Supplementary Information for:

GluN2B and GluN2D NMDARs dominate synaptic responses in the adult spinal cord

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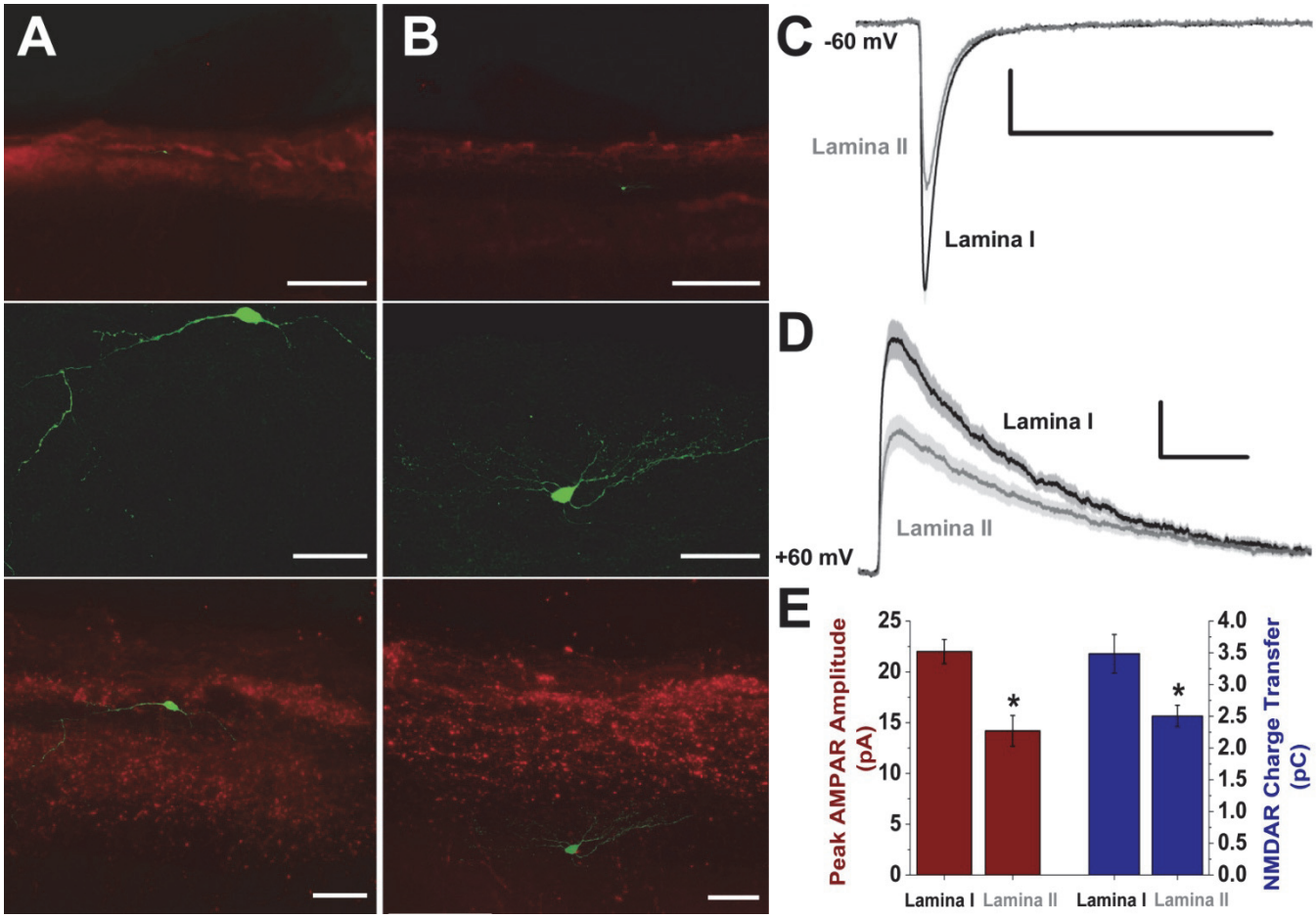
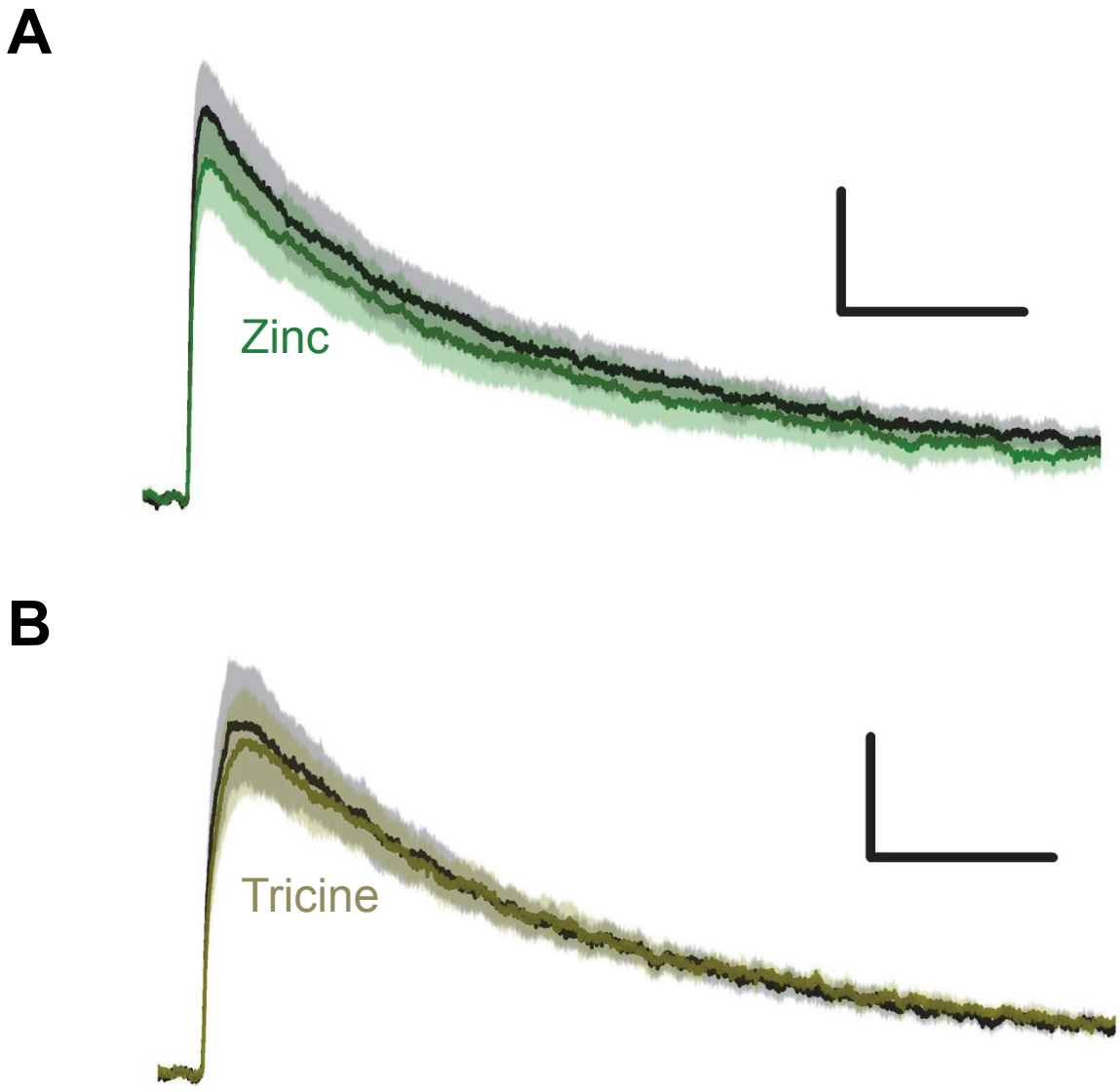
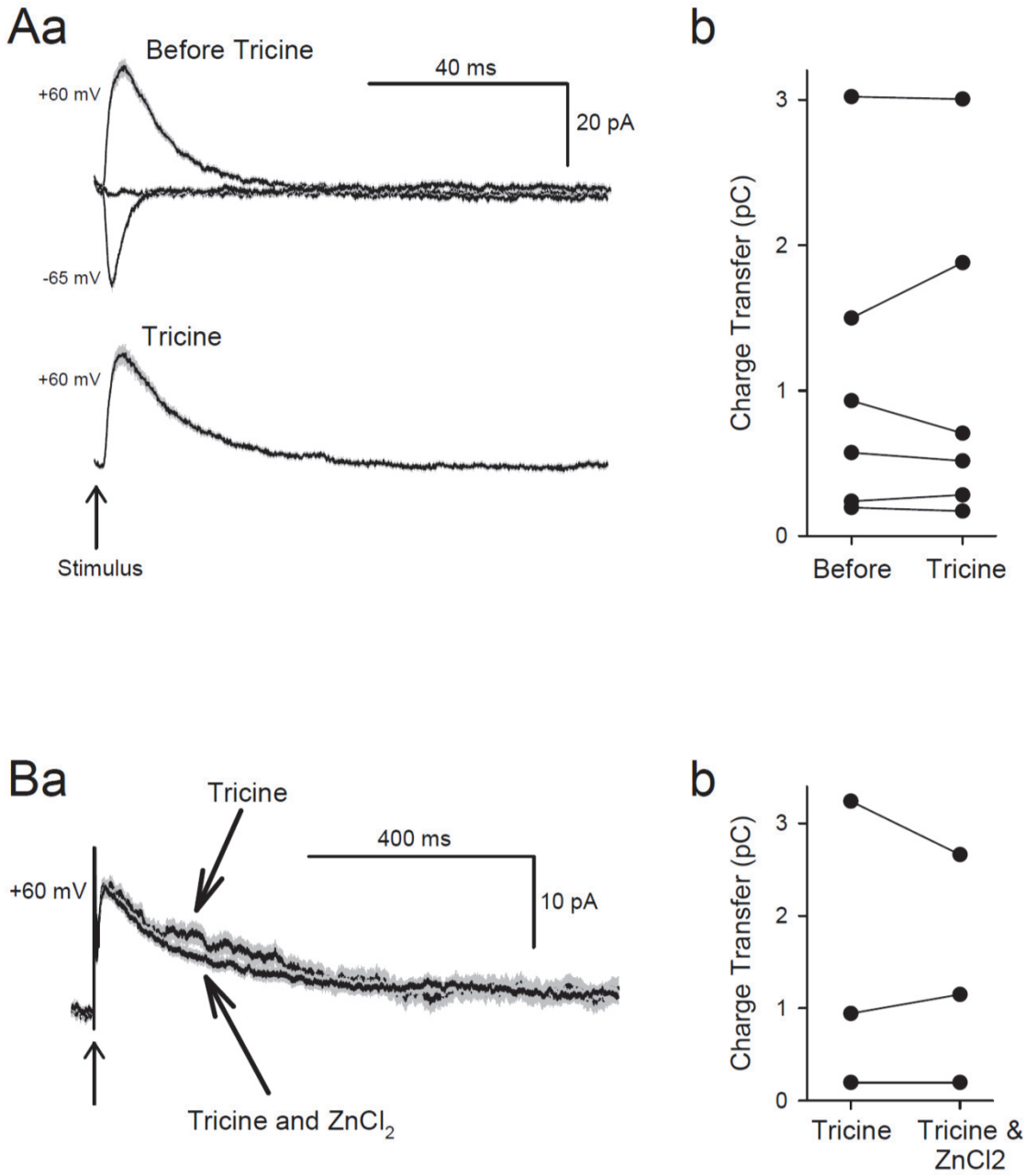


Supplementary Figure 1: Both AMPAR- and NMDAR- mEPSCs are significantly larger in lamina I neurons compared to lamina II neurons.
A,B) Representative images illustrating the location of a recorded lamina I neuron (**A**) and a recorded lamina II neuron (**B**) under both 50 x (*top*) and 200 x (*bottom*) magnification. The two-photon images of the lucifer yellow-filled neurons (*middle*) were manually scaled and superimposed onto epi-fluorescence stacks of slices fixed and stained for CGRP (red) labelling. Note the location of the lamina I neuron (*left*) in the dorsal region of CGRP staining and the location of the lamina II_{inner} neuron outside the CGRP stained dorsal horn region (*right*). Scale bars = 300 μm, 50 μm, and 100 μm for top, middle, and bottom panels, respectively. **C**) *Left*, averaged mEPSC traces at -60 mV demonstrating the smaller AMPAR mEPSC amplitude in LII neurons (grey) compared to LI neurons (black). **D**) Averaged mEPSC traces at +60 mV demonstrating a smaller NMDAR mEPSC amplitude in lamina II neurons compared to lamina I neurons. Trace scale bar x axes = 100 ms, y axes = 5 pA. All traces are presented as mean (darker line) ± standard error (lighter shaded area). **E**) Histograms comparing peak AMPAR mEPSC amplitude (at -60 mV, red) and NMDAR-mediated charge transfer (at +60mV, blue) between lamina I and lamina II neurons.



Supplementary Figure 2: Effects of exogenous and endogenous zinc on mEPSCs in lamina I neurons.
A) In the presence of 10 mM tricine (black), perfusion of 40 μM $ZnCl_2$ results in 200 nM free extracellular Zn^{2+} (green) (Paoletti *et al*, 1997). Administration of 200 nM free Zn^{2+} resulted in a small, significant inhibition of mEPSCs in lamina I neurons (*Control* Q = 2.50 ± 0.43 pC, n = 6; *200 nM zinc* Q = 2.16 ± 0.48 pC, n = 6, p = 0.025). **B**) Buffering endogenous Zn^{2+} with 10 mM tricine (olive green) does not affect mEPSCs. *Control* Q = 2.50 ± 0.30 pC, n = 4; *10 mM tricine* Q = 2.52 ± 0.27 pC, n = 4, p = 0.93. All traces are presented as mean (darker line) ± standard error (lighter shaded area). Trace scale bar x axes = 100 ms, y axes = 5 pA



Supplementary Figure 3: Effects of endogenous and exogenous zinc on uEPSCs in lamina I neurons.

A) Buffering endogenous Zn^{2+} with 10 mM tricine does not affect uEPSCs. **a)** Top**,** averaged traces from a lamina I neuron showing successful unitary EPSCs and synaptic failures at +60 mV as well as successful uEPSCs recorded at -65 mV. Bottom, administration of 10 mM tricine had no effect on uEPSC (+60 mV) amplitude or charge transfer for the same cell shown above**. b)** Plot of tricine-induced changes in NMDAR charge transfer in individual neurons demonstrating that tricine had no significant effect on NMDAR charge transfer for uEPSCs. **Ba**) In the presence of 10 mM tricine, perfusion of 40 μM $ZnCl_2$ results in 200 nM free extracellular Zn^{2+} (Paoletti *et al*, 1997). Averaged uEPSC traces from a lamina I neuron showing that administration of 200 nM free Zn^{2+} resulted in a small inhibition (18% reduction in charge transfer). **b**) Plot of Zn^{2+}-induced changes in NMDAR charge transfer in individual neurons demonstrating that the inhibitory effect of Zn^{2+} on NMDAR responses was only observed in one of three lamina I neurons.