. Sections were then washed, dehydrated and embedded in epoxy resin. Ultrathin sections (~ 60 nm) perpendicular to the cortex were made (Ultrotome E, Reichert-Jung/Leica), mounted on electron microscopy grids and contrasted with solutions of lead citrate and 0.5% uranyl acetate (Ultrastain 1 and 2, Laurellab, Brindis, France). Sections were observed with a transmission electron microscope (Tecnai G2 F20 S-TWIN, Malvern, Eindhoven, Netherlands) and digital images were acquired as TIFF files with a mega view 3 camera (Soft Imaging System/Olympus). The g-ratio for single labelled axons (longitudinally or transversally cut) was calculated from calibrated electron microscopy images as the diameter of the axon divided by the total diameter of the axon image. In parallel, sections were processed for electron microscopy with a BrightVision Poly-HRP-Goat-Anti-rabbit IgG (Immunologic, Duiven, The Netherlands). Tissues from wild-type and Kir4.1−/− animals were processed in parallel. In Kir4.1−/− sections, surrounding astrocytic processes exhibited gold particles, indicating successful labelling of the sections (Supplementary Fig. 4a–d). All other steps were performed as described above.
Potassium concentration estimation in the soma s-OL cleft. To estimate the extracellular K⁺ concentration [K⁺]e in the cleft region between the L5 soma and s-OL, we used an analytical solution as a function of cleft height (d). Our previous outside-out recordings from rat layer 5 pyramidal neuron somata showed that the K⁺ charge per AP is ≈ 5.3 μC μm⁻² (ref. 24). The measured spacing between a neuron and s-OL is probably affected by aldehyde fixation and therefore we use a constant (96485.336521 μC mol⁻³ Na⁺ representing glial, segmented with the same spatial resolution as the neuron. Each neuronal AP firing, we used a previous published computational model available at https://neuron.janelia.org. The model was mostly unchanged and run as published at ModelDB, except that spatial segmentation was increased 10-fold to obtain 3-μm segments. To mimic different intercellular distances, we varied the fraction of extracellular volume divided by intracellular volume, between 0.005 and 1.0 (Supplementary Fig. 6c). For clarity, these values were expressed as intercellular distance (d) between glia and the soma (with a diameter of 10 μm) and thus ranged between 12 and 2,000 nm. Final simulations were run with an intracellular volume fraction (x) of 0.045. To mimic glial Kir4.1 reduction, we adjusted Kir between 0.001 pS μm⁻² (Ba²⁺-block) and 0.10 pS μm⁻² (control density). Current injections were of short duration and ranged between 0 and 110 pA. The temperature in the simulations was nominally set to 33 °C and simulation time steps (dt) set to 25 μs.

Statistics. Data are given as mean ± s.e.m and the number of experiments and the corresponding statistical test is indicated. Data were statistically analysed with the Shapiro–Wilk test and when positive, we applied a paired or unpaired t-test subsequently. Not normally distributed data or experiments with more than two groups were performed by a Kruskal–Wallis test or ANOVA where appropriate.

References
1. Henneberger, C., Papouin, T., Oliet, S. H. R. & Rusakov, D. A. Long-term potentiation depends on release of D-serine from astrocytes. Nature 463, 232–236 (2010).
2. Min, R. & Nevian, T. Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nat. Neurosci. 15, 746–753 (2012).
3. Chavkin, J. et al. Heterosynaptic long-term depression mediated by ATP released from astrocytes. Glia 61, 178–191 (2013).
4. Morquette, P. et al. An astrocyte-dependent mechanism for neuronal rhythmogenesis. Nat. Neurosci. 18, 844–854 (2015).
5. Tong, X. et al. Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington’s disease model mice. Nat. Neurosci. 17, 694–703 (2014).
6. Wang, F. et al. Astrocytes modulate neural network activity by Ca²⁺-dependent uptake of extracellular K⁺. Sci. Signal. 5, ra26 (2012).
7. Vit, J.-P., Ohara, P. T., Bhargava, A., Kelley, K. & Jasmin, L. Silencing the Kir4.1 potassium channel subunit in satellite glial cells of the rat trigeminal ganglion results in pain-like behavior in the absence of nerve injury. J. Neurosci. 28, 4161–4171 (2008).
8. Baalman, K. et al. Axon initial segment-associated microglia. J. Neurosci. 35, 2283–2292 (2015).
9. Peters, A., Palay, S. L. & Webster, H. D. The Fine Structure of the Nervous System: Neurons and Their Supporting Cells (Oxford Univ. Press, 1991).
10. Takasaki, C. et al. Cytochemical and cytological properties of perineuronal oligodendrocytes in the mouse cortex. Eur. J. Neurosci. 32, 1326–1336 (2010).
11. Kruger, L. & Maxwell, D. S. Electron microscopy of oligodendrocytes in normal rat cerebral. Am. J. Anat. 118, 411–435 (1966).
12. Ludwin, S. K. The perineuronal satellite oligodendrocyte. A role in the genesis of myelin. Acta Neuropathol. 47, 49–53 (1979).
13. del Rio Hortega, P. Tercera aportación al conocimiento morfológico e interpretación funcional de la oligodendroglia. Mem. Real Soc. Esp. Hist. Nat. 14, 5–122 (1928).
14. Szuchet, S. et al. The genetic signature of perineuronal oligodendrocytes reveals their unique phenotype. Eur. J. Neurosci. 34, 1906–1922 (2011).
15. Butt, A. M. in Neuroglia (eds Kettenmann, H. & Ransom, B. R.) 62–73 (Oxford Univ. Press, 2013).
16. Nave, K.-A. Myelination and support of axonal integrity by glia. Nature 468, 244–252 (2010).
17. Taniike, M. et al. Perineuronal oligodendrocytes protect against neuronal apoptosis through the production of lipocalin-type prostaglandin D synthase in a genetic demyelinating model. J. Neurosci. 22, 4885–4896 (2002).
18. Cole, M. H. P. & Stuart, G. J. Signal processing in the axon initial segment. Neuron 73, 235–247 (2012).
19. Cole, M. H. P. First node of Ranvier facilitates high-frequency burst encoding. Neuron 71, 671–682 (2011).
20. Beltramo, R. et al. Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. Nat. Neurosci. 16, 227–234 (2013).
21. Bergles, D. E., Roberts, J. D., Somogyi, P. & Jahr, C. E. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405, 187–191 (2000).
22. Karadottir, R., Cavelier, P., Bergersen, L. H. & Attwell, D. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438, 1162–1166 (2005).
23. Gipson, K. & Bordey, A. Analysis of the K⁺ current profile of mature rat oligodendrocytes in situ. J. Membr. Biol. 189, 201–212 (2002).
24. Hallermann, S., de Kock, C. P. J., Stuart, G. J. & Cole, M. H. P. State and location dependence of action potential metabolic cost in cortical pyramidal neurons. Nat. Neurosci. 15, 1007–1014 (2012).
25. Maldonado, P. P., Vélez-Fort, M., Levavasseur, F. & Angulo, M. C. Oligodendrocyte precursor cells are accurate sensors of local K⁺ in mature gray matter. J. Neurosci. 33, 2432–2442 (2013).
26. Dujic, B., Casper, K. B., Phlipot, B. D., Chin, L.-S. & McCarthy, K. D. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J. Neurosci. 27, 11354–11365 (2007).
27. Kalsi, A. S., Greenwood, K., Wilkin, G. & Butt, A. M. Kir4.1 expression by oligodendrocytes in situ. J. Neurosci. 29, 1326–1336 (2009).
28. Maglione, M. et al. Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia 58, 1104–1117 (2010).
29. Ransom, B. R. & Kettenmann, H. Electrical coupling, without dye coupling, between mammalian astrocytes and oligodendrocytes in cell culture. Glia 3, 258–266 (1990).
30. Menichella, D. M. et al. Genetic and physiological evidence that oligodendrocyte gap junctions contribute to spatial buffering of potassium released during neuronal activity. J. Neurosci. 26, 10984–10991 (2006).
35. Kole, M. H. P. & Stuart, G. J. Is action potential threshold lowest in the axon? Nat. Neurosci. 11, 1253–1255 (2008).

36. Somjen, G. G., Kager, H. & Wadman, W. J. Computer simulations of neuron-glial interactions mediated by ion flux. J. Comput. Neurosci. 25, 349–365 (2008).

37. Kager, H., Wadman, W. J. & Somjen, G. J. Seizure-like afterdischarges simulated in a model neuron. J. Comput. Neurosci. 22, 105–128 (2007).

38. Hattoo, A. M. & Nelson, S. B. Layer V neurons in mouse cortex projecting to different targets have distinct physiological properties. J. Neurophysiol. 98, 3330–3340 (2007).

39. van Landeghem, F. K. H., Weiss, T. & von Deimling, A. Expression of PACAP and glutamate transporter proteins in satellite oligodendrocytes of the human CNS. Regul. Pept. 142, 52–59 (2007).

40. Vostrikov, V. M., Uranova, N. A. & Orlovskaya, D. D. Deficit of perineuronal oligodendrocytes in the prefrontal cortex in schizophrenia and mood disorders. Schizophr. Res. 94, 273–280 (2007).

41. Wang, F., Xu, Q., Wang, W., Takano, T. & Nedergaard, M. Bergmann glia modulate cerebellar Purkinje cell bistability via Ca2+–dependent K+ uptake. Proc. Natl Acad. Sci. USA 109, 7911–7916 (2012).

42. Kofuji, P. & Newman, E. A. Potassium buffering in the central nervous system. Neuroscience 129, 1045–1056 (2004).

43. Holthoff, K. & Witte, O. W. Directed spatial potassium redistribution in rat neocortex. Glia 29, 288–292 (2000).

44. Pannasch, U., Derangeon, M., Chever, O. & Rouach, N. Astroglial gap junctions shape neuronal network activity. Commun. Integr. Biol. 5, 248–252 (2012).

45. Sykova, E. & Nicholson, C. Diffusion in brain extracellular space. Physiol. Rev. 88, 1277–1340 (2008).

46. Poopalasundaram, S. et al. Glial heterogeneity in expression of the inwardly rectifying K+ channel, Kir4.1, in adult rat CNS. Glia 30, 362–372 (2000).

47. Bockenhauer, D. et al. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCN10 mutations. N. Engl. J. Med. 360, 1960–1970 (2009).

48. Neusch, C., Rozengurt, N., Jacobs, R. E., Lester, H. A. & Kofuji, P. Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. J. Neurosci. 21, 5429–5438 (2001).

49. Haj-Yasein, N. N. et al. Evidence that compromised K+ spatial buffering contributes to the epileptogenic effect of mutations in the human Kir4.1 gene (KCN10). Glia 59, 1635–1642 (2011).

50. Griesmann, S. et al. Characterization of panglial gap junction networks in the thalamus, neocortex, and hippocampus reveals a unique population of glial cells. Cereb. Cortex 25, 3420–3433 (2014).

51. Ma, B. et al. Gap junction coupling confers iso-potentiality on astrocyte synctium. Glia 64, 214–226 (2016).

52. Kofuji, P. et al. Kir potassium channel subunit expression in retinal glial cells: implications for spatial potassium buffering. Glia 39, 292–303 (2002).

53. Pessa, M., Tucker, S. J., Lee, K., Bond, C. T. & Adelman, J. P. Subunit positional effects revealed by novel heteromeric inwardly rectifying K+ channels. EMBO J. 15, 2980–2987 (1996).

54. Tanemoto, M., Kittaka, N., Inanobe, A. & Kurachi, Y. In vivo formation of a proton-sensitive K+ channel by heteromeric subunit assembly of Kir5.1 with Kir4.1. J. Physiol. 525(Pt 3): 587–592 (2000).

55. Zhang, Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947 (2014).

56. Hamilton, N. B., Kolodziejczyk, K., Kougoumtzidou, E. & Attwell, D. Proton-gated Ca(2+)-permeable TRP channels damage myelin in conditions mimicking ischaemia. Nature 529, 523–527 (2016).

57. Pérét, F., Radeke, C. M. & Vandenbark, C. A. Primary structure and characterization of a small-conductance inwardly rectifying potassium channel from human hippocampus. Proc. Natl Acad. Sci. USA 91, 6240–6244 (1994).

58. Xu, N.-L. et al. Nonlinear dendritic integration of sensory and motor input during an active sensing task. Nature 492, 247–251 (2012).

59. Larkum, M. A cellular mechanism for cortical associations: an organizing principle for the cerebral cortex. Trends Neurosci. 36, 141–151 (2013).

60. Hirrlinger, P. G. et al. Expression of reef coral fluorescent proteins in the central nervous system of transgenic mice. Mol. Cell. Neurosci. 30, 291–303 (2005).

61. Attwell, D. & Iles, J. F. Synaptic transmission: ion concentration changes in the synaptic cleft. Proc. Natl Acad. Sci. USA 106, 115–131 (1979).

62. Hardy, R. J., Lazzarini, R. A., Colman, D. R. & Friedrich, V. L. Cytoplasmic and nuclear localization of myelin basic proteins reveals heterogeneity among oligodendrocytes. J. Neurosci. 46, 246–257 (1996).

63. Bortone, D. S., Olsen, S. R. & Scanziani, M. Translaminar inhibitory cells recruited by layer 6 corticothalamic neurons suppress visual cortex. Neuron 82, 474–485 (2014).

64. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).

65. van den Pol, A. N. & Gorcs, T. Synaptic relationships between neurons containing vasopressin, gastrin-releasing peptide, vasovagal intestinal polypeptide, and glutamate decarboxylase immunoreactivity in the suprachiasmatic nucleus: dual ultrastructural immunocytochemistry with gold-substituted silver peroxidase. J. Comp. Neurol. 252, 507–521 (1986).

66. Eldred, W. D., Zucker, C., Karten, H. J. & Yazdilla, S. Comparison of fixation and penetration enhancement techniques for use in ultrastructural immunocytochemistry. J. Histochem. Cytochem. 31, 285–292 (1983).

67. Korogod, N., Petersen, C. C. H. & Knott, G. W. Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation. Elife 4, e05793 (2015).

68. van Heukelom, J. S. The role of the potassium inward rectifier in defining cell membrane potentials in low potassium media, analysed by computer simulation. Biophys. Chem. 50, 345–360 (1994).

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Conceptualization of the study by M.K. Design of the study and experiments A.B. and M.K. Performance of the experiments, analysis of the data and writing of the manuscript A.B., J.K. and M.K.

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