Supporting Information

Yang et al. 10.1073/pnas.1107998108

SI Materials and Methods

Recording Loci for Brain Slices. After detailed acute auditory mapping, multiple lesion marks were made in auditory cortex (AI) of naïve animals. A Nissl stain indicated that low and high characteristic frequency (CF) areas of AI can be consistently located by landmarks of adjacent hippocampal and thalamic structures (Fig. S1*A*). We recorded from pyramidal neurons in layers 2–3, 200–500 μm below pia, and 2.3–2.7 mm dorsal to the dorsal end of the Rhinal fissure. They were also filled with Biocytin and stained for morphological examination. All recorded pyramidal neurons displayed a regular firing pattern in response to current steps. They also displayed typical morphological features such as spiny apical and basal dendrites, pyramidal somas, and axonal projections to deep layers (Fig. S1*B*).

Measuring Synaptic Properties. We measured miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) in the presence of 1 μM tetrodotoxin (TTX) and appropriate synaptic blockers (100 μM picrotoxin, or 20 μM DNQX and 100 μM D-AP5, respectively). mEPSCs were measured with K-gluconate–based internal solution at −75 mV, and mIPSCs were recorded with CsCl-based solution at −60 mV. Both mEPSCs and mIPSCs were completely abolished by their corresponding antagonists (20 μM DNQX, $n = 4$, and 100 μM picrotoxin, $n = 3$). We did not observe any differences in serial resistance (SR) or root mean square (rms) noise of the sealed pipette between the naïve and trauma groups in low-CF mEPSCs (SR, naïve, 24.8 ± 1.44: trauma, 22.4 ± 1.10, $P = 0.27$; rms, naïve, 1.15 ± 0.04: trauma, 1.04 ± 0.05, $P = 0.09$), high-CF mEPSCs (SR, naïve, 22.9 ± 1.2: trauma, 23.3 ± 1.38, $P = 0.83$; rms, naïve, 1.07 ± 0.05: trauma, 1.09 ± 0.06, $P = 0.77$), low-CF mIPSCs (SR, naïve, 23.4 ± 1.18: trauma, 23.0 ± 0.96, $P = 0.78$; rms, naïve, 1.28 ± 0.05: trauma, 1.35 ± 0.03, $P = 0.25$) and high-CF mIPSCs (SR, naïve, 24.3 ± 1.19: trauma, 25.3 ± 1.77, $P = 0.62$; rms, naïve, 1.27 ± 0.05: trauma, 1.25 ± 0.05, $P = 0.86$).

To measure spontaneous inhibitory postsynaptic currents (sIPSCs) and tonic $GABA_A$ receptor-mediated currents, we replaced external TTX with 5 mM QX-314 in the internal solution. After stable baseline recording, 100 μM gabazine was applied, and the resulting outward shift of baseline current was measured as the magnitude of tonic inhibition. No differences were observed in SR and rms between the naïve and trauma groups, both in low-CF sIPSCs (SR, naïve, 17.9 ± 0.94: trauma, 17.7 ± 0.89, $P = 0.86$; rms, naïve, 1.50 ± 0.08: trauma, 1.66 ± 0.07, $P = 0.15$) and high-CF sIPSCs (SR, naïve, 17.9 ± 0.94: trauma, 19.1 ± 0.87, $P = 0.69$; rms, naïve, 1.53 ± 0.07: trauma, 1.56 ± 0.06, $P = 0.74$).

Immunohistochemistry. AI slices were fixed overnight at 4 °C in 4% paraformaldehyde in 120 mM phosphate buffer, pH 7.4, equilibrated in 30% sucrose in PBS and embedded in Tissue-Tek (Sankura Finetek). Frozen sections (16 μm) were air-dried onto positively charged glass slides. After air drying, sections were washed in PBS with 0.2% Tween-20 (PBTw) and incubated for 3 h in a solution of 10% normal goat serum in PBTw (PTN). The following antibodies were used as primary antibodies: Mouse monoclonal antibody against NeuN (Millipore) was used at 1:1,000; rabbit polyclonal antibody against GAD_{65} (Santa Cruz) was used at 1:1,000. Sections were incubated overnight at 4 °C with the primary antibodies, followed by incubation with corresponding secondary antibodies conjugated with Alexa Fluor 488 or Alexa Fluor 568 for 3 h at room temperature. After rinsing with PBS, the sections were mounted with Fluoromount-G (Southern Biotech) and viewed under a laser confocal microscope (Carl Zeiss). Unbiased stereological principles were followed to quantify cell and puncta densities. Confocal images were systematically and randomly sampled in three dimensions from the primary auditor cortex. Images were smoothed with a median filter and thresholded to remove background pixels. The filter parameters and the threshold value were fixed between all groups for each experimental condition. A computer program was then used to identify and count the number of pixel clusters in the entire image area to obtain the area density, which is reported.

Western Blotting. The isolated AI tissues were prepared by homogenization in lysis buffer containing 50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40 and protease inhibitor mixture (Roche). After gentle rotation for 3 h at 4 °C, homogenates were centrifuged at 14,000 × *g* for 60 min at 4 °C and the supernatants were collected. Protein lysate (100 μg) was dissolved in SDS sample buffer containing 5% β-mercaptoethanol and heated to 95 °C for 5 min. Equal amount of proteins were loaded on 10% SDS/PAGE and transferred onto nitrocellulose membranes. Membranes were blocked in 1× Western Blocking Reagent (Roche) in PTN for 1 h at 4 °C and incubated with 1:500 dilution of the anti-GAD65 or anti-α-tubulin primary antibody in PTN overnight at 4 °C. Afterward, the blots were washed with PTN and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit or anti-mouse (1:5,000) (Jackson ImmunoResearch) in PTN for 2 h at 4 °C. Immunoreactive bands were observed by using an enhanced chemiluminiscence system. GAD65 band intensities were normalized to the band intensities of α-tubulin, which was used as a loading control.

Statistics. Statistical differences were determined using paired Student's *t* test for behavioral data, and Tukey ANOVA test for electrophysiological data; the significance level was set at 5%. Corrections are made for multiple comparisons. Data were presented as mean ± SEM.

Chemicals. Concentrated stock solutions of pharmacological agents were prepared and diluted in ACSF to a final concentration before use. TTX (1 μM), QX314 (sodium channel blocker; 1 mM), DNQX (AMPA receptor blocker; 20 μM), CGP 55845 ($GABA_B$ blocker; 10 μM), SR 95531 ($GABA_A$ blocker; 100 μM), Vigabatrin (an inhibitor of GABA transminase; 150 mg/kg), and NO711 (a GABA uptake blocker; 3 mg/kg) were purchased from Tocris; picrotoxin (a blocker for $GABA_A$ receptor; 200 μM) and THIP hydrochloride (4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride; 1 μM) purchased from Sigma. These drugs were bath applied in final concentrations.

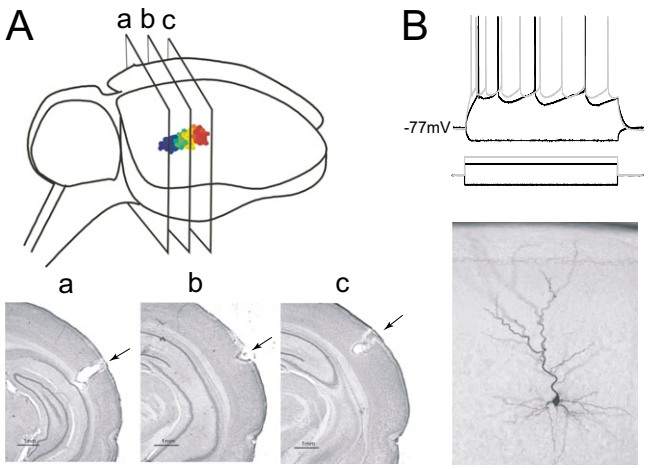

Fig. S1. Auditory cortical slice preparation and recording. Related to Fig. 2. (*A*) Schematic of slice preparation from high- and low-CF areas of the primary auditory cortex (A1). Lesion marks (as indicated by the arrows) were made after dense acute cortical mapping to identify high-, middle- and low-CF areas of A1. They can be consistently identified by nearby landmarks of the hippocampal and thalamic structures. (*B*) Cortical pyramidal neurons were recorded. They were first identified by their regular firing pattern, filled with Biocytin, and later verified by their morphology.

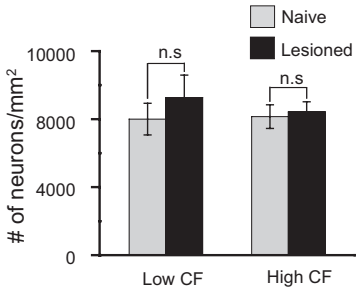

Fig. S2. Density of auditory cortical neurons labeled with NeuN green was not altered by hearing lesion. Related to Fig. 5. n.s., not significant.