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Supplementary Materials for

Delta glutamate receptors are functional glycine- and D-serine–gated cation channels in situ

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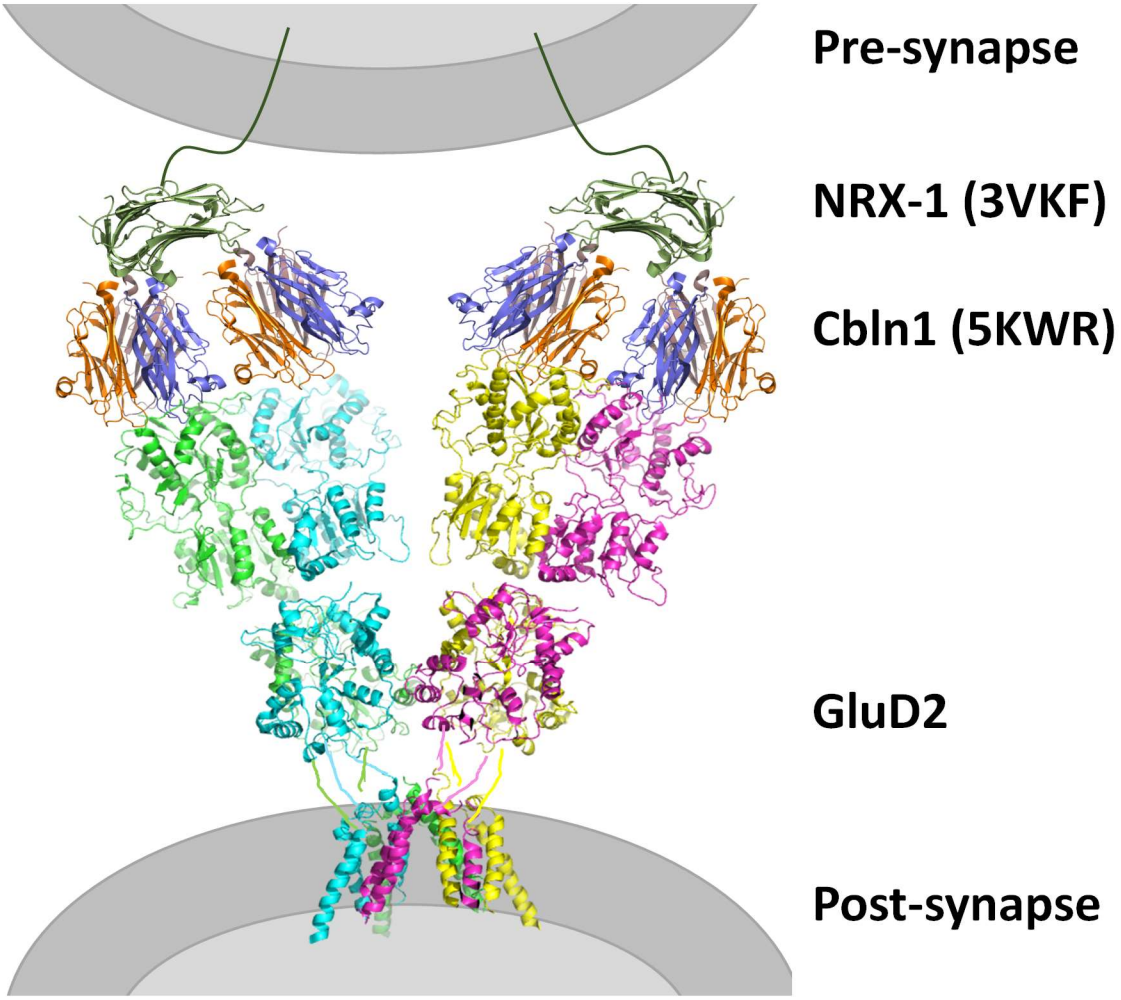

Fig. S1. GluD2 structure with hypothesized trans-synaptic connection. Schematic diagram of the arrangement of GluD2 receptors (modeled on GluD1, PDB:6KSS), Cbln1, and NRX1 within a synapse based on prior structures of individual components.

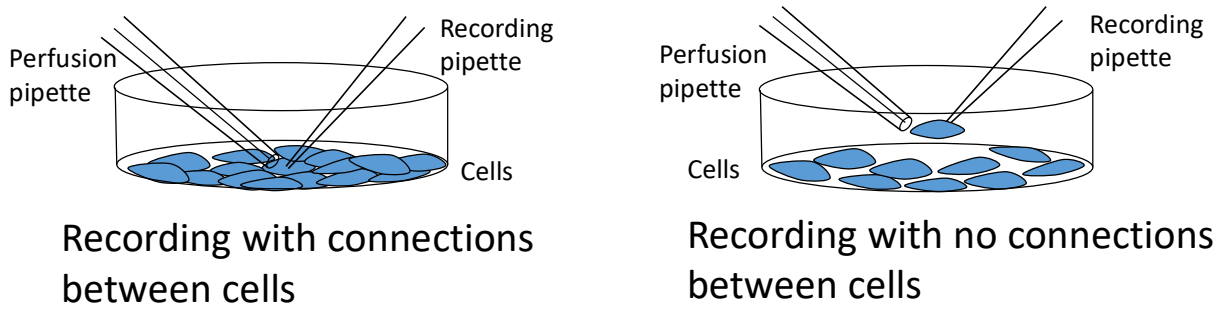

Fig. S2. (Top) Illustration showing configuration for recordings with connections and without connections. For recordings with connections the recordings were performed with cells/neurons attached to the dish so that the connections were maintained, and for recordings without connections an individual cell/neuron was lifted from the dish and brought close to perfusion device.

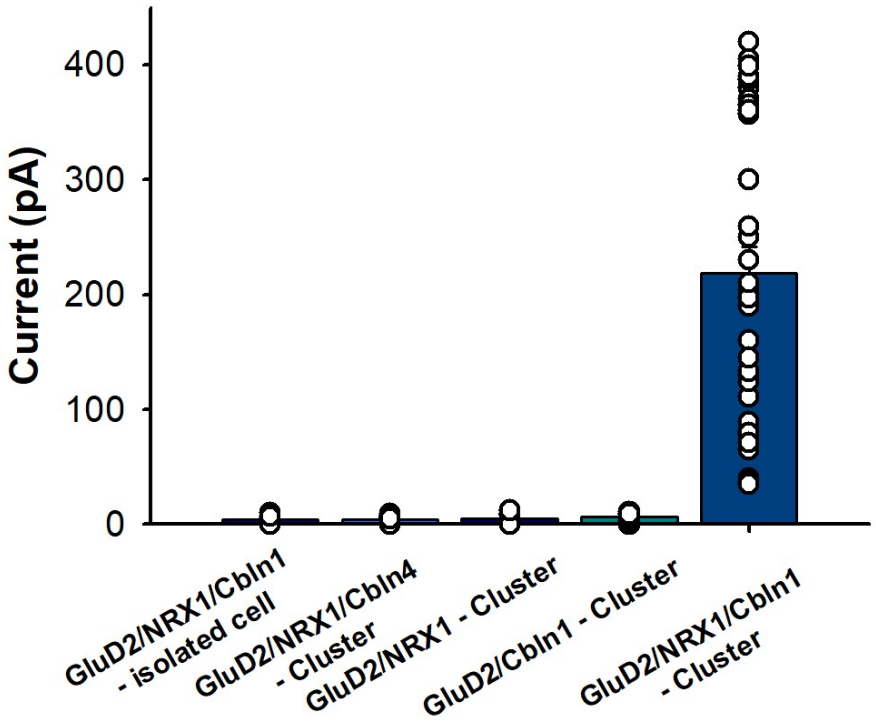

Fig. S3. Glycine activated maximal currents in HEK-293 cells expressing GluD2 receptors. Normalized bar graph showing individual measurements for Glycine-activated currents in cells expressing GluD2 with either Cbln1 or NRX1 in clusters, Cbln4 and NRX1 in clusters, or Cbln1 and NRX1 in isolated cells, compared to GluD2 with Cbln1 and NRX1 in clusters. P = 0.00005 between GluD2/NRX1/Cbln1-isolated cell and GluD2/NRX1/Cbln1-Cluster showing statistical significance.

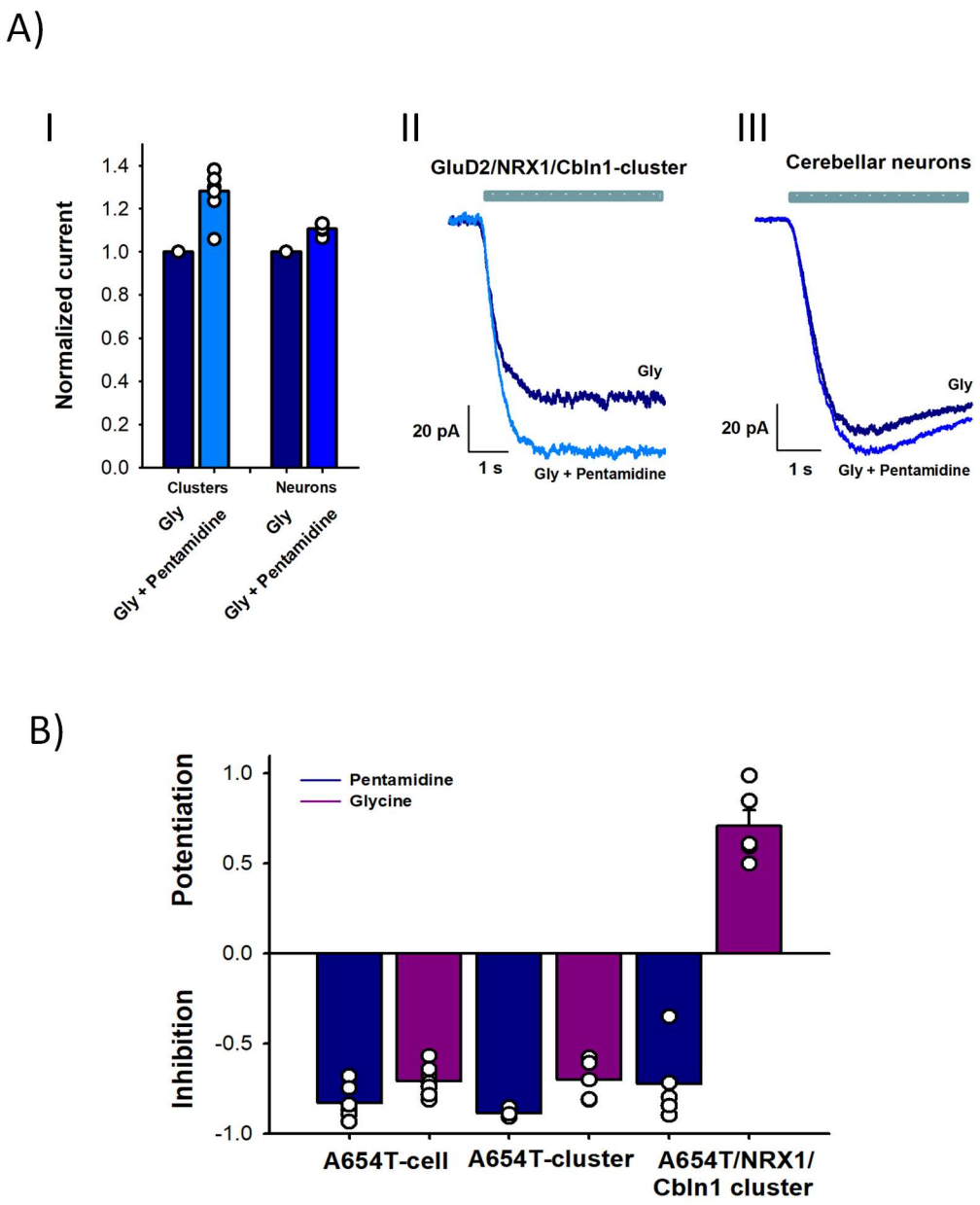

Fig. S4. Effect of pentamidine. A) I) Normalized bar graph showing extent of potentiation of currents mediated by 10 mM glycine due to application of 100 μM pentamidine in GluD2 with Cbln1 and NRX1 in clusters and in neurons representative currents for the same are shown in **II** and **III**.

B) Normalized bar graph showing individual measurements for currents recorded upon application of 100 μM pentamidine or 10 mM glycine from isolated cells, cells in clusters in the absence of NRX1 and Cbln1, and cell in clusters in the presence of NRX1 and Cbln1.

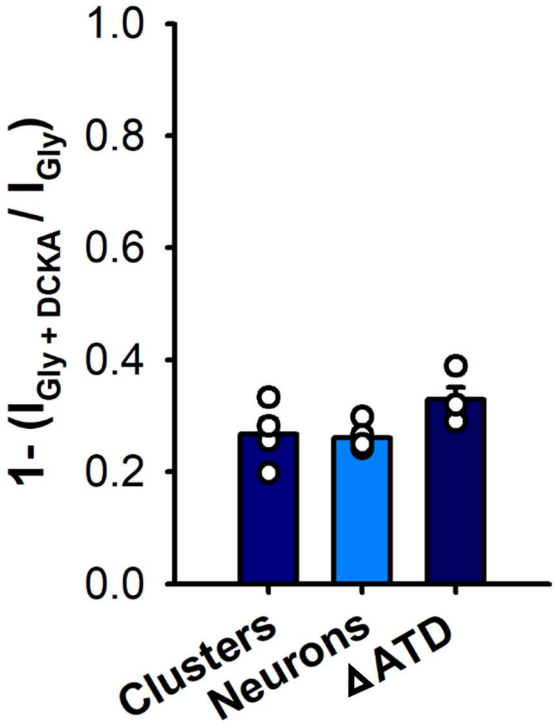

Fig. S5. Inhibition of glycine activated currents by DCKA. Normalized bar graph showing extent of inhibition of 10 mM glycine mediated currents due to application of 500 μM DCKA in GluD2 with Cbln1 and NRX1 in clusters, neurons, and GluD2-ΔATD receptors.

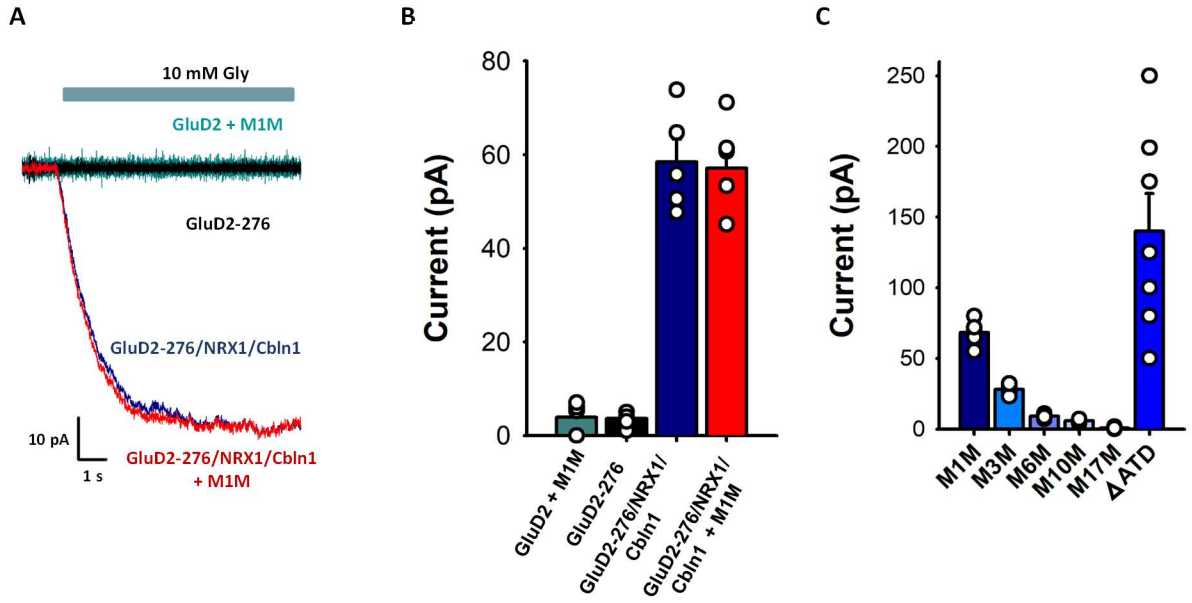

Fig. S6. Effect of cross-linkers on GluD2 receptor activation by glycine. (A) Representative electrophysiological current measurements upon application of 10 mM glycine, with GluD2 (R276C) with Cbln1 and NRX1 in clusters due to 10 mM glycine in the presence and absence of M1M, GluD2 receptors (without cysteine at 276) showing no currents with M1M, GluD2 (R276C) without M1M, (B) bar graphs showing currents by 10 mM glycine for all measurements shown in Figure S6A. (C) Whole cell currents with 10mM glycine with GluD2 (R276C) for all measurements with cross-linkers and ΔATD.

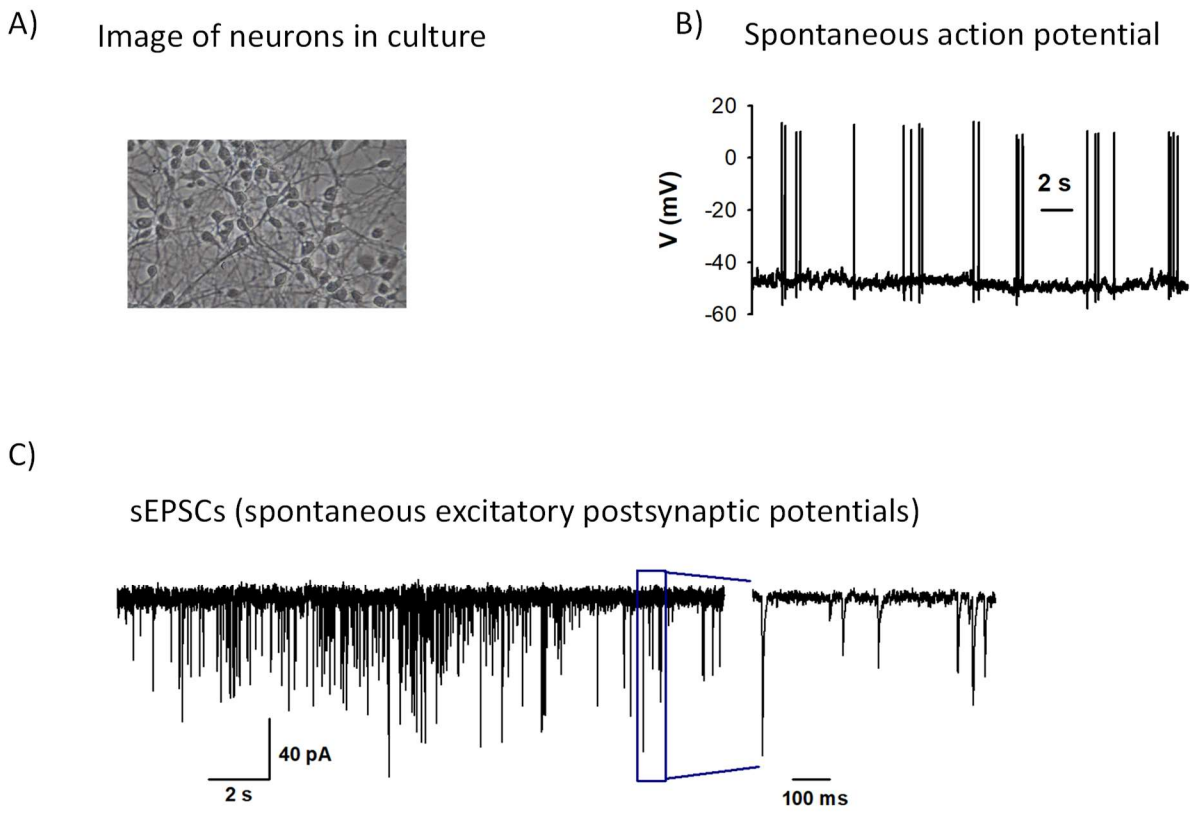

Fig. S7. Neuronal activity. A) Image showing the neurons with connections. **B)** Spontaneous action potentials recorded from a cerebellar neurons, bathed in physiological solution, using whole-cell current clamp. **C)** Representative recording showing spontaneous EPSCs, exhibit a broad range of amplitudes. sEPSCs were isolated by application of bicuculline and Mg^{2+}. Action potentials firing was suppressed by the addition of 1 μM TTX to the bath. The panel on the right show an expanded time scale from the left.

Ligand Concentration (mM)	Clusters Glycine	Clusters D-serine	Clusters 0.5 mM Gly + DCKA	Clusters 2 mM Gly + DCKA	Neurons Glycine
0.001			5		
0.01	4	4	7	4	3
0.1	5	4	7	5	4
0.25			4		
0.4			4		
0.5			5	5	
0.75					
1	5	5		4	6
1.5					
2				4	
2.5				4	
5	6	5			7
8	4	4			
10	10	7			7
15	3	4			

Table S1: Number of measurements. Current measurements for each point in the concentration response curves for Figure 1E, 1H and Figure 3E