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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 the inferior colliculus (IC) and the 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 the subcortical auditory nuclei.

Key words: 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 the 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 the 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 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 the tonotopic map following the manipulation of early acoustic experience. One study reported AI-like tonotopic changes in the central nucleus of the 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, 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 the tonotopic organizations of both ICC and AI in rat pups reared in an acoustic environment of repetitive tone pips, which have been previously shown to induce a change in the 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, and one train every 2 s) were played 24 h a day to the animals during a period from postnatal day 9 (P9) to P25 (Fig. 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.

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Abbreviations: AI, primary auditory cortex; CF, characteristic frequency; IC, inferior colliculus; ICC, central nucleus of inferior colliculus; MGN, medial geniculate nucleus.

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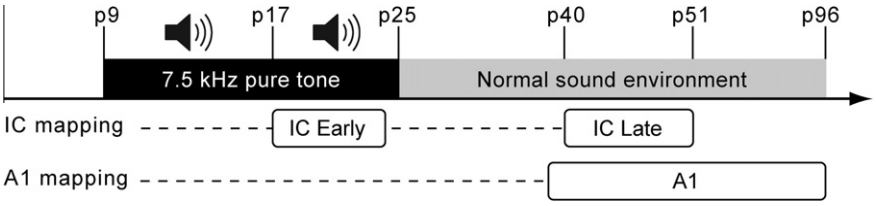

Fig. 1. Timeline of the experiments. Rats were exposed to 7.5-kHz pure tone pips between P9 and P25. Recordings were made from the inferior colliculus (IC) in an Early (P17–P24) or Late (P40–P51) mapping window. All AI mappings were performed in P37–P96 rats.

AI mapping

The AI of sound-exposed and control animals were mapped at comparable ages between P37 and 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 the cerebrospinal fluid and reduce brain pulsations. A craniotomy and durectomy was performed over the 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, Alachua, FL, USA) 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 μm). Electrical signals were amplified and recorded for 333 ms surrounding each stimulus presentation (Tucker–Davis Technologies RX5). Prior to each recording block, search stimuli (white noise bursts, 60 dB SPL, 25 ms duration, repeated at 3 Hz) 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 eight intensities (0–70 dB SPL, 10 dB spacing) were presented in a 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 ± 14) were made in the area of AI (mean area 2.41 ± 0.44 mm^2), corresponding to roughly 215 μm 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 ± 2.22, naïve, $n = 3$, mean age P18.33 ± 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 ± 3.44, naïve, $n = 3$, mean age P47 ± 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 stereotaxically 1-mm lateral and 1-mm rostral to lambda. A 16-channel polytrode (NeuroNexus Technologies; 1 shank, 50 μm contact spacing, 177 μm^2 contact site, model A1x16-3mm-50-177; or 4 shanks, 125-μm shank spacing, 50-μm contact spacing on each shank, 177 μm^2 contact site, model A4x4-3mm-50-125-177, Ann Arbor, MI, USA) 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 the 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 μm for 4-shank, or 200 μm for 1-shank polytrode) in a single track to record the entire dorsal–ventral extent of the 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 25-ms pure tone pips of 51 frequencies (1–32 kHz, 0.1 octave spacing, repeated three times) and eight 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 responses are evoked at threshold: the lowest sound intensity that activates the neuron. CF and tuning bandwidth at 30 dB 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 kHz ± 0.2 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).