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Supplemental Information

High-Frequency Microdomain Ca^{2+} Transients and Waves during Early Myelin Internode Remodeling

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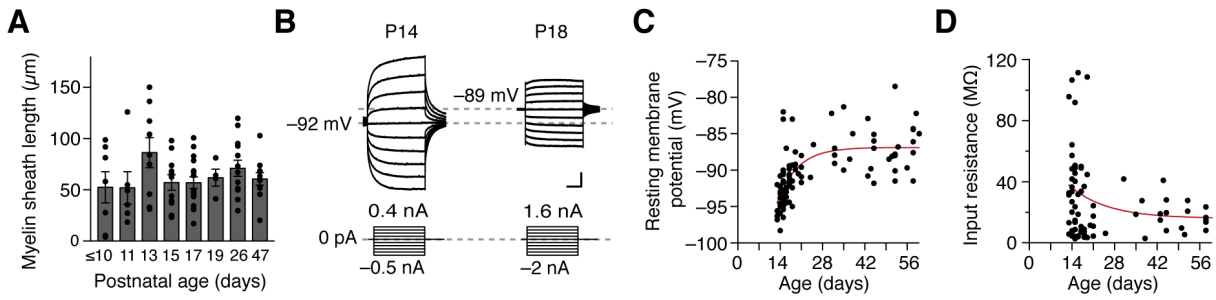

Figure S1 Oligodendrocytes undergo developmental changes in membrane properties. Related to Figures 1 and 3.

(**A**) Internodal length does not change during L5 axon myelin maturation (p = 0.24, ANOVA). The plot shows bins of 2 days. (**B**) Whole-cell current-clamp recordings of oligodendrocytes at P14 and P18 in response to hyperpolarizing and depolarizing current injections. Note the large difference in input resistance and membrane potential. Scale bars 5 mV and 0.2 s. (**C**) OL resting membrane potential is correlated with postnatal age (Pearson r = 0.59, p = 0.0001). Data were fitted with a single exponential equation ($y = -54.72 \times e^{(-0.15 \times x)} - 86.88$). (**D**) OL input resistance as a function of age (Pearson r = –0.21, P = 0.06). Data were fitted with a single exponential equation ($y = 62.97 \times e^{(0.08\,x)} + 16$).

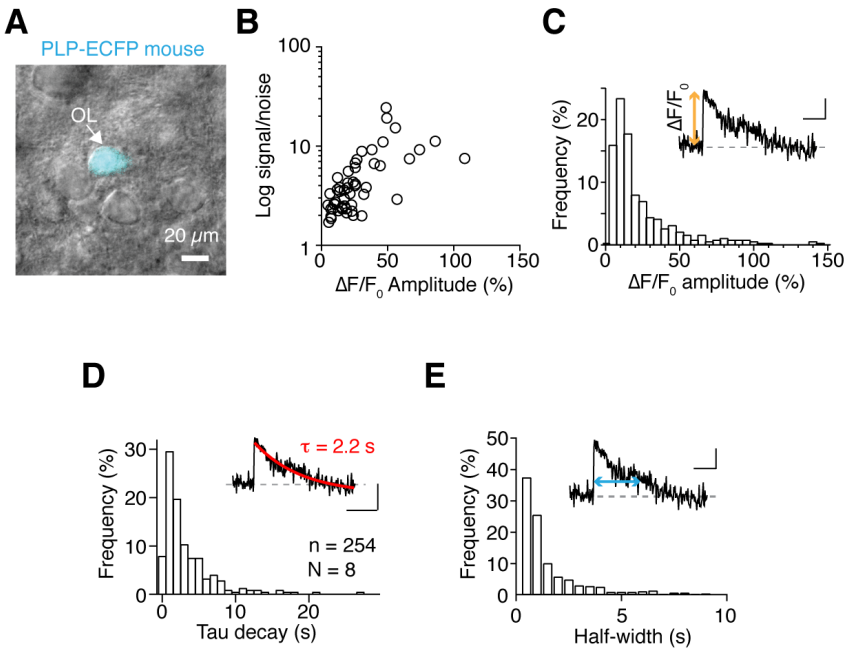

Figure S2 Properties of Ca^{2+} transients in the myelin sheath. Related to Figure 2.

(**A**) Combined oblique contrast and CFP fluorescence image showing a single OL (cyan) at P14 (arrow). Scale bar 20 μm. (**B**) Semi-logarithmic plot showing the signal-to-noise ratio versus event amplitude for randomly selected events (n = 51) from 12 different OLs and experiments. (**C**) Histogram of ΔF/F change for single Ca^{2+} transients (n = 390 events, N = 22 OLs). (**D**) Histogram of the decay time constant for Ca^{2+} events. Inset, typical Ca^{2+} transient; decay fitted with single exponential equation. Scale bars 5% ΔF/F and 1 s. (n = 254 events, N = 8 OLs) (**E**) Histogram of the half-width of myelin Ca^{2+} events (n = 427 events, N = 10 OLs).

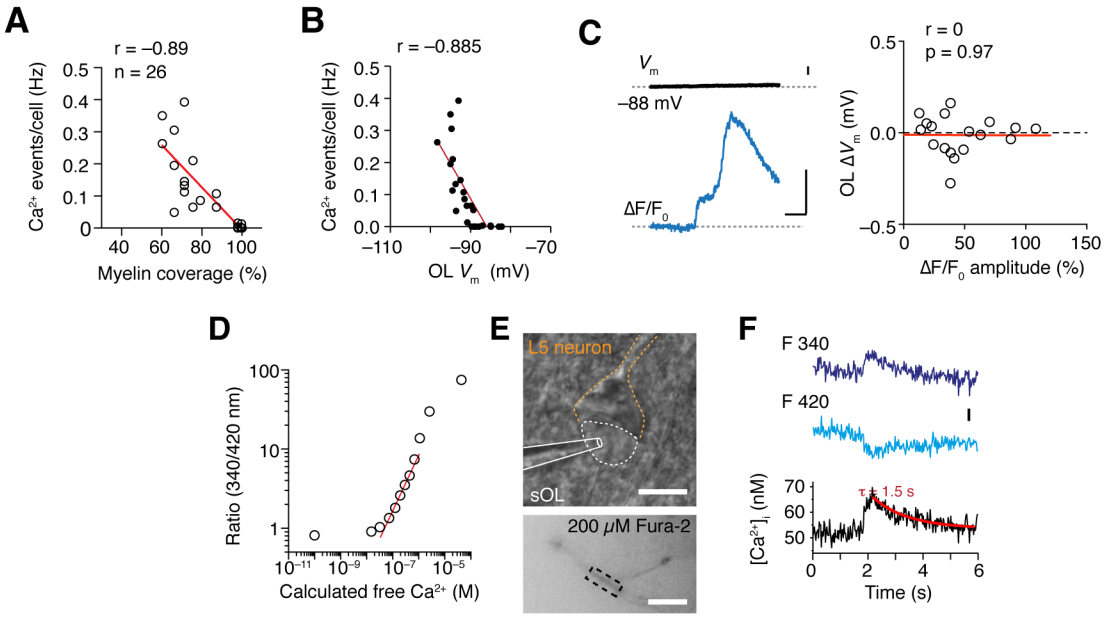

Figure S3 Developmental changes of Ca^{2+} events and determination of Ca^{2+} concentration. Related to Figure 1 and 3.

(**A**) Plot showing the negative correlation between myelin coverage and myelin Ca^{2+} event frequency (Spearman r = –0.890, p = 0.0001, n = 26 matched pairs). (**B**) Calcium event frequency is highly correlated with OL resting membrane potential (Spearman r = –0.885, p = 0.0001, n = 25 cells, N = 17 mice). (**C**) *Left*, example trace of a large Ca^{2+} event (blue). Note that the somatic resting membrane potential does not change. Scale bars, 1 mV, 20% ΔF/F and 2 s. *Right*, summary data illustrate no correlation between Ca^{2+} change and membrane potential (Pearson r = 0, p = 0.97, 18 large amplitude events from 11 cells). (**D**) Calibration curve of calculated free Ca^{2+} of the intracellular solution plotted against the obtained ratio of 340/420 nm fluorescence. The linear part of the fura-2 Ca^{2+} response is indicated with a linear fit. Fluorescence values were measured on the same system with the solutions used for experiments and measured in the tip of pipettes with low pressure applied to keep the concentration constant. Background subtraction was applied to the raw data before calculating the ratio. (**E**) *Top*, oblique contrast image of a satellite OL (sOL) in direct contact with a L5 neuron. *Bottom*, fura-2 epifluorescence image of the same cell showing two processes with a box indicating the location of the event shown in **F**. Scale bars 10 µm. (**F**) *Top*, background subtracted traces that show the opposite response of the fura-2 Ca^{2+} indicator at excitation wavelengths of 340 and 420 nm. Scale bar 100 arbitrary units (AU). *Bottom*, the processed corresponding trace shown as 340/420 ratio and as calibrated Ca^{2+} concentration. Overlaid in red is a single exponential fit to the decay.

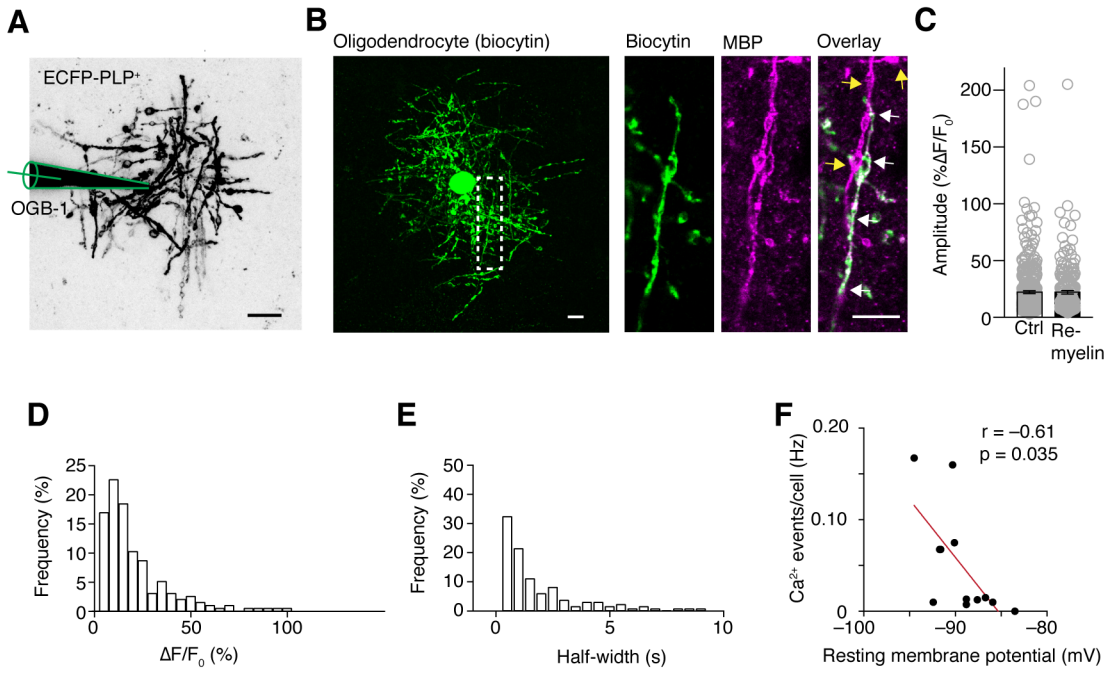

Figure S4 Remyelinating oligodendrocytes express MBP with myelin Ca^{2+} transients similar to early development. Related to Figure 4.

(**A**) Example *z*-projected confocal image of an OL filled with OGB-1 in the cuprizone-induced remyelinating somatosensory cortex. Scale bar 20 µm. (**B**) During whole-cell recording a single oligodendrocyte was filled with biocytin (green) and post-hoc labeled for MBP. The box indicates the processes shown in higher magnification on the right, revealing co-localization (white arrows) with myelin basic protein (MBP, magenta). An adjacent internode from another OL is not co-labeled for biocytin (yellow arrows). Two oligodendrocytes were recovered and both showed positive labeling. Scale bars 10 µm. (**C**) Summary plot of $\Delta F/F_0$ amplitude for transients during development and remyelination show no difference (MW-test, p = 0.47). (**D**) Histogram distribution of the $\Delta F/F_0$ of myelin Ca^{2+} transients in remyelinating oligodendrocytes (n = 195 events, N = 10 OLs). (**E**) Histogram of the half-width of Ca^{2+} events of remyelinating oligodendrocytes (n = 163 events, N = 8 OLs). (**F**) Similar as during at early postnatal ages, the Ca^{2+} event rate is correlated with the OL resting membrane potential (Spearman r = –0.61, p = 0.035, n = 12 OLs, N = 10 animals).

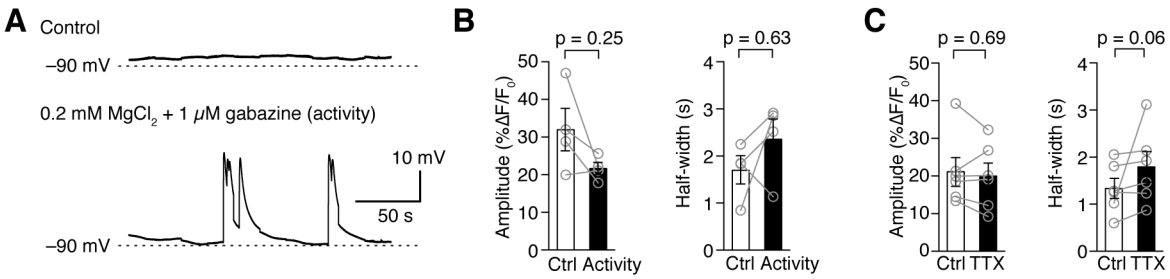

Figure S5 Ca^{2+} transient amplitude and half-width in myelin are not modulated by neuronal activity. Related to Figure 5.

(**A**) Current-clamp recordings from an OL in control conditions (top) and after pharmacological increase of activity (bottom). During increased activity, several large depolarization episodes can be seen reflecting increased neuronal firing. Traces were recorded with 4-seconds gaps and were concatenated for display. (**B**) Summary plots for Ca^{2+} event amplitude and Ca^{2+} event half-width for control and increased activity conditions. No changes were observed in 4 OLs. (**C**) Summary plots of Ca^{2+} event amplitude and half-width during control and in the presence of the sodium channel blocker tetrodotoxin (TTX) show no alterations (n = 6 OLs).

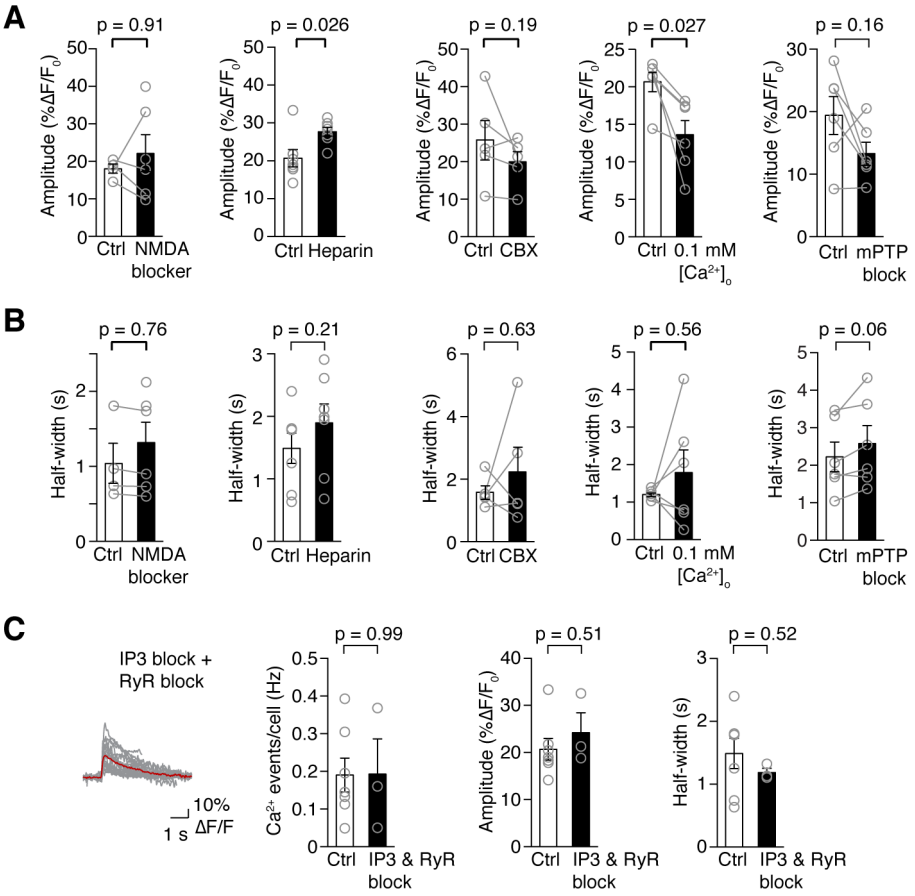

Figure S6 Effect of pharmacological blockers on microdomain Ca^{2+} transients. Related to Figure 6.

*(***A**) Population data showing amplitudes of Ca^{2+} transients before (open bars) and after wash in of indicated blockers (black bars). Heparin was used intracellularly and experiments could not be performed paired (Mann-Whitney test). NMDA blockers are pooled data of paired control-DAP5 and intracellular MK-801 experiments (Mann-Whitney test). All other experiments were conducted paired (Wilcoxon signed rank test). P values of the statistical tests are given in the figure. (**B**) Summary plots of the half-width in control and in the presence of indicated blockers. The half-width did not change with any of the tested pharmacological compounds. Results of statistical test are given in the figure. The same tests as in **A** were used for the different experiments. (**C**) *Left*, In the presence of the intracellular IP_3R blocker heparin and intracellular ryanodin receptor (RyR) blockers dantrolene and ruthenium red, no alterations in myelin Ca^{2+} transients are observed. *Right*, Summary plots for Ca^{2+} event frequency, amplitude and half-width show no difference to control cells (all Mann-Whitney tests). For **A-C**, individual data points depict one experiment. Mean ± SEM are shown for all summary plots.