

Physiological role for GABA_A receptor desensitization in the induction of long-term potentiation at inhibitory synapses

Martin Field^{1,2}, Valentina Dorovykh¹, Philip Thomas¹ & Trevor G Smart^{1,*}

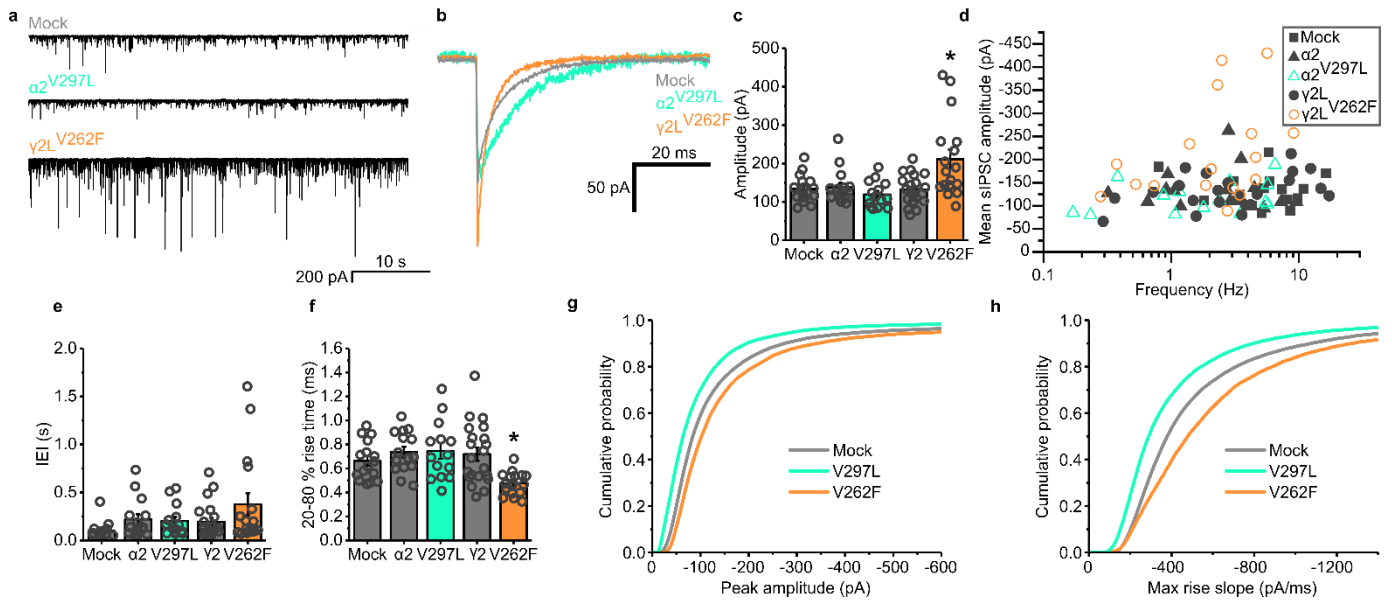
¹ Department of Neuroscience, Physiology & Pharmacology, UCL, Gower Street, London, United Kingdom, WC1E 6BT

² Current affiliation: Department of Pharmacology, University of Oxford, Oxford, United Kingdom, OX1 3QT

*Corresponding author – Trevor G Smart (t.smart@ucl.ac.uk)

Supplementary information

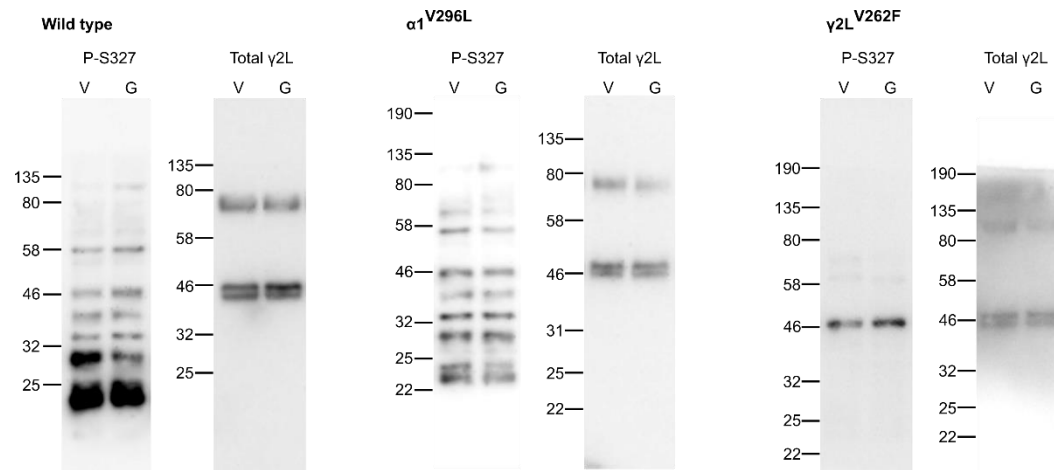
Supplementary figure 1



Supplementary Figure 1. $\gamma 2^{V262F}$ expression increases sIPSC amplitudes at physiological temperature. **a** Representative sIPSCs recorded at 37°C from voltage clamped neurons expressing the indicated constructs (Mock transfection, black; $\alpha 2^{V297L}$, green; $\gamma 2^{V262F}$, orange). **b** Average sIPSC unscaled waveforms and **c**, summary bar graph for mean sIPSC amplitudes: $n = 16$ (mock transfected), 15, 14, 21, and 17 neurons, for each construct as they appear on the abscissa (one-way ANOVA: $F_{(4, 78)} = 6.67$, $p = 0.00011$; Tukey test (mock vs $\alpha 2^{V297L}$): $p = 0.96$; Tukey test (mock vs $\gamma 2^{V262F}$): $p = 0.0022$). **d** Mean sIPSC amplitude plotted against average sIPSC frequency for each construct (see symbol key). **e** Median IEL for all cells (one-way ANOVA: $F_{(4, 78)} = 3.49$, $p = 0.011$; Tukey test (mock, $\gamma 2^{V262F}$): $p = 0.0099$). **f** Mean 20-80% rise times for all cells (one-way ANOVA: $F_{(4, 78)} = 5.51$, $p = 0.00059$; Tukey test ($\gamma 2^{V262F}$): $p = 0.0024$). **g** Mean cumulative probability distributions of the amplitudes and **h** maximum rate of rise slopes of all sIPSCs recorded. $n = 54111$ events from 16 cells (Mock), 24531 events from 14 cells ($\alpha 2^{V297L}$), and 24663 events from 17 cells ($\gamma 2^{V262F}$). Source data are provided as a Source data file for Supplementary Fig 1.

Supplementary Table 1: mutagenesis details

<u>Mutation</u>	<u>Forward primer</u>	<u>Reverse primer</u>
$\alpha 2^{V297L}$	CTGTTCTCTGCCCTAATTGAATTTGCA	AAACGCATAACAAACAGCTATAAACCAAGTCC
	<u>References</u>	
$\alpha 1^{V296L}$	Gielen, M., Thomas, P., and Smart, T.G. (2015). The desensitization gate of inhibitory Cys-loop receptors. <i>Nature Communications</i> 6 , 6829, doi.org/10.1038/ncomms7829.	
$\gamma 2L^{V262F}$	Gielen, M., Thomas, P., and Smart, T.G. (2015). The desensitization gate of inhibitory Cys-loop receptors. <i>Nature Communications</i> 6 , 6829, doi.org/10.1038/ncomms7829.	
$\gamma 2L^{S327A}$	Krishek, B. J. et al. Regulation of GABA _A receptor function by protein kinase C phosphorylation. <i>Neuron</i> 12 , 1081–1095 (1994).	



Whole blots for the panels in Fig. 8j. All molecular weight markers are given in kDa.