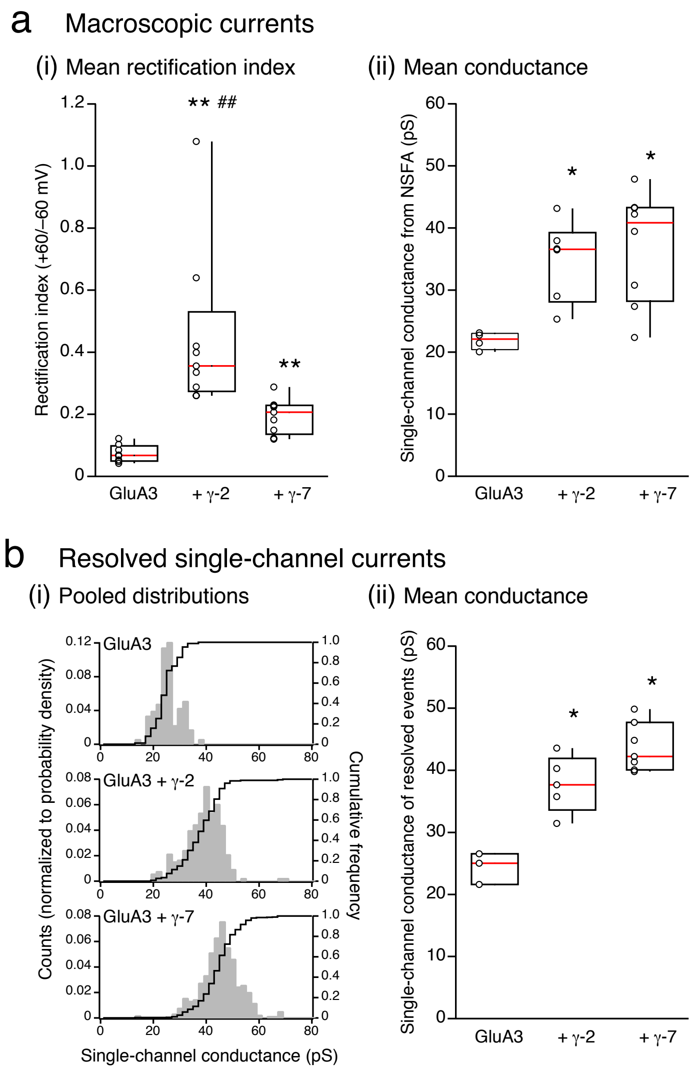
Channel properties reveal differential expression of TARPed and TARPless AMPARs in *stargazer* neurons

Cécile Bats, David Soto, Dorota Studniarczyk, Mark Farrant and Stuart G. Cull-Candy

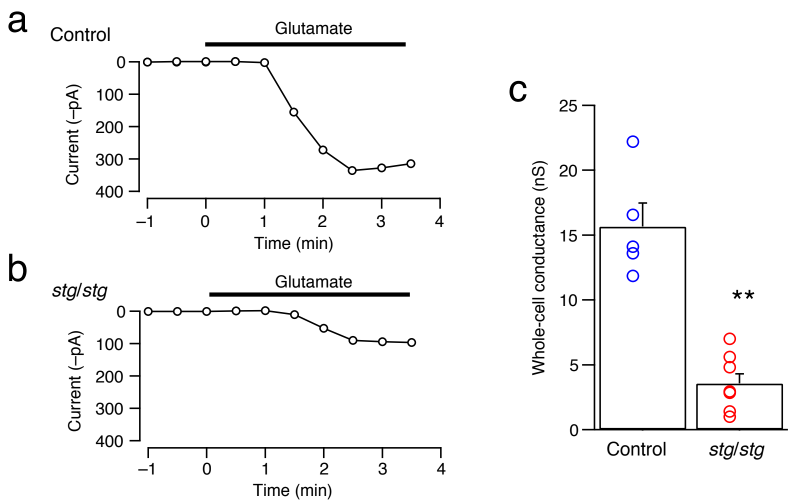
Department of Neuroscience, Physiology and Pharmacology
University College London, Gower Street, London WC1E 6BT U.K.

Figures

Tables



Supplementary Fig. 1: Properties of homomeric GluA3 CP-AMPARs determined from macroscopic responses and single-channel currents.
(**a**) Macroscopic responses to 10 mM glutamate recorded in outside-out patches from tsA201 cells were used to determine (i) the rectification index (+60/–60 mV), and (ii) weighted-mean single-channel conductance (from NSFA). (**b**) Measurements from resolved single-channel currents in the tail of macroscopic responses: (i) Pooled distributions of channel conductance determined from all-point amplitude histograms of selected events (see **Fig. 6**), (ii) Co-expression with γ-2 and γ-7 increased mean single-channel conductance. Group comparisons were performed using a Kruskal-Wallis rank-sum test followed by pair-wise Wilcoxon rank-sum tests with Holm's sequential Bonferroni correction. Asterisks denote statistical significance compared to GluA3 alone (**P*<0.05, ***P*<0.001); ## indicates significant difference from γ-7 (*P*<0.001).



Supplementary Fig. 2: Glutamate-evoked whole-cell currents from control and *stg*/*stg* stellate cells.
(**a**) Time course of response to bath-applied glutamate (100 μM) in the presence of CTZ (100 μM) and D-AP5 (20 μM) in a representative control stellate cell (–20 mV). (**b**) Time course of glutamate-evoked current in a representative *stg*/*stg* stellate cell. (**c**) Pooled data showing the reduced whole-cell conductance in *stg*/*stg* stellate cells (n = 5 control cells from 2 animals and 8 *stg*/*stg* cells from 3 animals; ** P<0.01).

	Control	***stg*/*stg***	*P*
eEPSC (acute slice)			
$RI_{(+40/–60)}$	0.39 ± 0.07 (9)	0.17 ± 0.02 (8) *	0.0061
qEPSC (acute slice)			
Amplitude at –80 mV (–pA)	44.8 ± 2.7 (17)	22.6 ± 1.0 (15) ***	5.9e–6
Amplitude *CV*	0.39 ± 0.07 (17)	0.27 ± 0.02 (15) ***	3.4e–6
10-90% Rise time (μs)	173 ± 9 (18)	196 ± 8 (8) *	0.026
$\tau_{\mathrm{w,\ decay}}$ (ms)	0.92 ± 0.06 (18)	0.86 ± 0.07 (8) n.s.	0.72
Peak conductance (–80 mV; pS)	623.4 ± 52.0 (6)	285.9 ± 11.9 (5) **	0.0081
Peak conductance (+60 mV; pS)	405.3 ± 40.4 (6) †	234.8 ± 13.5 (5) ¶ *	0.014
$RI_{(+60/–80)}$ (count matched)	0.66 ± 0.02 (6)	0.79 ± 0.07 (5) n.s.	0.12
$RI_{(+60/–80)}$	0.35 ± 0.08 (6)	0.09 ± 0.03 (5) **	0.0043
Frequency (+60/–80 mV)	0.58 ± 0.07 (6)	0.14 ± 0.03 (5) **	0.0081
Single-channel conductance (pS)	30.6 ± 3.7 (18)	16.1 ± 1.1 (8) **	0.0017
N_p	24.9 ± 3.6 (18)	19.9 ± 1.5 (8) n.s	0.66
mEPSC (dissociated culture)			
$RI_{(+60/–80)}$	1.14 ± 0.30 (5)	0.47 ± 0.10 (9) ***	0.001

Supplementary Table 1: Basic properties of EPSCs in control and *stg*/*stg* stellate cells. Data are presented as mean ± s.e.m. (from *n* cells). Statistical significance was determined using the non-parametric Wilcoxon rank sum test (unpaired). Asterisks denote significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Paired comparisons were made using the Wilcoxon signed rank test: † P = 0.036 versus –80 mV, ¶ P = 0.059 versus –80 mV. Note, as synaptic channel conductance was determined in the presence of strontium (see **Methods**), the absolute conductance value should not be compared directly with previously published values obtained in solutions containing calcium. Recordings from tsA201 cells showed that the weighted-mean single-channel conductance (determined by NSFA) was reduced by approximately 20% in strontium for both GluA1 and GluA1/2 combinations (data not shown).

	Control	***stg/stg***	*P*
qEPSC PhTX-433 block			
(PhTX/control, –80 mV)			
Charge (per PF stim.)	0.57 ± 0.08 (5)	0.22 ± 0.05 (6) *	0.022
Amplitude	0.79 ± 0.08 (5)	0.85 ± 0.02 (6) n.s.	0.17
Frequency	0.75 ± 0.11 (5)	0.26 ± 0.05 (6) *	0.0081
Excised somatic patches			
$\tau_{\text{w, des}}$ (ms)	3.07 ± 0.43 (11)	2.49 ± 0.28 (8) n.s.	0.48
Steady-state (% peak)	2.97 ± 0.76 (9)	2.87 ± 0.69 (10) n.s.	0.91
$RI_{(+60/-60)}$	0.41 ± 0.06 (9)	0.25 ± 0.02 (10) *	0.02
Channel conductance (NSFA) (pS)	27.5 ± 1.2 (9)	26.2 ± 3.2 (8) n.s	0.47
Channel conductance (Resolved†) (pS)	35.2 ± 1.8 (8)	31.6 ± 2.2 (7) n.s.	0.40

Supplementary Table 2: Properties of synaptic and extrasynaptic AMPARs in control and *stg/stg* stellate cells. Data are presented as mean ± s.e.m. (from *n* cells). Statistical significance was determined using the non-parametric Wilcoxon rank sum test (unpaired). Asterisks denote significance: * $P < 0.05$. †Note, direct measurement of single-channel current amplitudes was performed in a subset of recordings (8 out of 9 control and 7 out of 8 *stg/stg* cells).

Single-channel conductance (pS)

	NSFA	Resolved	*n*	*P*
GluA3	22.4 ± 0.5	24.4 ± 1.5	3	0.500
GluA3 + γ-2	36.6 ± 2.3	37.8 ± 2.1	5	0.625
GluA3 + γ-7	38.5 ± 3.3	43.7 ± 1.5	7	0.578

Supplementary Table 3: Single-channel conductance of recombinant homomeric GluA3 AMPARs from non-stationary fluctuation analysis and measurement of directly-resolved channel events. Data are presented as mean ± s.e.m. (from *n* outside-out patches from tsA201 cells), and represent weighted mean single-channel conductance from NSFA of macroscopic responses, and measurements from multiple all-point amplitude histograms of selected events in the tail of the same macroscopic currents. Paired comparisons were made using the Wilcoxon signed rank test.