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SPECTRAL INTEGRATION IN PRIMARY AUDITORY CORTEX: LAMINAR PROCESSING OF AFFERENT INPUT, *IN VIVO* AND *IN VITRO*

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Abstract—Auditory cortex neurons integrate information over a broad range of sound frequencies, yet it is not known how such integration is accomplished at the cellular or systems levels. Whereas information about frequencies near a neuron's characteristic frequency is likely to be relayed to the neuron by lemniscal thalamocortical inputs from the ventral division of the medial geniculate nucleus, we recently proposed that information about frequencies spectrally distant from characteristic frequency is mainly relayed to the neuron via "horizontal" intracortical projections from neurons with spectrally-distant characteristic frequencies [J Neurophysiol 91 (2004) 2551]. Here we test this hypothesis by using current source density analysis to determine if characteristic frequency and spectrally-distant non-characteristic frequency stimuli preferentially activate thalamocortical and horizontal pathways, respectively, in rat auditory cortex. Characteristic frequency stimuli produced current source density profiles with prominent initial current sinks in layers 3 and 4—the termination zone of lemniscal inputs from medial geniculate nucleus. In contrast, stimuli three octaves below characteristic frequency produced initial current sinks mainly in the infragranular layers. Differences between current source density profiles were only apparent for initial current sinks; profiles for longer-latency current sinks evoked by characteristic frequency and non-characteristic frequency stimuli overlapped to a greater degree, likely due to shared mechanisms of intracortical processing or to longer-latency thalamocortical contributions (lemniscal and nonlemniscal). To identify current source density profiles produced by activation of lemniscal thalamocortical inputs alone, we utilized the mouse auditory thalamocortical slice preparation. Electrical stimulation of the medial geniculate nucleus *in vitro* produced major current sinks in cortical layers 3/4, and excitation spread horizontally from this point throughout primary auditory cortex to produce current sinks in multiple cortical layers. These data support the hypothesis that relay of thalamocortical information throughout auditory cortex via horizontal intracortical projections may be the basis of broad spectral integration *in vivo*.

Key words: current source density, thalamocortical, horizontal projections, cortical layers, rat, mouse.

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Abbreviations: ACSF, artificial cerebrospinal fluid; ACx, auditory cortex; BF, best frequency; CF, characteristic frequency; CSD, current source density; DAB, 3′3′-diaminobenzidine; MGv, ventral division of the medial geniculate nucleus; SPL, sound pressure level.

Converging evidence has shown that single neurons in primary auditory cortex (ACx) may integrate information over a very broad spectral range. For example, pure tones that are several octaves outside the frequency receptive field of a neuron in primary ACx can modify multiunit responses to stimuli within the receptive field (Schulze and Langner, 1999). Conversely, optical (intrinsic signal) imaging has shown that a single-frequency stimulus can influence neurons throughout much, if not all, of ACx (Bakin et al., 1996). Moreover, intracellular recordings have shown that subthreshold synaptic inputs to a single ACx neuron can be elicited by tones spanning a range of up to five octaves (the maximum range tested; Kaur et al., 2004). These studies all show that single neurons in primary ACx may receive inputs subserving much of the audible spectrum. Given the potential involvement of primary ACx in the processing of spectrally complex acoustic stimuli (e.g. Kadia and Wang, 2003; Schreiner et al., 2000), it is important to understand the mechanisms of spectral integration in ACx neurons.

The main (lemniscal) input to primary ACx comes from the ventral division of the medial geniculate body (MGv) (Cetas et al., 1999; de Venecia et al., 1998; Huang and Winer, 2000; Kimura et al., 2003; Romanski and LeDoux, 1993; Vaughan, 1983). It seems likely that this input directly mediates cortical responses to characteristic frequency (CF, stimulus frequency with the lowest threshold) and other stimuli that are spectrally close to CF. For example, paired recordings of neurons in the MGv and primary ACx show that cells with correlated discharge have CFs within one-third of an octave (Miller et al., 2001). Similarly, studies combining physiological recordings with anatomical tracing show that MGv neurons project to cortical regions containing neurons with similar CFs (Budinger et al., 2000; Imig and Morel, 1984; Winer et al., 1999). If, as these data imply, axons of MGv neurons do not diverge to contact neurons throughout ACx, but rather project to a restricted portion of primary ACx, then thalamocortical projections are not solely responsible for the broad spectral integration observed in functional studies.

Physiological studies can help identify the location of synapses mediating auditory responses. In particular, current source density (CSD) analyses reveal the laminar location of current sinks—putative synapse locations—underlying auditory-evoked responses. Studies show that best frequency (BF, stimulus frequency eliciting the largest magnitude response) or click stimuli produce current sinks in layer 4 and lower layer 3 of primary ACx (Barth and Di, 1990; Fishman et al., 2000a,b, 2001; Muller-Preuss and

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Mitzdorf, 1984; Steinschneider et al., 1992, 1998), a laminar location that coincides with the terminal field of thalamocortical axons from MGv (Cetas et al., 1999; de Venecia et al., 1998; Huang and Winer, 2000; Kimura et al., 2003; Romanski and LeDoux, 1993; Vaughan, 1983). However, the tone-evoked current sink in layers 3/4 decreases in magnitude as the stimulus frequency diverges from BF, disappearing in ~1–2 octaves (60 dB sound pressure level, SPL; Fishman et al., 2001; Steinschneider et al., 1998). These data suggest, again, that lemniscal thalamocortical inputs mainly mediate responses to CF and near-CF stimuli.

The pathways mediating cortical responses to stimuli that are spectrally distant from CF remain unclear. We recently proposed that spectral integration in ACx involves long-distance intracortical ("horizontal") pathways (Kaur et al., 2004). In that study, we selectively suppressed cortical (but not thalamocortical) neurons by cortical injection of the inhibitory agonist muscimol, and determined the effect on responses to CF and nonCF stimuli. For CF stimuli, muscimol partly suppressed initial response components (the first ~10 ms of the response) and fully suppressed longer-latency response components, consistent with inhibition of local cortical neurons but not thalamocortical inputs. In contrast, for spectrally-distant nonCF stimuli, muscimol at times fully suppressed both initial and longer-latency response components, suggesting major involvement of intracortical pathways. Muscimol had intermediate effects in some cases, especially for responses to spectrally less-distant stimuli. We therefore proposed that direct thalamocortical inputs contribute to the initial response component to CF stimuli, whereas longer-latency response components to CF stimuli involve local cortical circuits. Responses (both initial and longer-latency components) to spectrally-distant nonCF stimuli mainly involve long-distance horizontal projections from neurons with spectrally-distant CFs (Kaur et al., 2004). (The involvement of horizontal projections in the acoustic-evoked response should increase with spectral distance from CF.) The present study represents a test of this hypothesis.

Horizontal pathways in ACx terminate in multiple cortical layers (Ojima et al., 1991; Matsubara and Phillips, 1988; Mitani et al., 1985; Wallace et al., 1991). In the present study, we use CSD analyses to compare the laminar location of inputs mediating CF vs. spectrally-distant nonCF information. We focus on the initial current sinks elicited by CF vs. nonCF stimuli to examine the differential contribution, if any, of thalamocortical pathways (longer-latency responses to either stimulus can have significant intracortical contributions). We first show that CF stimuli produce initial current sinks in layers 3/4, whereas nonCF stimuli three octaves below CF produce initial current sinks largely outside of layers 3/4, in infragranular layers. To specifically examine the intracortical relay of lemniscal thalamocortical input alone, we turn to the auditory thalamocortical slice preparation (Cruikshank et al., 2002) and show that thalamic inputs to layers 3/4 are subsequently relayed to multiple cortical layers via horizontal projections in ACx. The results support the hypothesis that thalamocortical inputs to a cortical neuron preferentially relay information about CF and near-CF stimuli, whereas horizontal intracortical inputs preferentially relay information about spectrally-distant stimuli from cortical neurons with spectrally-distant CFs. This integration of thalamocortical and intracortical inputs could underlie spectral integration in ACx neurons.

EXPERIMENTAL PROCEDURES

In vivo methods

Surgical procedure. Adult male Sprague–Dawley rats (Charles River Laboratories, Hollister, CA, USA) weighing 250–500 g were used in this study. All procedures were in accordance with the University of California, Irvine, animal use regulations and the NIH guide for the Care and Use of Laboratory Animals. The number of animals used and their suffering was minimized. Stereotaxic surgeries were carried out as detailed in Kaur et al. (2004), but briefly, animals were anesthetized with 1.5 g/kg urethane i.p. (Sigma, St. Louis, MO, USA) and 10 mg/kg xylazine i.p. (Phoenix Pharmaceuticals, St. Joseph, MO, USA) and subsequently administered 0.6 mg/kg atropine i.p. (Phoenix). The animal was placed in a sound-attenuating chamber (model AC-3, IAC, Bronx, NY, USA) and the head was secured in a stereotaxic frame (model 923, Kopf Instruments, Tujunga, CA, USA) using blunt earbars (Kopf). A midline incision was made and the skull was cleared and secured. A craniotomy was performed over the right ACx and the exposed cortex was kept moist with warmed saline. In most experiments, a Polaroid picture was taken of the exposed cortex through the surgical microscope (Carl Zeiss, Thornwood, NY, USA), which helped with the reconstruction of recording sites. After experiments animals were killed with a lethal dose of anesthesia; in some experiments the anesthesia overdose was followed by transcardial perfusion with 0.9% saline followed by 4% paraformaldehyde in preparation for anatomical procedures.

Electrophysiology. Glass microelectrodes filled with 1 M NaCl (~1 MΩ impedance) were used to obtain surface responses to click stimuli and to determine CF of recording sites in layer 4 (600 μm depth). For CSD measurements, a 16-channel silicon multiprobe was used to simultaneously sample field potentials throughout layers 1–6 (probe obtained from University of Michigan Center for Neural Communication Technology (Ann Arbor, MI, USA); 100 μm separation between recording sites; each site 177 μm^2, ~2–3 MΩ impedance at 1 kHz). The multiprobe was visually aligned to be as orthogonal as possible to the cortical surface. The 16-channel multiprobe was lowered until the first channel was just visible on the surface of the brain (the laminar CSD profile was then obtained for depths of 100–1400 μm) or until the second channel was on the surface of the brain (CSD profile obtained from 0 to 1300 μm). Neural activity in each channel was filtered, amplified (1 Hz-10 kHz, AI-401, Axon Instruments, Foster City, CA, USA), digitized at 5 kHz (Digidata 1322A, Axon Instruments) and stored on a computer (Macintosh G4, Apple Computer). Data acquisition was triggered 100 ms before acoustic stimulation, responses were averaged and viewed online and analyzed off-line (AxoGraph, Axon Instruments). The local electroencephalogram was monitored continuously on the oscilloscope.

Acoustic stimuli. Acoustic stimuli were digitally synthesized and controlled using MALab (Kaiser Instruments, Irvine, CA, USA) and a dedicated computer (Macintosh PowerPC, Apple Computer) and delivered through an electrostatic speaker (ES-1 with ED-1 driver, Tucker-Davis Technologies, Gainesville, FL, USA) positioned ~3 cm in front of the left ear. For calibration (SPL in dB re: 20 μPa) a microphone (model 4939 microphone and Nexus amplifier; Bruel and Kjaer, Norcross, GA, USA) was positioned in place of the animal at the tip of the left earbar. Pure tones were 100 ms in duration with 10 ms linear rise and fall ramps. CF and