Supporting Information

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SI Materials and Methods

Animals. Mice of wild-type (WT), transgenic (Nxph1-GFP$^{tg/-}$), or Nxph1-deficient (KO) genotypes were used in this study (See *Animals*). Nxph1 KO mice have been reported previously (1) and were crossed into GAD65-GFP transgenics (2) to facilitate recognition of GABAergic neurons. For overexpression of Nxph1-GFP under the Thy1.2 promoter, a transgenic vector was constructed using plasmid pCMVD2 [full-length Nxph1 (3)]. EGFP was inserted (junctional amino acid sequence: Nxph1 . . . PYFPSGPGPG*MVSKGEE ...EGFP... GMDELYKPG*stop . . . Nxph1 3′-UTR) and transferred to pEX21 (4) to generate pThyNxph1C′EGFP. Transgenic mice were produced by pronucleus injection, and founder mice and progeny were genotyped by PCR.

Quantitative RT-PCR. Tissue blocks were isolated from frontal brain slices under visual control from both the nucleus reticularis thalami (NRT) and the ventrobasal nucleus from postnatal day 7 (P7) and P61 Nxph1$^{+/+}$/GAD65$^{tg/+}$ mice, and RNA was isolated with the RNAzol-reagent (WAK-Chemie). Quantitative RT-PCR (qRT-PCR) using the LightCycler 480 SYBR Green I Master protocol (Roche Diagnostics) was performed with the following gene-specific primers for Nrxn3α (forward) GCACCATCAAAGTG-AAGGCCACTC and (reverse) GCCCAGATACATGTCCCC-CTCCA, for Nxph1 TGTGCCAACCTAACAAATGG (forward) and AGGCTCCTGAAGATCTGTGG (reverse), and for β-actin TGACCCTGAAGTACCCCATTGA (forward) and gggtcatctttt-cacggttgg (reverse). For internal control, β-actin reactions were used as a standard for all calculations. All reactions for each gene and corresponding control reactions were performed on a single 96-well plate. Quantitative standard reactions showed only minimal variation in qPCR conditions between the plates. Each cycle threshold value of a gene-specific reaction was calculated relative to the β-actin control reaction. Expression levels in P7 mice samples were set to 1, and the expression in samples was calculated as *x*-fold expression of P7.

1. Missler M, Hammer RE, Südhof TC (1998) Neurexophilin binding to alpha-neurexins. A single LNS domain functions as an independently folding ligand-binding unit. *J Biol Chem* 273(52):34716–34723.
2. López-Bendito G, et al. (2004) Preferential origin and layer destination of GAD65-GFP cortical interneurons. *Cereb Cortex* 14(10):1122–1133.
3. Missler M, Südhof TC (1998) Neurexophilins form a conserved family of neuropeptide-like glycoproteins. *J Neurosci* 18(10):3630–3638.
4. Zhang W, et al. (2005) Extracellular domains of alpha-neurexins participate in regulating synaptic transmission by selectively affecting N- and P/Q-type Ca2+ channels. *J Neurosci* 25(17):4330–4342.

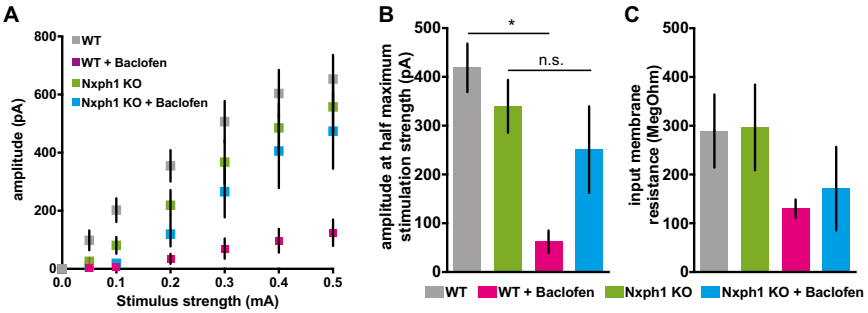

Fig. S1. Nxph1 knockout neurons in the NRT show a reduced response to the $GABA_B$ receptor ($GABA_BR$) agonist baclofen. (*A* and *B*) Evoked inhibitory synaptic responses (eIPSCs) were recorded without and with 30 μM baclofen in the bath solution. Input–output relationships between the electrically evoked amplitudes and the stimulation strengths (*A*) as well as the amplitudes of eIPSCs at half-maximum stimulation strength (*B*) were analyzed. Although there is no significant difference in eIPSCs between WT and KO cells without baclofen (colored gray and green; see also *Results*), we observed that baclofen significantly reduced eIPSCs in WT NRT neurons (magenta) in agreement with published results (1), but Nxph1 KO cells responded less to addition of agonists (blue). (*C*) The input resistance of neurons treated with baclofen dropped to 45% of controls and caused an enormous variability of eIPSCs, likely due to activation of $GABA_BR$-associated GIRK channels and deterioration of space clamp properties (2, 3). Therefore, the anaylsis of synaptic inputs originating from extrasomatic sites is not reliable enough under these conditions to apply the agonist to other protocols such as paired-pulse depression in the NRT. Data are means ± SEM (collected from 18 to 10 neurons per condition). Significance of differences was tested using Student *t* test for unpaired values. Levels are indicated as $*P < 0.05$. n.s., not significant.

1. Ulrich D, Huguenard JR (1996) GABAB receptor-mediated responses in GABAergic projection neurones of rat nucleus reticularis thalami in vitro. *J Physiol* 493(Pt 3):845–854.
2. Bettler B, Kaupmann K, Mosbacher J, Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. *Physiol Rev* 84(3):835–867.
3. Gassmann M, Bettler B (2012) Regulation of neuronal GABA(B) receptor functions by subunit composition. *Nat Rev Neurosci* 13(6):380–394.

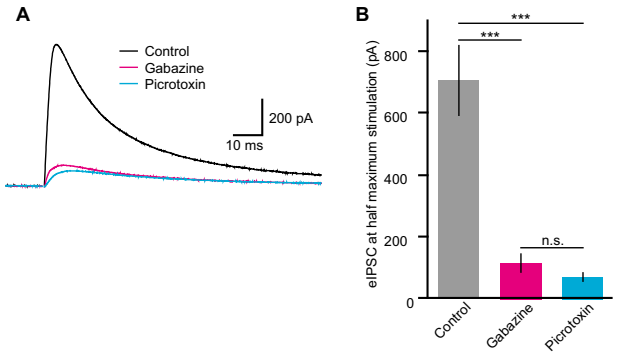

Fig. S2. GABA-mediated synaptic transmission is abolished by intracellular application of pharmacological blockers in WT neurons. (*A*) Averaged current traces of eIPSCs activated by single electrical pulses at 0.1 mA observed after intracellular application of picrotoxin (blue) or gabazine (pink) and under the control condition (black, no inhibitor present). (*B*) Quantification of eIPSC amplitudes revealed significant reduction after intracellular application of picrotoxin or gabazine. Data are means ± SEM (collected from 11 neurons per condition). Significance of differences was tested for both blockers against control using Student *t* test for unpaired values. Levels are indicated as ***$P < 0.001$.

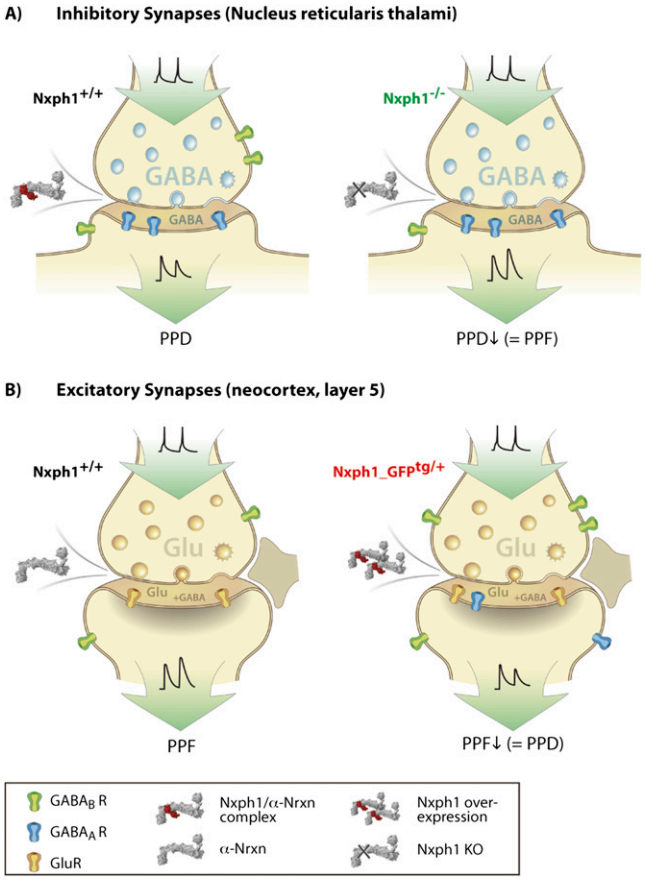

Fig. S3. Graphical summary of the role of Nxph1 as analyzed by knockout (Nxph1$^{-/-}$) and transgenic (Nxph1_GFP$^{tg/+}$) mouse models. (*A*) Analysis of Nxph1-deficient (KO) inhibitory terminals reveals decreased paired-pulse depression (PPD) at inhibitory synapses in the NRT. The phenotype can be mimicked by blocking presynaptic $GABA_BR$ in wild-type neurons, suggesting that fewer functional receptors are present in KO neurons. (*B*) To test the conclusion from KO neurons that Nxph1 supports $GABA_BR$, we expressed the molecule ectopically at excitatory terminals in the neocortex, which normally do not contain Nxph1. Our results show that presynaptic $GABA_BR$ and postsynaptic $GABA_AR$ together cause the diminished facilitation at transgenic excitatory synapses because the phenotype could be reversed by pharmacologically blocking $GABA_BR$ with CGP-55845, and a complete rescue was achieved by additional blocking of post-synaptic $GABA_AR$ with intracellular picrotoxin or gabazine.

Table S1. Characterization of NRT neurons

Properties	WT	Nxph1 KO	*P* value
Input resistence (MΩ)	286 ± 32.9	258 ± 45.2	0.62
Resting potential (mV)	−65 ± 1.9	−64 ± 2.2	0.64
Capacity (pF)	30.94 ± 4.14	38.56 ± 6.36	0.32
Time LTS (ms)	175.2 ± 25.3	147.9 ± 31.8	0.53
Amplitude LTS (mV)	22.9 ± 4.1	15.9 ± 1.1	0.19
N sodium spikes	3.56 ± 0.69	2.75 ± 0.73	0.47
Adaptation ratio	2.18 ± 0.18	1.93 ± 0.29	0.45
AP threshold (mV)	−38.62 ± 1.44	−36.00 ± 1.41	0.21
N (cells/mice)	7–16/16	4–12/10	

Passive cell properties of NRT neurons from WT and Nxph1-deficient (KO) mice were analyzed at P14–16, and characterization of LTS properties was performed by current-clamp recordings. Parameters analyzed included the adaptation ratio of tonically generated sodium spikes during depolarizing current steps (which includes analysis of the frequencies of sodium spikes generated during depolarization steps and their phasic or tonic behavior). LTS analysis included documentation of the sizes of the amplitudes, width of the LTS, and the number of action potentials generated. All data are means ± SEM. Significance of differences in the data were tested by Student *t* test for unpaired values. *P* values indicate level of significance. LTS, low-threshold spikes; AP, action potential.