Supplementary Materials for

Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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Movie S1. Optogenetic stimulation of M2 motor cortex elicits unidirectional ambulation. Video of the behavioral response in a P35 Thy1::ChR2 mouse to optogenetic stimulation of M2 motor cortex. After attachment of the implanted fiber optic ferrule in the right hemisphere to a mono fiber patch cord connected to a 100-mW 473-nm DPSS laser system, pulses of light were administered (~1 mW output at tip of fiber optic) at 20 Hz every 2 minutes for 30 seconds. The animal behaves freely during the "light off" period. When the "light on" period ensues, the animal circles to the left continuously until the next "light off" period.

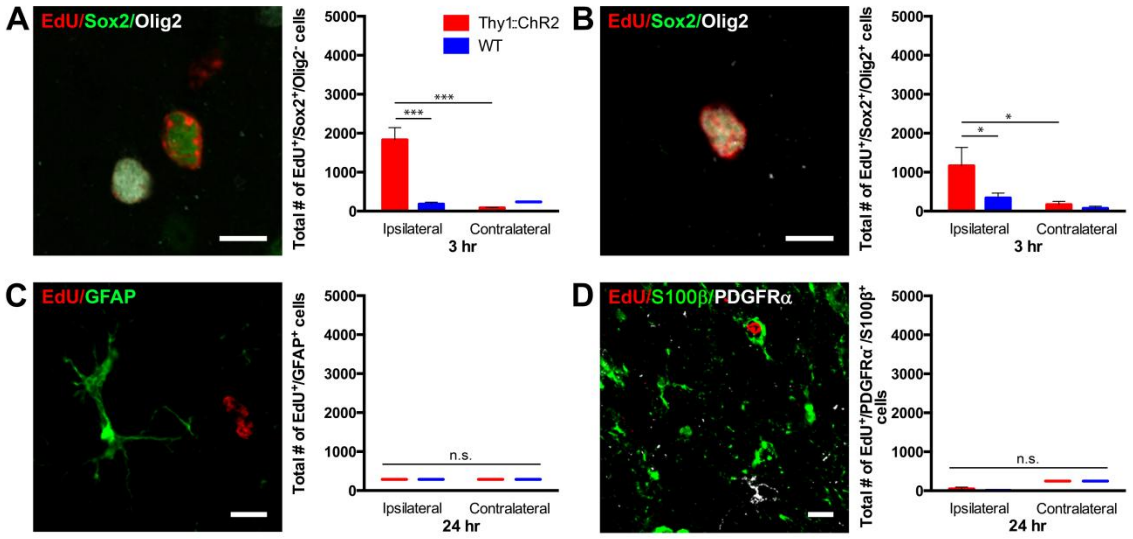

Fig. S1. Neuronal activity promotes NPC but not astrocyte proliferation. (A-D) Representative confocal micrographs of EdU^+ cells (red) and cell identity markers, together with total number of EdU-marked cells in M2 of a given cell identity within the first day following optogenetic stimulation at P35. **(A)** $EdU^+/Sox2^+/Olig2^-$ cells expressing a neural precursor cell (NPC) phenotype three hours following optogenetic manipulation. Sox2 (green) marks heterogeneous neural precursor cell populations in the postnatal mouse cortex, including a neurosphere-forming population (26), oligodendroglial lineage ($Olig2^+$, white) precursors **(B)** and astrocytic cell populations expressing markers such as GFAP **(C)** and S100β **(D)** (58). EdU did not co-localize with $GFAP^+$ (green) astrocytes **(C)** nor $S100\beta^+/PDGFR\alpha^-$ astrocytes **(D)**, measured at 24 hours. **(D)** Confocal micrograph illustrating a rare $EdU^+/S100\beta^+$ astrocyte (S100β = green, PDGFRα = white). In all graphs, red bars = Thy1::ChR2 mice and blue bars = WT littermate controls identically manipulated. $*P < 0.05$, $**P < 0.01$,$***P < 0.001$. n.s. indicates $P > 0.05$. Error bars, SEM. Scale bars = 10 μm. C-D: Value of (—) over x-axis = 0 ± 0.

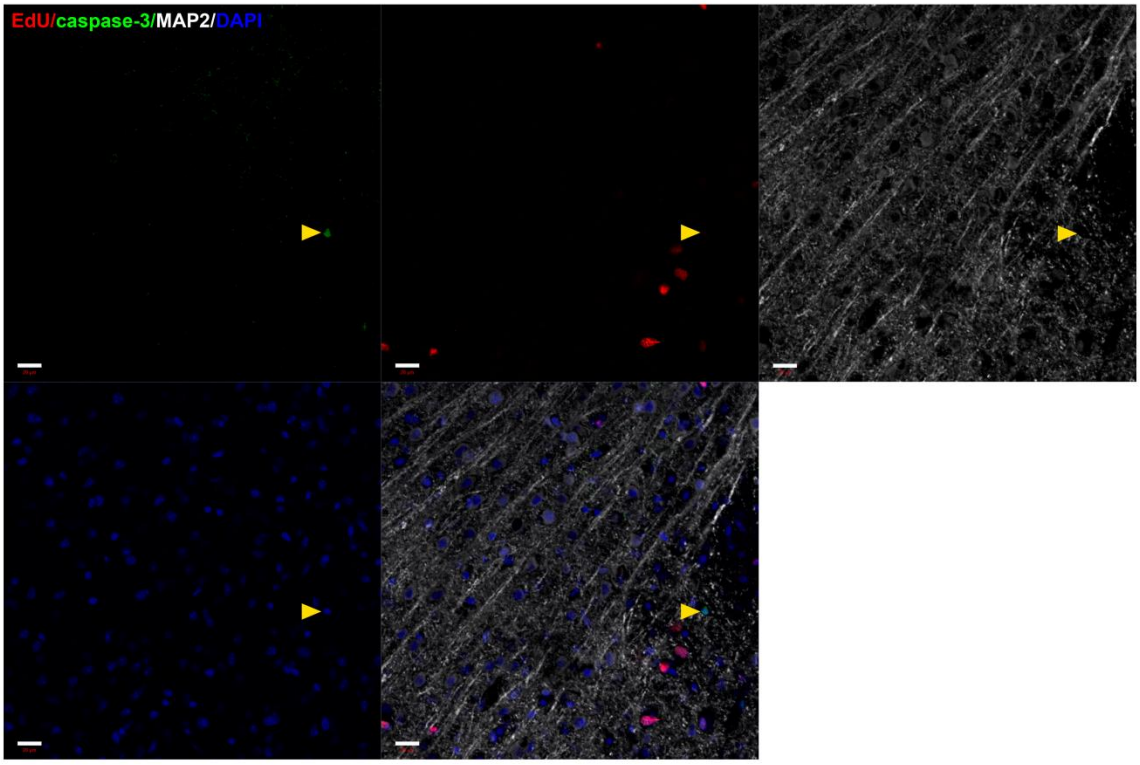

Fig. S2. Optogenetic stimulation did not cause apoptotic neuronal cell death. Confocal micrograph demonstrating cleaved caspase-3 immunostaining indicates rare cleaved caspase-3 apoptotic nuclei in the M2 cortex three hours following optogenetic stimulation at 20 Hz for 30 seconds every two minutes for 30 minutes. The single caspase-3^{+} (green) cell found in this section within the premotor cortex is indicated by a yellow arrowhead; it does not co-localize with the neuronal marker MAP2 (white), nor were any apoptotic neurons observed. EdU = red; DAPI = blue. Scale bar = 20 μm.

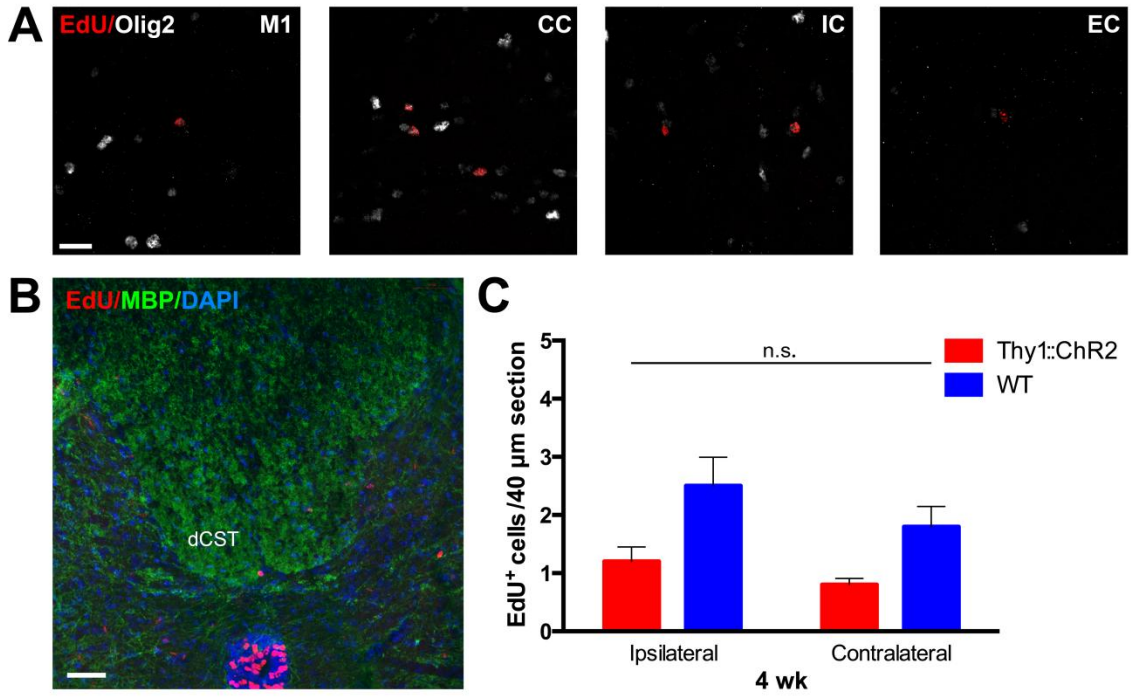

Fig. S3. Proliferating cells disperse, but are not found at the level of the cervical spinal cord. (A) By four weeks following optogenetic stimulation EdU-marked cells remain largely localized to area M2 and subcortical projections, but have also dispersed to some degree such that $EdU^+/Olig2^+$ cells are found in the neighboring primary motor cortex (M1), corpus callosum (CC) and internal capsule (IC); an $EdU^+/Olig2^-$ cell is shown in the external capsule (EC). **(B)** Confocal photomicrograph of a transverse section through the cervical spinal cord of a Thy1::ChR2 mouse 4 weeks post-stimulation. There is not an increase in the number of EdU^+ (red) cells detected in the spinal cord dorsal corticospinal tract (dCST). MBP = green; DAPI = blue. Scale bar = 50 µm. **(C)** Density of EdU^+ cells (average number of cells per 40-µm section) in the dorsal corticospinal tracts of the cervical spinal cord four weeks following optogenetic stimulation in Thy1::ChR2 (n = 4) or WT (n = 3) mice. Note that the corticospinal tract decussates at the level of the medullary pyramids, and thus the stimulated corticospinal tract is contralateral to the optogenetically-stimulated premotor cortex. Error bars, SEM; n.s. indicates $P > 0.05$.

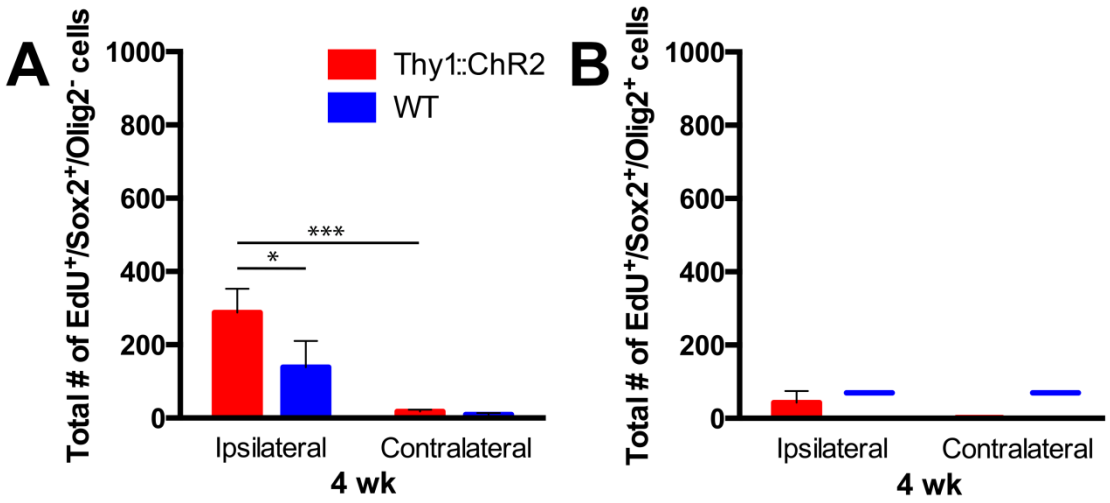

Fig. S4. EdU-marked Sox2$^+$ cells in M2 cortex at 4 weeks

(A-B) Sox2$^+$ cells evident four weeks following the completion of the 7-day optogenetic stimulation paradigm (P35-P42) in Thy1::ChR2 (n = 4) or WT (n = 3) mice. **(A)** EdU$^+$ neural precursor cells (EdU$^+$/Sox2$^+$/Olig2$^-$) and **(B)** oligodendroglial precursor cells (EdU$^+$/Sox2$^+$/Olig2$^+$) quantified in the M2 cortex. In all graphs, red bars = Thy1::ChR2 mice and blue bars = WT littermate controls identically manipulated. *P < 0.05, ***P < 0.001. Error bars, SEM. Value of (—) over x-axis = 0 ± 0.

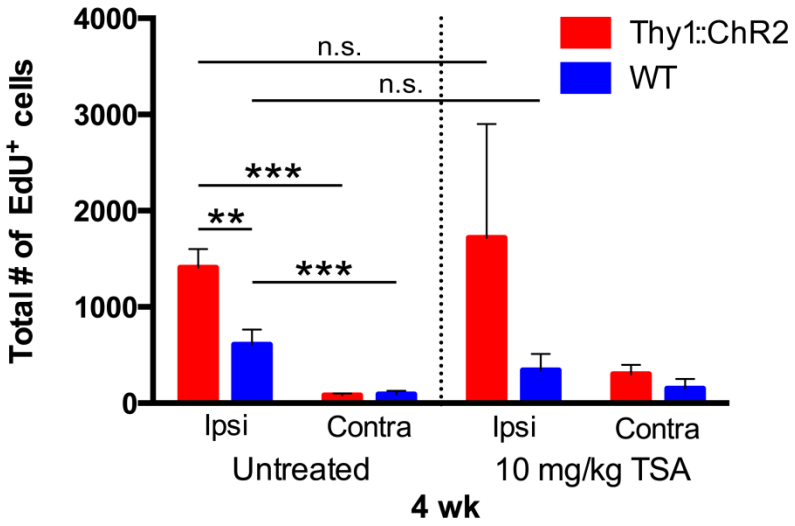

Fig. S5. Treatment with trichostatin A (TSA) does not affect total numbers of EdU$^+$ cells. Administration of the HDAC inhibitor trichostatin A (TSA) during the 7-day stimulation paradigm does not affect the total number of EdU$^+$ cells detected four weeks following the end of the stimulation paradigm. Data from non-drug-treated animals (presented in Fig. 4A) are repeated here for reference in the left-hand side of the graph. In all graphs, red bars = Thy1::ChR2 mice and blue bars = WT littermate controls identically manipulated. n = 4 Thy1::ChR2 mice, 3 WT mice; $^{**}P < 0.01$, $^{***}P < 0.001$, n.s. indicates $P > 0.05$, Error bars, SEM.

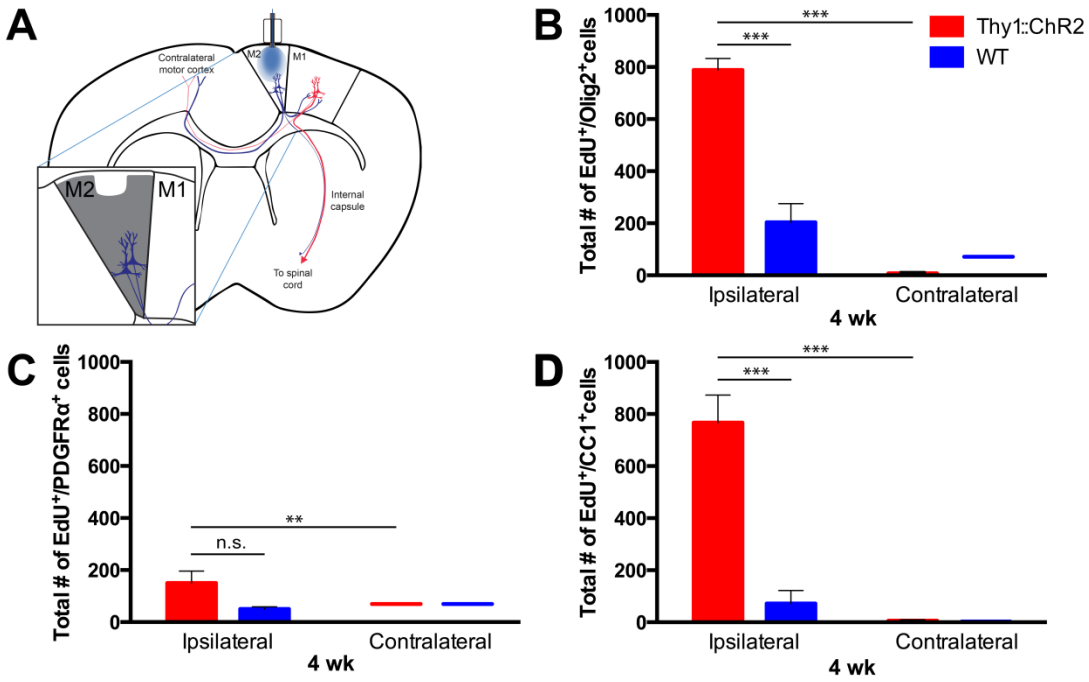

Fig. S6. Neuronal activity promotes oligodendrogenesis in the adult M2 cortex
Thy1::ChR2 and WT optogenetically-stimulated mice sacrificed 4 weeks following a 7-day stimulation paradigm (P84-P90). **(A)** Schematic illustrating the premotor cortex region quantified (shaded grey). **(B-D)** Quantification of cell identity markers of EdU-marked surviving cells quantified in adult M2 cortex reveals **(B)** persistent EdU^+ oligodendroglial lineage cells ($Olig2^+$), **(C)** with diminution of the oligodendrocyte precursor cell ($PDGFR\alpha^+$) population and **(D)** concomitant appearance of EdU-marked mature $CC1^+$ oligodendrocytes. In all graphs, red bars = Thy1::ChR2 mice and blue bars = WT littermate controls identically manipulated. n = 4 Thy1::ChR2 mice, 3 WT mice; $^*P < 0.05$, $^{**}P < 0.01$,$^{***}P < 0.001$. n.s. indicates $P > 0.05$. Error bars, SEM. Value of (—) over x-axis = 0 ± 0.

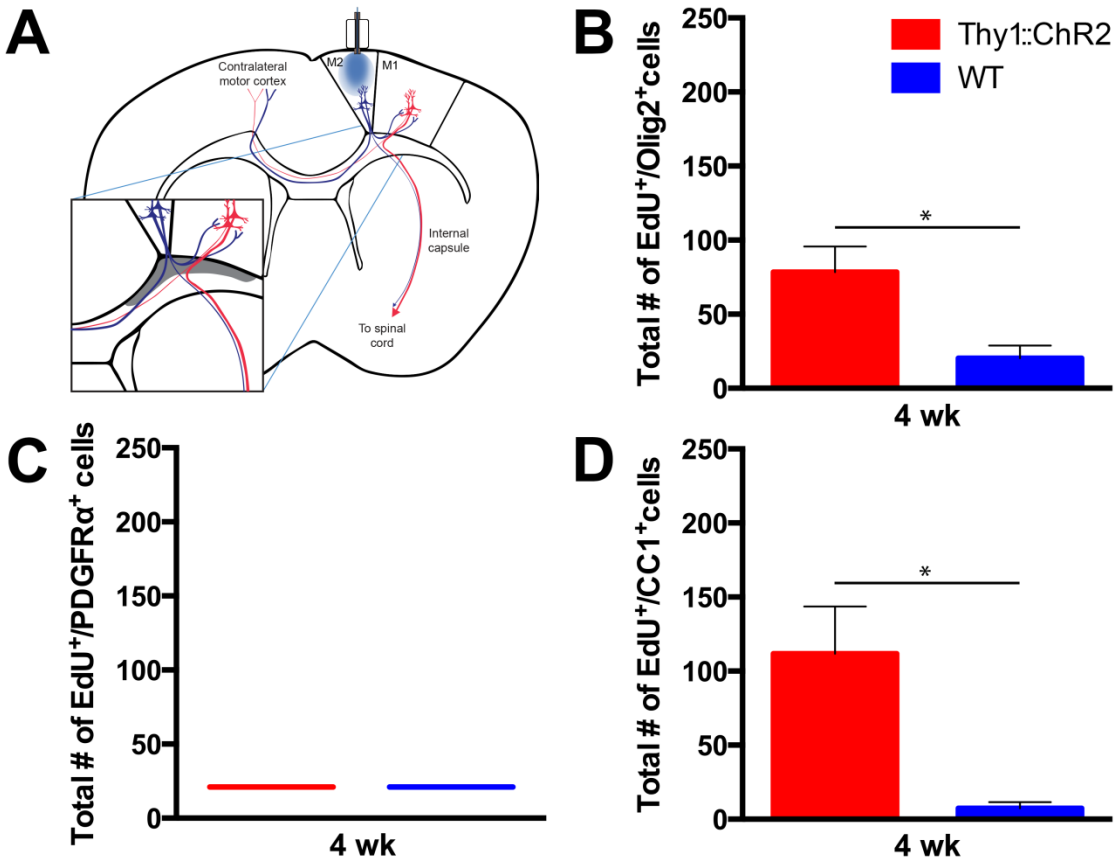

Fig. S7. Neuronal activity promotes oligodendrogenesis in the adult corpus callosum Thy1::ChR2 and WT optogenetically-stimulated mice sacrificed 4 weeks following a 7-day stimulation paradigm (P84-P90). **(A)** Schematic illustrating the corpus callosum region quantified (shaded grey). **(B-D)** Quantification of cell identity markers of EdU-marked surviving cells quantified in adult corpus callosum reveals **(B)** persistent EdU^+ oligodendroglial lineage cells ($Olig2^+$), **(C)** with diminution of the oligodendrocyte precursor cell ($PDGFR\alpha^+$) population and **(D)** concomitant appearance of EdU-marked mature $CC1^+$ oligodendrocytes. In all graphs, red bars = Thy1::ChR2 mice and blue bars = WT littermate controls identically manipulated. n = 4 Thy1::ChR2 mice, 3 WT mice; *$P < 0.05$; Error bars, SEM. Value of (—) over x-axis = 0 ± 0.

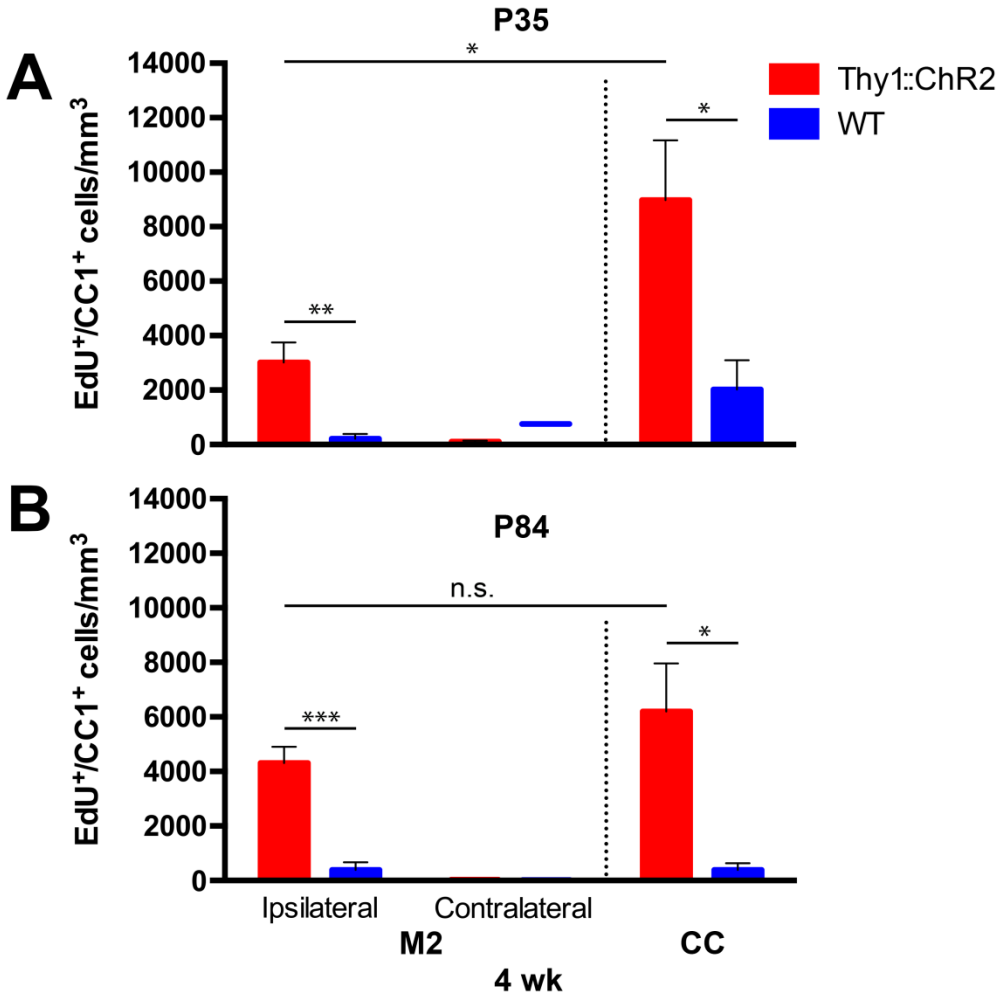

Fig. S8. EdU$^+$ oligodendrocytes normalized to tissue volume
The total number of EdU$^+$/ CC1$^+$ oligodendrocytes quantified 4 weeks following the end of the 7-day stimulation paradigm was normalized to the total volume (in cubic millimeters) of M2 cortex or corpus callosum (CC) for animals optogenetically manipulated at P35 **(A)** or P84 **(B).** In all graphs, red bars = Thy1::ChR2 mice and blue bars = WT littermate controls identically manipulated. *P < 0.05, **P < 0.01, ***P < 0.001. Error bars, SEM. Value of (—) over x-axis = 0 ± 0.

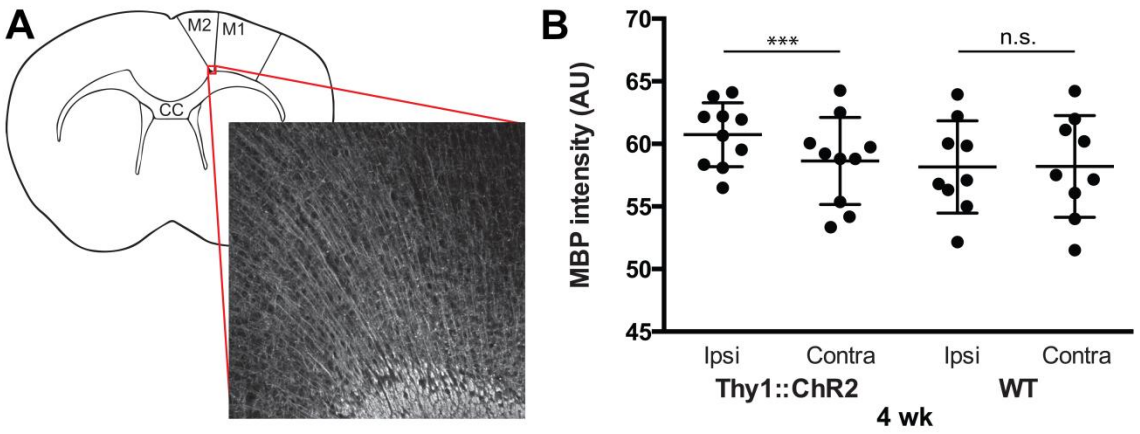

Fig. S9. MBP Expression. (A) Confocal photomicrograph (100X) of MBP^+ M2 fibers entering the corpus callosum. **(B)** Intensity measurements of MBP expression comparing ipsilateral to contralateral sides within a given slice. MBP intensity measured in the projections from M2 as they enter the corpus callosum in the region shown was increased on the side ipsilateral to optogenetic stimulation as compared to the contralateral side in Thy1::ChR2 mice; there was no significance observed between ipsilateral and contralateral sides in identically manipulated WT mice. N = 10 Thy1::ChR2 and n = 9 WT; ***P<0.001, n.s. indicates P > 0.05.

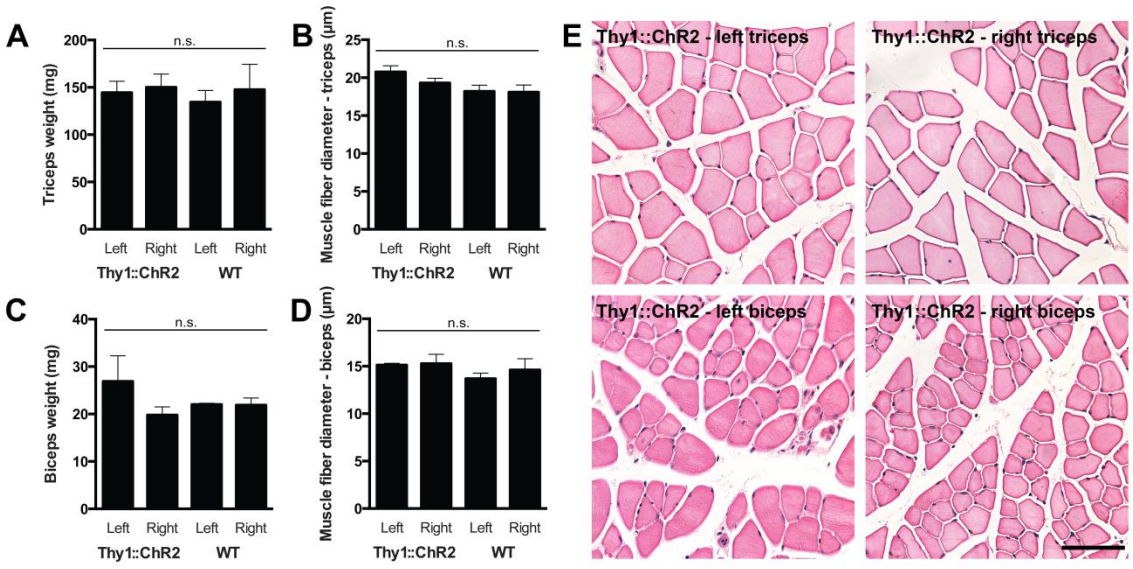

Fig. S10. The optogenetic stimulation paradigm does not affect muscle mass or myofiber morphology. We found no difference in triceps **(A** and **B)** or biceps brachii **(C** and **D)** mass or myofiber diameter between limbs or between groups. **(E)** Low-powered light photomicrographs of representative H&E-stained left and right triceps and biceps muscles from stimulated Thy1::ChR2 mice. Error bars, SEM. *n* = 4 mice/group. Scale bar = 50 µm. n.s. indicates *P* value > 0.05.

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