

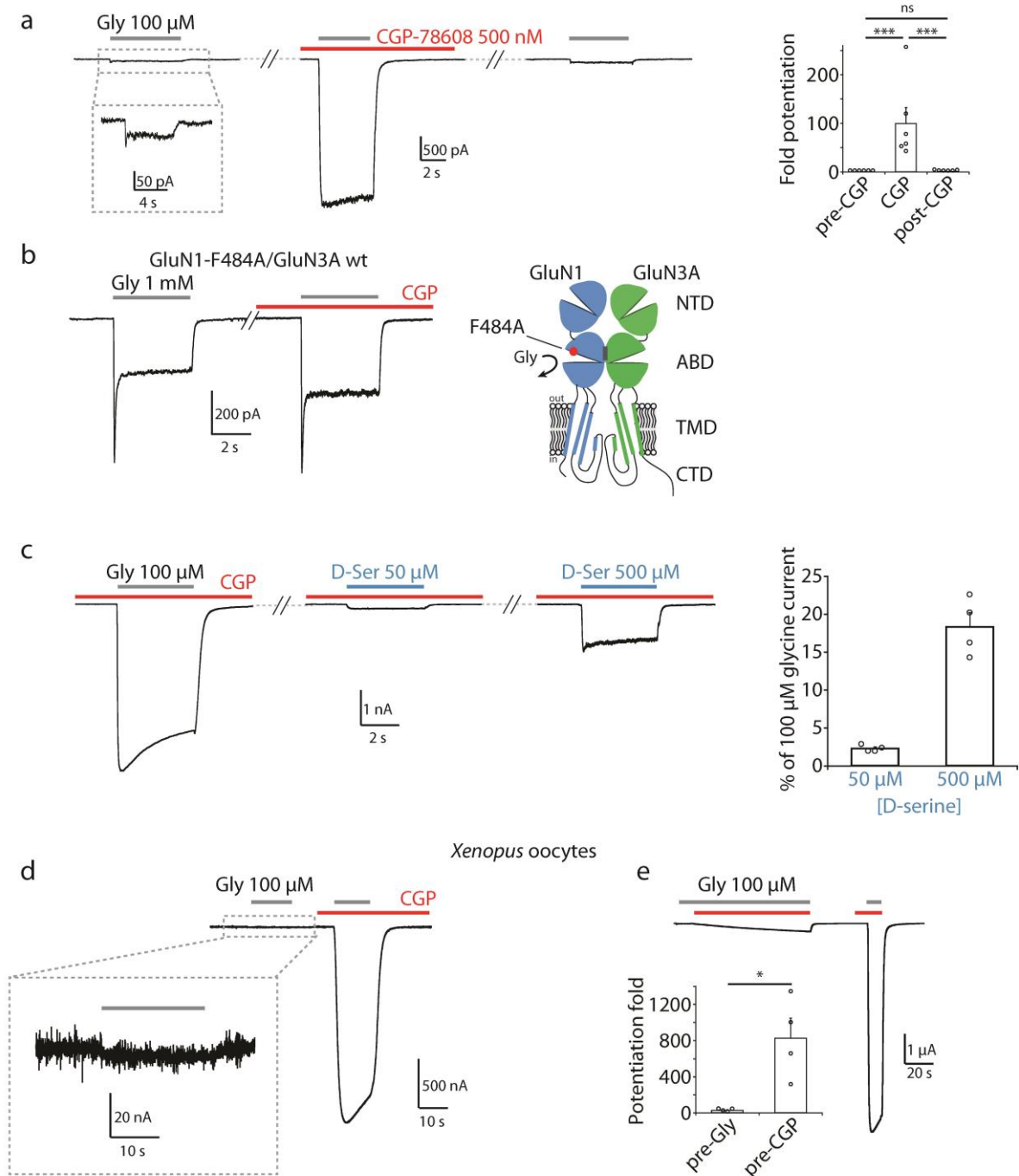
Unmasking GluN1/GluN3A excitatory glycine NMDA receptors

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Supplementary Figure 1: Properties of CGP-78608 potentiation

(a) CGP-78608 potentiation is fully reversible. Recording were performed on the same cell. n.s. $P=1.0$; *** $P<0.001$; Student's t -test ($n=6$).

(b) GluN1/GluN3A receptors harboring glycine binding disabled GluN1 subunits (GluN1-F484A mutation) are insensitive to CGP-78608 (500 nM). The fast desensitization of current responses observed upon glycine application likely stems from residual binding of glycine on mutant GluN1 ABD (see ref 1).

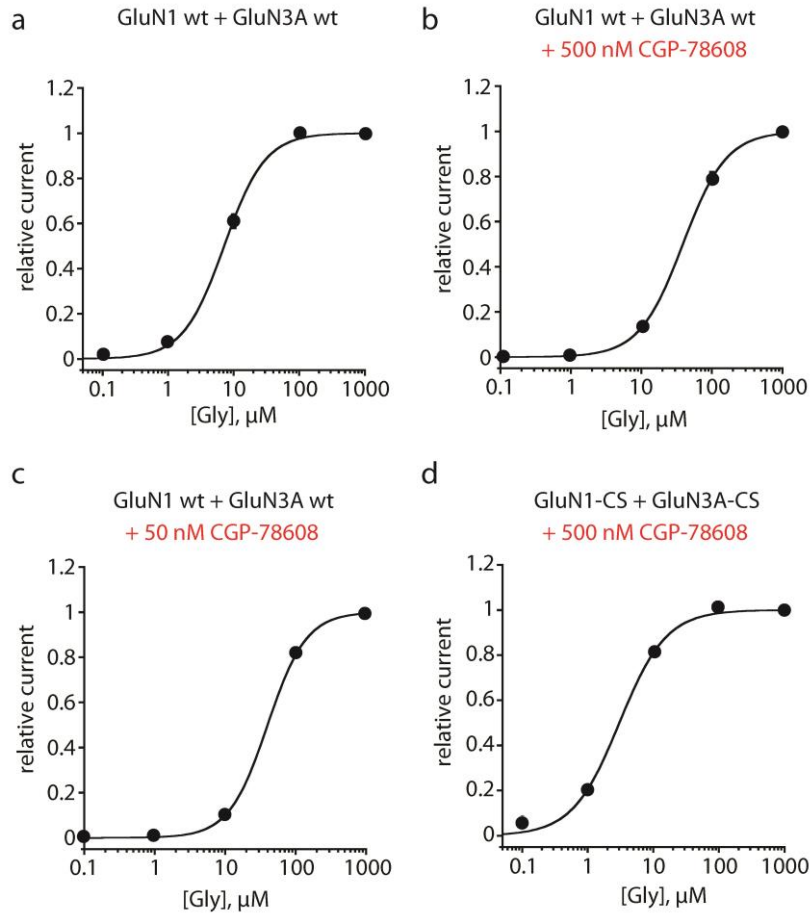
(c) D-serine has lower efficacy than glycine at GluN1/GluN3A receptors. CGP-78608 is applied at 500 nM. Bar graph (n=4).

(d) Pre-application of CGP-78608 (500 nM) massively potentiates excitatory glycine GluN1/GluN3A responses recorded in *Xenopus* oocytes.

(e) CGP-78608 potentiation is state-dependent. Potentiation by CGP-78608 (500 nM) is greatly reduced when the compound is applied after, rather than before, application of glycine (pre-Gly vs post-Gly, respectively). Bar graph: potentiation measured at steady-state.

* $P=0.029$, Mann-Whitney (n=4).

Recordings from HEK cells (panels a to c) or *Xenopus* oocytes (d and e).



Supplementary Figure 2: Glycine sensitivity of wild-type and redox mutant GluN1/GluN3A receptors.

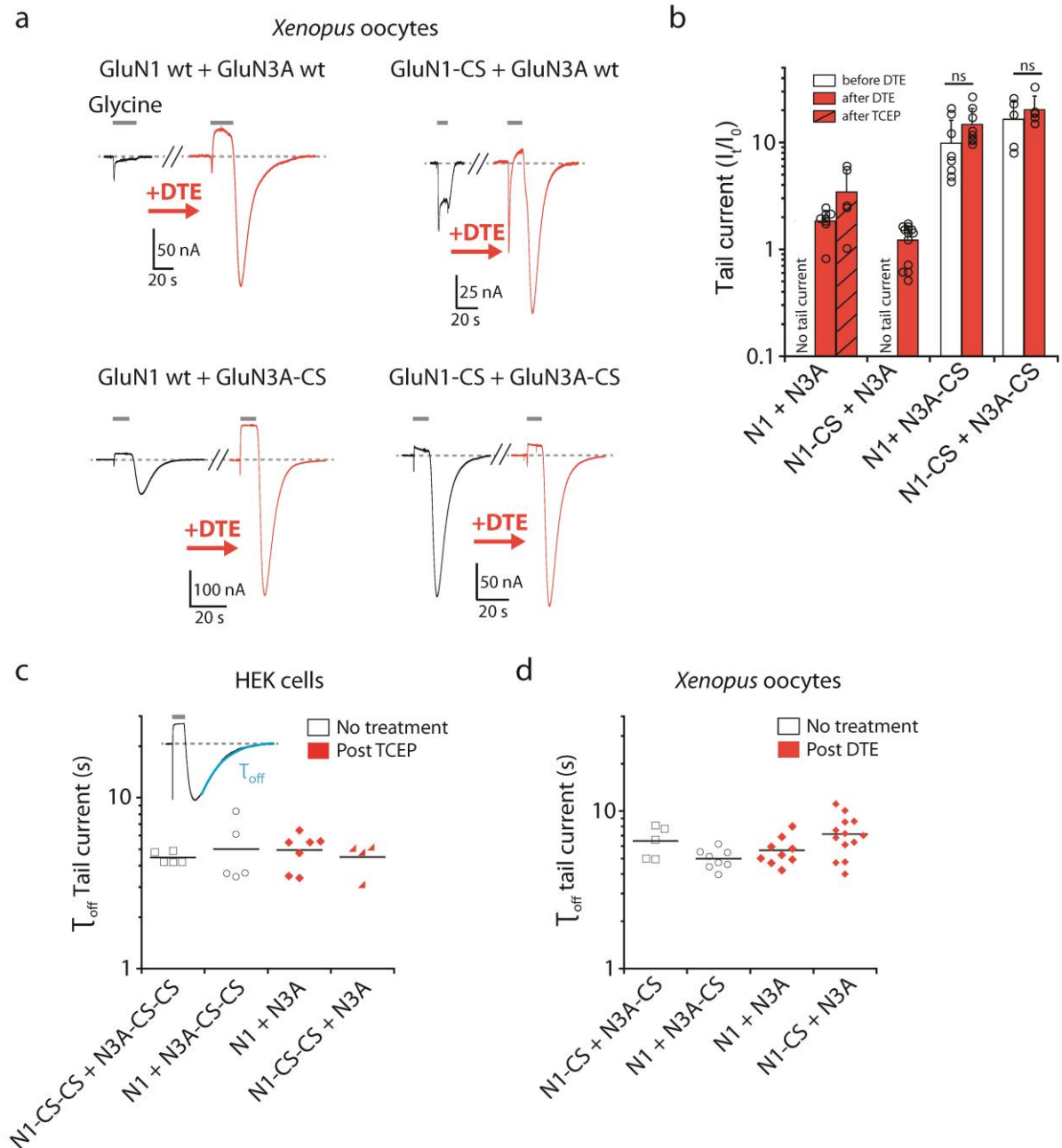
(a) Glycine concentration-response curve obtained for wild-type (wt) receptors. $EC_{50} = 7.1 \pm 0.4 \mu M$, $n_H = 1.39$ ($n=5-15$).

(b) Glycine concentration-response curve for wild-type receptors in presence of 500 nM CGP-78608. $EC_{50} = 38.9 \pm 0.8 \mu M$, $n_H = 1.47$ ($n=6$).

(c) Same as in (b) but in presence of 50 nM CGP-78608. $EC_{50} = 40 \pm 0.8 \mu M$, $n_H = 1.58$ ($n=9$).

(d) Glycine concentration-response curve for GluN1-CS/GluN3A-CS receptors in the presence of 500 nM CGP-78608. $EC_{50} = 3.0 \pm 0.3 \mu M$, $n_H = 1.21$ ($n=6$).

All recordings were performed in *Xenopus* oocytes and currents measured at the peak.



Supplementary Figure 3: Tail current kinetics of receptors lacking the redox-sensitive GluN3A ABD disulfide bridge

(a) Effect of DTE treatment (5 mM, 20 min) on wild-type (wt) mutant GluN1/GluN3A receptors expressed in *Xenopus* oocytes. Each pair of current traces corresponds to responses before and after DTE treatment on the same cell. Glycine was applied at 100 μ M.

(b) Quantification of tail currents as described in Figure 2c. n.s. $P=0.105$, Mann-Whitney and $P=0.453$, Student's t -test respectively ($n=5-13$).

(c) Tail current off kinetics measured on mutant receptors expressed in HEK cells. Receptors containing wild-type GluN3A subunits were treated with TCEP. N1-CS-CS refers to the GluN1 ABD C744S-C798S mutant subunit and N3-CS-CS to the GluN1 ABD C859S-C913S

mutant subunit. Mean values from the different groups are not significantly different ($P=0.891$, One way ANOVA on ranks, [n=4-7]).

(d) Same as in (c) but from *Xenopus* oocytes recordings, and with DTE used as a reducing agent instead of TCEP. Mean values from the different groups are not significantly different ($P=0.062$, One way ANOVA on ranks, [n=5-13]).

Supplementary references

1. Kvist, T., Greenwood, J. R., Hansen, K. B., Traynelis, S. F. & Brauner-Osborne, H. Structure-based discovery of antagonists for GluN3-containing N-methyl-D-aspartate receptors. *Neuropharmacology* **75**, 324-336 (2013).