

## Supplemental DATA

## The glycine transporter GlyT2 controls the dynamics of synaptic vesicle refilling in inhibitory spinal cord neurons

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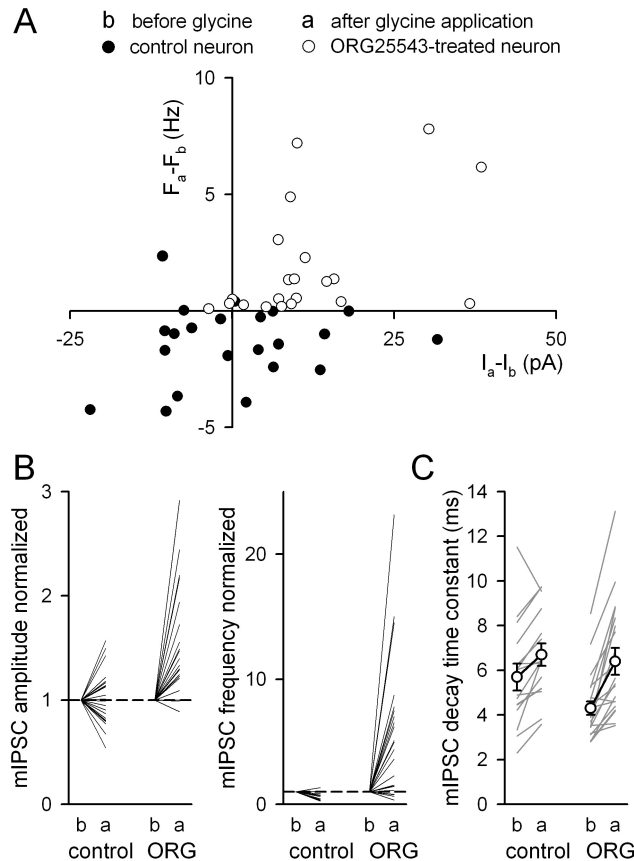
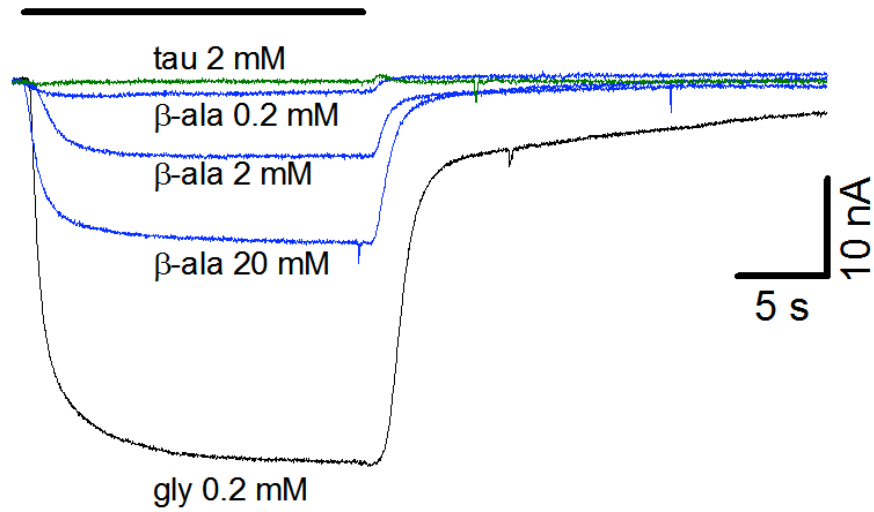


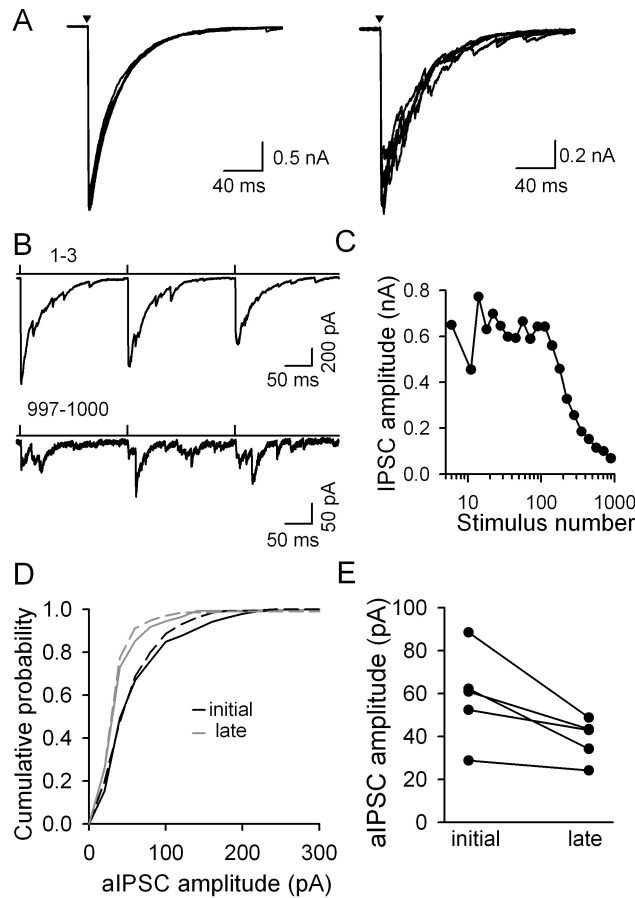
Figure S1. **Change in glycinergic mIPSC amplitude and frequency following glycine application in control and ORG25543-treated cells.**

**(A)** The difference in glycinergic mIPSC amplitude before (b) and after (a) glycine application (100  $\mu$ M, 10 min, in the presence of 3, 10 or 30  $\mu$ M strychnine) plotted against the difference in mIPSC frequency before (b) and after (a) glycine application for each control (closed circles,  $n = 22$ ) and ORG25543-treated (open circles,  $n = 23$ ) neurons. **(B)** Relative change in mIPSC amplitude (left), frequency (middle) and decay time constant (right) recorded from control neurons and ORG25543-treated neuron before (b) and after (a) glycine application. For amplitude and frequency bar graphs, data are normalized by the values before glycine application. (One pair of ORG25543-treated neurons was removed from the mIPSC frequency graph as its change in frequency was  $> 20$ ).



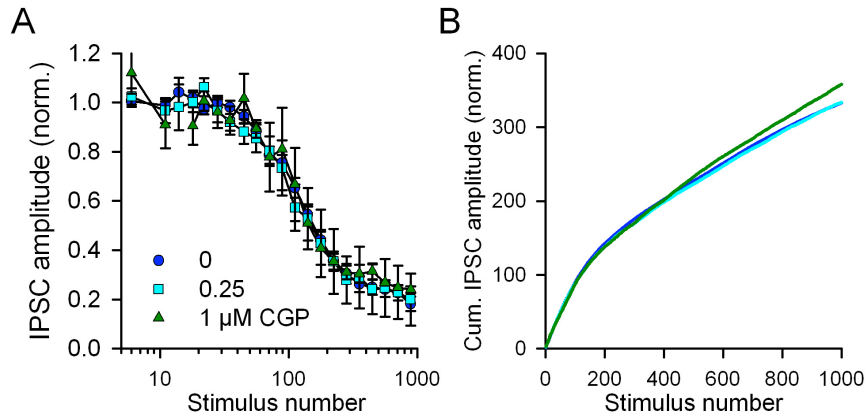
**Figure S2.  $\beta$ -alanine is a low-affinity, low-efficacy substrate of GlyT2.**

To examine whether  $\beta$ -alanine and taurine were substrates of GlyT2, the transporter currents evoked in *Xenopus* oocytes expressing GlyT2 by the application (solid bar) of these amino-acids were compared to that evoked by glycine. In contrast to taurine (2 mM),  $\beta$ -alanine (0.2, 2 and 200 mM, blue traces) was able to evoke small steady-state currents ( $n = 3$ ). At saturating concentration (200 mM, not shown) the current evoked by  $\beta$ -alanine was 51 % of the current evoked by a saturating glycine concentration (2 mM, not shown). We deduced from the amplitudes of these transporter currents that in the experimental condition of figures 4D-E, GlyT2-mediated uptake of  $\beta$ -alanine at 200  $\mu$ M represented only 4.6 % of glycine uptake (100  $\mu$ M).



**Figure S3. Change in glycine quantal size during high frequency stimulation.**

**(A)** Superimposed traces of glycinergic IPSCs ( $n = 5$ ) recorded in the presence of  $\text{Ca}^{2+}$  (left traces) or  $\text{Sr}^{2+}$  (right traces) show the presence of asynchronous events in  $\text{Sr}^{2+}$ . **(B)** Traces of asynchronous IPSCs recorded at the onset (top panel) and at the end (bottom panel, note the change in scale) of a 5 Hz stimulation. The solid line represents the time when the APs were triggered. **(C)** Time course of the amplitude of IPSCs recorded in  $\text{Sr}^{2+}$  during a 5 Hz train (Fig. 7D). **(D)** Cumulative probability distribution of asynchronous IPSC amplitudes recorded in the presence of  $\text{Sr}^{2+}$  from the pair shown in B and C (dashed line) and the average distribution from 5 pairs of neurons (solid line) at the onset (black lines) and at the end of the train (gray line;  $p > 0.05$ ). **(E)** Variation in glycinergic asynchronous IPSC mean amplitude recorded in the presence of  $\text{Sr}^{2+}$  during the initial and the late periods ( $p > 0.05$ ).



**Figure S4. GABA<sub>B</sub> receptors are not involved in the depression induced by a train of 1000 APs triggered at 5 Hz.**

To examine whether GABA<sub>B</sub> receptors were involved in the depression observed during a 5 Hz stimulation train, we recorded trains in the presence or absence of CGP5845 (3-N[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid) (0.25 or 1 μM), a selective antagonist of GABA<sub>B</sub> receptors. **(A)** Time course of the peak amplitude of glycinergic IPSCs recorded at 5 Hz in pairs of neurons in the absence (blue circle, n = 16), or the presence of 0.25 (cyan square, n = 6) and 1 μM (green triangle, n = 3) of CGP5845. IPSC amplitudes were normalized to the average of 30 IPSCs (from the 3<sup>rd</sup> to the 32<sup>nd</sup> IPSC). **(B)** Cumulative IPSC amplitude during a 5 Hz stimulation train recorded from pairs of control neurons in the absence (blue line, n = 16) or the presence of 0.25 (cyan line, n = 6) and 1 μM (green line, n = 3) of CGP5845.

## Supplementary Methods

Xenopus oocyte electrophysiology: Human GlyT2 was subcloned as indicated by Roux and Supplisson (2000). cRNAs were transcribed *in vitro* using the T7 mMessage-mMachine kit (Ambion, Austin, TX) and 50 ng were injected into Xenopus oocytes using a nanoliter injector (World Precision Instruments, Sarasota, FL). *Xenopus laevis* oocytes were prepared and incubated as indicated by Roux and Supplisson (2000). Two-electrode voltage clamp was performed using an O-725C amplifier (Warner Instrument, Hamden, CT). Currents were sampled at 10 Hz using a Digidata 1322A and pClamp8 software (Molecular Devices, Sunnyvale, CA). Glass electrodes were filled with a 3 M KCl solution and had typical resistances of 1 M $\Omega$ . The extracellular recording solution contained (in mM): NaCl 100, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1, HEPES 5, pH 7.2 adjusted with KOH.

Asynchronous synaptic responses were induced when presynaptic neurons were stimulated at 0.1 or 5 Hz and Ca<sup>2+</sup> was substituted by an equimolar concentration of Sr<sup>2+</sup> in the external solution. Data were analyzed off-line with custom-made routines written for the Igor PRO analysis environment (WaveMetrics, Lake Oswego, OR). A standard algorithm was used to detect asynchronous IPSCs. It consisted of a threshold detection performed on a discrete time derivative of the recorded trace. Asynchronous events were measured during a 100 msec period beginning 5 msec after the peak of AP to eliminate synchronous synaptic responses. Asynchronous events were examined only when a synchronous response evoked was observed.