Excitatory glycine responses of CNS myelin mediated by NR1/NR3 'NMDA' receptor subunits

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Supplemental material:

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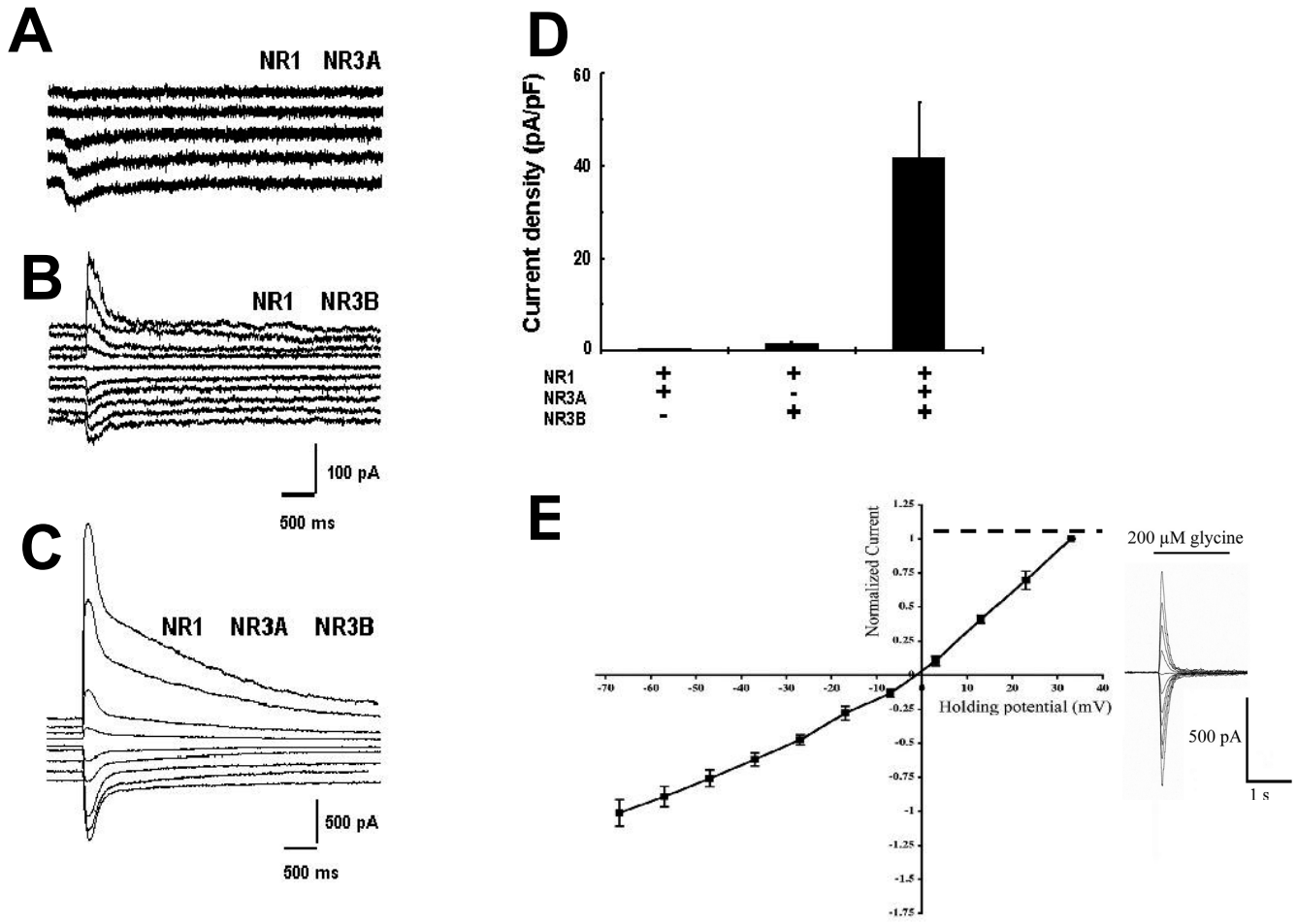
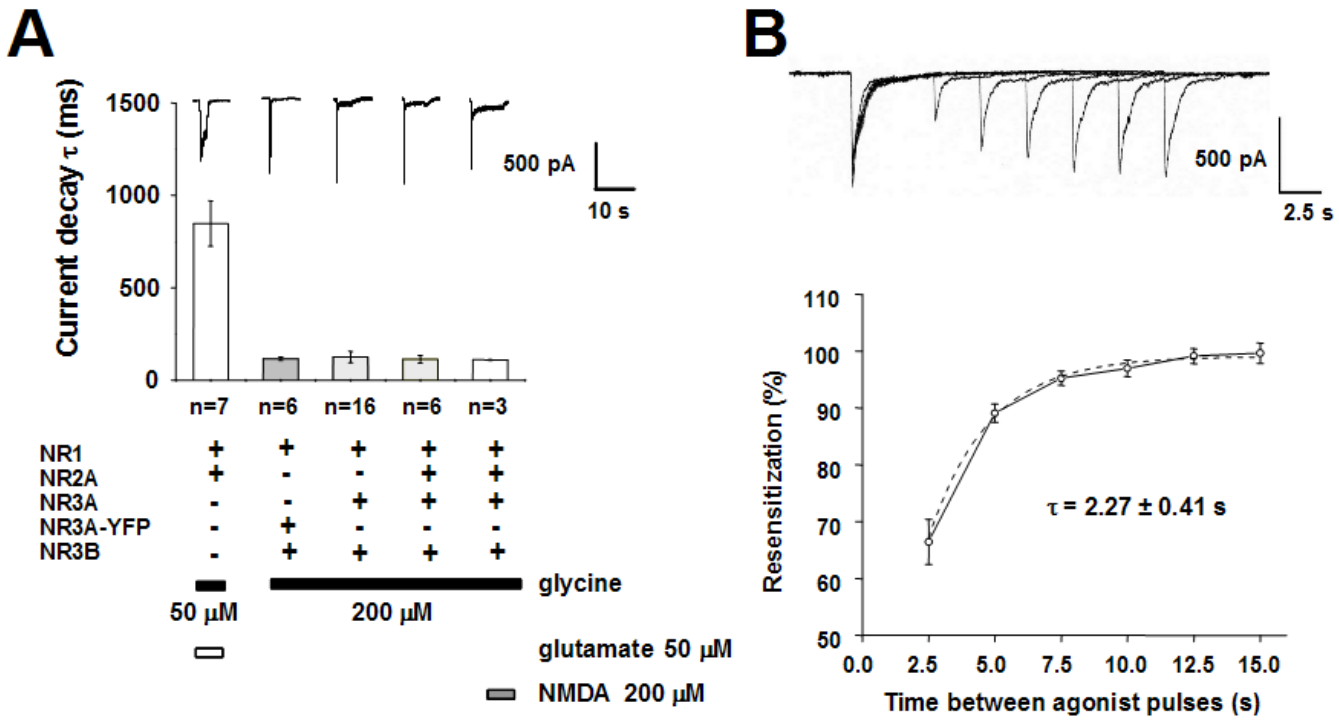
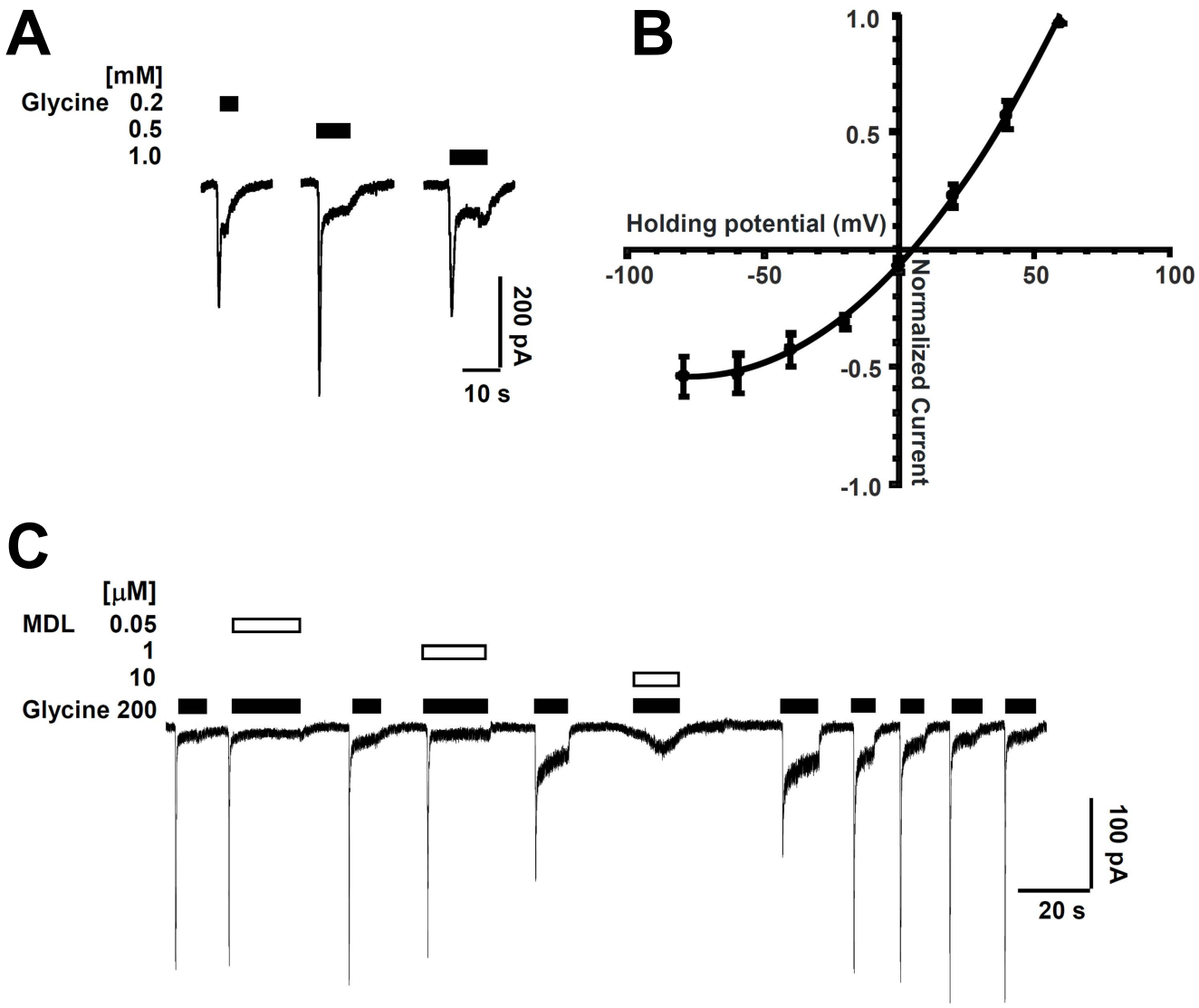


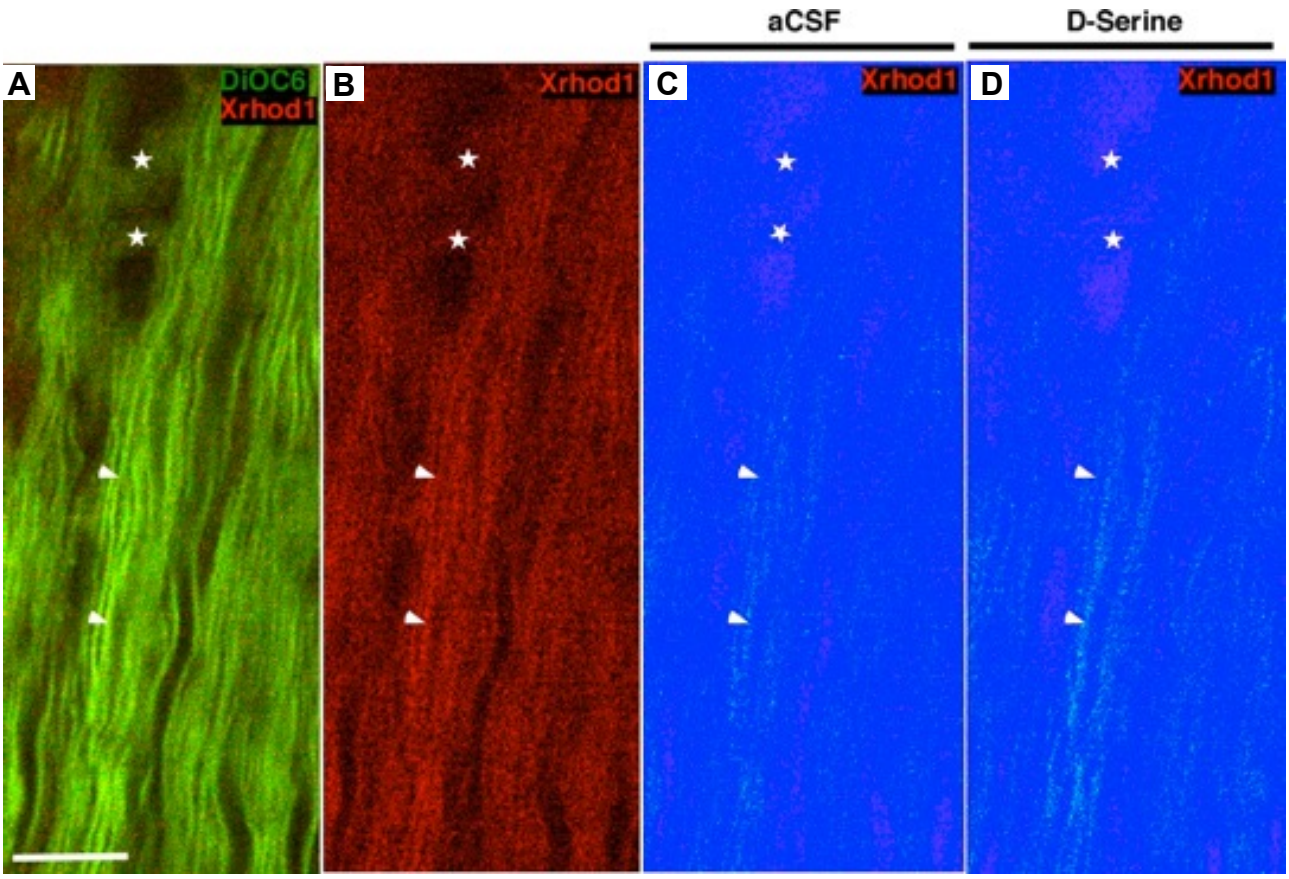
Supplementary Figure 1: Functional expression of recombinant NR1/NR3 receptors after transient co-transfection in mammalian HEK293 cells. HEK293 cells were transfected with the following subunit combinations: NR1 (NR1 or NR1-GFP) plus NR3A (NR3A or NR3A-YFP), or NR1 (NR1 or NR1-GFP) plus NR3B. When expression was assayed 24 to 72 hours post-transfection, we detected functional receptors activated by glycine in about 10% of all GFP-positive cells. ***A-C,*** Whole-cell responses recorded at different membrane potentials from HEK293 cells transduced with ***A,*** NR1/NR3A (1:2 ratio), ***B,*** NR1/NR3B (1:2 ratio), or ***C,*** NR1/NR3A/NR3B subunits (1:2:2 ratio). Whole-cell currents in response to a 300 ms application of glycine. ***D,*** Mean current density of whole-cell responses to glycine (n = 8 for each condition). ***E,*** Current-voltage (I/V) relationship for cells transfected with NR1/NR3A-YFP/NR3B. Recordings were performed during application of glycine at holding potentials between -80 and +30 mV. Mean current-density values were very small in response to application of glycine concentrations of 10 µM up to 1 mM for NR1/NR3A (0.3 pA/pF) and NR1/NR3B (1.5 ± 0.5 pA/pF) receptors (n = 5 each). NR1/NR3A/NR3B receptors yielded a larger mean current density of 41 ± 12 pA/pF (n = 10). Glycine-evoked currents were excitatory in nature since they reversed at ~0 mV in the presence of symmetrical cation and asymmetrical anion solutions on either side of the membrane (*inset at right*; n = 6).



Supplementary Figure 2: NR1/NR3 receptors expressed in HEK293 cells have distinctive desensitization and re-sensitization kinetics. ***A,*** We analyzed the kinetics of NR1/NR3 receptors by comparing the decay of NMDA and/or glycine-evoked whole-cell currents in HEK293 cells transfected with NR1/NR3A/NR3B subunits versus classical NR1/NR2A NMDARs. Use of a fast application system revealed currents with a large rapidly desensitizing component (91 ± 1.8% of peak amplitude; n=15) and a smaller but persistent non-desensitizing component (9 ± 1.8% of peak amplitude; n=15) for NR1/NR3 receptors. The desensitizing component in NR1/NR3 receptor-mediated responses had mean decay time constants of about 100 ms compared to 800 ms for NR1/NR2A receptors (Supplementary Table 1). *B,* Recovery of glycine-evoked current from desensitization. Complete recovery (re-sensitization) was achieved 7.5 s after the end of the conditioning pulse ($\tau_{1/2}$ = 2.27 ± 0.41 s). To analyze recovery from desensitization, we applied test applications of 200 μM glycine at increments of 2.5 seconds to cells expressing NR1/NR3A/NR3B receptors. We observed ~ 93% recovery of the control response within 5 seconds of the first application of agonist and complete recovery within 7.5 s of the first agonist application. Recovery from desensitization occurred with a time constant τ = 2.27 ± 0.41 s (*n* = 6). No currents were observed when glycine was applied to non-transfected cells (*n* = 11) or to cells transfected with a single subunit (NR1, NR3A or NR3B; *n* = 17; data not shown). Holding potential, -60 mV.



Supplementary Figure 3: Pharmacological properties of recombinant NR1/NR3 receptors expressed in HEK293 cells. Application of 200-μM glycine evoked currents in about 60% of cells (threshold of response, ~100 μM glycine). ***A,*** Concentrations of 200 μM to 1 mM glycine produced desensitization, with progressively smaller peak amplitude and tail currents at higher concentrations. ***B,*** Glycine-evoked currents were observed in the presence of 1 mM external Mg^{2+} at a holding potential of -60 mV, although some degree of blockade developed at more negative potentials. ***C,*** MDL-105,519 [(E)-3-(2-phenyl-2-carboxyethenyl)-4, 6-dichloro-1H-indole-2-carboxylic acid], an antagonist of the NR1 glycine binding-site (Baron et al. 1996), exhibited a long-lasting, dual-effect on glycine-evoked currents, inhibiting peak current but potentiating steady-state current. The glycine-binding site antagonist 5,7-dichlorokynurenic acid (5,7-dichloro-4-hydroxyquinoline-2-carboxylic acid; DCKA; Baron et al. 1991) had similar dual effects on glycine-evoked currents (data not shown). Effects were concentration-dependent and reversible. Complete recovery of peak and steady-state responses from antagonist usually occurred within 3 min. Holding potential, -60 mV.



Supplementary Figure 4: D-serine induces Ca^{2+} accumulation in the myelin compartment. ***A,*** Two photon-excited image of the rat optic nerve co-loaded with ion insensitive DiOC6 (green) to visualize myelin (arrowheads) and oligodendrocytes (stars) and with the calcium-sensitive dye Xrhod1 (red). ***B,*** Same image as ***A*** showing only Xrhod1 channel. ***C,*** Same image as in ***B*** showing Xhod1 fluorescence in pseudocolor during exposure to normal oxygenated aCSF. ***D,*** D-Serine application induced Ca^{2+} accumulation in the cytoplasmic compartment of myelin (arrowheads) but not in oligodendrocytes (stars). Scale bar, 10 μm.

Supplemental Table 1

Decay time constants of glycine or glycine/glutamate evoked currents.

Subunit composition	Agonist(s)	Decay time constant, ms
NR1/NR2A *n* = 7	Glycine 20 μM Glutamate 50 μM	846.5 ± 122.9
NR1/NR3A-YFP/NR3B *n* = 6	Glycine 200 μM	116.0 ± 9.4
NR1/NR3A/NR3B *n* = 16	Glycine 200 μM	124.2 ± 30.5
NR1/NR2A/NR3A/NR3B *n* = 6	Glycine 200 μM	112.8 ± 20.43
NR1/NR2A/NR3A/NR3B *n* = 3	Glycine 200 μM NMDA 100 μM	109.1 ± 1.5

Supplemental References

Baron BM, Siegel BW, Harrison BL, Gross RS, Hawes C, Towers P (1996) [^{3}H]MDL 105,519, a high-affinity radioligand for the *N*-methyl-D-aspartate receptor-associated glycine recognition site. J Pharmacol Exp Ther 279: 62-68.

Baron BM, Siegel BW, Slone AL, Harrison BL, Palfreyman MG, Hurt SD (1991) [^{3}H]5,7-dichlorokynurenic acid, a novel radioligand labels NMDA receptor-associated glycine binding sites. Eur J Pharmacol 206: 149-154.