Supplementary Information for:

KEY BINDING INTERACTIONS FOR MEMANTINE IN THE NMDA RECEPTOR

Walrati Limapichat, Wesley Yu, Emma Branigan, Henry Lester, and Dennis Dougherty

LEGENDS TO SUPPLEMENTAL DATA

Supplemental Figure 1: Memantine fold shifts (IC_{50}(mutant)/IC_{50}(wild type)) of mutant NMDA receptors containing a conventional or an unnatural mutation in the transmembrane region. Abbreviation used are F4W, 2,3,4,5-fluoro-Trp; Cha, cyclohexylalanine; hGln, homoglutamine; F3-Phe, 3,4,5-fluoro-Phe. *, Conventional mutations performed through the nonsense-suppression method. Unnatural amino acid mutagenesis was performed using established protocols.[1]

Supplemental Figure 2. Representative response curves. A. Wild type receptor exposed to 5.6 μM memantine (solid line). B. N1-645N exposed to 100 μM memantine (solid line).

Supplemental Table 1: Glutamate EC_{50} ± s.e.m. and Hill constant of wild-type and mutant NMDA receptors. Hill equation was use to fit data: $I/I_{max} = 1/(1 + (EC_{50}/[A])^{n_H})$, where I_{max} is the current response to agonist activation, I is the agonist-activated current measured in the presence of antagonist at the concentration (A).

NMDA Receptor	Glutamate EC_{50}	Hill Constant	n [1]	EC_{50}(mutant)/EC_{50}(wild type)
	μM			
Wild type	1.94 ± 0.04	1.7	8	1.00
GluN1 Mutants				
N616Q	0.47 ± 0.01	1.6	11	0.24
N616D	1.3 ± 0.02	1.8	14	0.65
V644T	2.0 ± 0.03	1.6	11	1.01
V644L	1.3 ± 0.02	1.5	10	0.67
V644N	1.7 ± 0.03	1.6	12	0.89
A645V	0.93 ± 0.03	1.6	8	0.48
A645L	0.60 ± 0.02	1.3	9	0.31
A645N	0.33 ± 0.01	2.1	7	0.17
V656N	0.20 ± 0.01	2.3	7	0.10
GluN2B Mutants				
N615D	2.9 ± 0.06	1.7	5	1.01
N616D	2.8 ± 0.04	1.5	6	1.45
A639N	0.63 ± 0.02	1.5	9	0.32
V640N	2.8 ± 0.10	1.6	9	1.42
L643N	1.2 ± 0.03	1.5	9	0.64
A644V	0.61 ± 0.05	1.2	5	0.31
A644L	0.76 ± 0.05	1.5	11	0.39
A644N	0.73 ± 0.01	1.7	9	0.37

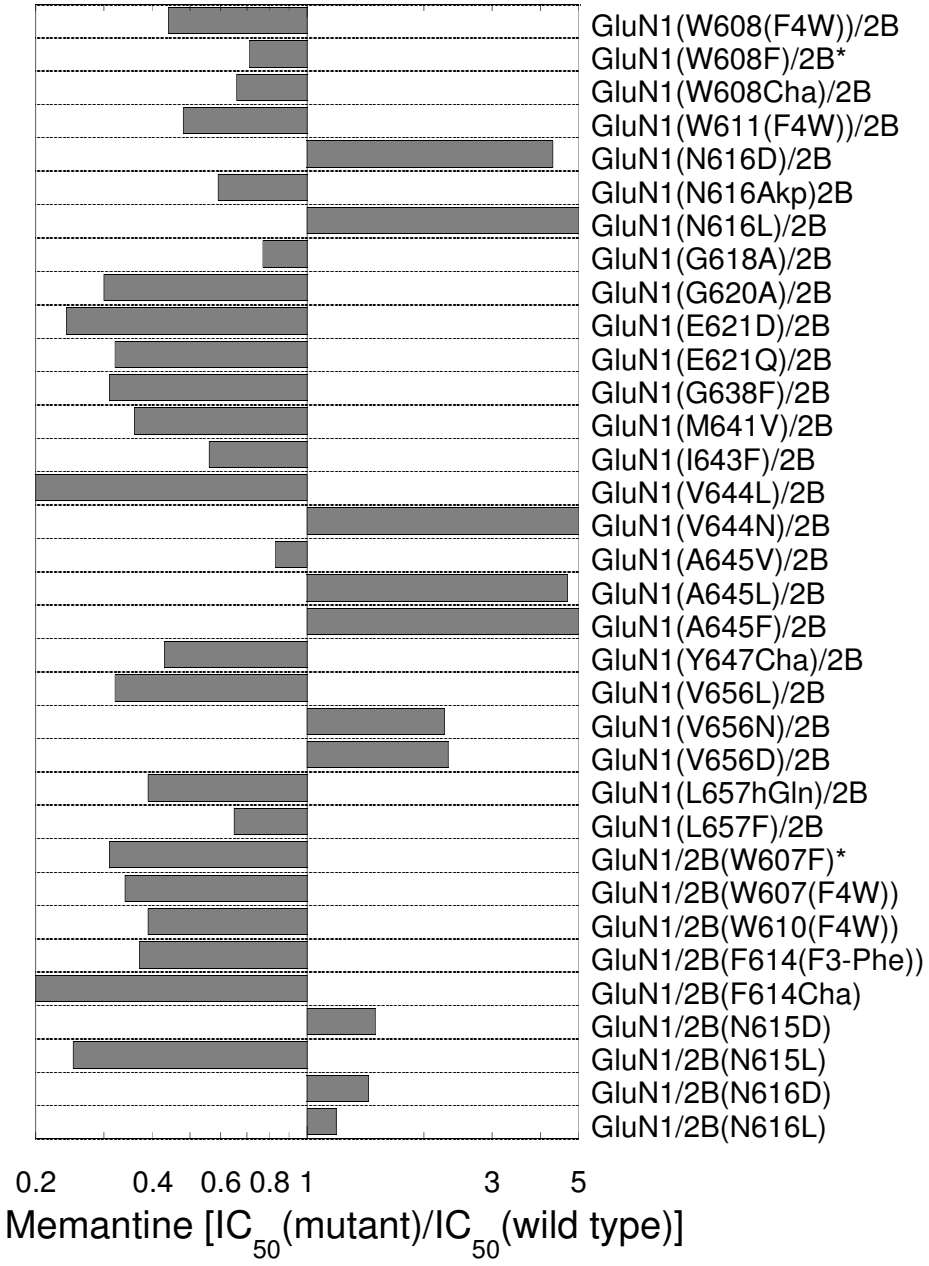
[1] n, number of oocytes

Supplemental Table 2: TMAm IC_{50} ± s.e.m. for wild type and mutant NMDA receptors.

NMDA Receptor	**TMAm IC_{50}**	***n*** [1]	**IC_{50}(mutant)/IC_{50}(wild type)**
	μM		
Wild type	3.4 ± 0.4	10	1.0
GluN1(N616Q)/2B	2.0 ± 0.08	9	0.6
GluN1(V644T)/2B	3.1 ± 0.4	10	0.9
GluN1(A645N)/2B	180 ± 11	12	53
GluN1/2B(N615D)	5.4 ± 1.1	13	1.6
GluN1/2B(N616D)	2.9 ± 0.4	8	0.9
GluN1/2B(V640N)	0.72 ± 0.1	8	0.2
GluN1/2B(A644N)	100 ± 7.4	11	30

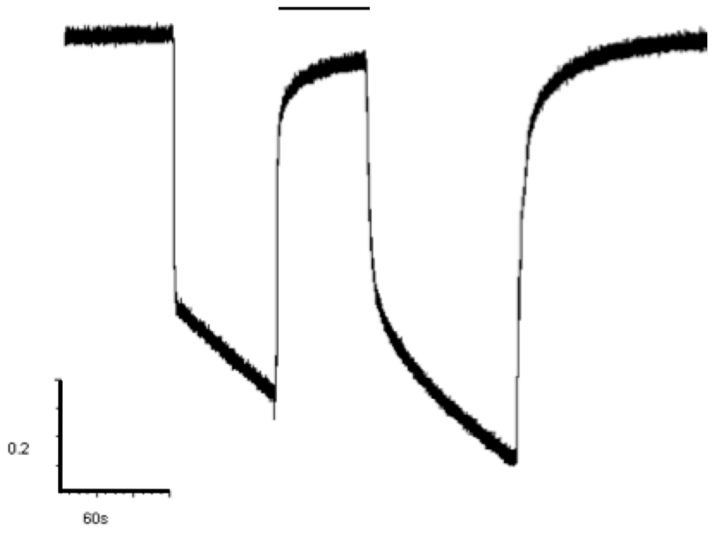
[1] *n*, number of oocytes

Supplemental Figure 1

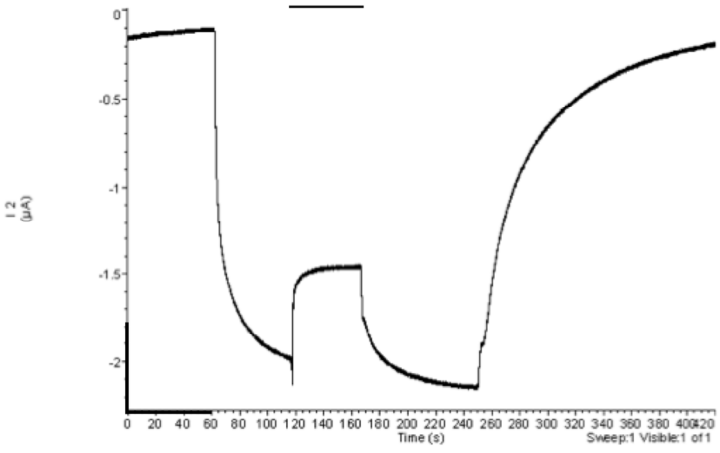


Supplemental Figure 2.

A.



B.



Reference:

(1) Nowak, M. W.; Gallivan, J. P.; Silverman, S. K.; Labarca, C. G.; Dougherty, D. A.; Lester, H. A., (1998) *In vivo* incorporation of unnatural amino acids into ion channels in a *Xenopus* oocyte expression system. *Methods Enzymol 293*, 504-529.