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Supplemental Information

Oligodendrocytes Support Neuronal Glutamatergic Transmission via Expression of Glutamine Synthetase

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SUPPLEMENTAL FIGURES

Figure S1

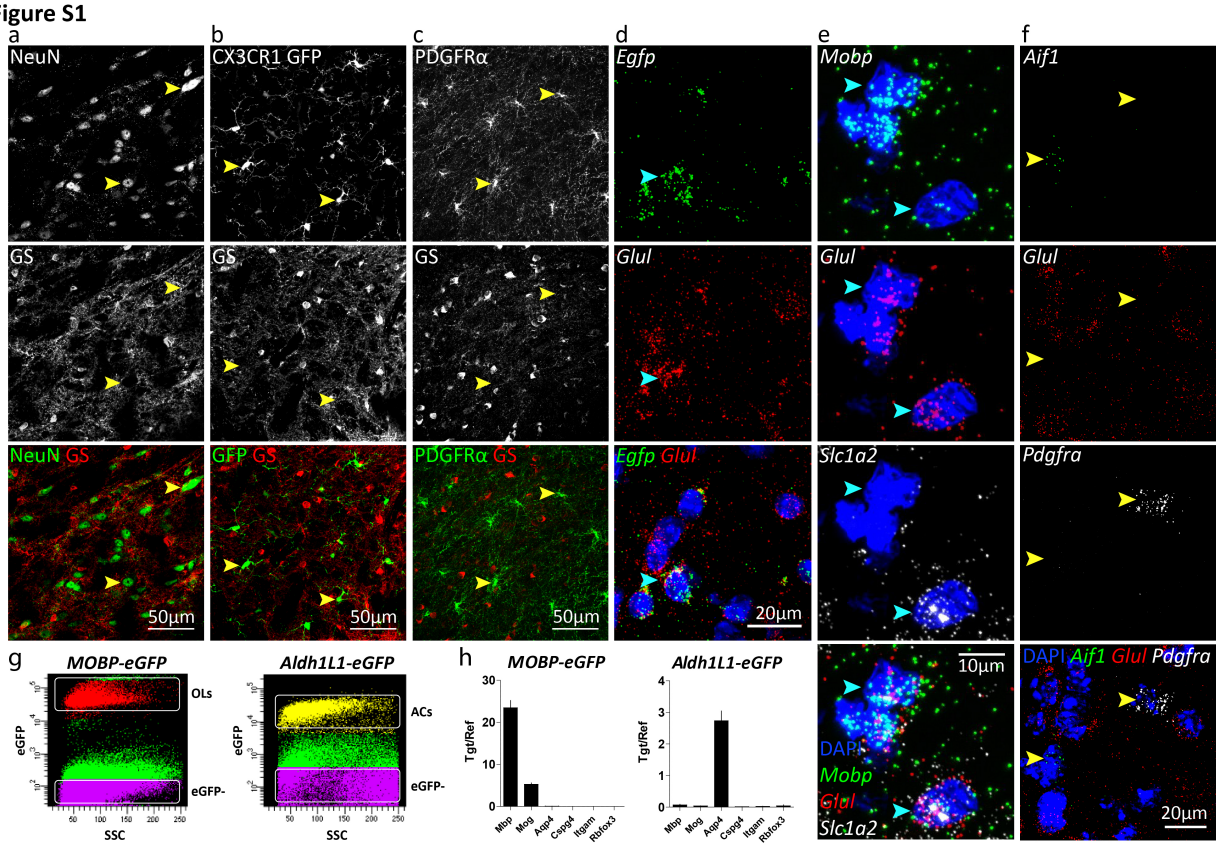

Figure S1 – Related to Figure 1: Oligodendrocytes express GS mRNA and protein. **a**, Immunostaining for GS and NeuN in the ventral midbrain of a P60 wild-type mouse. **b**, Immunostaining for GS in the ventral midbrain of a P60 CX3CR1 GFP mouse. **c**, Immunostaining for GS and PDGFRα in the ventral midbrain of a P60 wild-type mouse. **a-c**, Quantification in Figure 1f. **d**, In situ hybridization for *Egfp* and *Glul* in the ventral midbrain of an *MOBP-eGFP* mouse. **e**, In situ hybridization for *Mobp*, *Glul*, and *Slc1a2* in the ventral midbrain of a wild-type mouse. Quantification in Figure 1h. **f**, In situ hybridization for *Aif1*, *Glul*, and *Pdgfra* in the ventral midbrain of a P60 wild-type mouse. **a-f**, Yellow arrowheads indicate GS- or *Glul*- cells, blue arrowheads indicate *Glul*+ cells. **g**, Example scatter plots from FACS experiments with one *MOBP-eGFP* mouse and one *Aldh1L1-eGFP* mouse; x-axis = side scatter (SSC), y-axis = FITC channel intensity (eGFP). **h**, qPCR quantification of cell-type specific transcripts in eGFP+ cells isolated from *MOBP-eGFP* or *Aldh1L1-eGFP* mice. Expression levels normalized to sample *Gapdh* transcript; n=6-9 animals per cell type.

Figure S2

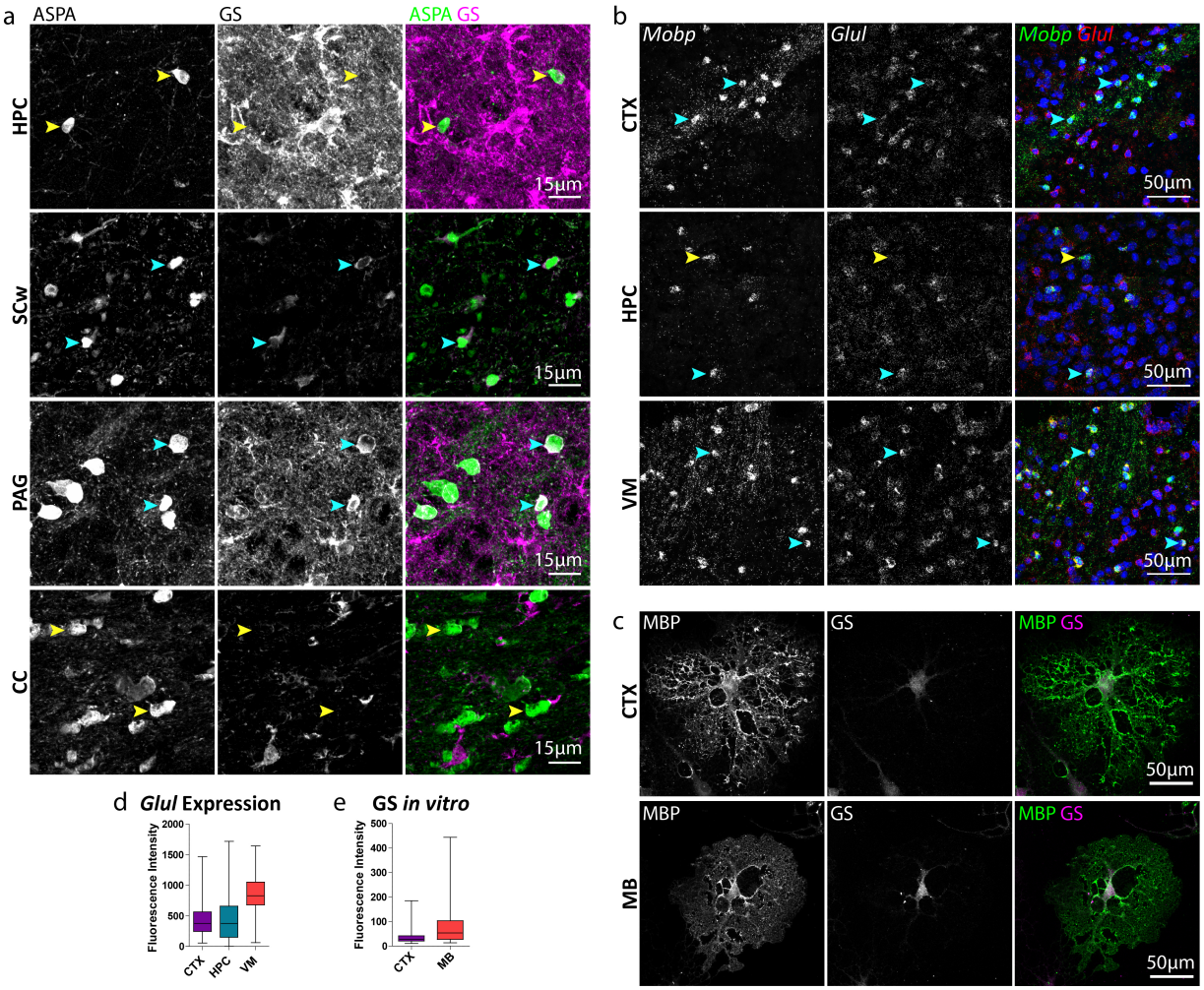

Figure S2 – Related to Figure 2: Oligodendrocyte GS expression appears at three weeks postnatally and is regionally heterogeneous. a, Immunostaining of ASPA and GS in dorsal hippocampus (HPC), spinal cord white matter (SCw), periaqueductal grey (PAG), and corpus callosum (CC) of P60 wild-type animals. Yellow arrowheads indicate GS- oligodendrocytes, blue arrowheads indicate GS+ oligodendrocytes. **b**, In situ hybridization for *Mobp* and *Glul* in cortex (CTX), hippocampus (HPC), and ventral midbrain (VM) of P60 wild-type mice. Blue arrowheads indicate *Glul*+ oligodendrocytes, yellow arrowheads indicate *Glul*- oligodendrocytes. **c**, Immunostaining of MBP and GS in DIV6 oligodendrocytes differentiated from oligodendrocyte progenitors isolated from P7 cortex (CTX) or P7 midbrain (MB). **d**, Box and whisker plots of *Glul* fluorescence in *Mobp*+ oligodendrocytes from each region; n=48-90 cells per region from 2 animals. **e**, Quantification of GS fluorescence within MBP+ oligodendrocytes; n=30 cells per group from 3 separate cultures.

Figure S3

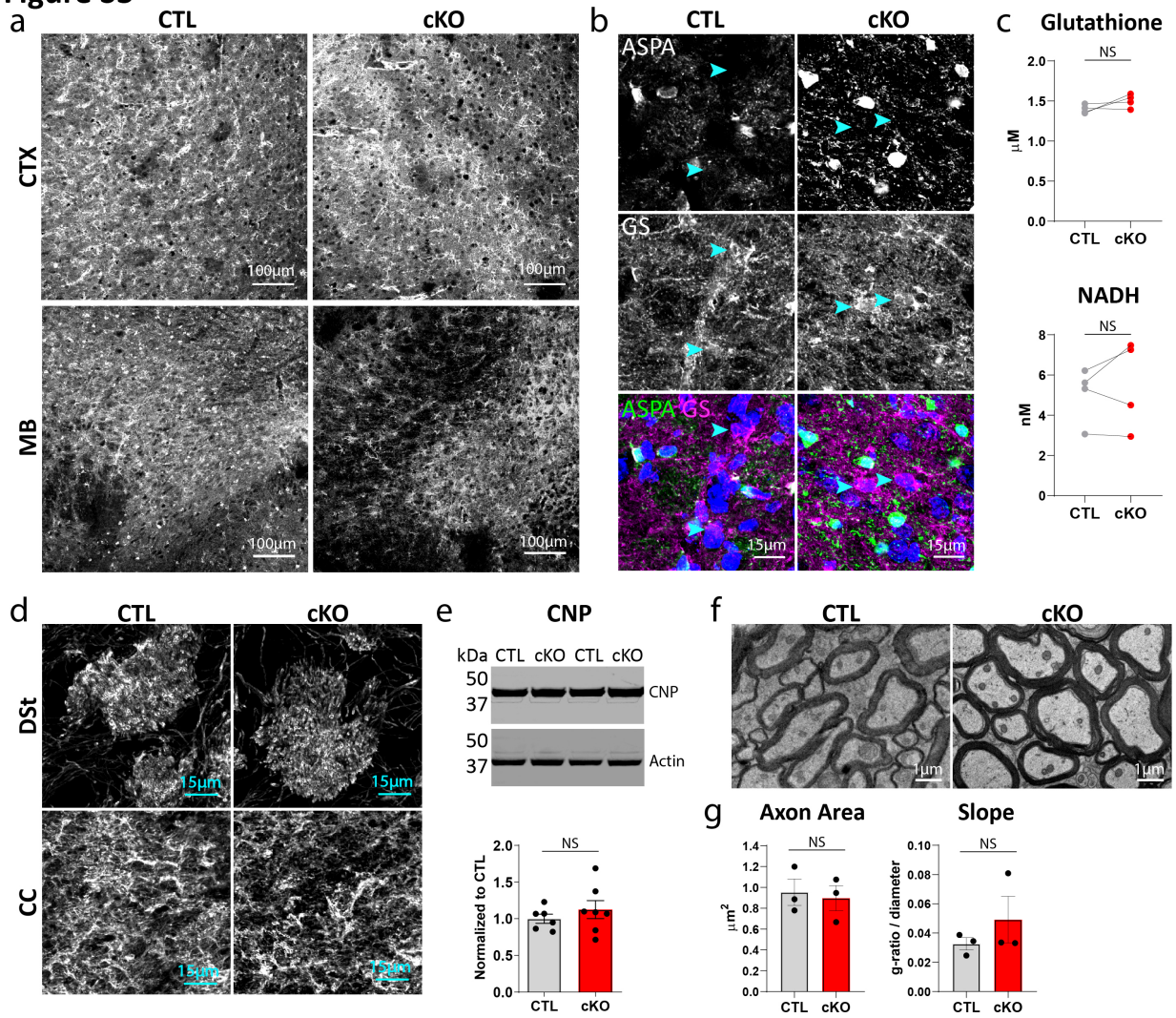

Figure S3 – Related to Figure 3: Mice lacking oligodendrocyte GS have reduced tissue levels of GS substrates but normal patterns of myelination. a, Immunostaining for GS in cortex (CTX) and midbrain (MB) of control (CTL) and conditional knockout (cKO) mice. **b**, Blue arrowheads indicate ASPA- GS+ astrocytes in CTL and cKO mice. **c**, Quantification of tissue glutathione and NADH in microdissected midbrains via colorimetric assays. One CTL and one cKO sample were processed in parallel per day. CTL and cKO values from the same day were paired for analysis. Glutathione paired t=1.684, df=3, p=0.1908; n=4 animals per group. NADH paired t=0.8253, df=3, p=0.4697; n=4 animals per group. **d**, Immunostaining for MBP in dorsal striatum (DSt) and corpus callosum (CC) of CTL and cKO mice. **e**, Western blot for CNP and loading control Actin in microdissected midbrain. Quantification of CNP protein relative to sample Actin. Unpaired t=0.8652, df=11, p=0.4054; n=6-7 animals per group. **f**, TEM images of myelinated axons in spinal cord of CTL and cKO mice. **g**, Quantification of axon area and slope of the best-fit line in linear regressions of axon diameter vs. g-ratio by animal. Axon area unpaired t=0.3318, df=4, p=0.7567. Slope unpaired t=1.011, df=4, p=0.3691; n=3 animals per group.

Figure S4

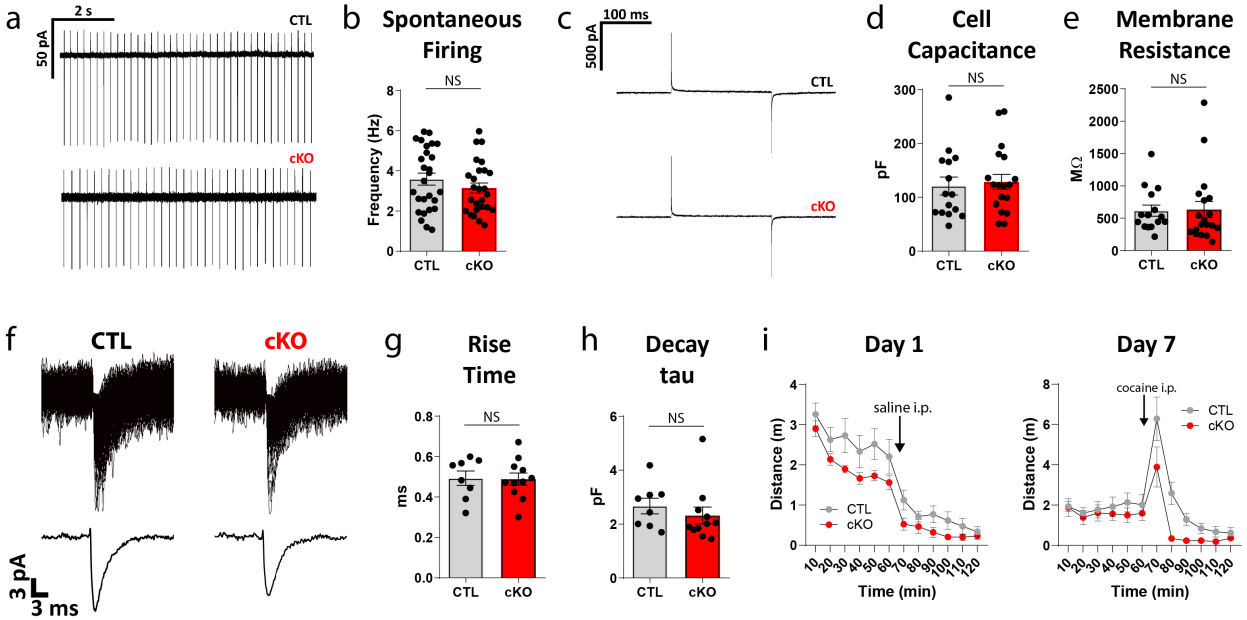

Figure S4 – Related to Figure 4: Oligodendrocyte GS deletion disrupts synaptic glutamate transmission in the midbrain and impairs cocaine-induced locomotor sensitization. a, Example cell-attached traces showing spontaneous dopamine neuron firing in CTL and cKO mice. **b**, Frequency of cell-attached spontaneous firing rate, unpaired t=1.129, df=51, p=0.2640; n=26-28 cells, 9 animals per group. **c**, Example traces of voltage-clamped neurons in response to a 5mV command step. **d**, Cell capacitance, unpaired t=0.3577, df=32, p=0.7229; n=15-19 cells, 5 animals per group. **e**, Membrane resistance, unpaired t=0.1098, df=32, p=0.9133; n=15-19 cells, 5 animals per group. **f**, Superimposed and averaged example traces of mEPSCs from CTL and cKO mice. **g**, mEPSC rise time, unpaired t=0.09417, df=17, p=0.9261; n=8-11 cells, 4 animals per group. **h**, mEPSC decay tau, unpaired t=0.8004, df=17, p=0.4345; n=8-11 cells, 4 animals per group. **i**, Distance traveled on Days 1 and 7 of locomotor sensitization behavior. Day 1 repeated measures ANOVA (time x genotype), main effect of time, $F(11,176)=77.8$, $p<0.0001$; main effect of genotype, $F(1,16)=2.925$, $p=0.1065$; interaction $F(11,176)=1.012$, $p=0.4372$, n=8-10 per group. Day 7 repeated measures ANOVA (time x genotype), main effect of time $F(11,176)=21.1$, $p<0.0001$; main effect of genotype $F(1,16)=3.706$, $p=0.0722$; interaction $F(11,176)=2.118$, $p=0.0212$; n=8-10 per group.

Table S1 – Related to Key Resources Table: Oligonucleotides

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Aqp4	Thermo Fisher Scientific	Cat# Mm00802131
Cspg4	Thermo Fisher Scientific	Cat# Mm00507257_m1
Gapdh custom probe primer forward sequence	Thermo Fisher Scientific	Sequence - GACAACTTTGGCATTGTGGAA
Gapdh custom probe primer reverse sequence	Thermo Fisher Scientific	Sequence – CACAGTCTTCTGGGTGGCAGTGA
Gapdh custom probe primer probe sequence	Thermo Fisher Scientific	Sequence – CTCATGACCACAGTCCA
Gls2	Thermo Fisher Scientific	Cat# Mm01164870_g1
Glul custom probe primer forward sequence	Thermo Fisher Scientific	Sequence – CAGGCTGCCATACCAACTTCA
Glul custom probe primer reverse sequence	Thermo Fisher Scientific	Sequence – TCCTCAATGCACTTCAGACCAT
Glul custom probe primer probe sequence	Thermo Fisher Scientific	Sequence – CAAGGCCATGCGGGA
Itgam	Thermo Fisher Scientific	Cat# Mm00434455_m1
Mbp	Thermo Fisher Scientific	Cat# Mm01262037_m1
Mbp	Thermo Fisher Scientific	Cat# Mm01266402_m1
Mog	Thermo Fisher Scientific	Cat# Mm00447824_m1
Rbfox3	Thermo Fisher Scientific	Cat# Mm01248771_m1