Tone-detection training enhances spectral integration mediated by intracortical pathways in primary auditory cortex

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ABSTRACT

Auditory-cued behavioral training can alter neural circuits in primary auditory cortex (A1), but the mechanisms and consequences of experience-dependent cortical plasticity are not fully understood. To address this issue, we trained adult rats to detect a 5 kHz target in order to receive a food reward. After 14 days training we identified three locations within A1: (i) the region representing the characteristic frequency (CF) 5 kHz, (ii) a nearby region with CF ~10 kHz, and (iii) a more distant region with CF ~20 kHz. In order to compare functional connectivity in A1 near to, vs. far from, the representation of the target frequency, we placed a 16-channel multiprobe in middle- (~10 kHz) and high- (~20 kHz) CF regions and obtained current-source density (CSD) profiles evoked by a range of tone stimuli (CF ± 1–3 octaves in quarter-octave steps). Our aim was to construct "CSD receptive fields" (CSD RFs) in order to determine the laminar and spectral profile of tone-evoked current sinks, and infer changes to thalamocortical and intracortical inputs. Behavioral training altered CSD RFs at the 10 kHz, but not 20 kHz, site relative to CSD RFs in untrained control animals. At the 10 kHz site, current sinks evoked by the target frequency were enhanced in layer 2/3, but the initial current sink in layer 4 was not altered. The results imply training-induced plasticity along intracortical pathways connecting the target representation with nearby cortical regions. Finally, we related behavioral performance (sensitivity index, d') to CSD responses in individual animals, and found a significant correlation between the development of d' over training and the amplitude of the target-evoked current sink in layer 2/3. The results suggest that plasticity along intracortical pathways is important for auditory learning.

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1. Introduction

A1 is organized topographically by frequency, yet its organization can be modified by auditory experience (Edeline, 2003; Irvine, 2007; Scheich, Brechmann, Brosch, Budinger, & Ohl, 2007; Weinberger, 2007). For example, frequency-discrimination training in adult monkeys can alter the tonotopic map of CF to increase the area devoted to frequencies relevant for training (Recanzone, Schreiner, & Merzenich, 1993). Similarly, single and multi-unit recordings show that frequency RFs can become biased towards stimuli that acquire behavioral significance during training (Bakin & Weinberger, 1990; Fritz, Shamma, Elhilali, & Klein, 2003), a phenomenon that presumably underlies frequency-specific plasticity of tonotopic maps. Studies have shown that training procedures can alter physiological response properties in ways that are related to the specific information acquired, implying that the nature of

cortical plasticity depends on task demands (Bieszczad & Weinberger, 2010a; Fritz, Elhilali, & Shamma, 2005; Polley, Steinberg, & Merzenich, 2006; Scheich et al., 2007). In some cases, the extent of map expansion correlates with improvements in behavioral performance and resistance to behavioral extinction, suggesting that the degree of plasticity may determine the strength of learning (Bieszczad & Weinberger, 2010b; Polley et al., 2006; Recanzone et al., 1993; Rutkowski & Weinberger, 2005). However, other studies found that improvements in auditory perception can occur in the absence of significant plasticity of tonotopy in A1 (Brown, Irvine, & Park, 2004). It appears that auditory training can lead to specific and selective changes in neuronal circuit properties in A1 that underlie improvements in behavioral performance; these changes may or may not produce changes in tonotopic maps.

Although a number of studies have demonstrated learning-related plasticity in A1, fewer have examined cellular and molecular mechanisms (Carpenter-Hyland, Plummer, Vazdarjanova, & Blake, 2010; Guo et al., 2012). Even the locus of plasticity is not fully established, since changes observed in A1 may not originate there but instead could be relayed from subcortical structures (Ma & Suga, 2009). One approach to better understand the nature and

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locus of plasticity in A1 is to determine the laminar profile of physiological changes using tone-evoked CSD analysis. Because CSD analysis can help to distinguish thalamocortical from intracortical contributions to evoked responses (Happel, Jeschke, & Ohl, 2010; Intskirveli & Metherate, 2012; Kawai, Lazar, & Metherate, 2007; Kawai, Kang, & Metherate, 2011), it can be used to address whether plasticity originates within A1 or exists already in thalamic inputs.

Thus, to examine intracortical and subcortical contributions to plasticity we applied CSD analysis to learning-induced plasticity in A1. The results show that tone-detection training altered responses at a cortical site with CF ~10 kHz (~1 octave above the target), producing plasticity of tone-evoked responses within cortical layers 2–4. At this cortical site, altered target-evoked current sinks in layer 2/3 implicate intracortical neural circuits, and altered sinks in layer 4 also were more likely associated with intracortical, rather than thalamocortical, synapses. Importantly, in individual animals the magnitude of the target-evoked intracortical, but not thalamocortical, responses correlated with the development of learned behavior. These results help elucidate the origins of learning-related plasticity in A1.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats 55–58 days old were obtained from a commercial breeder (Charles River). All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

2.2. Operant training

Animals were handled daily for a minimum of 3 days before the start of behavioral training. Prior to tone-detection training, rats underwent behavioral shaping to nose poke for food pellets (45 mg pellets, Bio-Serv) using a operant chamber placed inside an acoustically shielded box (Habitest, Coulbourn Instruments). The nose poke and pellet dispenser were controlled using custom software (LabView, National Instruments). The animal was trained to hold a nose poke for progressively longer durations, up to 800 ms, in order to receive a food pellet. Most of each animal's daily allotment of food was obtained during training sessions, and each session was terminated when the animal earned ~200 pellets, or after 1 h. Upon return to the home cage, each animal was given an additional 4 g of food. On non-training days (weekends) animals received 16 g of food. Animals had unlimited access to water, were weighed daily, and during training remained at ~85–95% of their pre-training weight. Nose-poke shaping continued for a minimum of 10 days until reliable 800 ms holds were maintained for three consecutive days, at which point tone-detection training began. In that task, animals were required to release the nose poke upon presentation of a 5 kHz target tone (50 dB, 25 ms) produced by a speaker (FF-1, Tucker Davis Technologies) located on the ceiling of the acoustic chamber (calibration performed using a microphone positioned near the floor of the chamber). The target tone was presented randomly at one of two times, either 400 ms or 800 ms after initiation of the nose poke. In either case, the nose poke had to be withdrawn within 400 ms of tone onset to be rewarded. Performance was evaluated using the sensitivity index (d') from signal detection theory, which is equal to the z-score difference between hit and false alarm rates. Thus, the behavioral metric (d') compares the animal's responses over the 400–800 ms window after nose-poke initiation. Some responses within this window follow tones at 400 ms (hits), and others do not follow a tone (false alarms, on trials that would have presented a tone at 800 ms). The probability of hits and false alarms is determined, and d' calculated as the z-score difference between the two probabilities (z-scores determined using the norminv() function in Matlab), i.e., $d' = z(\text{hits}) - z(\text{false alarms})$.

2.3. Surgical procedure

Rats were anesthetized with urethane (0.7 g/kg i.p., Sigma) and xylazine (13 mg/kg i.p., Phoenix Pharmaceutics), placed in a sound-attenuating chamber (AC-3, IAC) and maintained at 37 °C. Anesthesia was supplemented at ~1 h intervals in order to maintain lack of reflex response to tail or paw pinch, with urethane (0.13 g/kg) and xylazine (1.3 mg/kg) administered via a catheter to avoid movement. The head was secured in a stereotaxic frame (model 923, Kopf Instruments) using blunt earbars. After a midline incision, the skull was cleared and secured using a custom head holder fixed onto the skull. A craniotomy was performed over the right temporal cortex and the exposed brain was kept moist with warmed saline. Subsequently, the earbars were removed to permit acoustic stimulation.

2.4. Electrophysiology and acoustic stimulation

For mapping A1, multiunit activity was recorded using a tungsten electrode. For CSD profiles, local field potentials (LFPs) were recorded using a 16-channel silicon multiprobe (~2–3 MΩ at 1 kHz for each 177 μm² recording site, 100 μm separation between recording sites; NeuroNexus Technologies), with the multiprobe advanced into the cortex until the first channel was just visible at the cortical surface. LFPs were filtered and amplified (1 Hz to 10 kHz, AI-401 or AI-405, CyberAmp 380; Axon Instruments), digitized and stored on a computer (Macintosh running AxoGraph software). Acoustic stimuli were digitally synthesized and controlled using MALab (Kaiser Instruments) and a computer (Macintosh) and delivered through a speaker (ES-1 or FF-1 with ED-1 driver, Tucker-Davis Technologies) positioned ~3 cm in front of the left ear. For calibration (sound pressure level, SPL, in dB re: 20 μPa) a model 4939 microphone and Nexus amplifier (Brüel and Kjaer) was positioned in place of the animal at the location of the left earbar tip. Tones were 100 ms in duration with 5 ms linear rise and fall ramps; range 1–40 kHz and −10 dB to 70 dB SPL. For data collection, stimuli were delivered at a rate of 1/s in sets of 25 trials.

2.5. Laminar CSD analysis

Tone-evoked CSD profiles were constructed and analyzed offline as described previously (Intskirveli & Metherate, 2012; Kaur, Rose, Lazar, Liang, & Metherate, 2005; Kawai et al., 2011). One-dimensional CSD profiles are the second spatial derivative of the LFP laminar profile (Muller-Preuss & Mitzdorf, 1984); conventionally, a current sink implies the location of underlying net synaptic excitation. The onset of a tone-evoked current sink was defined as the time at which the response crossed a threshold 2 standard deviations above baseline. The middle-layer sink with shortest onset latency (typically 500–700 μm depth) was designated the initial sink and assigned to "layer 4" for purposes of averaging across animals, and its initial amplitude considered a reflection of monosynaptic thalamocortical input (Intskirveli & Metherate, 2012; Kawai et al., 2011); see Section 4.1.

2.6. Statistical analyses

All averaged data are reported ± s.e.m. Statistical comparisons were performed using Microsoft Excel or SPSS 18.0. Tests of