Table S1. Effects of MTS modification on receptors incorporating NR1wt and the indicated NR2 mutant

NR2 mutant	Relative current (mean ± s.d.)		
	post MTSEA	post MTSET	post MTS-PtrEA
NR2B mutant			
H127C	1.13 ± 0.03 (n=5)	1.29 ± 0.06 (n=5)	1.58 ± 0.10 (n=8)
H127A	0.93 ± 0.01 (n=7)	0.84 ± 0.03 (n=5)	0.74 ± 0.01 (n=5)
Y282C	2.1 ± 0.1 (n=10)	4.4 ± 0.6 (n=10)	5.5 ± 1.0 (n=33)
Y282S	0.91 ± 0.02 (n=4)	0.92 ± 0.03 (n=4)	0.92 ± 0.02 (n=4)
NR2A mutant			
H128C	0.74 ± 0.01 (n=6)	0.83 ± 0.02 (n=8)	0.91 ± 0.10 (n=10)
H128S	0.80 ± 0.01 (n=4)	0.76 ± 0.01 (n=4)	0.84 ± 0.07 (n=6)
Y281C	1.02 ± 0.02 (n=12)	1.09 ± 0.04 (n=6)	0.95 ± 0.03 (n=6)
Y281A	0.60 ± 0.06 (n=12)	0.58 ± 0.04 (n=6)	0.58 ± 0.03 (n=5)

Table S2. pH sensitivity of receptors incorporating NR1wt and the indicated NR2 construct

NR2 construct	рН _{IC50}	
NR2Awt	6.93 ± 0.02 (n=9)	
NR2Bwt	7.50 ± 0.03 (n=5)	
NR2A-ΔNTD	7.11 ± 0.09 (n=9)	
NR2B-ANTD	7.14 ± 0.03 (n=6)	
NR2A-(2B NTD+L)	7.26 ± 0.03 (n=4)	
NR2B-(2A NTD+L)	7.02 ± 0.02 (n=4)	
NR2Dwt	7.52 ± 0.03 (n=10)	
NR2D-(2A NTD+L)	7.13 ± 0.01 (n=5)	

	(N-Terminal Domain)		
NR2A	ONAAAEKGPPALNIAVLLGHSHDVTERELRNLWGPEQATGLPLDVNVVALLMNRTDPKSLITHVCDLMSGARIHGLVFGDDTDOEAVAOM	112	
NR2B	SKARSQKSPPSIGIAVILVGTSDEVAIKDAHEKDDFHHLSVVPRVELVAMNETDPKSIITRICDLMSDRKIQGVVFADDTDQEAIAQI	111	
NR2C	-LGAGQG-EQAVTVAVVFGSSGPLQTQARTRLTSQNFLDLP-LEIQPLTVGVNNTNPSSILTQICGLLGAARVHGIVFEDNVDTEAVAQL	120	
NR2D	AVGGGTGGARPLNVALVFSGPAYAAEAARLGPAVAAAVRSPGLDVRPVALVLNGSDPRSLVLQLCDLLSGLRVHGVVFEDDSRAPAVAPI	126	
	(N-Terminal Domain)		
VID 0 V		202	
ND2B	IDFISOUTIFIEDSINGASMINADESSENGACESENCOLS SWINTNEEDWINFSSWITTPEGADELSFLATTUDISSEVENTES	202	
NR2C	LIDEVGSOFHVETI STOCCSVIVI TEKEDGSET (JEGVSTEOLOVI, EKVI FEVDWSTESVITTSTEDSVI TELEVSVORTKOTTSUS	210	
NR2D	LDFLSAQTSLPIVAVHGGAALVLTPKEKGSTFLQLGSSTEQQLQVIFEVLEEYDWTSFVAVTTRAPGHRAFLSYIEVLTDGSLVGWEHRG	216	
NR2A	VITLDTSFEDAKTOVOLKKTHSSVILLVCSKDEAVLILSEARSLGLTGYDFFWIVPSLVSGNTELTPKEFPSGLISV	279	
NR2B	VILLIDMSLDDCDSKIONOLKKLOSDIILLIVCKKERATVIERVANSVCLUGVGVWUVP-SUVACDTDTVPSEPPTCLISV		
NR2C	VLTLELGPGGPRARTORLLROVDAPVLVAYCSREEAEVLFAEAA0AGLVGPGHVWLVPNLALGSTDAPPAAFPVGLISV	289	
NR2D	ALTLDPGAGEAVLGAQLRSVSAQIRLLFCAREEAEPVFRAAEE AGLTGPGYVWFMVGPQLAGGGGSGVPGEPLLLPGGSPLPAGLFAV	304	
	(N-Terminal Domain)		
NR2A	S <mark>YDDW</mark> DYSLEARVRDGLGILTTAASSMLEKFSYIPEAKASCYGOAEKPETPLHTLHOFMVNVTWDGKDLSFTEEGYOVHPRLVVIVLNKD	369	
NR2B	SYDEWDYGLPARVRDGIAIITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINVTFEGRNLSFSEDGYQMHPKLVIILLNKE	370	
NR2C	VTESWRLSLRQKVRDGVAILALGAHSYRRQYGTLPAPAGDCRSHPGPVSPAREAFYRHLLNVTWEGRDFSFSPGGYLVRPTMVVIALNRH	379	
NR2D	RSAGWRDDLARRVAAGVAVVARGAQALLRDYGFLPELGHDCRTQNRTHRGESLHRYFMNITWDNRDYSFNEDGFLVNPSLVVISLTRD	392	
	-(N-Terminal Domain) - linker (ABD S1 segment)		
NR2A	REWEKVGKWENOTLSLRHAVWPRYKSFSDCEPDDNHLSIVTLEEAPFVIVEDIDPLTETCVRNTVPCR-KFVKINNSTNEG-MNVKKCCK	457	
NR2B	RKWERVGKWKDKSLOMKYYVWPRMCPETE-E0EDDHLSIVTLEEAPFVIVESVDPLSGTCMRNTVPCO-KRIISENKTDEEPGYIKKCCK	458	
NR2C	RLWEMVGRWDHGVLYMKYPVWPRYSTSLOPVVDSRHLTVATLEERPFVIVESPDPGTGGCVPNTVPCRROSNHTFSSG-DLTPYTKLCCK	468	
NR2D	RTWEVVGSWEQQTLRLKYPLWSRYGRFLQPVDDTQHLTVATLEERPFVIVEPADPISGTCIRDSVPCRSQLNRTHSPPPDAPRPEKRCCK	482	
	Region of NR2 subunits Region of NR2 subunits Region of NR2 subunits Residues of the NTD that wore mutated into evoteine	05	
	constructs chimeras. including both for MTS experiments	63	
•	Region of NR2 subunits the NTD and the linker		
	exchanged in «short»between the NTD andNTD hinge regionschimerasABD S1 segment		

(N-Terminal Domain)

Figure S1. Sequence alignment of the NTD+linker region of NR2A-D subunits

The indicated α -helices (red) and β -strands (green) of NR2 subunits were predicted as described in ref. 25.





Representative bursts of openings (left) and shut-time distribution histograms (right) from outside-out patches expressing either NR1wt/NR2Awt, NR1wt/NR2Bwt, NR1wt/NR2A-(2B NTD+L) and NR1wt/NR2B-(2A NTD+L). The value of Tcrit used to define bursts is indicated for each histogram.



Figure S3. Kinetics of MK-801 inhibition reveal that NMDAR Po heterogeneity is controlled by the NR2 NTD+linker region

a Kinetics of inhibition by 50 nM MK-801 of receptors incorporating NR1wt together with NR2Awt ($\tau_{on} = 2.0$ s), NR2Bwt (6.9 s), NR2A-(2B NTD+L) (5.1 s) or NR2B-(2A NTD+L) (2.8 s). **b** Bar graph showing the time constant of inhibition by 50 nM MK-801 of receptors incorporating NR1wt together with NR2Awt ($\tau_{on} = 2.1 + - 0.1$ s [n=6]), NR2Bwt (6.9 + - 0.7 s [n=10]), NR2A- Δ NTD (6.8 + - 0.6 s [n=6]), NR2B- Δ NTD (6.7 + - 1.0 s [n=4]), NR2A-(2B NTD) (3.8 + - 0.3 s [n=6]), NR2B-(2A NTD) (10.2 + - 0.5 s [n=6]), NR2A-(2B NTD+L) (5.7 + - 0.4 s [n=6]), NR2B-(2A NTD+L) (3.1 + - 0.2 [n=9]) or NR2A-(2D NTD+L) (31.4 + - 4 .9 s [n=10]) (**p<0.001). **c** Bar graph showing the time constant of inhibition by 200 nM MK-801 of receptors incorporating NR1wt together with NR2Dwt (36 + - 3 s [n=5]), NR2D- Δ NTD (5.5 + - 0.7 s [n=6]) or NR2D-(2A NTD+L) (1.9 + - 0.2 [n=5]). Error bar represent s.d.



Figure S4. Locking open the NR2-NTD increases the onset of MK-801 inhibition

a Recordings showing the inhibition by 50 nM MK-801 of NR1wt/NR2B-Y282C receptors in the presence of 100 μ M glutamate and 100 μ M glycine before (left panel) and after (right panel) modification by 0.2 mM MTS-PtrEA. *Inset:* onsets of MK-801 inhibition shown in panel *a* were fitted with a single exponential (τ_{on} = 9.7 s before and 30 s after MTS-PtrEA). **b** The MTS-induced relative change of the MK-801 on-rate is plotted versus the MTS-induced relative change of the NR1wt/NR2A-Y281A (0.60 +/- 0.07 [n=4] vs 0.60 +/- 0.02 [n=12] with MTSEA, respectively), NR1wt/NR2A-Y281C (1.10 +/- 0.13 [n=4] vs 1.02 +/- 0.06 [n=12] with MTSEA, respectively), NR1wt/NR2B-H127C (1.24 +/- 0.21 [n=4] vs 1.58 +/- 0.10 [n=8] with MTS-PtrEA, respectively) and NR1wt/NR2B-Y282C (1.15 +/- 0.12 [n=4] vs 2.1 +/- 0.11 [n=9] with MTSEA, respectively; and 2.5 +/- 0.4 [n=4] vs 5.5 +/- 1.0 [n=33] with MTS-PtrEA, respectively). The line represents a linear regression fit of the data points. The R value of the fit is 0.98. Error bars represent s.d.



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Figure S5. Locking open the NR2-NTD increases single-channel activity

a Single-channel recordings of an outside-out patch from a HEK cell expressing NR1wt/NR2B-Y282C receptors, in the presence of 100 µM glutamate and 100 µM glycine, before (left) and during (right) application of 0.2 mM MTSET (same patch). Bottom traces display time-expanded views. b All-points amplitude histograms from the patch recorded in a. Data (gray filling) were fitted with multiple gaussian components (red lines), amplitude of which enabled the calculation of N*Po before and during MTSET application. Similar results were obtained with 3 patches, yielding a mean potentiation of Po by MTSET of 3.4 ± 0.5 (s.e.m.). Data were filtered at 5 kHz for analysis and at 1 kHz for display.



Figure S6. Effect of MTS modification of NR1wt/NR2B-Y282C receptors on glutamate and glycine sensitivities

a Glutamate dose-response curves of NR1wt/NR2B-Y282C receptors in the presence of 100 μ M glycine before (EC₅₀ = 0.38 μ M, n_H = 1.2 [n = 9]; black-filled circles) and after (EC₅₀ = 0.9 μ M, n_H = 1.0 [n = 9]; open squares) modification by 0.2 mM MTS-PtrEA. **b** Glycine dose-response curves of NR1wt/NR2B-Y282C receptors in the presence of 100 μ M glutamate before (EC₅₀ = 0.4 μ M, n_H = 1.5 [n = 4]; black-filled circles) and after (EC₅₀ = 0.4 μ M, n_H = 1.5 [n = 9]; open squares) modification by 0.2 mM MTS-PtrEA. Error bars represent s.d.



Figure S7. MTS-PtrEA modification rate of NR1wt/NR2B-Y282C receptors is faster in the absence of agonists than in their presence

a Recordings showing the modification of NR1wt/NR2B-Y282C receptors by 20 μ M MTS-PtrEA in the absence of glutamate and glycine. **b** The onsets of MTS-PtrEA modification were fitted with a single exponential ($\tau_{on} = 30 + /-6 \text{ s}$, [n=5]). **c** Mean reaction rate of MTS-PtrEA modification at NR1wt/NR2B-Y282C in the absence or in the presence of 100 μ M glutamate and 100 μ M glycine (1700 +/- 300 M⁻¹.s⁻¹ [n=5] and 280 +/- 50 M⁻¹.s⁻¹ [n=17], respectively) (**p<0.001, Student's t-test). Error bars represent s.d.

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Figure S8. Effect of NR2-NTD mutations on receptor activity

a Bar graph showing the MTSEA-induced potentiations of receptors incorporating NR1-A652C together with NR2A-H128S (3.8 +/- 0.2, [n=7]), NR2A-Y281A (6.0 +/- 0.5, [n=7]), NR2B-H127A (42 +/- 2, [n=5]) or NR2B-Y282S (87 +/- 7, [n=3]). **b** Bar graph showing the onset of inhibition by 50 nM MK-801 of receptors incorporating NR1wt together with NR2A-H128C (2.4 +/- 0.2 s, [n=5]), NR2A-Y281C (4.6 +/- 0.2 s, [n=4]), NR2B-H127C (11.4 +/- 0.5 s, [n=4]) or NR2B-Y282C (29.1 +/- 0.9 s, [n=4]). Error bars represent s.d.



Figure S9. Effect of MTS modification at NR2B-H127, NR2A-Y281 and NR2A-H128 mutated receptors

a Recordings from NR1wt/NR2B-H127C and NR1wt/NR2B-H127A receptors during treatment by 0.2 mM of the indicated MTS compounds. **b** Recordings from NR1wt/NR2A-H128C, NR1wt/NR2A-Y281A and NR1wt/NR2A-Y281C receptors during treatment by 0.2 mM of the indicated MTS compound. Note that the MTS-potentiating effects are specific to the cysteine mutation introduced, since no potentiation were observed with the non-reactive serine or alanine control mutants, but rather an inhibition (likely due to the modification of an endogenous cysteine).