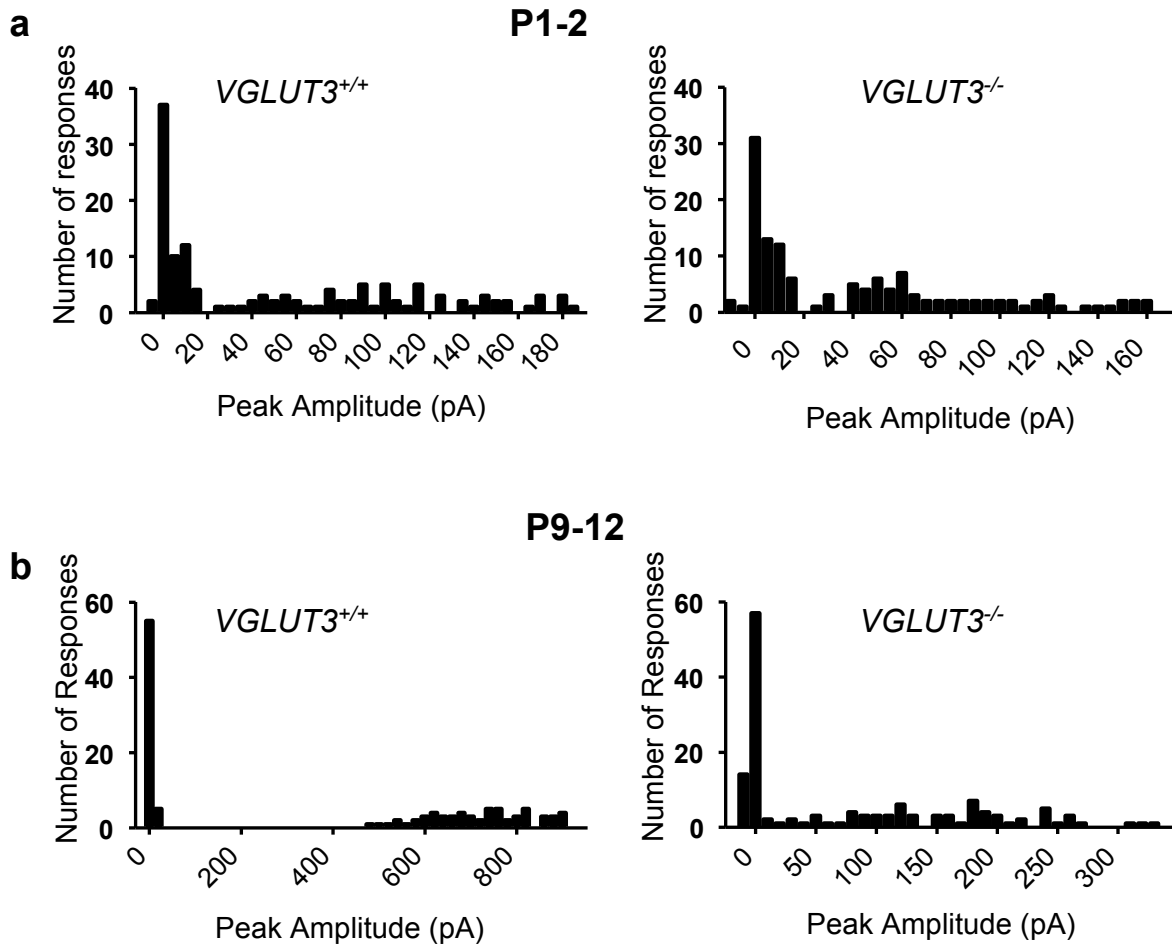


Glutamate co-release at GABA/glycinergic synapses is crucial for the refinement of an inhibitory map

Jihyun Noh, Rebecca P Seal, Jessica A. Garver, Robert H Edwards & Karl Kandler

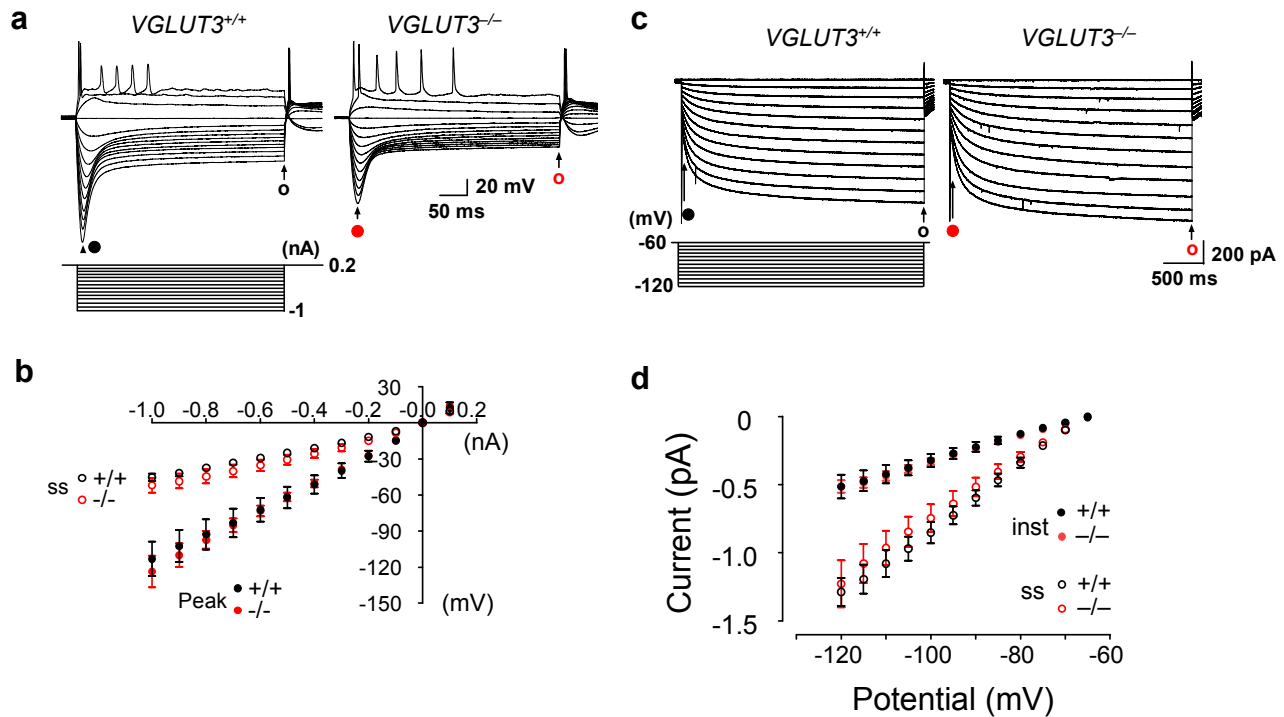
Supplemental Figure 1



Supplemental figure 1.

Amplitude histograms of minimal stimulation responses. **(a)** Amplitude histogram of responses in P1-2 mice (shown in Fig 2a). **(b)** Amplitude histogram of responses at P9-12 (shown in Figure 2b).

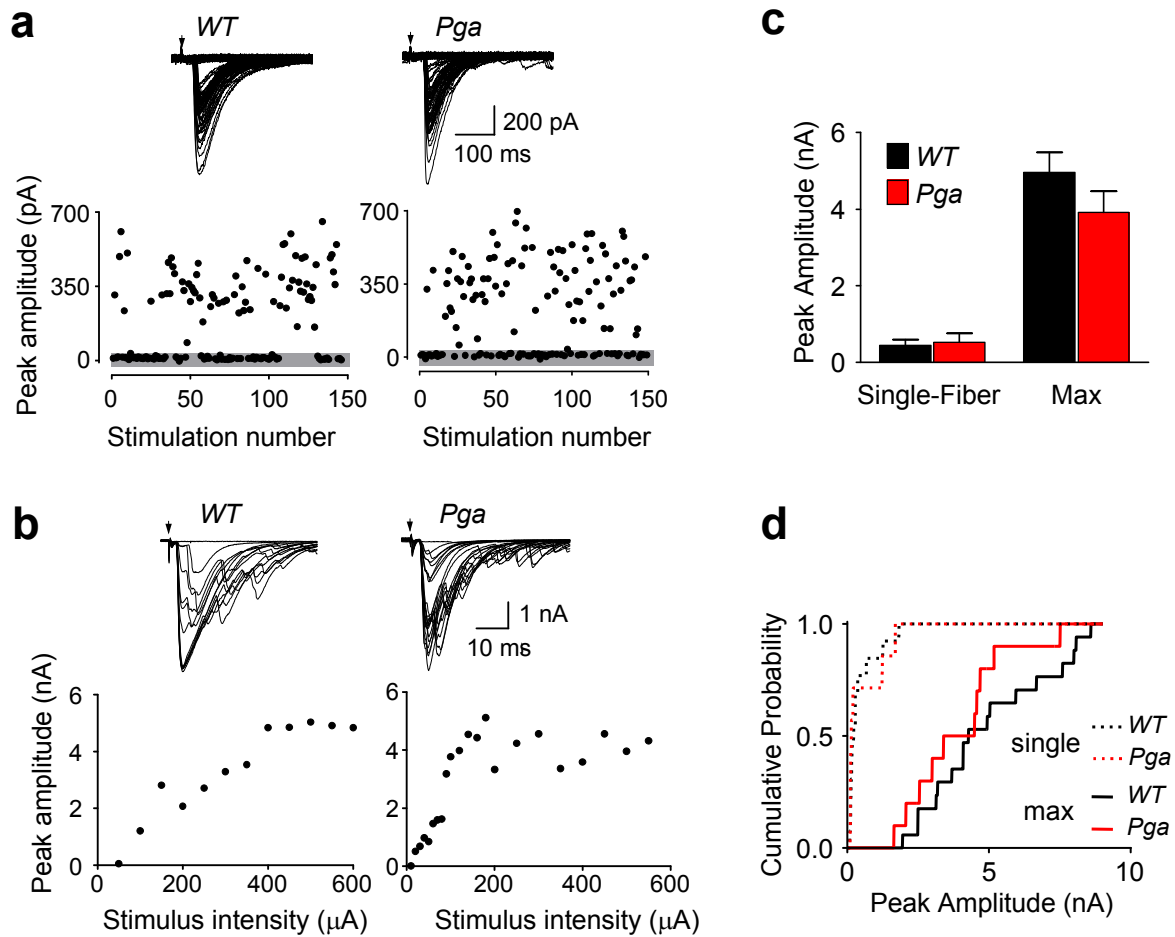
Supplemental Figure 2



Supplemental Figure 2

Membrane properties of LSO neurons are not different between *VGLUT3*^{-/-} and *VGLUT3*^{+/+} mice. (a) Membrane voltage responses of LSO neurons (P9–12) to current injections. Negative currents generated a slowly relaxing hyperpolarization 'sag', typical for LSO neurons. (b) Current-voltage relationship for LSO neurons. Inward rectification in response to hyperpolarizing current pulses was present in each case (*VGLUT3*^{+/+}, black, $n = 4$; *VGLUT3*^{-/-}, red, $n = 5$; closed circle, peak point; open circle, steady-state: *ss*). (c) LSO neurons from both *VGLUT3*^{+/+} and *VGLUT3*^{-/-} expressed hyperpolarizing-activated currents (I_h). I_h was activated by 3 s, hyperpolarizing voltage steps from -120 mV to -60 mV in 5 mV increments (*inset*, voltage commands). (d) Current-voltage relationship illustrates I_h showing instantaneous currents (*inst*, closed circles) and steady state currents (*ss*, open circle; *VGLUT3*^{+/+}, black, $n = 4$; *VGLUT3*^{-/-}, red, $n = 5$). In these experiments, the internal pipette solution contained (in mM): 54 D-potassium gluconic acid, 56 KCl, 1 MgCl₂, 1 CaCl₂, 10 HEPES, 11 EGTA, 0.3 Na-GTP, 2 Mg-ATP, 5 QX-314 and 0.3 % biocytin (pH 7.2, 280 mOsm/l).

Supplemental Figure 3



Supplemental figure 3 Minimal and maximal stimulation of MNTB-LSO fibers in *WT* and *Pachanga* mice aged P9-12 (*Pga*, Otoferlin knockout; Schwander *et al.*, *J. Neurosci.* **27**,2163-2175, 2007). **(a)** Examples of single fiber responses. *Inset*: Superposition of 150 consecutive traces. **(b)** Examples of stimulus-response relationships. **(c)** Single-fiber and maximal synaptic current amplitudes were not significantly different between *WT* and *Pga* mice (Single-fiber response: *WT*, 444 ± 145 pA, $n = 13$; *Pga*, 519 ± 251 pA, $n = 7$; $P > 0.7$; Student's t-test. Maximal response: *WT*, 5.0 ± 0.5 nA, $n = 17$; *Pga*, 3.9 ± 0.6 nA, $n = 10$; $P > 0.2$; Student's t-test). **(d)** Cumulative probability histograms for single-fiber and maximal responses in *WT* and *Pga* mice (Single-fiber, $P > 0.5$; Max., $P > 0.6$; Kolmogorov-Smirnov test).