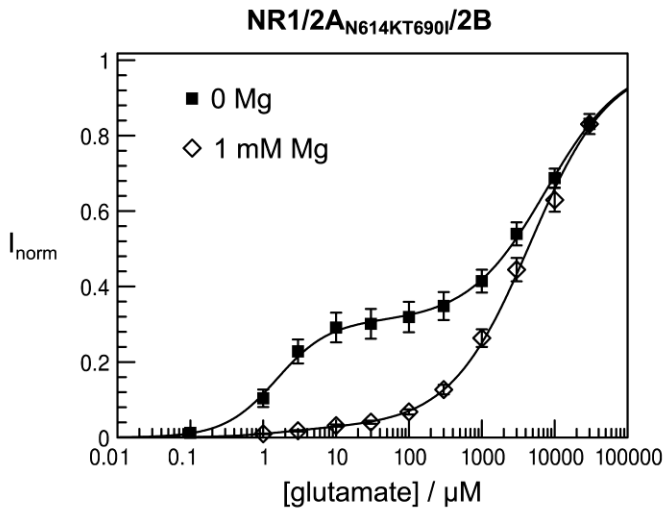
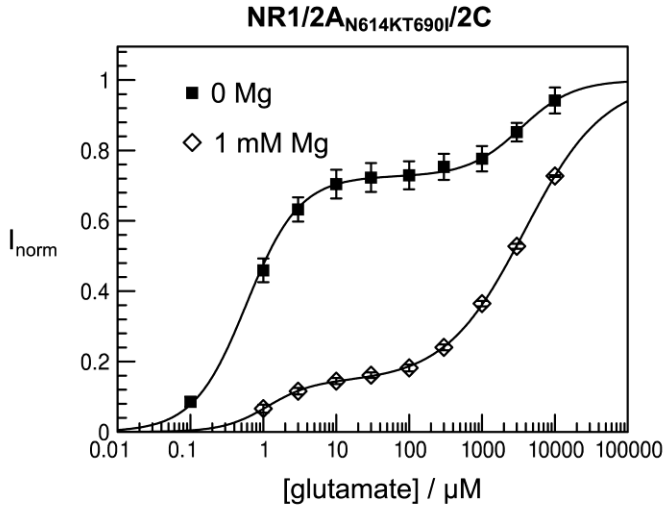
A

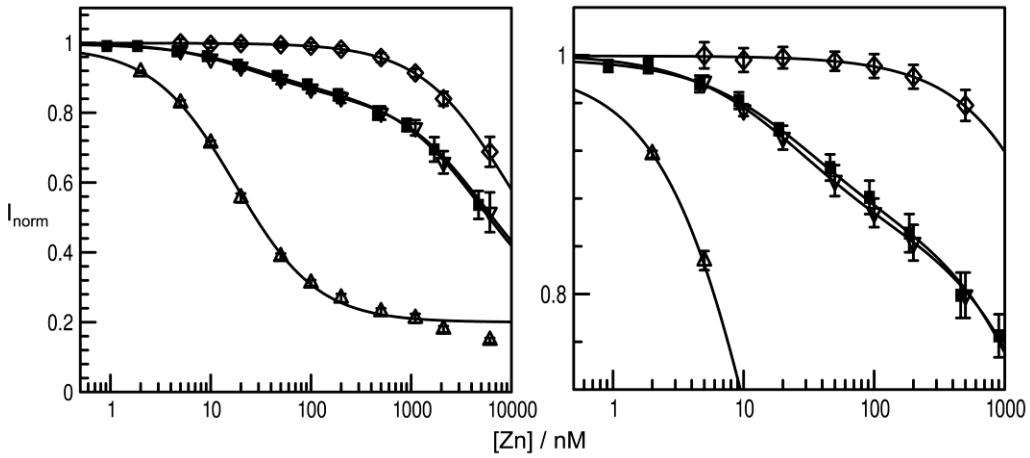

NR1/2A$_{N614KT690I}$/2B
0 Mg
1 mM Mg
I_{norm}
[glutamate] / µM

B


NR1/2A$_{N614KT690I}$/2C
0 Mg
1 mM Mg
I_{norm}
[glutamate] / µM

Supplementary Figure 1: Pharmacological isolation of NR1/2A/2B and NR1/2A/2C triheteromeric NMDA receptors

(A) Glutamate concentration-response curves for oocytes expressing NR1, $NR2A_{N614KT690I}$ and NR2B subunits and recorded in the absence (solid squares) or presence (open diamonds) of 1 mM Mg. Each point shows the average response from 4-10 cells. In the absence of Mg two components are readily distinguished; the first component has an affinity identical to that of NR1/2B wt receptors (EC_{50} = 1.5 ± 0.4 μM, relative area = 30.7 ± 0.3 %, n_H = 1.21 ± 0.17) while the second has an affinity intermediate between that of NR1/2B wt and all-mutant $NR1/NR2A_{N614KT690I}$ receptors (see Fig.2) and corresponds to the triheteromeric $NR1/2A_{N614KT690I}$/2B receptors (EC_{50} ~ 7.5 mM, relative area = 69.3 ± 15 %, n_H = 0.82 ± 0.23). In the presence of 1 mM Mg, the NR1/2B wt component is almost completely abolished (relative area = 2.6 ± 1.2 %) leaving an almost pure triheteromeric receptor population (EC_{50} = 4.6 ± 1.9 mM, relative area = 97.4 ± 12.7 %, n_H = 0.79 ± 0.11). **(B)** Glutamate concentration-response curves for oocytes expressing NR1, $NR2A_{N614KT690I}$ and NR2C subunits and recorded in the absence (solid squares) or presence (open diamonds) of 1 mM Mg. Data are from 3-12 cells. In the absence of Mg two components are readily distinguished; the first component has an affinity identical to that of NR1/2C wt receptors (EC_{50} = 0.6 ± 0.1 μM, relative area = 72.7 ± 2.0 %, n_H = 1.12 ± 0.1) while the second has an affinity intermediate between that of NR1/2C wt and all-mutant $NR1/NR2A_{N614KT690I}$ receptors (see Fig.2) and corresponds to the triheteromeric $NR1/2A_{N614KT690I}$/2C receptors (EC_{50} 3.4 ± 2.3 mM, relative area = 27.3 ± 8.1 %, n_H fixed = 1). In the presence of 1 mM Mg, the NR1/2C wt component is markedly reduced, but not completely abolished (EC_{50} = 1.1 ± 0.2 μM, relative area = 14.2 ± 5.0 %, n_H = 1.44 ± 0.44). The majority of current in the presence of Mg is carried by the triheteromeric receptor population (EC_{50} = 3.8 ± 0.5 mM, relative area = 85.8 ± 8.3 %, n_H = 0.79 ± 0.23). Error bars represent standard errors.



Supplementary Figure 2: The glutamate binding mutation $NR2A_{T690I}$ has little effect on Zn inhibition via the NR2-NTDs

Shows data from Fig. 4B (see legend for details), with the Zn concentration-inhibition curve (in 1mM Mg at -80 mV) recorded from cells expressing NR1, NR2A and $NR2A_{H128SN614K}$ subunits superimposed (▽, n=13 cells). Under these recording conditions, approximately 90% of the current is carried by triheteromeric $NR1/2A/2A_{H128SN614K}$ receptors (see Results). Note that this curve is almost identical to that recorded from $NR1/2A/2A_{H128SN614KT690I}$ receptors (■) indicating that inclusion of the T690I glutamate binding mutation has little effect on Zn inhibition via the NR2A-NTD of the associated NR2A wt subunit. Right-hand panel shows a close-up of the inhibition of triheteromeric receptors in the nM range. Fitted parameters for ▽ were: IC_{50} = 25.0 ± 4.6 nM, 5.6 ± 1.0 µM, relative area 19.0 ± 1.4 %, 81.1 ± 1.4 %, n_H fixed = 1. Error bars represent standard errors.