Immediate manifestation of acoustic trauma in the auditory cortex is layer specific and cell type dependent

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Submitted 18 August 2015; accepted in final form 28 January 2016

Novák O, Zelenka O, Hromádka T, Syka J. Immediate manifestation of acoustic trauma in the auditory cortex is layer specific and cell type dependent. J Neurophysiol 115: 1860–1874, 2016. First published January 28, 2016; doi:10.1152/jn.00810.2015.—Exposure to loud sounds damages the auditory periphery and induces maladaptive changes in central parts of the auditory system. Diminished peripheral afferentation and altered inhibition influence the processing of sounds in the auditory cortex. It is unclear, however, which types of inhibitory interneurons are affected by acoustic trauma. Here we used single-unit electrophysiological recording and two-photon calcium imaging in anesthetized mice to evaluate the effects of acute acoustic trauma (125 dB SPL, white noise, 5 min) on the response properties of neurons in the core auditory cortex. Electrophysiological measurements suggested the selective impact of acoustic trauma on inhibitory interneurons in the auditory cortex. To further investigate which interneuronal types were affected, we used two-photon calcium imaging to record the activity of neurons in cortical layers 2/3 and 4, specifically focusing on parvalbumin-positive (PV+) and somatostatin-positive (SST+) interneurons. Spontaneous and pure-tone-evoked firing rates of SST+ interneurons increased in layer 4 immediately after acoustic trauma and remained almost unchanged in layer 2/3. Furthermore, PV+ interneurons with high best frequencies increased their evoked-to-spontaneous firing rate ratios only in layer 2/3 and did not change in layer 4. Finally, acoustic trauma unmasked low-frequency excitatory inputs only in layer 2/3. Our results demonstrate layer-specific changes in the activity of auditory cortical inhibitory interneurons within minutes after acoustic trauma.

ACUTE ACOUSTIC TRAUMA (AAT) results in complex alterations of central auditory processing that do not simply reflect the traumatically diminished peripheral input. Central alterations may contribute to the development of tinnitus (Roberts et al. 2010), a clinical condition with unclear pathogenesis and no available effective treatments.

In the primary auditory cortex (AC), exposure to loud sound is immediately followed by increased stimulus-driven activity, synchrony, and bursting, accompanied by complex changes of the receptive fields (RFs) usually associated with downward shifts of characteristic frequencies (Norena et al. 2003; Syka et al. 1994). RF modifications can be explained by unmasking latent inputs that are normally suppressed by feedforward lateral inhibition (Eggermont and Roberts 2004). Acoustic trauma can, however, increase and decrease inhibition in distinct parts of the RFs, which cannot be explained by unmasking alone (Scholl and Wehr 2008). Changes in inhibition also follow partial deafferentation caused by exposure to ototoxic drugs or by aging (Llano et al. 2012). Both of these conditions can lead to the onset of tinnitus, which suggests that maladaptive inhibitory plasticity might be a shared mechanism in the development of the condition (Wang et al. 2011). A homeostatic increase of gain compensating for the diminished input has been proposed as a common purpose of such plasticity (Norena 2011; Yang et al. 2011).

Inhibitory microcircuits in the auditory cortex are involved in shaping both temporal and spectral selectivity and intensity tuning (Zhang et al. 2011). Recent studies have shown specific computational functions and target specificity for two major inhibitory interneuron subclasses in the brain, parvalbumin (PV)- and somatostatin (SST)-positive neurons (Kepecs and Fishell 2014). PV cells participate in gain modulation across sensory systems (Atallah et al. 2012; Hamilton et al. 2013; Kato et al. 2013) and influence feature selectivity and perceptual discrimination in the visual cortex (Lee et al. 2012). SST+ cells suppress the activity of PV+ cells (Cottam et al. 2013), and their input synapses show unique short-term facilitation (Beierlein et al. 2003). In the auditory cortex, PV interneurons seem to be involved mainly in providing broadly tuned feedforward inhibition and SST neurons probably mediate more narrowly tuned feedback inhibition (Li et al. 2014). Because of these functional differences it can be expected that PV+ and SST+ neurons will be differently affected by the AAT. However, the extent to which the different classes of cortical interneurons are affected by acoustic trauma is unknown.

Various types of cortical neurons are organized into specific circuits positioned across the cortical layers. Cortical interneurons likely serve layer-dependent distinct functions (Li et al. 2014). Their input/output patterns often differ substantially (Atencio and Schreiner 2010; Liu et al. 2007) as well as the amount of direct thalamocortical afferentation they receive (Beierlein et al. 2003). PV+ neurons receive direct thalamic input (Moore and Wehr 2013); however, at least in the AC, there is currently no evidence for the direct thalamic input to SST+ neurons. Both classes of interneurons are innervated by axons of cortical pyramidal cells (Harris and Shepherd 2015). PV+ and SST+ neurons synapse mainly on excitatory cells, but with different subcellular localization, onto soma and onto dendrites, respectively. PV+ neurons also inhibit other PV+ neurons (Pfeffer et al. 2013), and SST+ neurons synapse on PV+ cells in layer (L)2/3 of the visual cortex (Cottam et al. 2013; Pfeffer et al. 2013). PV+ and SST+ neurons constitute a large portion of all GABAergic interneurons in the AC. In the rat AC PV+ and SST+ interneurons constitute 30 ± 4% and 25 ± 2%, respectively, of interneurons in L2/3 and 66 ± 5%...
and 27 ± 2% in L4 (Ouellet and de Villers-Sidani 2014). The functional architecture of the AC thus implies different patterns of noise-induced changes across cortical layers.

Here we evaluated the impact of AAT on the activity of different neuronal populations across the upper layers of the AC. Using single-unit recordings and calcium imaging in vivo we were able to determine the subtype- and layer-specific effects of AAT on the sensory processing of sounds in the AC.

MATERIALS AND METHODS

Experimental Animals

For electrophysiological experiments and measurements of auditory brain stem responses (ABRs) we used C57BL/6J mice (n = 25, Jackson Laboratory, stock no. 000664). To selectively label interneurons for imaging experiments in vivo we crossed tdTomato reporter mice (Jackson Laboratory, stock no. 007908) with mice expressing cre-recombinase under specific promoters characteristic for given classes of interneurons. PV-Cre/tdT mice (n = 25) with selectively labeled PV+ interneurons were generated by crossing Pvalb-IRES-Cre mice (Jackson Laboratory, stock no. 00869) with tdTomato mice. Analogously, SST-Cre/tdT mice (n = 30) with selectively labeled SST+ interneurons were generated by crossing Sst-IRES-Cre mice (Jackson Laboratory, stock no. 013044) with tdTomato mice. We used young adult (6–12 wk, 25–35 g) male and female mice: 25 PV-Cre/tdT mice and 30 SST-Cre/tdT mice in total.

Surgical Procedures

All experimental procedures were approved by the Ethics Committee of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, and followed the guidelines of the Declaration of Helsinki.

Anesthesia was induced with 2% isoflurane with oxygen, and animals were then injected subcutaneously with a mixture of ketamine (35 mg/kg) and xylazine (6 mg/kg). The animal was placed onto a heated pad (38°C), and its eyes were covered with eye-protective ointment. To facilitate breathing and prevent endotracheal secretion in the animal, atropine (1 mg/kg) was injected subcutaneously. Throughout the entire experiment, pure oxygen was delivered via a custom-made gas mask (Smith and Bolon 2006) to maintain high oxygen saturation (>95%), which was continuously monitored with a pulse oximeter (mouseSTAT, Kent Scientific). To prevent brain swelling, dexamethasone (2.5 mg/kg) was injected subcutaneously.

Lidocaine (2%, 40 µl) was injected subcutaneously over the parietal area, skin was removed, and a custom-made head holder was attached to the skull with cyanoacrylate glue (UHU Super Glue). For electrophysiological experiments, the temporal muscle was removed and a craniotomy (~4-mm diameter) and durotomy were performed over the right AC. For imaging experiments, after removal of the temporal muscle a plastic protective well was attached to the right temporal bone with cyanoacrylate glue and a small craniotomy (~1.5- to 2-mm diameter) was performed over the right AC.

Auditory Brain Stem Response and Distortion Product Otoacoustic Emission Measurement

ABR and distortion product otoacoustic emission recordings. Sound-evoked ABRs were recorded before AAT, immediately after AAT, and 24 h, 2 days, 7 days, and 14 days after AAT in an anechoic chamber. The animal was placed onto a heated pad (38°C) in the middle of the chamber, and its head was fixed. Three stainless steel needlelike recording electrodes were placed subcutaneously. The positive recording electrode was placed over the interparietal and occipital bones (vertex), and the negative and grounding electrodes were placed at the neck muscles. A TDT (Tucker Davis Technologies) System III setup was used for acoustic stimulation and signal acquisition. The data were amplified and digitized with a Medusa RA16PA preamplifier and RA4LI headstage (A/D sample rate 25 kHz). The digitized signal was sent to a Pentusa RX5-2 base station via optic fibers and processed further in a computer. Free-field tone stimuli were generated in the TDT system, amplified with a custom-made amplifier, and presented from a two-way loudspeaker system (Jamo woofer and SEAS T25CF 002 tweeter with smooth responses; ±5 dB). Speakers were placed 70 cm in front of the animal’s head. AAT was induced by loud noise generated by a custom-made broadband noise (BBN) source, amplified with a Transiwatt 140P amplifier, and presented with a piezoelectric speaker (Motorola KSN1025A, ±5 dB in the frequency range 2–33 kHz; Fig. 1A), which was positioned above the animal’s head. The acoustic system was calibrated with an Brüel & Kjær microphone, a ZC0020 preamplifier, and a Brüel & Kjær 2231 Sound Level Meter.

In distortion product otoacoustic emission (DPOAE) measurement, stimuli were presented to the ear canal with two custom-made piezoelectric stimulators connected to the probe with a low-noise microphone system (Etymotic probe ER-10B+, Etymotic Research). The signal from the microphone was analyzed with TDT System III (RP2 processor, sampling rate 100 kHz). DPOAEs were successively recorded in both ears of each animal.

Acoustic stimulation. Baseline ABR measurement was followed by a 5-min period of intense BBN stimulation (125 dB SPL), followed by successive ABR measurements. During ABR measurements, the animals were stimulated with tone bursts (frequency 2–40 kHz, duration 5 ms with 2 ms rise-fall times) of decreasing intensity (10-dB

Fig. 1. Assessment of peripheral auditory changes induced by acute acoustic trauma (AAT). A: frequency spectrum of the traumatic broadband noise. B and C: auditory brain stem response (ABR) thresholds (B) and distortion product otoacoustic emission (DPOAE; C) measured before the AAT and immediately (0 h), 24 h, 2 days, 7 days, and 14 days after the AAT (n = 4 mice).
Single-Unit Electrophysiology

**Data analysis**. The signal was band-pass filtered (300-3,000 Hz) and averaged with TDT BioSig software. The hearing threshold at each frequency was determined as the lowest intensity at which the ABR signal was clearly distinguishable from noise. In some mice we did not observe brain stem responses after AAT at the highest frequency (40 kHz). Even at 100 dB SPL there was no signal that could be distinguished from noise. Therefore, we did not include the values for 40 kHz in further analysis.

### Single-Unit Electrophysiology

**Extracellular single-unit recordings**. Electrophysiological recordings were conducted in an anechoic chamber. Sound-evoked single-unit responses were recorded in the right AC of anesthetized mice. The location of the AC core was estimated from the position of the best frequency (BF) gradient (Stiebler et al. 1997); response latencies and responsiveness to tonal stimuli were obtained from coarse mapping performed at the beginning of a recording session. Neural activity was recorded from the middle layers (200–400 μm below pia) with 4 × 1 iridium tetrodes (150-μm intertetrode distances, 11-μm² contact area, A4x1-tet-3mm-150-121, NeuroNexus Technologies).

The signal was amplified and digitized (sampling rate 25 kHz) by a Medusa RA16PA preamplifier and band-pass filtered (300-6,000 Hz) with Pentusa RX5-2 (TDT). Spikes from the raw recorded data traces were detected with thresholding (>4 SD of signal mean) and clustered with KlustaKwik (Harris et al. 2001) based on five spike waveform parameters (energy, peak, time, valley, and first principal component). Only those clusters that remained well separated throughout the entire experiment (BBN pulse stimulation, followed by acoustic trauma period and the second BBN stimulation) were included in the analysis. Single units were further checked manually with MClust (MClust-4.0, A. D. Redish; http://redishlab.neuroscience.umn.edu/MClust/MClust.html), and only clusters with an L ratio <0.5 and isolation distance >12 (Harris et al. 2001; Schmitzer-Torbert et al. 2005) were included in the analysis.

To confirm the recording depth estimates, we made small electrolytic lesions in a subset of mice (n = 4) with a custom-made device (current pulse 0.6 mA for 10 s). Mice were then transcardially perfused with 20 ml of saline followed by 40 ml of 4% paraformaldehyde, the brains were explanted, postfixed in 4% paraformaldehyde for 2–3 days, sliced with a cryostat into 40-μm thick sections, and stained with Nissl stain, and the recording depth was then evaluated by light microscopy (Fig. 2H).

**Acoustic stimulation and noise exposure**. The stimuli consisted of 100-ms BBN bursts (100 pulses, 65 dB SPL, 1,500-ms interstimulus interval). The AAT was induced with white noise (5-min duration, 125 dB SPL) delivered via a calibrated piezoelectric speaker (Motorola KSN1025A) positioned next to the animal’s head. The acoustic system was calibrated with a B&K 4939 microphone, a ZC0020 preamplifier, and a B&K 2231 Sound Level Meter. Data recordings before and after AAT took ~5 min. The after-AAT recordings started within 1 min after the AAT.

**Data analysis**. Clustered data were analyzed with custom scripts written in MATLAB. Spontaneous activity was calculated as the mean firing rate [spikes/second (sp/s)] in a 100-ms window directly preceding stimulus presentation, and evoked activity was calculated as the mean firing rate in a 115-to 200-ms time window following stimulus presentation. The statistical significance of sound-evoked responses was evaluated with the Wilcoxon signed-rank test (P < 0.05). For each neuron we tested, on a trial-by-trial basis, whether the evoked

![Typical spike waveforms](image)

**Fig. 2.** Electrophysiological characterization of neuronal activity before and after the AAT. Example peristimulus time histograms (PSTHs) of neurons displaying persistent responsiveness (A, Persistent), cessation of significant responsiveness (B, Silenced), and emergence of significant responsiveness (C, Unmasked) before (gray areas) and after (colored lines) 5-min loud noise exposure. Arrows indicate where the full width at half-maximum (FWHM) was measured. D–F: changes in spontaneous activity (D), evoked activity (E), and response jitter, calculated as the FWHM (F), were evaluated for the 3 different groups of neurons. G: Silenced neurons had significantly narrower spike waveforms compared with other subgroups of neurons. Right: typical waveforms aligned according to their maxima. H: a coronal brain slice in Nissl staining (described in MATERIALS AND METHODS) showing recording depths ~200–400 μm below pia. *P < 0.05, **P < 0.01, ***P < 0.001. n.s., Nonsignificant.
activity increased significantly compared with the corresponding spontaneous activity. On the basis of significance of their responsiveness, neurons were divided into four groups, those that were significantly responsive both before and after the AAT (Persistent), those that were significantly responsive only before the AAT (Silenced), those that were significantly responsive only after the AAT (Unmasked), and neurons unresponsive under both conditions (unresponsive neurons were not included in further analysis). Response jitter was calculated as full width at half-maximum (FWHM) of a sound-evoked peristimulus time histogram (PSTH) computed in a 115- to 200-ms time window after stimulus onset. Spike width was defined as the time difference between the spike peak (maximum waveform deflection) and spike valley (minimum deflection following the peak) of the mean spike waveform for each neuron.

Two-Photon Calcium Imaging

Experimental layout. Calcium imaging was performed with an Ultima IV two-photon scanning microscope (Prairie Technologies), controlled by PrairieView software, equipped with a Chameleon Ultra II laser (Coherent). Imaging experiments were performed with a ×20 objective (XLUMPLN 20XW, Olympus). The laser wavelength was set to 950 nm for all measurements, and the pulse precompensation was set accordingly. The laser pumping unit was positioned inside a separate custom-made soundproof box that minimized any ambient noise.

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evoked to spontaneous ratio (PT-ESR). Evoked-to-spontaneous ratios were calculated as particular EFR divided by SFR.

Results of statistical tests were corrected for multiple comparisons with Bonferroni correction where necessary. Error bars in figures represent SE unless stated otherwise.

RESULTS

Distinct Effect of Acoustic Trauma on Peripheral Hearing Function

Acoustic trauma affects the entire auditory system. To estimate the extent to which trauma affected the afferentation into the central auditory system we first evaluated the effects of AAT (Fig. 1A) on the auditory periphery. We measured the thresholds of ABR and DPOAE before AAT—a 5-min exposure to 125 dB SPL BBN (Fig. 1, B and C)—and after 0 h, 24 h, 2 days, 7 days, and 14 days in four mice. Hearing thresholds were substantially elevated after AAT, especially at frequencies with the lowest before-AAT thresholds (Fig. 1B). DPOAE practically disappeared immediately after AAT (Fig. 1C). Over the following 14 days DPOAE almost returned to pre-AAT values, but ABR threshold improved only poorly (−10 dB) and remained elevated (~30 dB above pre-AAT values).

Despite the increase in thresholds, however, ABRs were still present, indicating a functional input into the central part of the auditory system.

Neurons Silenced After AAT Had Narrower Spikes

We then evaluated the effect of AAT on spiking activity of single neurons in the AC. We used 4 × 1 iridium tetrodes to record spiking activity of several neurons simultaneously. The intensity of the BBN pulses was set to 65 dB SPL. In contrast to the ABR thresholds, this intensity proved to be sufficient to evoke moderate responses in the AC (see DISCUSSION). Altogether we recorded spiking sound-evoked responses from 93 well-isolated and reliably reidentified neurons (from 20 mice, see MATERIALS AND METHODS). In further analysis we included only neurons whose cluster quality fulfilled defined isolation criteria both before and after the AAT.

Individual neurons displayed different responses to BBN before and after the 5-min noise exposure. Almost half of the neurons (n = 42, 45%) responded significantly both before and after the AAT (Persistent neurons; Fig. 2A), a subset of neurons were silenced after the AAT, i.e., responded significantly only before the AAT (n = 13, 14%) (Silenced neurons; Fig. 2B), while another subset (n = 22, 24%) displayed response significance only after the AAT (Unmasked neurons; Fig. 2C). The rest of the neurons (n = 16, 17%) were not responsive to acoustic stimulation (unresponsive not shown).

Only neurons with persistent responsiveness displayed significantly increased spontaneous activity after the AAT (Fig. 2D; before: 5.14 ± 0.53 sp/s, after: 7.68 ± 0.93 sp/s; Wilcoxon signed-rank test, P < 0.01), while the spontaneous activity of Silenced and Unmasked neurons did not change significantly (Fig. 2D; before: 3.4 ± 1.1 sp/s and 5.7 ± 1.6 sp/s, after: 2.75 ± 0.80 sp/s and 6.97 ± 1.65 sp/s, respectively; Wilcoxon signed-rank test, P > 0.05). The evoked activity of Persistent neurons did not change (Wilcoxon signed-rank test, P > 0.05; Fig. 2E). The Silenced neurons decreased their evoked activity to the level of spontaneous activity, whereas Unmasked neu-

eurons displayed a significant increase in the evoked activity after AAT (Fig. 2E; before: 12.8 ± 1.3 sp/s, 6.7 ± 2.4 sp/s, and 5.5 ± 1.4 sp/s, after: 13.1 ± 1.5 sp/s, 3.07 ± 0.91 sp/s, and 11.2 ± 2.5 sp/s, respectively; Wilcoxon signed-rank test, P > 0.05, P > 0.05, and P < 0.001, respectively). The response jitter, estimated from the FWHM of sound-evoked PSTH, increased in neurons with persistent responsiveness (Fig. 2F; before: 37.2 ± 3.1 ms, after: 52.3 ± 6.2 ms; Wilcoxon signed-rank test, P < 0.01). Note that the assignment of neurons to different groups was based on their responsiveness before and after AAT (in terms of significant change of firing rate before and after the stimulus presentations), not on changes in evoked firing rates compared before and after AAT (see MATERIALS AND METHODS). Overall changes in neuronal activity (Fig. 2, D–F) corresponded with the definition of each group of neurons.

Interestingly, Silenced neurons had significantly narrower spikes than other responsive neurons (Fig. 2G; for the Persistent, Silenced, and Unmasked groups: 0.855 ± 0.020 ms, 0.758 ± 0.030 ms, and 0.835 ± 0.038 ms, respectively; Wilcoxon rank sum test, Bonferroni correction, P < 0.013 for Persistent vs. Silenced, P < 0.013 for Silenced vs. Unmasked, P > 0.013 for Persistent vs. Unmasked), suggesting that neurons that stopped responding after the AAT were mostly fast-sporing interneurons (Beierlein et al. 2000; Moore and Wehr 2013). Electrophysiological identification of interneurons in vivo, however, can be rather ambiguous, and their precise identification requires a complementary method (Lima et al. 2009; Pinault 1996). Therefore, to detect the identity of cortical interneurons possibly affected by AAT, we then turned to calcium imaging in vivo.

Layer- and Class-Dependent Differences in Sound-Evoked Responses After AAT

We used calcium imaging in transgenic mice to measure sound-evoked responses and resolve the identities of the two main types of interneurons positioned across the upper cortical layers of AC (Fig. 3). Neurons in the AC were bulleted up with OGB-1 for calcium imaging. To identify PV+ and SST+ cortical interneurons in vivo, we used transgenic PV-Cre/tdT and SST-Cre/tdT mice (see MATERIALS AND METHODS), in which the respective subclasses of interneurons expressed tdTomato red fluorescent protein. Individual neurons loaded with OGB-1 were unambiguously reidentified after the AAT, and interneurons expressing the red fluorescent protein (PV+, SST+) were clearly distinguishable from the rest of the population (Fig. 3, B and C). Note that labeled interneurons were also loaded with OGB-1 and calcium activity was recorded in the green channel, whereas the red channel was used for interneuron identification only. In each field of view the green-only population of neurons (PV, SST) consisted mainly of pyramidal neurons. For example, any PV population of cells in our FOVs—a complement population to the labeled PV+ cells—would contain ~83% pyramidal neurons, ~8% SST interneurons, and ~9% other, less frequent types of interneurons (Pfeffer et al. 2013; Rudy et al. 2011).
Tone-Responsive Neurons Shifted Their Best Frequencies After AAT

Neurons in the AC lowered their BFs after the AAT (Fig. 4). Across the whole population (n = 766 neurons), the mean BF shift was −0.65 oct (BF before 11.20 ± 0.13 kHz, BF after 8.6 ± 0.14 kHz; mean ± SE, P < 0.001, Wilcoxon signed-rank test). BF shifts induced by acoustic trauma were present in both L2/3 and L4. The mean BF shift in L2/3 (n = 406 neurons; Fig. 4A) was −0.66 oct (BF before 11.15 ± 0.16 kHz, BF after 8.52 ± 0.16 kHz; mean ± SE, P < 0.001, Wilcoxon signed-rank test). In L4, the mean BF shift across all neurons (n = 360; Fig. 4B) was −0.64 oct (BF before 11.25 ± 0.20 kHz, BF after 8.71 ± 0.22 kHz; mean ± SE, P < 0.001, Wilcoxon signed-rank test). The mean BF shifts of interneurons in both L2/3 and L4 (Fig. 4) differed neither from the mean BF shifts in respective layers nor from each other (P > 0.05).

Interestingly, neurons with the lowest BFs (BFs < 4 kHz) shifted their BFs toward higher frequencies in L2/3 (P < 0.01, Wilcoxon signed-rank test; Fig. 4A, right) but not in L4 (P > 0.05, Wilcoxon signed-rank test; Fig. 4B, right). BFs > 4 kHz shifted toward lower frequencies in both L2/3 and L4 (P < 0.001, Wilcoxon signed-rank test).

Acoustic Trauma Increased Tone-Evoked Responses Below BF

AAT also induced changes in the frequency tuning of individual neurons in the AC (Fig. 5). Individual neurons displayed pronounced disinhibition bands on the flanks of their V-shaped RFs after the AAT. Three example RFs are shown in Fig. 5A. Besides an increase in the response threshold around the BF, neurons in L2/3 typically increased their responses at the low-frequency side of their RF (Fig. 5A, left). In some L2/3 neurons responses increased at the high-frequency side of the RF (Fig. 5A, center). On the other hand, neurons in L4 mostly displayed smaller RFs after the AAT (Fig. 5A, right). These changes induced by the AAT were also apparent across neuronal populations in L2/3 and L4 (Fig. 5, B and C), mainly at and below the pre-AAT BF.

Neurons in L2/3 and L4 were affected differently by the AAT (Fig. 5B). The differential curve was narrower in L4; on the other hand, L2/3 neurons showed more pronounced disinhibition at lower frequencies. Consistent with the dominant BF shifts, responses to lower frequencies (−2−2.5 oct below the BF) increased in L2/3 neurons (+10 ± 2%, averaged across intensities; Fig. 5C, left). All types of neurons decreased their responses around pre-AAT BF (Fig. 5C; Wilcoxon signed-rank test, P < 0.0014). Furthermore, we divided neurons within
both layers into three groups according to their original BFs (Fig. 6). The AAT had the smallest impact on responses of low-frequency neurons in L2/3 and L4. Responses of medium- and high-frequency neurons, however, decreased overall after the AAT in both L2/3 and L4.

Only medium- and high-frequency neurons in L2/3 consistently displayed increased tone-evoked responses below BF. The greatest overall increase was present in L2/3 high-frequency neurons (9%, averaged across the used intensities).

**Distinct Groups of AC Inhibitory Interneurons Were Differentially Affected by AAT**

Finally, we evaluated changes in five different parameters of neuronal activity after the AAT (see Materials and Methods): SFR, PT-EFR, BBN-EFR, PT-ESR, and BBN-ESR.

To compare changes in the activity of PV+ and SST+ neurons among different mice we used PV− and SST− (td-Tomato negative) neuronal populations as an internal control in each mouse. We divided our data according to layer and BF. The main significant results are shown in Fig. 7 and further in the tables.

SFRs increased significantly in SST+ neurons (P < 0.05; Table 1). Across all frequencies SFRs increased significantly only in L4 (P < 0.01) and but not in L2/3 (Fig. 7A, Table 1). With respect to the three frequency bands, the increase in SFR was significant only for SST+ neurons with low- and mid-frequency BFs (Table 2). SFRs of PV+ neurons did not change significantly after the AAT.

Along with SFRs SST+ neurons increased their PT-EFRs in L4 (P < 0.01; Fig. 7B, Table 3). Interestingly, BBN-EFR of SST+ neurons did not change (Table 4). Evoked firing rates of PV+ neurons remained unchanged (Fig. 7B, Table 3). The only changes in PV+ neurons were observed in PT-ESR and BBN-ESR of high-frequency neurons (P < 0.05; Fig. 7, C and D, Tables 5 and 6).

**DISCUSSION**

**Results Overview**

We studied the effect of acute AAT on the activity of defined neuronal populations in the mouse AC. Using single-unit electrophysiological recordings we demonstrated that cortical neurons that were silenced after the AAT had significantly narrower spikes than other sound-responsive neurons. Tone-responsive neurons in the AC lowered their BFs after the AAT, which was caused both by partial peripheral deafferentation in the vicinity of the pre-AAT BF and disinhibition at lower frequencies. Our main results include the observed rise in SFR and EFR of SST+ interneurons in L4 at lower and middle frequencies and a rise in ESR of PV+ interneurons in L2/3 on higher frequencies. Our results suggest a selective layer-dependent impact of the AAT on the activity of cortical interneurons.
Calcium Imaging In Vivo

In vivo two-photon calcium imaging is a widely used technique for simultaneously recording the activity of many neurons (Denk et al. 1990; Huber et al. 2012; Issa et al. 2014; Svoboda and Yasuda 2006). Here we used OGB-1, a calcium indicator characterized by relatively bright basal fluorescence and high signal-to-noise ratio. Thanks to its low Ca\(^{2+}\) dissociation constant, \(K_d \sim 170–180\) nM (Gersbach et al. 2009), OGB-1 reliably detects single action potentials (Bandyopadhyay et al. 2010). We used a Two-Photon Processor (Tomek et al. 2013), a freely available software package, to process data from two-photon calcium imaging and estimate positions and numbers of action potentials. The software uses the peeling algorithm (Grewe et al. 2010) to detect action potential-related events. Using the optimal set of internal parameters of the used algorithm, we reduced the risk of data contamination with subthreshold-event-related calcium fluxes (Bandyopadhyay et al. 2010). To minimize any possible contamination of experimental data by signal from neuropil (Lutcke and Helmchen 2011) we used a high-numerical aperture objective (NA \( \sim 0.95\)) whose back aperture was overfilled with the laser beam. Therefore, the contamination from neuropil should be generally low, <10% in neurons sectioned in the middle (Gobel and Helmchen 2007).

Impact of Acoustic Trauma on Auditory Processing

Most previous studies used loud pure tones as a traumatic stimulus (Kimura and Eggermont 1999; Norena et al. 2003;

Fig. 5. Frequency tuning changes in the AC after AAT. A: example receptive fields (RFs) before and after AAT: disinhibition at lower frequencies (left), disinhibition at higher frequencies (center), and no clear disinhibition (right). RFs became smaller in all 3 cases. B: mean difference in tuning curves before and after the AAT for L2/3 and L4 neurons. C: average pre-AAT tuning curves (blue), post-AAT tuning curves (dashed red), and differential curves (green) for both layers and 3 classes of neurons; Bonferroni correction for multiple comparisons. *\(P < 0.0014\), Wilcoxon rank sum test (B) or Wilcoxon signed-rank test (C).
Loud pure-tone stimulation damages regions of the cochlea that respond to frequencies equal or higher than that of the traumatic frequency (Ruegger et al. 2013). We chose 125-dB BBN as it corresponds better to blunt real-world traumatic acoustic exposures than a pure-tone-induced trauma. BBN was used only in a few studies, and in none of them was the activity in the AC recorded with a single-cell resolution (Groeschel et al. 2011; Kim et al. 2007; Syka et al. 1994; Syka and Rybalko 2000).

Fig. 6. Changes in frequency tuning of AC neurons after the AAT with respect to their BFs. Average pre-AAT tuning curves (blue), post-AAT tuning curves (dashed red), and differential curves (green) across all neurons are shown for L2/3 and L4; Bonferroni correction for multiple comparisons. *P < 0.0014, Wilcoxon signed-rank test.

Fig. 7. Changes in activity parameters of AC neurons after AAT. A: changes in spontaneous firing rate (SFR) of PV−, PV+, SST−, and SST+ neurons in L4. B: changes in pure-tone-evoked firing rate (PT-EFR) of PV−, PV+, SST−, and SST+ neurons in L4. C: changes in broadband noise evoked-to-spontaneous firing rate (BBN-ESR) of PV−, PV+, SST−, and SST+ neurons in L2/3. D: changes in pure-tone evoked-to-spontaneous ratio (PT-ESR) of PV−, PV+, SST−, and SST+ neurons in L2/3. Two-tailed two-sample permutation test, Bonferroni correction for multiple comparisons; n = 2 for A and B and n = 6 for C and D. *P < 0.05, **P < 0.01.
We performed our study in mice under ketamine-xylazine anesthesia. The anesthesia could influence excitability and the presumed central effects of the AAT, as such overstimulation and possible accompanying plasticity. Ketamine itself has a rather complex influence on the spontaneous and evoked activity in the auditory thalamus and cortex (Zurita et al. 1994); however, administration of a mixture of ketamine and xylazine decreases both spontaneous and evoked activity (Kisley and Gerstein 1999; Syka et al. 2005). Therefore, anesthesia during the AAT should have a rather protective influence on neuronal activity. We kept the level of anesthesia constant during the experiment, and thus the effects of the AAT described in our study were recorded under comparable anesthetic conditions.

We observed broad ABR threshold shifts, mostly manifested at higher frequencies (Fig. 1B). The magnitude of the ABR threshold shift with a maximum of ~40 dB at 8–16 kHz was comparable to previously published results (Komiya and Eggert 2000; Norena et al. 2003). DPOAE practically disappeared immediately after the AAT. During the following 14 days DPOAE almost returned to pre-AAT values, but the ABR threshold improved only slightly (~10 dB) and remained elevated (~30 dB above the pre-AAT values). Although the noise intensity we used for AAT induction was similar to intensities used in other studies, we used a much shorter noise intensity we used for AAT induction was similar to 30 dB above the pre-AAT values). Although the noise intensity we used for AAT induction was similar to intensities used in other studies, we used a much shorter traumatic duration. Most other studies used traumatic exposure lasting 1 h or more (Norena and Eggert 2003; Sun et al. 2008; Tan et al. 2007). All components of the auditory periphery could be affected by the AAT during the short period in which we recorded data from the AC. The peripheral deterioration is most likely a mix of the temporary and persistent effects of acoustic trauma. The observed persistence of ABR threshold elevation and the recovery of DPOAE suggest that our acoustic trauma protocol also caused some primary degeneration with spared outer hair cells (Kujawa and Liberman 2009).

Interestingly, the elevated peripheral threshold exceeds the thresholds of neurons in the AC in our data. This is probably caused by the fact that ABR measurement generally shows lower sensitivity compared with, e.g., behavioral estimation of the thresholds. ABR measurements tend to overestimate the hearing threshold by up to 20 dB SPL, especially at lower frequencies (Stapells and Oates 1997; Werner et al. 1993).

Heterogeneous reactions of cortical neurons after acoustic trauma have been consistently reported (Norena et al. 2010). Together with altered thalamocortical afferentation such effect of AAT on cortical neurons is probably also a consequence of altered interactions between different inhibitory interneuron subtypes and excitatory neurons as proposed previously (Norena and Eggert 2003), which is further supported by our data showing layer dependency of the AAT in the AC. Increased spontaneous and evoked activities in the Persistent group of our electrophysiological data (Fig. 2) may be caused by diminished tonic and phasic perisomatic inhibition, respectively, provided by PV+ cells (Hu et al. 2014). In vivo cortical whole cell recordings revealed no change in SFR up to 1 h after the AAT (Scholl and Wehr 2008). The discrepancy may be caused by the fact that the authors recorded neuronal activity across most cortical layers (135–770 μm deep). To the best of our knowledge, changes in spike-timing precision after AAT have not been addressed previously. The decreased spike-timing precision and prolonged firing may reflect altered PV neuron responses (Hu et al. 2014) or may be a manifestation of changes in subcortical processing (Eggermont and Roberts 2004). Spike widths for the Silenced neuronal subgroup (pu-

### Table 1. AAT-induced changes in SFR, PT-EFR, BBN-EFR, PT-ESR, and BBN-ESR of PV− and SST+ neurons compared with PV− and SST− neurons

<table>
<thead>
<tr>
<th></th>
<th>SFR5/SFR0 Count</th>
<th>PT-EFR</th>
<th>BBN-EFR</th>
<th>PT-ESR</th>
<th>BBN-ESR</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2/3</td>
<td>1.12 1.17**</td>
<td>0.94</td>
<td>9.99**</td>
<td>0.64</td>
<td>9.99**</td>
</tr>
<tr>
<td>L4</td>
<td>1.12 1.17**</td>
<td>1.02</td>
<td>11.3**</td>
<td>0.98</td>
<td>1.84***</td>
</tr>
<tr>
<td>L2/3/4</td>
<td>1.12 1.17**</td>
<td>1.15**</td>
<td>0.99**</td>
<td>0.99</td>
<td>1.24***</td>
</tr>
</tbody>
</table>

Values are AAT-induced changes in SFR of PV− and SST+ neurons compared with PV− and SST− neurons, respectively. Neurons were classified according to their best frequencies. Significant differences: "**P < 0.01, ***P < 0.001, ns, Nonsignificant.

### Table 2. AAT-induced changes in SFR of PV+ and SST+ neurons compared with PV− and SST− neurons

<table>
<thead>
<tr>
<th></th>
<th>Low Frequencies</th>
<th>Middle Frequencies</th>
<th>High Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFR5/SFR0 Count</td>
<td>SFR5/SFR0 Count</td>
<td>SFR5/SFR0 Count</td>
<td>SFR5/SFR0 Count</td>
</tr>
<tr>
<td>L2/3</td>
<td>1.16 1.00**</td>
<td>0.96   1.13**</td>
<td>1.16 2.33**</td>
</tr>
<tr>
<td>n</td>
<td>67</td>
<td>32</td>
<td>105</td>
</tr>
<tr>
<td>L4</td>
<td>1.41 1.39**</td>
<td>0.94   1.87**</td>
<td>1.03 1.43**</td>
</tr>
<tr>
<td>n</td>
<td>28</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>L2/3/4</td>
<td>1.26 1.26**</td>
<td>0.97   1.50**</td>
<td>0.99 1.84***</td>
</tr>
<tr>
<td>n</td>
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<td>65</td>
<td>122</td>
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</tbody>
</table>
Table 3.  AAT-induced changes in PT-EFR of PV+ and SST+ neurons compared with PV− and SST− neurons

<table>
<thead>
<tr>
<th>Activity After AAT</th>
<th>PV−</th>
<th>PV+</th>
<th>SST−</th>
<th>SST+</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFR5/EFR0 Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2/3</td>
<td>1.15</td>
<td>0.94ns</td>
<td>0.66</td>
<td>0.89*</td>
</tr>
<tr>
<td>n</td>
<td>92</td>
<td></td>
<td>9</td>
<td>64</td>
</tr>
<tr>
<td>L4</td>
<td>1.17</td>
<td>1.19*</td>
<td>1.14</td>
<td>1.73**</td>
</tr>
<tr>
<td>n</td>
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<td>4</td>
<td>32</td>
</tr>
<tr>
<td>L2/3</td>
<td>1.15</td>
<td>1.10ns</td>
<td>0.90</td>
<td>1.31*</td>
</tr>
<tr>
<td>n</td>
<td>92</td>
<td></td>
<td>9</td>
<td>64</td>
</tr>
</tbody>
</table>

Values are AAT-induced changes in PT-EFR of PV+ and SST+ neurons compared with PV− and SST− neurons respectively. Neurons were classified according to their best frequencies. Significant differences: *P < 0.05, **P < 0.01, ***P < 0.001. ns, Nonsignificant.

distinct classes of interneurons might be related to previously published frequency-dependent increase/decrease of inhibition after AAT (Scholl and Wehr 2008).

Possible Mechanisms Underlying Changes in Neuronal Activity After AAT

AAT generally impairs all levels of the auditory system (Eggermont and Roberts 2004), and its impact should always be considered throughout the whole pathway. Although the auditory periphery is somewhat protected against excessive auditory stimulation (Peterson and Liden 1972; Wen et al. 2009; Zheng et al. 1997, 1999), a traumatic stimulus, such as 125-dB BBN, probably still evokes excessive firing rates across auditory nerve fibers and the activity spreads further within the ascending auditory pathway. However, only firing characteristics of spiral ganglion neurons during the traumatic stimulus were described (Cody and Johnston 1980). The maximum discharge rate of an auditory nerve fiber does not depend on its characteristic frequency (Taberner and Liberman 2005). Within the subcortical part of the central auditory pathway, however, sound stimuli are processed extensively and the RFs of individual neurons are shaped by local inhibitory minar connections between regular-spiking cells in L2/3 (Douglas and Martin 2007; Watkins et al. 2014). This model was previously proposed in Eggermont and Roberts (2004).

Finally, we investigated changes in response parameters of different classes of neurons after the AAT. The most prominent changes were observed in low- and middle-frequency SST+ neurons in L4 and in high-frequency PV+ neurons in L2/3. The difference in the observed impact of the AAT on these two
tative fast-spiking neurons) are similar to widths recorded from optogenetically identified PV+ cells (Moore and Wehr 2013).

Downward shifts of preferred frequencies were repeatedly reported previously (Kimura and Eggermont 1999; Komiyaw and Eggermont 2000; Norena et al. 2003, 2010). At first glance, this might seem to be a sole consequence of the peripheral threshold shift at higher frequencies. However, several studies provided evidence that a decrease of inhibition at lower frequencies is also involved (Norena et al. 2000; Norena and Eggermont 2000). Our data are consistent with these results and extend them further. Using calcium imaging we observed mostly downward shifts of BFs accompanied by disinhibition at a lower-frequency part of the RF (Figs. 4–6). The shifted RFs thus occupied parts of the stimulus space that had not been included in the initial RFs, suggesting that the post-AAT BF shifts are not a simple result of peripheral threshold shifts. All neurons underwent similar BF shifts; the BF shifts of PV+ and SST+ neurons were the same as in the rest of the neuronal population. We observed marked disinhibition in L2/3, especially in high-frequency neurons, but we did not observe any disinhibition in L4. These layer-dependent differences had not been described previously. The observed effect might be related to opposite AAT impact on lower frequencies (Norena et al. 2010) and more prominent intralaminar connections between regular-spiking cells in L2/3 (Douglas and Martin 2007; Watkins et al. 2014). This model was previously proposed in Eggermont and Roberts (2004).

Finally, we investigated changes in response parameters of different classes of neurons after the AAT. The most prominent changes were observed in low- and middle-frequency SST+ neurons in L4 and in high-frequency PV+ neurons in L2/3. The difference in the observed impact of the AAT on these two distinct classes of interneurons might be related to previously published frequency-dependent increase/decrease of inhibition after AAT (Scholl and Wehr 2008).

Table 4.  AAT-induced changes in BBN-EFR of PV+ and SST+ neurons compared with PV− and SST− neurons

<table>
<thead>
<tr>
<th>Activity After AAT</th>
<th>PV−</th>
<th>PV+</th>
<th>SST−</th>
<th>SST+</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFR5/EFR0 Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2/3</td>
<td>0.69</td>
<td>0.85**</td>
<td>0.60</td>
<td>0.57**</td>
</tr>
<tr>
<td>n</td>
<td>46</td>
<td></td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>L4</td>
<td>1.44</td>
<td>0.96**</td>
<td>1.47</td>
<td>1.68**</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td></td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>L2/3</td>
<td>0.93</td>
<td>0.90**</td>
<td>1.02</td>
<td>1.12**</td>
</tr>
<tr>
<td>n</td>
<td>67</td>
<td></td>
<td>8</td>
<td>63</td>
</tr>
</tbody>
</table>

Values are AAT-induced changes in BBN-EFR of PV+ and SST+ neurons compared with PV− and SST− neurons, respectively. Neurons were classified according to their best frequencies. ns, Nonsignificant.
response characteristics of neurons in the AC (Norena et al. 2003, 2010; Norena and Eggermont 2003; Tomita et al. 2004; for review see Eggermont 2015). These studies have demonstrated that changes in responsiveness of the AC neurons after AAT are particularly attributable to alterations in both peripheral afferentation and local cortical inhibition (Norena et al. 2003; Norena and Eggermont 2003; Scholl and Wehr 2008). We have previously described a difference between the central and the peripheral effect of AAT in guinea pigs and rats (Popelar et al. 1987; Syka and Rybalko 2000), and we have also shown that there is a time and dosage dependency influencing the central effect (Popelar et al. 2008; Syka et al. 1994). Interestingly, the alteration in inhibition seems to be more complex, as inhibition can be both decreased and increased with respect to stimulus frequency (Norena et al. 2003; Scholl and Wehr 2008).

Here we focused on the role of PV+ and SST+ cells in changes in cortical activity induced by AAT. Although recent evidence suggests that at least some patterns of canonical microcircuits are shared between different sensory cortices (Kepecs and Fishell 2014), some current data on the role of PV+ and SST+ neurons are available only from the somatosensory or visual cortex and might not be easily extrapolated to the auditory cortex. The two types of interneurons have distinct profiles of postsynaptic partners. SST+ interneurons nonreciprocally inhibit PV+ interneurons, as has been shown in the somatosensory (Xu et al. 2013) and visual (Pfeffer et al. 2013) cortices. During high cortical activity SST+ interneurons inhibit regular spiking neurons as well (Beierlein et al. 2003; Kapfer et al. 2007). PV+ fast-spiking basket cells are involved in feedforward inhibition, notably in regulation of thalamocortical transmission onto L4 regular-spiking cells (Cruikshank et al. 2007; Isaacson and Scanziani 2011). Other substantial differences between SST+ and PV+ interneurons are in short-term plasticity on both input and output synapses. While most cortical synapses desensitize, excitatory synapses onto SST+ neurons strongly facilitate (Beierlein et al. 2003; Reyes et al. 1998). This facilitation is persistent even for longer periods, >10 min (Chen et al. 2009; Lu et al. 2007). Paradoxically, synapses of thalamocortical axons targeting L4 PV+ interneurons, the cells regulating the timing and amount of thalamocortical input (Cruikshank et al. 2007), desensitize most strongly (Beierlein et al. 2003). SST+ neurons probably do not receive any direct thalamic afferentation (Beierlein et al. 2003; Reyes 2011).

Here we hypothesize that SST+ cells could serve as a key component to counteract excessive cortical activity because of their unique features: the supralinearity of their recruitment and facilitation of input synapses from regular-spiking neurons. During the traumatic acoustic stimulus SST+ neurons and their synapses might be affected. Dendrite-targeting inhibitory cells, for example, deteriorate in experimental epilepsy models (Binaschi et al. 2003; Cossart et al. 2001; Silberberg and Markram 2007). We observed higher reactivity (higher SFR and PT-EFR) in low- and middle-frequency SST+ interneurons in L4 after AAT, compared with complementary neurons. These cells could suppress the activity of PV+ cells in similar frequency bands in L4 and might disinhibit the low-frequency part of RF. However, we did not observe any significant change in activity of PV+ neurons in L4, although the absolute evoked PV+ activity in L2/3 was presumably lower at specific frequencies after the AAT (Tables 2–6). On the higher frequencies, the effect was the opposite.

Acoustic trauma is tightly associated with the onset of tinnitus, probably via altered inhibition (Roberts et al. 2010). Our results show that distinct types of inhibitory interneurons are differentially affected by intense noise exposure. Whether the changes in inhibitory subsystems are adaptive and compensatory or caused by detrimental overactivation of the AC during intense noise exposure is unclear. We observed neither immediate cell death nor morphological changes of the investigated interneurons. Our data point at a possible effect of maladaptive plasticity in the following circuit: thalamocortical relay cell → regular-spiking cell in L4 → SST+ cell in L4 →

### Table 5. AAT-induced changes in PT-ESR of PV+ and SST+ neurons compared with PV− and SST− neurons

<table>
<thead>
<tr>
<th></th>
<th>Low Frequencies</th>
<th>Middle Frequencies</th>
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<tr>
<td></td>
<td>ESR5/ESR0</td>
<td>ESR5/ESR0</td>
<td>ESR5/ESR0</td>
</tr>
<tr>
<td></td>
<td>PV− PV+</td>
<td>STT− SST+</td>
<td>PV− PV+</td>
</tr>
<tr>
<td>PV−</td>
<td>0.72 0.88**</td>
<td>0.68 0.49**</td>
<td>0.85 1.15*</td>
</tr>
<tr>
<td>L2/3/4</td>
<td>0.96 0.91**</td>
<td>0.81 0.75**</td>
<td>0.88 1.01*</td>
</tr>
</tbody>
</table>

Values are AAT-induced changes in PT-ESR of PV+ and SST+ neurons compared with PV− and SST− neurons, respectively. Neurons were classified according to their best frequencies. Significant differences: *P < 0.05, **P < 0.01. ns, Nonsignificant.

### Table 6. AAT-induced changes in BBN-ESR of PV+ and SST+ neurons compared with PV− and SST− neurons

<table>
<thead>
<tr>
<th></th>
<th>Low Frequencies</th>
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<td></td>
<td>ESR5/ESR0</td>
<td>ESR5/ESR0</td>
<td>ESR5/ESR0</td>
</tr>
<tr>
<td></td>
<td>PV− PV+</td>
<td>STT− SST+</td>
<td>PV− PV+</td>
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<tr>
<td>PV−</td>
<td>0.85 0.79**</td>
<td>0.93 0.97**</td>
<td>0.92 0.94**</td>
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<td>L2/3/4</td>
<td>0.92 0.76**</td>
<td>0.81 0.75**</td>
<td>0.92 0.94**</td>
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</table>

Values are AAT-induced changes in BBN-ESR of PV+ and SST+ neurons compared with PV− and SST− neurons, respectively. Neurons were classified according to their best frequencies. Significant differences: *P < 0.05, **P < 0.01. ns, Nonsignificant.
PV+ cell in L2/3 → pyramidal cell in L2/3. Addressing the precise nature of circuit changes after or even during acoustic trauma is, however, beyond the scope of this study. Whether the traumatic changes in cortical processing are transient or persistent also remains an open question that can be answered with the use of the chronic cranial window technique, which enables the study of properties of individual neurons over weeks or months with a cellular or even dendritic resolution (Holtmaat et al. 2009).

REFERENCES


