

Correction to “The NR1 M3 Domain Mediates Allosteric Coupling in the *N*-Methyl-D-aspartate Receptor”

In the above article [Blanke ML and VanDongen AM (2008) *Mol Pharmacol* **74**:454–465], incorrect figures were printed. The correct figures appear below.

The online version of this article has been corrected in departure from the print version.

The printer regrets this error and apologizes for any confusion or inconvenience it may have caused.

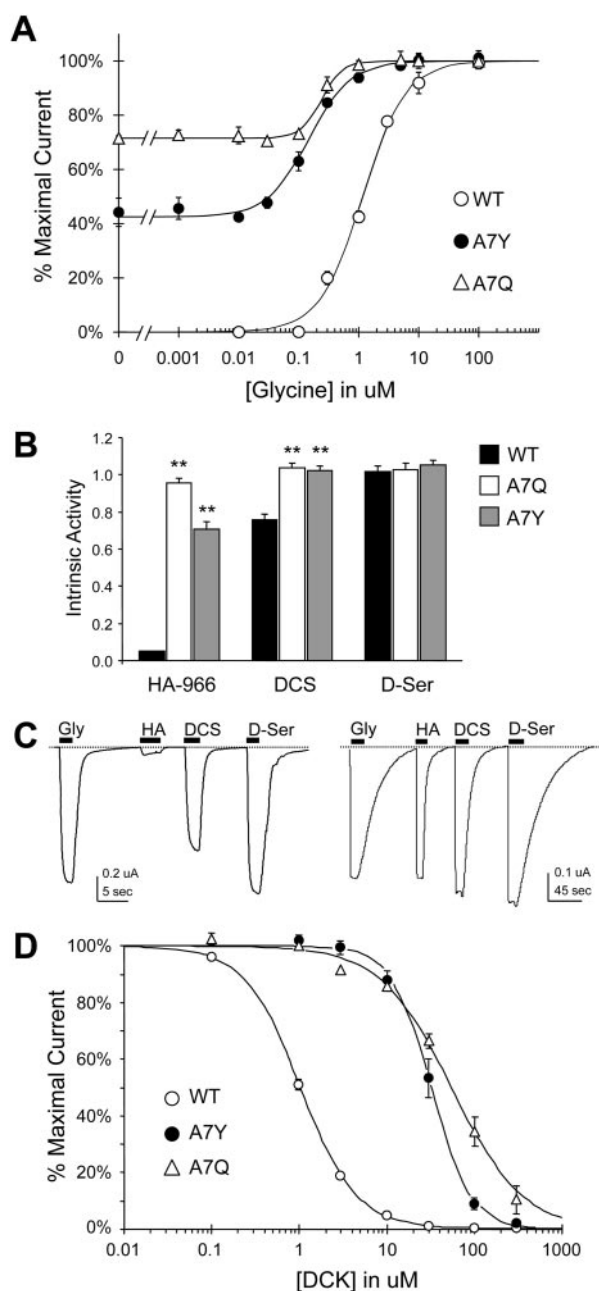


Fig. 2. Altered activation equilibrium in NR1 A7Q and A7Y. **A**, glycine concentration-response curves in the presence of 100 μ M L-glutamate. Both A7Q (Δ) and A7Y (\bullet) exhibited significant glycine-independent current (71 and 44%, respectively) and increased sensitivity to glycine. Concentration midpoints were 0.24 μ M for A7Q and 0.15 μ M for A7Y, compared with 1.16 μ M for WT. Hill coefficients were 2.5 for A7Q and 1.2 for A7Y, compared with 1.2 for WT. **B**, response to glycine-site partial agonists, calculated as a fraction of full agonist response. HA-966 (500 μ M), 1 mM DCS, and 100 μ M D-serine were coapplied with 100 μ M glutamate. Both A7Q and A7Y displayed increased activity in response to HA-966 and DCS, but not D-serine. Intrinsic activities for WT, A7Q, and A7Y were as follows: HA-966: 0.05 ± 0.00 , 0.96 ± 0.02 , and 0.71 ± 0.04 ; DCS: 0.76 ± 0.03 , 1.04 ± 0.02 , and 1.02 ± 0.02 ; and D-Ser: 1.02 ± 0.03 , 1.03 ± 0.07 , and 1.06 ± 0.03 . **C**, representative whole cell traces illustrating the response of WT (left) and A7Q (right) to glycine-site partial agonists in the presence of 100 μ M glutamate. A7Q receptors exhibit increased partial agonist responses and markedly slow deactivation. **D**, inhibition by DCK, a competitive glycine-site antagonist, in the presence of 10 μ M glycine and 100 μ M glutamate. Both mutants were significantly less sensitive to DCK inhibition. IC_{50} values were 51.5 μ M for A7Q and 31.7 μ M for A7Y, compared with 1.04 μ M for WT. Hill coefficients were 1.1 for A7Q, 2.3 for A7Y, and 1.5 for WT. Calculated K_D values were as follows: A7Q (1.21), A7Y (0.47), and WT (0.11). *, $p < 0.05$ (significant); **, $p < 0.01$ (highly significant).

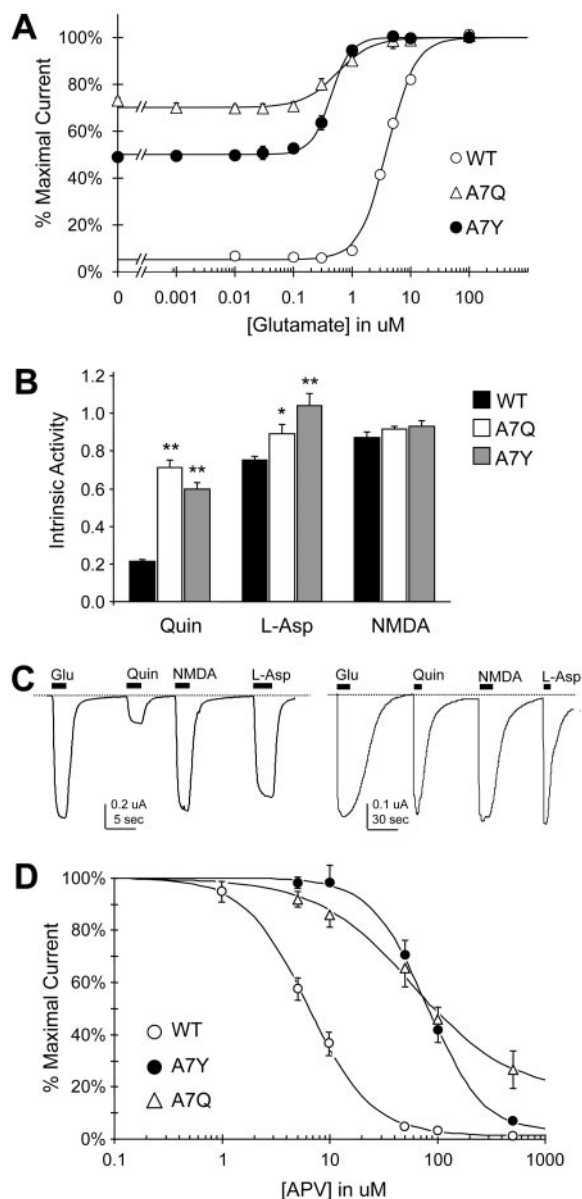


Fig. 4. A7Q and A7Y affect the NR2 ligand binding domain. **A**, glutamate concentration-response curves in the presence of 100 μM glycine. Both A7Q (Δ) and A7Y (\bullet) exhibited significant glutamate-independent current (73 and 49%, respectively) and increased sensitivity to glutamate. Concentration midpoints were 0.57 μM for A7Q and 0.45 μM for A7Y, compared with 4.05 μM for WT. Hill coefficients were 1.5 for A7Q, 2.6 for A7Y, and 1.9 for WT. **B**, response to glutamate-site partial agonists, calculated as a fraction of full agonist response. Quinolinic acid (10 mM; Quin), 1 mM NMDA, and 100 μM L-aspartate (L-Asp) were coapplied with 100 μM glycine. Both mutants displayed increased activity in response to quinolinic acid and L-aspartate, but not NMDA. Intrinsic activities for WT, A7Q, and A7Y were as follows: Quin: 0.22 ± 0.01 , 0.71 ± 0.04 , and 0.60 ± 0.03 ; L-Asp: 0.75 ± 0.02 , 0.89 ± 0.05 , and 1.04 ± 0.06 ; and NMDA: 0.87 ± 0.03 , 0.91 ± 0.02 , and 0.93 ± 0.03 . **C**, representative whole cell traces illustrating the response of WT (left) and A7Q (right) to glutamate-site partial agonists in the presence of 100 μM glycine. **D**, inhibition by APV, a competitive glutamate-site antagonist, in the presence of 100 μM glycine and 10 μM glutamate. Both mutants were significantly less sensitive to APV inhibition. IC_{50} values were 86.4 μM for A7Q and 83.5 μM for A7Y, compared with 6.22 μM for WT. Hill coefficients were 0.8 for A7Q, 2.1 for A7Y, and 1.3 for WT. Calculated K_D values were as follows: A7Q (4.66), A7Y (3.60), and WT (1.79). *, $p < 0.05$ (significant); **, $p < 0.01$ (highly significant).

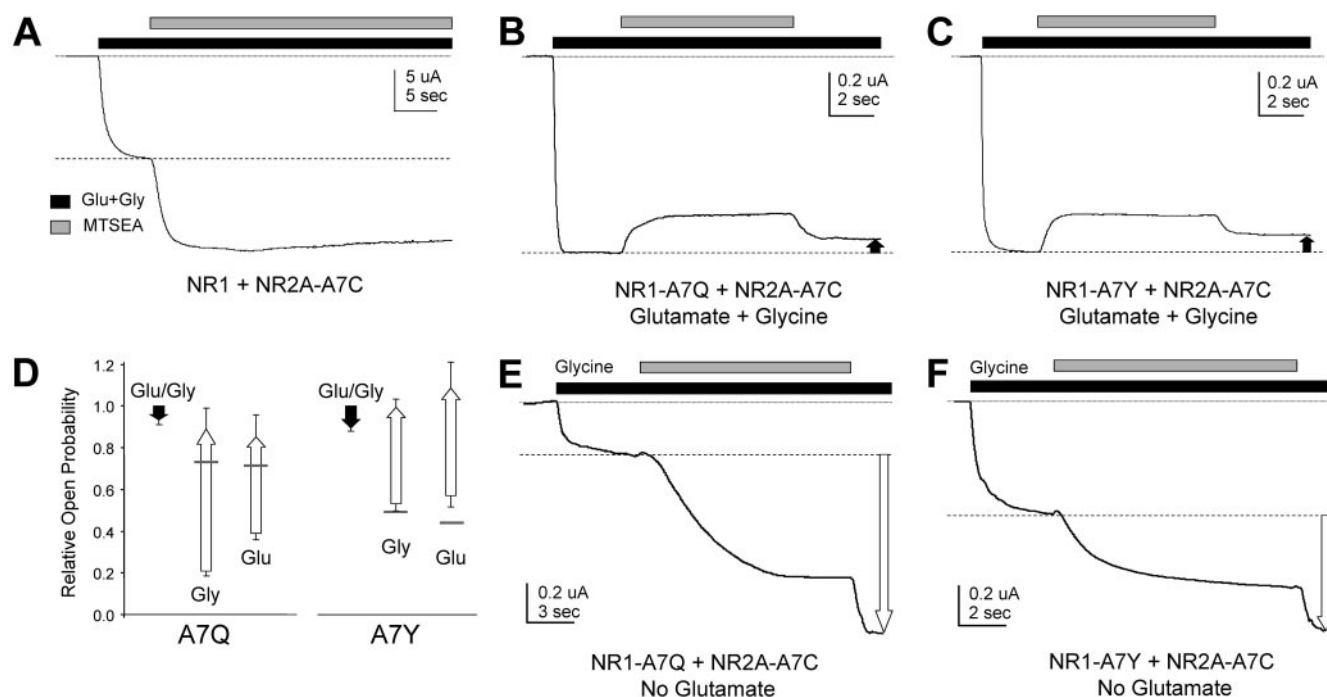


Fig. 5. A7Q and A7Y increase accessibility of the NR2 M3 domain. NR1 subunits were coexpressed with NR2-A7C, an activation-dependent reporter of M3 accessibility. A, representative whole cell trace showing modification of the NR2-A7C reporter by 0.5 mM MTSEA, a thiol-modifying reagent, in the presence of 100 μ M glycine and 100 μ M glutamate. MTSEA modifies and potentiates A7C-containing receptors only in the presence of agonist, increasing channel P_o to approximately 1.0. B, modification of NR2-A7C coexpressed with NR1 A7Q produced only a small amount of irreversible inhibition (filled arrow). Transient inhibition reflects open channel block by the positively charged MTSEA. C, modification of NR2-A7C coexpressed with NR1 A7Y, showing a small inhibition similar to the A7Q response. D, bar graph illustrating the correlation between channel activation and MTSEA modification. The y-axis represents relative P_o , with the maximal response to full agonist assigned a value of 1.0 in each mutant. Inhibition and potentiation are depicted with closed and open arrows, respectively. Gray bars denote the percentage of single-agonist current observed when the mutants were coexpressed with WT NR2 (Figs. 2A and 4A); coexpression with NR2-A7C resulted in decreased (A7Q) or increased (A7Y) single-agonist activation. E, modification of NR2-A7C coexpressed with NR1 A7Q, in the presence of 100 μ M glycine only. F, modification of NR2-A7C coexpressed with NR1 A7Y, in the presence of 100 μ M glycine only. -Fold potentiation of covalent modification after full agonist activation: WT (2.7 ± 0.3), A7Q (0.93 ± 0.02), and A7Y (0.89 ± 0.01); after activation with 100 μ M glycine: A7Q (4.23 ± 0.48) and A7Y (1.86 ± 0.08); and after activation with 100 μ M glutamate: A7Q (2.20 ± 0.27) and A7Y (1.91 ± 0.22).

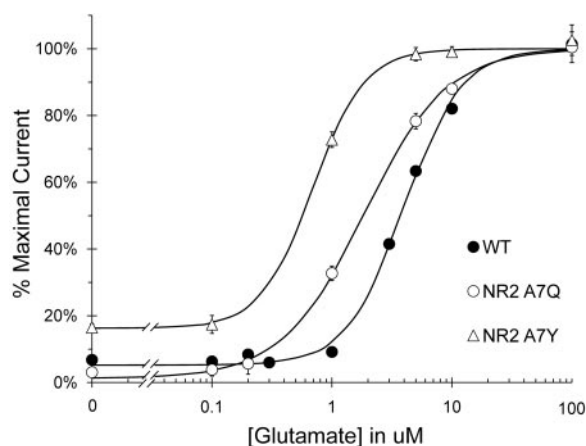


Fig. 6. NR1 gain-of-function phenotypes are not conserved in NR2. Q and Y substitutions were introduced at the A7 position in the NR2 subunit, and glutamate concentration-response curves were determined in the presence of 100 μ M glycine. NR2 A7Q exhibited no significant glycine only current ($3 \pm 1\%$), whereas NR2 A7Y displayed only a small amount ($16 \pm 1\%$, compared with $7 \pm 1\%$ for WT). Glutamate concentration midpoints were 2.03 μ M (NR2 A7Q) and 0.69 μ M (NR2 A7Y), Hill coefficients were 1.2 (NR2 A7Q) and 2.0 (NR2 A7Y).

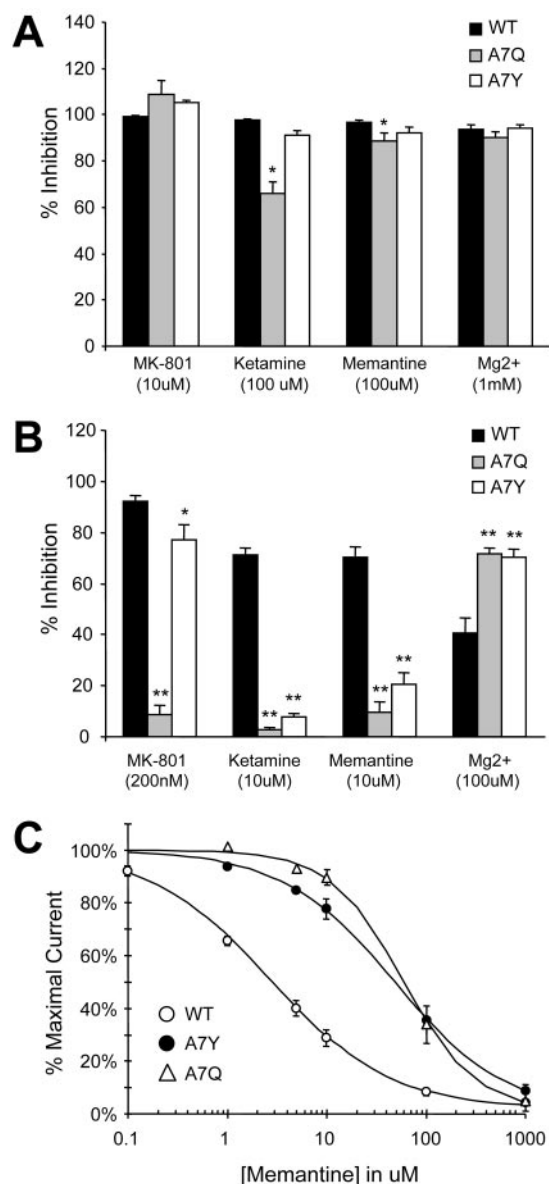


Fig. 7. Decreased sensitivity to pore blockers in A7Q and A7Y. A, Percentage of inhibition by various channel blockers was measured in the presence of 100 μ M glycine and 100 μ M glutamate. Inhibition of A7Q and A7Y was similar to WT with all antagonists tested except ketamine, which was less effective at inhibiting A7Q. B, both mutants exhibited decreased sensitivity to lower concentrations of MK-801, ketamine, and memantine, but increased sensitivity to low magnesium. C, memantine concentration-inhibition curves obtained in the presence of 100 μ M glycine and 100 μ M glutamate. Both mutants were significantly less sensitive to inhibition by memantine. IC_{50} values were 59.0 μ M for A7Q and 48.3 μ M for A7Y, compared with 2.67 μ M for WT. Hill coefficients were 1.2 for A7Q, 0.8 for A7Y and 0.7 for WT. *, $p < 0.05$ (significant); **, $p < 0.01$ (highly significant).

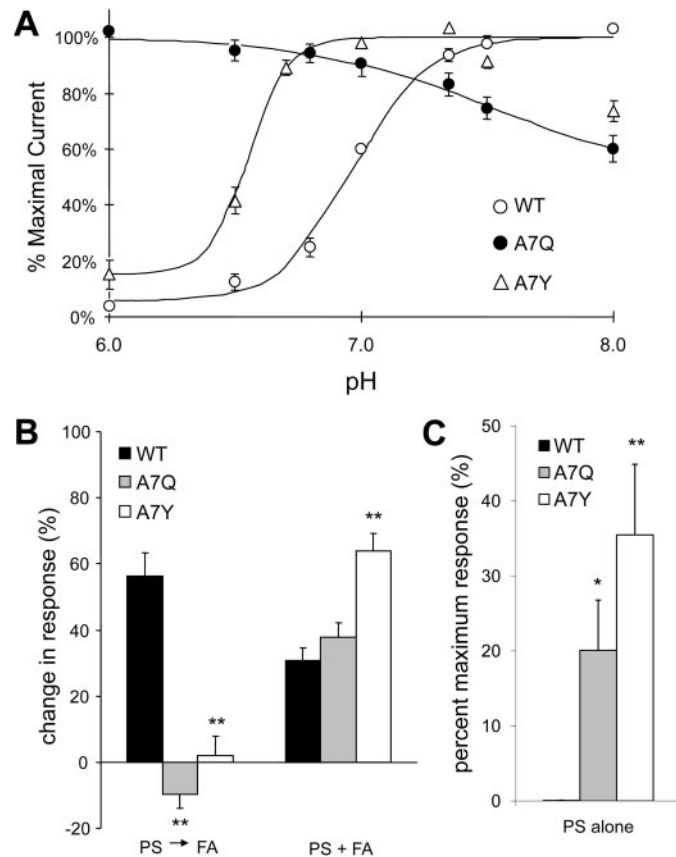


Fig. 8. Altered response of A7Q and A7Y to allosteric modulators. A, proton inhibition curves in the presence of 100 μ M glycine and 100 μ M glutamate. WT receptors are highly sensitive to pH modulation, with a pK_a value of 6.97. Decreased proton inhibition was observed in A7Y ($pK_a = 6.59$), whereas A7Q was insensitive to proton block and inhibited by high pH. Inhibition of A7Y was also seen at high pH values, which were not used in calculating the pK_a for that mutant. B, current potentiation by 100 μ M PS, applied either before or in the presence of agonist. PS preapplication yielded $\sim 50\%$ potentiation of the WT full agonist response but had little effect on A7Q or A7Y (WT: $56 \pm 7\%$; A7Q: $-10 \pm 5\%$; and A7Y: $2 \pm 6\%$). However, concurrent treatment with PS and full agonist restored potentiation in both mutants (WT: $31 \pm 4\%$; A7Q: $38 \pm 4\%$; and A7Y: $64 \pm 5\%$). C, application of 100 μ M PS was found to partially activate both A7Q and A7Y even in the absence of agonist, whereas having no effect on WT (WT: $0.1 \pm 0.0\%$; A7Q: $16 \pm 6\%$; and A7Y: $30 \pm 10\%$). *, $p < 0.05$ (significant); **, $p < 0.01$ (highly significant).