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Repeated exposure to a tone transiently alters spectral tuning bandwidth of neurons in the central nucleus of inferior colliculus in juvenile rats

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Abstract

Early acoustic experience changes tonal frequency tuning in inferior colliculus (IC) and primary auditory cortex. The contributions of IC plasticity to cortical frequency map reorganization are not entirely clear. While most cortical plasticity studies exposed animals to pulsed tones, studies of IC plasticity used either noise or a continuous tone. Here we compared the effects of repeated exposure to single-frequency tone pips on cortical and IC frequency representations in juvenile rats. We found that while tone exposure caused a long-lasting increase in cortical representations of the exposure frequency, changes to IC neurons were limited to a transient narrowing of tuning bandwidth. These results suggest that previously documented cortical frequency map reorganization does not depend on similar changes in subcortical auditory nuclei.

Keywords

Subcortical plasticity; inferior colliculus; auditory cortex; bandwidth; tonotopic map; polytrode

Introduction

Early acoustic experience can lead to long lasting changes in neural response properties (Sanes and Constantine-Paton, 1985, Zhang et al., 2001, Chang and Merzenich, 2003, de Villers-Sidani et al., 2007, Han et al., 2007, Insanally et al., 2009, Kim and Bao, 2009, Barkat et al., 2011). Previously, we demonstrated that exposing a rat to 7-kHz pure tone during the auditory critical period increased the number of neurons tuned to 7 kHz in primary auditory cortex (AI). This developmental plasticity has lasting effects on the animal's sensory perception and behavior (Han et al., 2007). Auditory cortex reflects the statistics of environmental sounds by modifying its sound representation, or the tonotopic map. However, we know surprisingly little about the original locus or loci of tonotopic map plasticity. In theory, altered frequency tuning observed in the auditory cortex could arise from a feed-forward manifestation of synaptic changes upstream of the auditory cortex, such as auditory brain stem nuclei (Sanes and Constantine-Paton, 1985, Poon and Chen, 1992, Yu et al., 2007). Should that be the case, molecular and cellular studies of auditory plasticity

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should focus first on subcortical auditory structures before studying downstream effects in the cortex.

Currently, limited reports are available on whether subcortical structures show AI-like reorganization of tonotopic map following manipulation of early acoustic experience. One study reported AI-like tonotopic changes in the central nucleus of inferior colliculus (ICC) following exposure to single continuous tone (Poon and Chen, 1992). Tuning bandwidth and other response properties of ICC neurons can also be changed by exposure to clicks and noises (Sanes and Constantine-Paton, 1983, Sanes and Constantine-Paton, 1985, Grecova et al., 2009, Bures et al., 2010) or paired pure tones (Yu et al., 2007). Oliver and colleagues examined ICC frequency maps following repeated tone exposure similar to that used in early cortical plasticity studies (Oliver et al., 2011). However, their methods of response analysis were different from those of the cortical studies, hindering a direct comparison with parallel cortical plasticity effects (see Discussion for details). In order to address this issue, we studied tonotopic organization of ICC after rearing rat pups in an acoustic environment of repetitive tone pips, which has been previously shown to induce a change in cortical tonotopic organization (Han et al., 2007).

Experimental Procedures

Sound exposure

All procedures used in this study were approved by the University of California Berkeley Animal Care and Use Committee. A litter of rat pups and dam (Sprague Dawley) was placed in an anechoic sound-attenuation chamber, and trains of pure tone pips (7.5 kHz, 60 dB SPL, 100-ms pip duration, 5-ms cosine squared ramp, six pips in a train at 6 Hz, one train every 2 s) were played 24 hours a day to the animals during a period from postnatal day 9 (P9) to P25 (Figure 1). This time window covers the critical period for spectral representation plasticity in the primary auditory cortex (AI) and has been used in previous studies (Insanally et al., 2009). After sound exposure, animals were returned to standard housing conditions. A control litter was maintained in the standard animal husbandry room.

AI mapping

The AI of sound-exposed and control animals were mapped at comparable ages between P37 to P96 (exposed, $n = 4$, mean P67.50, SD 20.04, naïve, $n = 4$, mean P52.75 SD 11.15). Rats were anesthetized with urethane (2.0 g/kg, i.p.). Atropine sulfate (0.1 mg/kg, s.q.) and dexamethasone (1 mg/kg, s.q.) were administered to reduce brain edema and viscosity of bronchial secretions. The anesthetized rat was placed in a custom head holder and a slit was made in the cisterna magna to drain cerebrospinal fluid and reduce brain pulsations. A craniotomy and durectomy was performed over right AI. A layer of silicon oil was applied to prevent brain desiccation. Sound stimuli were generated by an audio signal processor (Tucker-Davis Technologies RX6) and delivered to the left ear from an enclosed cannulated speaker (Tucker-Davis Technologies Electrostatic Speaker, Coupler model) through a tube. The speaker was calibrated to have <3% harmonic distortion and flat output in the entire frequency range (Tucker-Davis Technologies SigCal32).

Multiunit responses of AI neurons were recorded using tungsten microelectrodes (FHC) advanced orthogonally to the cortical surface to the cortical layer IV (450–600 μ m). Electrical signals were amplified and recorded for 333ms surrounding each stimulus presentation (Tucker-Davis Technologies RX5). Prior to each recording block, search stimuli (white noise bursts, 60dB SPL, 25ms duration, repeated at 3Hz) were played to identify sound-evoked multiunit responses. Multiunit responses were defined as voltage changes that exceed the mean amplitude of the baseline electrical trace by two standard

deviations. Thresholds for multiunit discrimination were set for each microelectrode before recordings. Pure tone pips of 51 frequencies (1–32 kHz, 0.1 octave spacing, 5-ms cosine-squared ramps, 25-ms duration, repeated three times) at 8 intensities (0–70 dB SPL, 10 dB spacing) were presented in pseudorandom order, and responses were used to reconstruct the frequency-intensity receptive field of each multiunit.

Electrode penetrations were made densely throughout the temporal cortex while avoiding surface blood vessels. On average, 71 penetrations ($SD \pm 14$) were made in the area of AI (mean area $2.41 \pm 0.44 \text{ mm}^2$), corresponding to roughly $215 \mu\text{m}$ between penetrations. Recording sites were marked on a magnified digital photograph of the cortex for later tonotopic map reconstruction.

ICC mapping

The inferior colliculus of 7.5 kHz-experienced and naïve rats was mapped at comparable ages in two time windows: an Early mapping window at the end of the acoustic exposure between P17 and P24 (exposed, $n = 4$, mean age $P19.25 \pm 2.22$, naïve, $n = 3$, mean age $P18.33 \pm 5.51$), and a Late mapping window at least 15 days after the acoustic exposure, between P40 and P51 (exposed, $n = 7$, mean age $P45.1 \pm 3.44$, naïve, $n = 3$, mean age $P47 \pm 2.65$). Anesthetics, surgical procedures and the speaker setup were identical to those used in the AI mapping.

Multiunit recording was made from the right inferior colliculus. A burr hole was made on the skull stereotactically 1mm lateral and 1mm rostral to lambda. A 16-channel polytrode (NeuroNexus Technologies; 1 shank, $50 \mu\text{m}$ contact spacing, $177 \mu\text{m}^2$ contact site, model A1x16-3mm-50-177; or 4 shanks, $125 \mu\text{m}$ shank spacing, $50 \mu\text{m}$ contact spacing on each shank, $177 \mu\text{m}^2$ contact site, model A4x4-3mm-50-125-177) was lowered vertically into the inferior colliculus while search stimuli were being played, until strong auditory evoked responses were observed. Penetrations were made at multiple locations of IC and the central nucleus of IC (ICC) was differentiated from other IC nuclei by its tonotopic organization and sharp frequency-intensity tuning. Only ICC multiunits were included in the analysis. Regular spacing of recording sites on each shank of the polytrode ensured unbiased sampling from the ICC. The polytrode was advanced in set distance intervals ($50 \mu\text{m}$ for 4-shank, or $200 \mu\text{m}$ for 1-shank polytrode) in a single track to record the entire dorsal-ventral extent of ICC. Undersampling of units at the beginning and the end of each electrode track was accounted for during the data analysis.

Frequency-intensity tuning curve of each multiunit was reconstructed from its responses to 25ms pure tone pips of 51 frequencies (1–32 kHz, 0.1 octave spacing, repeated three times) and 8 sound pressure levels (0 – 70 dB SPL, 10 dB steps). The tonotopic axis was defined as the dorso-ventral recording site depth relative to the most dorsal ICC site encountered. Tonotopy of ICC was compared between the 7.5 kHz-experienced and naïve animals to examine the effect of tone rearing on spectral representations.

Data analysis

Off-line data analysis and statistical tests were conducted using MATLAB (MathWorks). The characteristic frequency (CF) of auditory cortical and ICC multiunits was determined as the frequency at which the lowest intensity sound evokes a neural response. CF and tuning bandwidth at 30dB above multiunits' firing threshold (BW30) were determined visually from the V-shaped response-frequency curve by experienced experimenters blind to the acoustic exposure conditions. Peak latency was defined as the poststimulus duration to the peak of the peristimulus time histogram. Peak firing rate was defined as the spike rate during the 2-ms surrounding this peak.

Since pure tone exposure has been associated with overrepresentation and an accompanying decrease in tuning bandwidth at the exposure frequency (Poon and Chen, 1992, Zhang et al., 1998, Han et al., 2007, Barkat et al., 2011), we hypothesized that similar frequency-specific effects would be observed in the present study. Therefore, we performed one-tailed t-tests on tonotopic frequency representation and tuning bandwidth at the exposure frequency ($7.5 \text{ kHz} \pm 0.2 \text{ octave}$) to determine the statistical significance of differences between sound-exposed and control groups.

Results

The total number of animals, their mean ages and the numbers of multiunits are reported in Table 1. The peak latency, firing threshold and peak response firing rate of the multiunits were not different between sound exposed animals and their age matched controls (Table 1).

ICC tonotopic representation is not altered following acoustic exposure

CFs of ICC multiunits were plotted as a function of dorso-ventral recording depth relative to the most dorsal ICC site. ICC exhibits smooth tonotopy from low to high CF dorso-ventrally, covering roughly 4 octaves over $1500 \mu\text{m}$. Overrepresentation of the exposure frequency was expected to manifest as an increased number of units tuned to $7.5 \text{ kHz} \pm 0.2 \text{ octaves}$. However, no differences were observed in the proportion of sites tuned to the exposure frequency when comparing between control and experimental groups for both Early and Late mapping windows (Figure 2).

ICC multiunit tuning bandwidth changes as a result of acoustic exposure

Tuning curve bandwidth (BW), the frequency range to which a multiunit responds, was measured at 30dB above multiunits' firing threshold (BW30) and compared between control animals and exposed animals mapped during the Early and Late mapping windows. Statistical analysis did not show any significant difference, possibly because tuning bandwidth varied substantially between animals. To identify potential frequency-specific effects, we normalized the BW30 within each animal and compared their Z-scores. Animals in the Early mapping group showed a significant narrowing of BW for sites tuned to the exposure frequency (one-tailed two sample t-test at $7.5\text{kHz} \pm 0.2 \text{ octave}$: $p = 0.0095$) (Figure 3a). No BW effects were observed when ICC was mapped in the Late mapping window (Figure 3b).

AI tonotopic representation changes following acoustic exposure

AI tonotopy was examined in the littermates that underwent the same acoustic rearing as the ICC animals. AI tonotopic axis, defined here as the straight line connecting the site of the lowest and highest CF in AI tonotopy, showed a clustering of units tuned around 7.5 kHz (Figure 4a). Acoustic rearing induced an enlargement of the cortical area tuned to the exposure frequency in AI (Figure 4b: one-tailed two sample t-test at $7.5\text{kHz} \pm 0.2 \text{ octave}$: $p=0.0062$), consistent with previous studies (Zhang et al., 1998, de Villers-Sidani et al., 2007, Han et al., 2007, Kim and Bao, 2009).

Bandwidths of AI frequency-intensity areas were examined at 30 dB above firing threshold. To be consistent with the ICC bandwidth analysis, we also Z-scored the BW30 for cortical responses. No differences were observed between sound-exposed and control animals (Figure 4c).

Discussion

Few studies have compared tonotopic map changes in both auditory cortex and inferior colliculus using exactly the same rearing paradigms. Here, we show that tonotopic map changes induced by pure tone acoustic rearing are limited to AI and absent in ICC under our experimental conditions. We conclude that tonotopic reorganization in AI following acoustic rearing does not depend on similar changes in ICC. This is consistent with previous findings that conductive hearing loss-induced tonotopic map plasticity occurs in AI but not in ICC (Popescu and Polley, 2010).

Further, we have demonstrated a reduction of receptive field bandwidth in ICC multiunits with characteristic frequencies around the acoustic exposure. Notably, this bandwidth effect was only present in our Early mapping groups (P17–24), and not in the Late mapping groups (P40–51), despite the identical acoustic exposure during the auditory critical period. These results suggest that the ICC bandwidth effects are transient, while AI tonotopic map change is persistent well after (>15days) the termination of acoustic rearing. Equivalent bandwidth effects were absent in AI in the present study. A previous study reported reduced tuning bandwidth after pure tone exposure in AI multiunits tuned to the exposure frequency (Han et al., 2007). The discrepancy may be due to differences in experimental procedures and methods of data analysis—in the previous report, animals had gone through behavioral tests before their AI were mapped, and the tuning bandwidth were derived from rate-frequency tuning curve instead of intensity-frequency tuning curve.

Suga and colleagues extensively documented a similar distinction between experience-induced tuning changes in IC and AI in the bat. For example, fear conditioning results in frequency tuning shifts for both cortical and ICC neurons, with changes in ICC neurons occurring earlier, but decaying much faster, than in cortical neurons, suggesting that transient ICC plasticity may contribute to long-term cortical reorganization (Gao and Suga, 1998, 2000). In those studies, plasticity was induced in adult animals using either electrical stimulation or behavioral conditioning. In the present study, we observed transient bandwidth change in the ICC immediately after tone exposure, but did not observe any tuning shifts in the ICC. It appears that, in both bats and rats, ICC exhibits transient experience-dependent spectral plasticity, whereas AI shows long-lasting reorganization of the frequency map.

Developmental plasticity in frequency tuning has been previously reported in the ICC. When mice were exposed to repetitive clicks, a stimulus that co-activates afferents across a broad frequency range, single ICC neurons became more broadly tuned to frequency (Sanes and Constantine-Paton, 1983, 1985, Grecova et al., 2009, Bures et al., 2010). Broadening of the ICC tuning bandwidths has also been reported in adult rats that were exposed to a 125 dB broadband noise for eight minutes at postnatal day 14 (Grecova et al., 2009, Bures et al., 2010). Similarly, when animals were exposed to two simultaneously played tones, ICC neurons became broadly tuned (Yu et al., 2007). Their primary bandwidth finding is in general agreement with our results. Taken together, these findings suggest that ICC neurons exhibit bidirectional bandwidth plasticity, narrowing or broadening depending on the stimuli. The discrepancy between results of the present study and those of Poon et al (Poon and Chen, 1992) may be due to differences in the sound exposure and unit sampling procedures.

Oliver and colleagues (Oliver et al., 2011) exposed juvenile rats to repetitive tone pips using stimulus parameters and exposure time windows that were similar to those used in the present and previous studies of auditory cortical plasticity (Zhang et al., 2001, de Villers-Sidani et al., 2007). They reported an increase in the number of ICC sites tuned to the

exposure frequency after exposing the rats to a 14-kHz tone using the stimulus parameters used by Zhang and colleagues (Zhang et al., 2001). However, it is unclear whether they found such effects in animals exposed to a 7-kHz tone following the stimulus parameters used by de Villers-Sidani and colleagues (de Villers-Sidani et al., 2007). After both exposure conditions, subsets of ICC neurons showed reduced responses to the exposure frequency (Oliver et al., 2011). The discrepancy between our results and those of Oliver and colleagues may be due to differences in animal strains (SD vs. Long Evans), recording electrodes (multi-site silicon probe vs. tungsten-in-glass), CF identification methods (“blind” off-line scoring vs. on-line searching aided by an oscilloscope and audio monitor), frequency scales (logarithmic vs. linear) and quantification of representational sizes (fixed CF bins vs. variable CF steps).

A negative finding of ICC tonotopic reorganization in the present study might be attributed to several confounding factors. For instance, the acoustic exposure length might have been too short for some animals in the Early mapping group, since the exposure was terminated by the ICC mapping. Since the rat ear canal opens at approximately P12, the shortest exposure length used in our study was ~5 days. AI tonotopic map changes can be induced by acoustic exposure for as short as 3 days (de Villers-Sidani et al., 2007). Another confounding possibility is that ICC undergoes a very brief transient tonotopic reorganization, which was not captured in our mapping interval. We mapped at a range of ages within the Early mapping group to maximize the chance of capturing such transient effects. Although our experimental design cannot completely rule out the possibility of transient ICC map changes, our results suggest that persistent changes in ICC are not required for the long-lasting AI map reorganization. Finally, ICC tonotopic reorganization might not be expressed under urethane anesthesia. Again, the fact that we were able to observe map changes in AI but not in ICC under identical anesthetic procedures suggests that the cortical map reorganization does not depend on an expression of associated map changes in ICC, if any.

Afferent fibers from ICC synapse at medial geniculate nucleus (MGN) of thalamus before reaching AI. Although we did not investigate plasticity of MGN, our ICC bandwidth finding is in line with a report on thalamocortical plasticity (Barkat et al., 2011). In that study, Barkat et al. exposed juvenile mice to repeated pure tone pips and contrasted the tonotopic organization at thalamus and AI. In the MGN, this manipulation led to a narrowing of bandwidth at the exposure frequency without changing the tonotopic representation, while the AI tonotopic map showed overrepresentation at the exposure frequency. Given these findings, it appears that robust tonotopic reorganization following repeated sound exposure is a reserved property of the cortex, and that this effect is not a simple readout from upstream subcortical auditory nuclei.

Efforts to identify the source of auditory plasticity is significantly complicated by the existence of extensive feedback projections from auditory cortex to ICC and other nuclei of the inferior colliculus (Saldana et al., 1996). There is compelling evidence that auditory cortex modulates spectral selectivity of lower auditory nuclei via feedback projections (Yan and Suga, 1996, Zhang et al., 1997). Moreover, stimulation of ICC neurons was found to cause a shift of the frequency tuning in nearby ICC neurons. This shift was abolished by cortical inactivation, suggesting that ICC stimulation must go through the cortex in order to produce effects on ICC itself (Zhang and Suga, 2005). Selective manipulation of auditory pathways using newer techniques (e.g. optogenetic) will reveal the exact nature of subcortical contributions to cortical tonotopic map reorganization and will help us gain further insight into the mechanisms of auditory pathway plasticity.

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Highlights

- Rat pups were exposed to pulsed tone from postnatal days 9 to 25.
- Exposure did not alter the frequency map in the central nucleus of inferior colliculus (ICC)
- Exposure led to transient narrowing of tuning bandwidth in ICC.
- Exposure changed frequency map in primary auditory cortex.
- Conclusion: cortical map reorganization is not due to subcortical changes.

Figure 1.

Timeline of the experiments. Rats were exposed to 7.5-kHz pure tone pips between P9 and P25. Recordings were made from the inferior colliculus (IC) in an Early (P17 – P24) or Late (P40 – P51) mapping window. All AI mappings were performed in rats P36 – P96.

Figure 2.

ICC tonotopic representation following acoustic exposure. The characteristic frequencies (CFs) for each IC site are plotted as a function of recording depth (scale shown at right). Multiple recording tracks are shown for some animals. To the right, histograms indicate the proportion of sites with CFs at the corresponding frequency. For all figures, error bars indicate standard error of the mean (SEM). The proportion of sites tuned near the exposure frequency ($7.5 \text{ kHz} \pm 0.2 \text{ oct}$) for exposed and control animals did not differ significantly for recordings made during both the Early and Late windows.

Figure 3. ICC bandwidth following acoustic exposure. Tuning bandwidth of IC sites measured 30 dB above firing threshold is plotted as a function of that site's characteristic frequency (CF) for control (blue) and exposed (red) animals. In exposed animals mapped in the Early window, a significant increase in tuning bandwidth was observed for sites tuned near the exposure frequency. No differences were observed in the Late mapping window.

Figure 4.

AI tonotopic representation following acoustic exposure. **A.** Left: representative tonotopic maps recorded from primary auditory cortex (AI) are shown. Polygons were generated using Voronoi tessellation of all AI recording sites. Warm and cool colors indicate sites tuned to high and low frequencies, respectively. Right: scatterplots showing characteristic frequency (CF) of each site as a function of its location along the tonotopic axis. **B.** A histogram showing the proportion of each map tuned to different frequency bands in control (blue) and exposed (red) animals. In exposed animals, significantly more of AI is tuned to the exposed frequency. **C.** Average bandwidths 30 dB above firing threshold (BW30) are displayed as a function of characteristic frequency (CF). No differences are present between control and exposed animals.

Table 1

Basic response properties of ICC and AI multiunits

	ICC Early mapping window (P17–P24)			ICC Late mapping window (P40–P51)			AI mapping (P37–P96)		
	control	exposed	p-value	control	exposed	p-value	control	exposed	p-value
n	3	4		3	7		4	4	
age (days)	18.33±3.89	19.25±1.28	0.78	47.00±1.87	45.14±1.40	0.43	52.75±6.44	67.50±11.57	0.25
multiunits (per animal)	370.67±155.78	458.00±100.30	0.58	85.33±6.53	182.86±88.75	0.47	67.50±10.80	73.75±5.46	0.57
Peak Latency (ms)	17.32±1.33	21.74±1.92	0.10	14.32±0.45	15.19±1.22	0.64	23.97±3.06	20.72±1.05	0.29
Firing threshold (dB SPL)	33.25±3.57	31.11±1.61	0.50	22.44±7.10	27.14±2.20	0.35	29.01±4.26	32.39±2.14	0.44
peak FR (Hz)	43.25±8.66	37.09±12.90	0.69	85.69±10.66	73.76±12.72	0.55	34.47±3.14	38.78±5.33	0.45

Data are presented as means ± SEM. P-values are reported from two-sample t-tests conducted between control and exposed animals