Supporting Information

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SI Materials and Methods

Insertion and Endocytosis Assays. Hippocampal neurons (14–17 d in vitro) expressing $\alpha2^{pHBBS}$ were assayed for the insertion of GABA$_A$ receptors (GABA$_A$Rs) over time (1). Neurons were first labeled with 10 μg/mL unlabeled bungarotoxin for 15 min at 15 °C to block existing cell surface receptors, and then washed three times in PBS at 15 °C followed by an incubation with 3 μg/mL of Alexa 594-conjugated bungarotoxin (Invitrogen) for 5–15 min. All incubations were performed in the presence of 150 μM tubocurarine (Sigma) to block bungarotoxin binding to endogenous acetylcholine receptors (2). At 5, 10, and 15 min, samples were removed, fixed, and permeabilized, and the total receptor population was labeled with anti-GFP antibody. Endocytosis assays were performed as previously (3) and described in *Results*. At 0, 7.5, and 15 min, the remaining surface Alexa594::Bgt was calculated according to the following formula: $100 \times [I_{tf}/I_{t0}]$, where I_{tf} represents the total surface fluorescence count (clustered and diffuse) at the given time point and I_{t0} is the starting fluorescence count. Images were acquired as described in live imaging, and MetaMorph was used to determine the average total cell surface fluorescence intensity along a 20-μm length from two to three proximal dendrites/neuron as previously described (1), blind to experimental condition.

Electrophysiology. Neurons were plated on 12-mm glass coverslips (German glass; VWR) coated with poly-L-lysine (0.5 mg/mL; Sigma) and cultured for 2–3 wk before the recordings. To measure mIPSCs, coverslips were placed in a recording chamber mounted on the stage of an inverted microscope and continually perfused with the following (in mM): 140 NaCl, 4.7 KCl, 10 Hepes, 11 glucose, 2 $MgCl_2$, and 2.5 $CaCl_2$ (adjusted to pH 7.4 with NaOH, 295–315 mOsm). For mIPSC recording, the extracellular solution was supplemented with 200 nM TTX, 10 μM CNQX, and 20 μM D-AP5 (Sigma). Borosilicate pipettes (3–6 MΩ) were filled with the following (in mM): 150 CsCl, 10 Hepes, 1.1 EGTA, 2 $MgCl_2$, 0.1 $CaCl_2$, 2 Mg^{2+}-ATP (adjusted to pH 7.2 with CsOH). Recordings were started 5–10 min after a stable whole-cell access was obtained. An Axopatch 200B amplifier and Digidata 1322A (Axon Instruments) software were used for pulse generation, data acquisition (10 kHz), and filtering (5 kHz, four-pole Bessel filter). Series resistance and membrane capacitance were partially compensated (40–60%). Miniature events were analyzed using pClamp 9.2 (Campfit; Axon Instruments) and MiniAnalysis 6.0.3 software (Synaptosoft). The decay phase was fitted with a monoexponential function (τ decay); to describe the rise time, we chose as the base 10–90% rise time.

1. Saliba RS, Pangalos M, Moss SJ (2008) The ubiquitin-like protein Plic-1 enhances the membrane insertion of GABA$_A$ receptors by increasing their stability within the endoplasmic reticulum. *J Biol Chem* 283(27):18538–18544.
2. Sekine-Aizawa Y, Huganir RL (2004) Imaging of receptor trafficking by using α-bungarotoxin-binding-site-tagged receptors. *Proc Natl Acad Sci USA* 101(49):17114–17119.
3. Jacob TC, et al. (2009) GABA$_A$ receptor membrane trafficking regulates spine maturity. *Proc Natl Acad Sci USA* 106(30):12500–12505.

Table S1. Effect of 0.5 μM flurazepam on GABA-induced currents (I_{GABA}) was measured in transfected HEK-293 cells for varying GABA$_A$R constructs

Subunit combination	0.5 μM flurazepam % (control)
α1β3γ2	167.5 ± 25.6
$\alpha1^{pHGFP}$β3γ2	187.5 ± 32.1
α2β3γ2	154.5 ± 21.7
$\alpha2^{pHGFP}$β3γ2	162.3 ± 14.9

Magnitude of I_{GABA} seen with 5 μM GABA (control) was compared with that seen with 5 μM GABA plus 0.5 μM flurazepam. $n = 4$ for each construct.