Single amino acid residue in the M4 domain of GluN1 subunit regulates the surface delivery of NMDA receptors

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SUPPLEMENTARY FIGURE LEGENDS

**Fig. S1** The expression of mutated NMDA receptors. (a) The amino acid sequence of the wild type GluN1 M4 domain is shown. The underlined amino acid residues were individually replaced with alanine residues. (b-c) COS-7 cells co-transfected with indicated GluN1/GluN2B subunits, cell homogenates were prepared and probed with rabbit anti-GFP antibody. Please note that similar protein expression levels were observed for the wild type and mutated receptors.

**Fig. S2** The responses of both the wild type and mutated NMDA receptors are completely inhibited by the specific inhibitors that were present in culture media. Electrophysiological recordings were performed on HEK293 cells transfected with full-length YFP-GluN1-1a/GluN2B and YFP-GluN1-1a-L830A/GluN2B receptors. NMDA receptor-mediated responses were elicited with a 20 s application of 100 μM glutamate (indicated by filled bar) with 1 mM MgCl₂ (a) or 1 mM d,l-2-amino-5-phosphonopentanoic acid (AP5) and 3 mM kynurenic acid (kyn; b; indicated by open bar) at membrane potential of -60 mV. Representative traces are shown.

**Fig. S3** The replacement of the GluN1-L830 residue with alanine decreases the surface expression of GluN1/GluN2A receptors. (a) Amino acid sequence of the GluN1 M4 domain with underlined amino acid residues that were individually substituted with alanine residues is shown. (b) Bar graph represents surface (black) and total (white) expression of NMDA receptors determined using a quantitative colorimetric assay on heterologous COS-7 cells expressing indicated full-length YFP-GluN1-1a/GluN2A subunits. Data show mean ± SEM; n = 9 in three experiments. *p<0.05 relative to control (YFP-GluN1-1a/GluN2A), ANOVA. (c) The replacement of the L830 residue within the GluN1 M4 domain does not alter the formation of functional GluN1/GluN2A receptors. Electrophysiological recordings were performed on HEK293 cells co-transfected with full-length YFP-GluN1-1a/GluN2A and YFP-GluN1-1a-L830A/GluN2A receptors. NMDA receptor-mediated responses were elicited with a 5 s application of 1 mM glutamate (indicated by filled bar); representative traces are shown. (d-e) Quantitative analysis of NMDA receptor responses revealed that the degree of desensitization (d), and the weighted deactivation time constant (τ_w; e), calculated as in Fig. 3, were not significantly different between YFP-GluN1-1a/GluN2A and YFP-GluN1-1a-L830A/GluN2A receptors; n = 6; p>0.05, t test.
Figure S1
Figure S2
Figure S3

a  GluN1 M4  812-NMAGFVMLVAGGIVAGIFLIFI-833

b

YFP-GluN1-1a/GluN2A  YFP-GluN1-1a-L830A/GluN2A  YFP-GluN1-1a-I831A/GluN2A

Relative YFP expression

Surface  Total

0.0  0.2  0.4  0.6  0.8  1.0  1.2

Table: Relative YFP expression

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YFP-GluN1-1a/GluN2A  YFP-GluN1-1a-L830A/GluN2A

Iss/Ip

0  50  100  150  200  250

Table: Iss/Ip

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Glu

YFP-GluN1-1a/GluN2A  YFP-GluN1-1a-L830A/GluN2A

0.6 nA

2 s

YFP-GluN1-1a-GluN2A

Glu

0.6 nA

2 s

YFP-GluN1-1a-L830A/GluN2A

Glu

0.6 nA

2 s

Figure S3