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Research Paper

Multiscale mapping of frequency sweep rate in mouse auditory cortex

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ABSTRACT

Functional organization is a key feature of the neocortex that often guides studies of sensory processing, development, and plasticity. Tonotopy, which arises from the transduction properties of the cochlea, is the most widely studied organizational feature in auditory cortex; however, in order to process complex sounds, cortical regions are likely specialized for higher order features. Here, motivated by the prevalence of frequency modulations in mouse ultrasonic vocalizations and aided by the use of a multiscale imaging approach, we uncover a functional organization across the extent of auditory cortex for the rate of frequency modulated (FM) sweeps. In particular, using two-photon Ca^{2+} imaging of layer 2/3 neurons, we identify a tone-insensitive region at the border of AI and AAF. This central sweep region behaves fundamentally differently from nearby neurons in AI and AII, responding preferentially to fast FM sweeps but not to tones or bandlimited noise. Together these findings define a second dimension of organization in the mouse auditory cortex for sweep rate complementary to that of tone frequency.

1. Introduction

Functional maps of sensory cortices have provided blueprints for key studies of cortical development and experience-dependent plasticity (Buonomano and Merzenich, 1998; de Villers-Sidani et al., 2008; Karmarkar and Dan, 2006). These maps often reflect the topology of the sensory organ. In auditory cortex, the parameter most commonly represented in these maps is the frequency of a pure tone, which arises from the mechanical transduction of sound waves in the inner ear (Moore, 1995; Von Békésy, 1960). The resulting spectral or tonotopic organization of the cochlea is mirrored throughout the auditory system (Hackett et al., 2011; Kaas, 2011). However, this narrowband representation is not enough for recognition of complex sounds. Rather, the auditory system must integrate across spectral and temporal dimensions to extract higher order features of sound. Such integration begins in subcortical areas (Clopton and Winfield, 1974; Covey and Casseday, 1999; Poon and Yu, 2000) but is refined via cortical processing (Zhang et al., 2003). The resulting functional specialization for various complex sound features (Chechik et al., 2006; Chi et al., 2005; King and Nelken, 2009; Rauschecker, 1998) has been observed in humans (Norman-Haignere et al., 2015) and non-human primates (Bendor and Wang, 2005; Rauschecker and Scott, 2009) but is not as well-understood in other animal models such as rodents (Linden et al., 2003).

Ethological factors play a key role in the nature of these organizational properties since auditory cortex is thought to develop based on the statistics of environmental sounds, especially those that carry behavioral relevance (Klug and Grothe, 2010; Singh and Theunissen, 2003). In mice, ultrasonic vocalizations used for communication contain complex acoustical features (Neunuebel et al., 2015). Frequency modulated (FM) sweeps are especially prevalent, appearing in the form of upward sweeps, downward sweeps, chevrons, and reverse chevrons (Grimsley et al., 2011; Holy and Guo, 2005; Portfors, 2007). More generally, frequency modulations are ubiquitous across species, present in the vocalizations of bats (Neuweiler, 1989), marmosets (Pistorio et al., 2006), macaques (May et al., 1989), and humans (Altmann and Gaese, 2014). Given this widespread behavioral relevance of FM sweeps, a specialization for processing of FM sweeps is predicted at the level of auditory cortex.

In fact, prior studies have pointed towards auditory cortex as critical for the discrimination of FM sweeps in many species including mice (Letzkus et al., 2011), rats (Rybalko et al., 2006), and

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Mongolian gerbils (Ohl et al., 1999). In bats, cortical circuits responsible for FM sweep processing, such as the FM-FM area involved in echolocation, have been implicated (Suga et al., 1983). In other species, certain regions prefer faster FM sweeps, such as a ventral region at the border of the primary auditory cortex (AI) and the secondary auditory cortex (AII) in cats (Mendelson et al., 1993), various lateral belt areas in rhesus monkeys (Tian and Rauschecker, 2004), and the anterior auditory field (AAF) in mice (Trujillo et al., 2011). In addition, regions of the ultrasonic field (UF) have been noted to prefer frequency modulations (Stiebler et al., 1997) and are selective for reversals in FM direction (Honma et al., 2013; Tsukano et al., 2016, 2015). While these studies highlight the potential specialization of cortical regions for FM sweep processing, a more comprehensive mapping of FM selectivity, which would facilitate the search for dedicated FM areas, is lacking.

Optical Ca^{2+} imaging opens the door for powerful studies on the functional architecture of cortical circuits by allowing for functional imaging with both large scale and single neuron resolution (Issa et al., 2014; Wekselblatt et al., 2016). In particular, a multiscale approach in transgenic mice provides for visualization of both global maps under widefield epifluorescence imaging and cellular-resolution maps under two-photon imaging in the same preparation. Here, we perform multiscale imaging in search of a functional organization for the rate of FM sweeps in the mouse auditory cortex. We find that tonotopic regions prefer slow-FM sweeps while fast-FM regions overlap with tone-insensitive regions in a manner that cannot be predicted from sensitivity to bandlimited noise. These global organizational features are consistent down to the single-neuron level, pointing towards a fine-scale organization of mouse auditory cortex for the processing of behaviorally-relevant complex features of sound.

2. Material and methods

2.1. Animal surgery

All animal procedures were approved by the Johns Hopkins Institutional Animal Care and Use Committee and have been described previously (Issa et al., 2014). Cre-dependent GCaMP3 mice (R26-lsl-GCaMP3, Ai38 from Jackson Labs, JAX no. 014538) (Zariwala et al., 2012) were crossed with Syn1-Cre mice (JAX no. 003966) (Zhu et al., 2001) or Emx1-Cre mice (JAX no. 005628) (Gorski et al., 2002), resulting in GCaMP3-Syn1 (Syn1-Cre; R26-lsl-GCaMP3) or GCaMP3-Emx1 (Emx1-Cre; R26-lsl-GCaMP3) mice to be used for imaging experiments. These transgenic mice allow for stable and widespread expression of GCaMP3 in neurons of the neocortex, thus facilitating functional imaging of neural activity. A total of 11 males and 7 females were used; all of the females were virgins. Under isoflurane anesthesia (1–2% in 0.5 L/min O_2), tissue overlying left auditory cortex was exposed and the skull was fixed with dental cement to a custom headpost. Body temperature was maintained near 37 °C as measured by a rectal probe. Lidocaine (2%) with epinephrine (1:100,000) was applied locally for analgesia. Dexamethasone (~5 mg/kg, i.p.) was administered for inflammation and normal saline (0.5 ml, i.p.) for dehydration. The skull was thinned by a high speed drill (Foredom MH-170) and covered with either 0.9% saline or petroleum jelly to increase transparency. At this point the mouse was restrained and given a few minutes to recover from anesthesia before performing widefield transcranial imaging, after which the mouse was returned to 1% isoflurane and a 1–2 mm craniotomy performed. To dampen pulsations, the craniotomy was filled with 1.5–2% agarose (A9793, Sigma-Aldrich) and a glass coverslip affixed with dental cement. For a subset of experiments (138 of 920 active neurons in this study), immediately following the craniotomy AM ester of Fluo-2 MA (TEFLabs) at a final dye concentration of ~0.75 mM was pressure injected (10–50 kPa) via glass pipette 200–300 μm below dura under two-photon imaging guidance. Fluo-2 provided brighter signal but was extruded from cells within 2–3 h, so for the majority of experiments we relied on GCaMP3 fluorescence. After waiting at least 10 min post-anesthesia, two-photon imaging was performed on unanesthetized restrained mice with the head rotated ~45° about the coronal axis to bring the surface of left auditory cortex perpendicular to the microscope objective.

For a subset of experiments, GP4.12 *Thy1*-GCaMP6s mice (Dana et al., 2014, p. 1) were implanted with chronic windows. Surgical procedures were identical to those described above for acute windows except that instruments were sterilized by autoclave and mice were given carprofen (5 mg/kg, s.c.) perioperatively for inflammation and buprenorphine (0.5 mg/kg, i.p.) postoperatively for pain. After 7–10 days, mice were habituated to head-fixation while running on a ball before imaging sessions began. Imaging habituated mice on the ball appeared to minimize both discomfort for the mice and motion artifacts of the imaging field. While running has been shown to suppress auditory cortical activity (Schneider et al., 2014), for the purposes of this study we did not examine the influence of running behavior on cortical responsiveness.

2.2. Imaging

Widefield imaging was performed using a GFP filter cube (460/50 excitation, 540/50 emission) and a white light source (X-cite 120Q or LED Engin LZ1-10CW00). GCaMP3 fluorescence was collected through a 10 × 0.25 NA objective (Olympus) by a Photometrics CoolSnap HQ camera, furnishing a 2 × 2 mm^2 field of view with a pixel size of 15 × 15 μm^2. Illumination power density was 0.25 mW/mm^2. For GCaMP6s, a Photometrics Evolve 512 camera was used instead.

Two-photon Ca^{2+} imaging was performed using an Ultima system (Prairie Technologies) with a mode-locked laser (Coherent Chameleon XR Ti:Sapphire) tuned to 950 nm and raster scanned at 5–12 Hz via a pair of galvanometer mirrors. Laser power at the sample was between 20 and 80 mW. Typically a 40 × 0.8 NA objective (Olympus) was used, although in some instances a 25 × 1.05 NA objective (Olympus XLPlan N) was used. Resolution along the *y*-axis was reduced by a factor of 4 × for faster imaging, yielding a final pixel size of 0.45 × 1.8 μm (or 0.7 × 2.9 μm with the 25 × objective). Dwell time was either 2 or 4 μs. Green emission channel (525/70 nm) was used for GCaMP3 or Fluo-2 fluorescence. Cells were imaged at depths of 150–430 μm, with the majority (73%) between 200 and 300 μm.

2.3. Auditory stimulation

The imaging set-up was located within a sound-treated room (Acoustical Solutions, Inc., AudioSeal ABSC-25). Ambient noise levels were measured using both a Bruel & Kjær ¼″ type 4939 microphone and a probe tube designed by G. Sokolich, which has a useful frequency response up to 100 kHz. With both instruments, noise was found to be below the threshold for mouse hearing, with spectrum levels over a 1 Hz band (dB *re*: 20 $\mu Pa/Hz^{1/2}$) of −10 dB or less. These calibrations have been previously published (Issa et al., 2014; Figure S4).

Sounds were delivered by a free-field speaker (Tucker-Davis Technologies, ES1) located 12 cm from the contralateral (right) ear. Using the two microphones mentioned above, intensity levels were flat within 15 dB between 3 and 96 kHz (Fig. 1C, left). Total harmonic distortions were measured during frequency sweeps and found to be in the range of −30 to −40 dB for 3–96 kHz (Fig. 1C,

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