High fidelity tonotopic mapping using swept source functional magnetic resonance imaging

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Abstract

Tonotopy, the topographic encoding of sound frequency, is the fundamental property of the auditory system. Invasive techniques lack the spatial coverage or frequency resolution to rigorously investigate tonotopy. Conventional auditory fMRI is corrupted by significant image distortion, sporadic acoustic noise and inadequate frequency resolution. We developed an efficient and high fidelity auditory fMRI method that integrates continuous frequency sweeping stimulus, distortion free MRI sequence with stable scanner noise and Fourier analysis. We demonstrated this swept source imaging (SSI) in the rat inferior colliculus and obtained tonotopic maps with ~2 kHz resolution and 40 kHz bandwidth. The results were vastly superior to those obtained by conventional fMRI mapping approach and in excellent agreement with invasive findings. We applied SSI to examine tonotopic injury following developmental noise exposure and observed that the tonotopic organization was significantly disrupted. With SSI, we also observed the subtle effects of sound pressure level on tonotopic maps, reflecting the complex neuronal responses associated with asymmetric tuning curves. This in vivo and noninvasive technique will greatly facilitate future investigation of tonotopic plasticity and disorders and auditory information processing. SSI can also be adapted to study topographic organization in other sensory systems such as retinotopy and somatotopy.

Introduction

Humans and many animal species rely on effective hearing to survive and prosper in a competitive world. The sense of hearing and the auditory system that processes acoustic information are critical for performing core functions such as communicating, finding food and avoiding danger. The auditory system is very adept at distinguishing among frequency components in a sound, which allows us to hear the fine differences in words and allows animals to distinguish the sounds of predators and prey. For example, humans can distinguish two pure tone sounds that differ by only 2 to 6 Hz even though the sounds are in the frequency range of 1 to 4 kHz (Longstaff, 2005; Malmierca et al., 2009). To enhance our abilities to perform these vital functions or to treat auditory disorders such as tinnitus (Muhlnickel et al., 1998) that reduce quality of life, we need to advance our understanding of frequency encoding in the auditory system.

Much of our knowledge of auditory function and frequency encoding comes from invasive studies (Ehret and Fischer, 1991). Sound pressure waves enter the ear and vibrate the ear drum. These vibrations are transmitted to the basilar membrane, whose motions produce periodic depolarization and hyperpolarization of hair cells (Longstaff, 2005), sending neuronal signals to the cochlear nucleus of the central auditory pathway. Axons carrying neuronal signals run from the cochlear nucleus to the superior olivary complex, then the lateral lemniscus, inferior colliculus (IC), medial geniculate body and the auditory cortex (Malmierca, 2003). At each structure of the auditory pathway, electrophysiology studies have observed that the majority of neurons have sharp frequency tuning curves, meaning these neurons are most sensitive to a narrow spectrum of sounds centered about a characteristic frequency (CF).

The findings of invasive electrophysiology and immunohistochemistry studies also suggest that neurons in a structure with similar CFs are positioned close together (Ehret and Fischer, 1991). This indicates the presence of tonotopic organization, a topographic encoding of frequency. The ideal technique for studying tonotopy would be sensitive to neuronal activity and be capable of mapping a
large field of view (FOV) with high spatial and frequency resolution. Unfortunately, the traditional techniques are not ideal. Electrophysiological recordings cannot achieve the continuous spatial coverage and large FOV needed to thoroughly study tonotopy. Immunohistochemistry techniques would require an infeasible number of animals to cover a broad frequency range and are difficult to use, if not inapplicable, in longitudinal investigations.

Functional magnetic resonance imaging (fMRI) has potential for tonotopic mapping. fMRI using the blood oxygenation level-dependent (BOLD) signal for endogenous contrast is widely used in brain mapping (Bilecen et al., 1998b; Cheung et al., 2012; Ogawa et al., 1990). BOLD fMRI is noninvasive and applicable to longitudinal human and animal studies. It is typically implemented with echo planar imaging (EPI) sequences, which provide adequately high spatial resolution (~1 mm in clinical scanners). However, EPI emits sporadic acoustic noise which adversely affects auditory fMRI (Seifritz et al., 2006). Auditory studies typically employ sparse temporal sampling paradigms, which use EPI sequences with long repetition time (on the order of 10 s), to reduce the adverse effects of scanner noise (Hall et al., 1999). This technique has proven useful, but it is highly time inefficient and like conventional EPI, images suffer from distortion and susceptibility induced signal loss (Jezzard and Balaban, 1995), which hamper studies in many fine structures in the auditory system. These limitations become more apparent at high magnetic fields and will restrain the growth of high resolution auditory fMRI.

Stimulation in fMRI tonotopy studies is typically presented in block-design paradigms (Baumann et al., 2011; Bilecen et al., 1998a). Block-design involves presenting a pure tone sound to the subject in an on–off pattern and using statistical analysis to identify brain regions where the BOLD signal correlates with the stimulus on–off timing. Recently, we applied a block-design paradigm to map tonotopic organization in the rat IC and map the ascending auditory pathway (Cheung et al., 2012). Block-design provides high statistical power and sensitivity. However, it cannot map tonotopic organization with high frequency resolution as only a limited number of pure tones can be presented in a study session. All together, the conventional fMRI techniques for tonotopic mapping using EPI and block-design suffer from image distortion, signal loss and low frequency resolution.

We develop a novel fMRI technique named magnetic resonance swept source imaging (SSI) that maps the tonotopic organization of auditory structures with high spectral and spatial resolution. Instead of EPI, SSI uses balanced steady state free precession (bSSFP), a fast MRI acquisition sequence that provides $T_2/T_1$ contrast without sparse temporal sampling (Lee et al., 2008; Zhou et al., in press), image distortion, susceptibility-induced signal loss and sporadic noise. Therefore, bSSFP avoids the time inefficiencies and image artifacts of EPI and is ideally suited for auditory fMRI studies. To improve upon block-design, SSI uses a frequency sweeping stimulation paradigm along with Fourier transformation analysis that maps tonotopic organization over a continuous frequency spectrum.

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**Fig. 1.** Left: Rat brain rendering showing the location of the inferior colliculi (ICs) in green. The rendering was built from high resolution MRI anatomical images covering the entire brain. The ICs are large ellipsoidal nuclei in the midbrain that serve as relay centers for all ascending inputs in the mammalian auditory system. Right: A single slice coronal anatomical image centered at bregma $-8.5$ mm covering both ICs. This slice localization was used in all tonotopic mapping experiments.

**Fig. 2.** Schematic of hemodynamic responses measured from a voxel by swept source imaging (SSI) during the sweep up and down scans. All neurons in a voxel were assumed to have the same characteristic frequency (CF). At $t_b$, which was directly related to the frequency encoded by the voxel, these neurons responded to a narrow range of stimulation frequencies about CF. Top: The neurons fired shortly after $t_b$, but the BOLD signal rose only after the hemodynamic delay time $t_H$. Bottom: When the frequency sweeping direction was reversed, the direction of $t_H$ was not changed. Two scans with opposite frequency sweeping directions were required because the phase information obtained after Fourier transformation in a single scan was not determined by CF alone. Phase also depended on the hemodynamic delay, the duration of the response and the shape of the response. The duration of the hemodynamic response depended on the frequency sweeping rate and the bandwidth of neurons within the voxel. By sweeping in opposite directions, the encoded frequency and the hemodynamic delay time could be decoupled for each voxel.
In this study, we first describe SSI and demonstrate its ability to map tonotopy in the rat IC. In normal animals, the new tonotopic maps show significantly higher frequency resolution and spatial fidelity compared with conventional fMRI maps. We subsequently apply SSI to study the IC of animals injured by early post-natal noise exposure (NE) and find that the tonotopic organization is significantly disrupted. We also observe with SSI the subtle effects of sound pressure level (SPL) on tonotopic maps, reflecting the neuronal responses associated with asymmetric tuning curves.

Methods

All animal experiments were approved by the local animal research ethics committee. Four different fMRI experiments were performed in this study: (1) mapping inferior colliculus tonotopy in normal animals with magnetic resonance swept source imaging; (2) studying the effect of increasing stimulus sound pressure level on the BOLD signal amplitude; (3) mapping tonotopic changes caused by early post-natal noise exposure; and (4) studying the effect of SPL on observed tonotopic maps. This section details the animal preparation, auditory stimulation setup, auditory stimulation paradigm, MRI acquisition and fMRI data analysis involved with each experiment.

Experiment #1: swept source imaging and tonotopic mapping in normal IC

Animal preparation

Normal male Sprague–Dawley rats (n = 8, 250 to 300 g) were prepared for fMRI sessions as described in our earlier studies (Cheung et al., 2012; Lau et al., 2011a, 2011b). In brief, animals were anesthetized using isoflurane (about 3% for induction and 1% for maintenance) and kept warm with circulating water throughout the experiment. Respiration rate, heart rate, oxygen saturation and rectal temperature were monitored (SA Instruments). Animals were positioned in a birdcage transmitt-only coil with 68 mm inner diameter (tapered to 2 mm in diameter over the last 7.5 cm) acrylic sound delivery tube. The narrow end of the tube was connected to a 6.5 cm long, 2 mm inner diameter flexible polyurethane tube that fitted into the right ear canal. Sound waveforms were measured from experiments using an omnidirectional condenser microphone (M50, Earthworks) and sampled using a recorder (FR2, Fostex). Spectra are presented in Supplementary Fig. S1. The left ear was occluded with an ear plug made of cotton wool and vaseline.

Auditory stimulation setup

The monaural sound stimuli presented to all animals were produced using an ultrasonic loudspeaker (L010, Kemo) driven by a power amplifier (Altitude 3600, Rhyme Acoustics) and a waveform generator (33120A, Hewlett-Packard). Sound waves were delivered to the rat via a 1 m long, 2 cm inner diameter (tapered to 2 mm in diameter over the last 7.5 cm) acrylic sound delivery tube. The narrow end of the tube was connected to a 6.5 cm long, 2 mm inner diameter flexible polyurethane tube that fitted into the right ear canal. All sound spectra were measured prior to experiments using an omnidirectional condenser microphone (M50, Earthworks) and sampled using a recorder (FR2, Fostex). Spectra are presented in Supplementary Fig. S1. The left ear was occluded with an ear plug made of cotton wool and vaseline.

Auditory stimulation paradigm

The auditory frequency sweeping stimulation paradigm used by SSI consisted of two 880-s scans. For the first scan, frequency was swept linearly from 1 to 40 kHz (sweep up) in 40 s and repeated 22 times. For the second scan, the frequency was instead swept from 40 to 1 kHz (sweep down). For all animals and both scans, the fMRI images acquired during the first two sweep cycles were discarded. The SSI experiment time was less than 30 min for each animal. The amplitude envelope of the frequency sweeping stimulus was recorded and shown in Supplementary Fig. S1. The sweeping paradigm is conceptually similar to paradigms used to study continuous frequency modulation. The locations of the voxels are shown on a MRI anatomical image of the IC. The error bars indicate the standard deviation across the 20 sweeping cycles and the red arrows point to the cycling frequency (1/40 s$^{-1}$ or 0.025 Hz) where coherence and phase were recorded. Time profiles from the sweep up (left) and down (right) scans showed that these two voxels were activated at different t opportunity, corresponding to different encoded frequencies.

MRI acquisition

Experiments were performed on a Bruker 7T PharmaScan scanner using a birdcage transmit-only coil with 72 mm inner diameter in combination with an actively decoupled receive-only quadrature surface coil (Chan et al., 2010; Lau et al., 2011a, 2011b). Balanced steady state free precession (bSSFP) functional images used by SSI were acquired from a single coronal slice covering the entire IC, centered at bregma = −8.5 mm (Paxinos and Watson, 1998) (Fig. 1), with gradient ramping time = 420 µs, TR/TE = 3.8/1.9 ms, flip angle = 30°, phase advance = 180°, field of view (FOV) = 32 × 32 × 120 mm$^3$, data matrix = 64 × 64 (zero-filled to 128 × 128) and NEX = 4 for an effective temporal resolution of 1 s (Zhou et al., in press). The IC was chosen as a target to demonstrate the tonotopic mapping capabilities of swept
source imaging because its tonotopic organization was relatively simple and well understood. bSSFP acquired 880 volumes (first 80 discarded) during each of the sweep up and down scans of SSI. bSSFP was compared with single-shot echo planar imaging used in conventional fMRI. EPI functional images were acquired from the same localization with TR/TE = 1000/28 ms, FOV = 32 × 32 × 120 mm³ and data matrix = 64 × 64 (zero-filled to 128 × 128). EPI acquired 410 volumes (first 50 s discarded) per block-design scan. High resolution anatomical images were also acquired with the same localization as the fMRI images. Fig. 1 illustrated the location and shape of the ICs in both hemispheres as observed from an anatomical image.

fMRI data analysis

fMRI images were first realigned with two dimensional rigid-body motion correction followed by linear detrending on a voxel level. The Fourier analysis of the images acquired by SSI was performed using custom software executed in Matlab (Mathworks). The coherence and phase spectra from each sweep were extracted from the time profiles at each voxel using Fourier transformation. The coherence spectrum was defined as the amplitude at a frequency divided by the square root of the sum of squared amplitude at all frequencies. Its value lies between zero and one and it reflects the consistency of BOLD signal fluctuations with a frequency. SSI specifically uses coherence and phase at the cycling frequency (1/40 s⁻¹ or 0.025 Hz). The average coherence (cmean) map of the two sweeps was obtained for each animal by the following formula: $c_{\text{mean}} = \sqrt{c_{\text{up}}^2 + c_{\text{down}}^2}/2$, with $c_{\text{up}}$ and $c_{\text{down}}$ the coherence maps from the two sweeps (at the cycling frequency). Because any delay in the temporal domain is directly related to the linear phase modulation in the Fourier domain, response time (RT, ranging from 0 to 40 s) could be determined as the phase at the cycling frequency (ranging from 1 to 40 kHz) was then computed as $((\text{RT}_\text{up} - \text{RT}_\text{down})/40 \times 39 \text{kHz} + 1 \text{ kHz})$ on the voxel level without further processing. SSI data from all eight animals were group averaged by interpolating the mean bSSFP image of each animal to $320 \times 320$ and normalizing them to a template animal. The resulting averaged coherence and tonotopic maps were transformed and combined accordingly. For comparison with SSI, we analyzed the block-design EPI images used in conventional fMRI tonotopy experiments with standard cross correlation analysis (Chan et al., 2010; Cheung et al., 2012; Lau et al., 2011b) to obtain correlation coefficients (r) that represent degree of activation.

Experiment #2: effect of increasing stimulus sound pressure level (SPL) on BOLD signal amplitude

The data acquisition and analysis procedures were the same as those for conventional fMRI in Experiment #1 except for the following differences: (i) bandlimited noise generated using in-house Matlab code was presented to the animals in place of pure tones (spectrum in Supplementary Fig. S1); (ii) four blocks were used per block-design scan, instead of six, and sound pressure level (SPL) was varied over a 36 dB range in 6 dB increments, instead of frequency; (iii) bSSFP scans acquired 290 volumes (first 50 s discarded), instead of 410; and (iv) seven scans, instead of nine, were performed per animal. Eight normal animals were studied (different from the group in Experiment #1). We further computed the BOLD signal amplitude after averaging the four blocks at each SPL by dividing the mean BOLD signal from 10 to 20 s after onset of stimulation by the average signal from 10 s before to onset of stimulation. The voxel with highest r at each SPL was identified. The signal amplitudes at these voxels were plotted against SPL and fitted to a line using least squares optimization and the squared correlation coefficient ($r^2$) was computed.
Experiment #3: tonotopic changes caused by early post-natal noise exposure

All SSI tonotopic mapping procedures were identical to those used in Experiment #1. A litter of rat pups (n = 8) along with their mother were placed in a separate cage for seven consecutive days with a 12 h light/dark cycle during their developmental stage (post-natal days 14 to 21). They were continuously exposed to broadband noise trains produced using a waveform generator (33120A, Hewlett-Packard) and played via a pair of tweeters (SSI Electronics). The SSI image was obtained at approximately 100 dB (see Supplementary Fig. S1 for the spectrum). The NE animals were returned to normal housing after the exposure period and were scanned at five months of age. No seizures were observed in these animals.

Experiment #4: effect of SPL on observed tonotopic maps

All SSI tonotopic mapping procedures were identical to those used in Experiment #1. Four different normal animals were used. Tonotopic mapping was performed in each animal by stimulating with the baseline SPL (as used in Experiments #1 and #3) and with an additional 12 dB. The effect of SPL was examined by taking the difference between two sets of tonotopic maps. Note that unlike in Experiment #2, tonotopic maps of the IC at multiple SPLs instead of the BOLD signal amplitudes were acquired.

Results

The findings of this study are organized according to the four experiments described in the Methods section.

Experiment #1: SSI and tonotopic mapping in normal IC

Tonotopic maps are acquired from the inferior colliculus of normal rats using swept source imaging and conventional fMRI to demonstrate the capabilities of the new imaging technique. Fig. 3 presents the typical time profiles and coherence spectra of acquired signals from two voxels in a representative animal. Prominent peaks were observed in all the coherence spectra, a measure of activation, at the cycling frequency. Time profiles from the sweep up and down scans show that these two voxels were activated at different times. The coherence and tonotopic maps computed from the time profiles in Fig. 3 are shown in Fig. 4. The maps are overlaid on the anatomical image shown in Fig. 1 without spatial coregistration error due to the distortion free bSSFP sequence used by SSI. Coherence and tonotopic maps were obtained from the amplitude and phase information after Fourier transformation of the time profiles at each voxel. In the sweep up scan, the response time (computed from phase) increased gradually from dorsolateral to ventromedial IC. When the sweeping direction was reversed (sweep down), the response time progression was reversed, indicating that the phase information from Fourier transformation was sensitive to and correlated with the frequency encoded by the voxel. The coherence maps were not significantly dependent on the sweeping direction. From the tonotopic map, dorsolateral IC encoded lower frequencies while ventromedial IC encoded higher frequencies. The encoded frequency increased monotonically from dorsolateral to ventromedial IC. This tonotopic map was in excellent agreement with the invasive findings illustrated in the right hemisphere (Malmierca et al., 2008). The location and shape of the iso-frequency bands (voxels encoding similar frequencies) at different stimulation frequencies are shown in video format in Video S1. The frequency resolution of SSI was estimated by taking the difference between the encoded frequencies of two adjacent voxels in the dorsolateral IC (3.6 kHz and 5.7 kHz shown in Fig. 5). The tonotopic map has 2.1 kHz frequency resolution, in the dorsolateral IC and along the dorsolateral–ventromedial direction, and 40 kHz bandwidth. A v-shaped tonotopic organization can also be observed at higher frequencies. Fig. 6 presents the grouped averaged tonotopic map acquired from normal animals (n = 8). The encoded frequency increased monotonically from dorsolateral to ventromedial IC and from dorsal to ventral IC. A v-shaped tonotopic organization is also observed at 16 kHz. SSI’s tonotopic map was in close agreement with the findings of electrophysiological recordings (Malmierca et al., 2008) (refer to Fig. 3 in the reference). Noninvasive SSI therefore provides accurate and precise tonotopic mapping over a broad frequency range.

Fig. 7 compares the tonotopic maps acquired using SSI and conventional fMRI (block-design stimulation paradigm and EPI acquisition sequence). Functional images acquired using single-shot EPI were significantly distorted, especially along the phase-encoding (dorsal–ventral) direction. This was not the case for bSSFP images acquired by SSI. The block-design activated regions were qualitatively consistent with those activated using frequency sweeping. Although distinct iso-frequency bands could also be identified using conventional fMRI, they failed to show the progressive changes in encoded frequency observed by SSI. SSI not only was more time efficient, but also yielded significantly higher frequency resolution and spatial fidelity than the conventional fMRI approach. These findings demonstrate that SSI is a significant advancement over conventional fMRI tonotopic mapping techniques.

Experiment #2: effect of increasing SPL on BOLD signal amplitude

Fig. 8 plots the sound pressure level dependence of the BOLD signal amplitude. The BOLD signal amplitude measured with a block-design paradigm was observed to increase almost linearly with the applied SPL ($r^2 = 0.98$ computed with linear regression). Such a relationship indicated that BOLD activations detected by SSI can be considered as the additional activation to the baseline activation caused by the continuous and relatively smooth bSSFP scanner acoustic
noise. One important attribute of SSI using bSSFP is the stable scanner noise compared to that of conventional EPI, which emits sporadic noise. This is shown in Supplementary Fig. S1. Compared to background noise recorded when the scanner is inactive, the noise time profile during EPI has a spike every TR. These spikes are not present in the bSSFP profile.

Experiment #3: tonotopic changes caused by noise exposure

Having demonstrated the improvements offered by SSI over conventional fMRI, we applied SSI to study the effects of early post-natal noise exposure on later tonotopic organization. Fig. 9 presents the coherence and tonotopic maps acquired from NE animals. These animals were reared in a loud acoustic noise environment from post-natal day 14 to 21. Invasive studies have shown that tonotopy was disrupted by early noise exposure (Pierson and Snyder-Keller, 1994), but noninvasive imaging studies have not been used to provide more detailed information about the injury and subsequent recovery. The coherence map acquired by SSI showed that the frequency sweeping stimulus did not activate the dorsolateral region of the IC responsible for encoding low frequencies. The tonotopic organization was in general significantly disrupted in NE animals. This was likely due to noise exposure during the critical period of tonotopic development. Of the eight rats exposed to noise, six showed a tonotopic map with significantly disrupted tonotopic organization and the remaining two showed maps similar to those of normal animals. This finding lays the groundwork for future longitudinal SSI studies of tonotopic development and plasticity.

Experiment #4: effect of SPL on tonotopic maps

SSI can also be applied to observe the subtle effects of sound pressure level on tonotopic maps. Fig. 10 presents the effect of changing stimulation SPL on IC frequency encoding in four normal animals. A neuron’s response to auditory stimuli of varying SPL and frequency is governed by its tuning curve (Hernandez et al., 2005). Voxels in the middle of the IC showed prominent increases in encoded frequency while those in the dorsal and ventral IC showed less prominent increases and decreases, respectively. Such changes of encoded frequency with increased SPL likely resulted from the overall asymmetric frequency tuning characteristics of auditory neurons in the IC. Thus, varying both SPL and frequency may enable SSI to probe variations in the tuning curves within and across structures of the auditory system.

Discussion

Swept source imaging mapped tonotopy with high fidelity at approximately 2 kHz resolution and 40 kHz bandwidth in 30 min. The resulting tonotopic maps from each animal yielded significantly higher frequency resolution and spatial specificity compared with maps acquired using conventional fMRI and were in excellent agreement with previous invasive findings (Huang and Fex, 1986; Webster et al., 1984). Using SSI, we observed that early post-natal noise exposure significantly disrupted the tonotopic organization and increasing stimulation sound pressure level increased the frequency encoded in dorsal and middle IC and decreased that encoded in ventral IC.

Comparison with existing brain mapping techniques

Tonotopy was first studied with invasive techniques such as electrophysiology and immunohistochemistry, which observed that iso-frequency layers in the IC have approximately the same extent and orientation as the dendritic lamellae (Faye-Lund and Osen, 1985). In the large central nucleus of the IC (CNIC), the iso-frequency layers run from dorsomedial to ventrolateral (Huang and Fex, 1986; Malmierca et al., 1993, 1995, 2008; Webster et al., 1984). The tonotopic maps acquired with SSI were in excellent agreement with these findings. SSI’s maps clearly demonstrated that iso-frequency bands run from dorsomedial to ventrolateral IC. In the external cortex of the IC, the tonotopic organization is different from that in the CNIC (Loftus et al., 2008; Malmierca et al., 2011). This difference may have been observed by the tonotopic map in Figs. 4 to 6. The v-shaped tonotopic organization at middle and high frequencies in the ventral IC is very similar to that in Fig. 5 of (Loftus et al., 2008).

More recently, functional imaging technologies such as fMRI and positron emission tomography (PET) have been used to map tonotopy in human auditory cortex. However, PET lacks the spatial specificity necessary to fully explore tonotopic organization in the auditory
cortex (Johnsrude et al., 2002). In comparison, the high spatial resolution of fMRI allows detailed examination of tonotopy in humans and animals such as rodents and primates. In a recent study, we performed tonotopic mapping in the rat IC using conventional block-design stimulation and EPI acquisition (Cheung et al., 2012). Low (7 kHz) and high acoustic frequencies (40 kHz) were encoded in the dorsolateral and ventromedial IC, respectively, while middle frequencies were encoded in between. Manganese enhanced MRI, another MRI technique that uses the metallic contrast agent manganese, has also been used to acquire frequency-specific information in mouse IC (Yu et al., 2005). Pure tone (40 kHz), bandlimited noise (20 to 50 kHz) and broadband noise (1 to 59 kHz) stimuli produced MRI signal enhancements in different regions of the mouse IC. However, our SSI approach provides higher frequency resolution than these in a significantly shorter period of time and is non-toxic. Its noninvasive nature permits human studies and longitudinal studies. SSI is well positioned to be the tonotopic mapping technique of choice in investigation of various auditory structures (see Supplementary Fig. S2) and (Cheung et al., 2012). It may solve scientific controversies such as local or global tonotopic organization in the primary auditory cortex (Castro and Kandler, 2010) and permit tonotopic mapping in human subcortical nuclei, where observing BOLD activation is challenging (Guimaraes et al., 1998).

**Improvements with MRI hardware advances**

SSI with existing MRI hardware is already an excellent tonotopic mapping technique, but it will benefit significantly from emerging MRI technological advances such as higher magnetic field scanners (Van der Linden et al., 2007), cryogenic radiofrequency probes (Baltes et al., 2011) and parallel imaging (Chappell et al., 2011; de Zwart et al., 2002). These advances will provide better sensitivity and reduce scanner noise during fMRI acquisition. In contrast, EPI based techniques suffer more significant image distortion at higher fields, which can limit the improvements that conventional fMRI tonotopic mapping techniques gain from hardware advances. SSI’s capabilities are well positioned to improve with rapidly advancing MRI hardware.

**IC tonotopy in post-natal noise exposure rats**

SSI noninvasively mapped, for the first time, tonotopy in early post-natal day 14 to 21 (P14 to P21) noise exposure animals and observed that the tonotopic organization was significantly disrupted (Fig. 9). Invasive studies have shown that the banded pattern of tonotopy exists before the onset of hearing (Gabriele et al., 2000) and extensive refinement of frequency encoding occurs over the proceeding 2 to 5 weeks (Bures et al., 2010). The period P9 to P28 (our study used P14 to P21) was shown to be important for IC tonotopic development (Oliver et al., 2011) while P11 to P13 was important for primary auditory cortex development (de Villers-Sidani et al., 2007). The damage observed by SSI was likely due to noise exposure during this critical period of development, which altered the representation of sound frequency and pressure level (Bures et al., 2010; Grecova et al., 2009). Note that intense noise exposure in general can disrupt the tonotopic organization in IC (Pierson and Snyder-Keller, 1994). SSI further observed that the dorsolateral IC normally encoding lower frequencies was not activated in the coherence map, indicating possible damage of frequency encoding in that region. The loss of low frequency encoding was likely caused by noise exposure during the early part of the critical period as neurons in the IC with lower characteristic frequencies (CFs) develop first (Romand, 1997). Also, there is growing evidence that suggests that auditory synapses in the brainstem express experience dependent activity (Kandler et al., 2009). In this study, noise exposure disturbed the frequency tuning of neurons.
that developed during the early post-hearing stage, supporting the claim that experience dependent activity is essential for tonotopic refinement in IC.

In contrast to our present findings, Izquierdo et al. (2008) exposed rats aged 96 to 341 days to pure tones at 5 or 8 kHz, between 110 to 121 dB and for 3.3 to 16 h. They observed that the normal stepwise representation of sound frequency in the IC was altered. Affected regions “appeared to be invaded by adjacent normal frequencies”, but there was still tonotopic organization. Further, the authors did not observe significant differences compared to controls in low frequency regions. The differences between this study and our present study may be related to the significant difference in the ages of exposed rats in the two studies. Also, the acoustic exposure durations, sound pressure level, and bandwidths (pure tone vs. broadband noise) were significantly different. Oliver et al. (2011) performed a complementary study where they exposed rats to 14 kHz pure tone sound at 60 to 70 dB (below injury threshold) from P9 to 28 for 14 to 18 h/day. They observed that more IC regions responded to 14 kHz. Therefore, unlike the damaging sounds presented in our study and in that by Izquierdo, a low SPL pure tone presented during the early post-natal period increases the representation of the pure tone frequency in the tonotopic map.

Varying sound pressure level to probe local auditory neuronal characteristics

We varied SSI’s stimulation SPL to observe the subtle effects of sound pressure level on tonotopic maps. We observed changes in encoded frequency across the IC (Fig. 10). The encoded frequency measured by SSI is related to the effective response time of BOLD activation. Response time depends on the properties of the stimulation paradigm, such as the frequency sweeping duration and mode (linear or logarithmic), and the complexity of the frequency tuning curves of neurons within the voxel (Fig. 2). Most of the frequency tuning curves are V-shaped and often asymmetric (Ehret and Schreiner, 2005) and hence, the neurons respond to a broader bandwidth of stimuli at higher stimulation SPL. We observed a general increase of encoded frequency in the dorsolateral IC with SPL, especially around the middle of the IC. This likely reflected the fact that neurons operated in the peripheral regions of their tuning curves at higher SPL and neurons with low CFs have more area under the tuning curve at frequencies above the CF. This lead to a shift in the effective response time measured by SSI because the center of the neurons’ bandwidth shifted to a higher frequency. Similarly, the encoded frequency in the ventral IC decreased to a lesser extent likely because neurons with high CFs have more area under the curve at frequencies below the CF. Significant heterogeneity of tuning curve shapes has been observed in the IC both within and across the iso-frequency lamina (Ehret et al., 2003; Hernandez et al., 2005). Our experimental results indicated that SSI is sensitive to these subtle changes in local frequency encoding. By manipulating SPL and the stimulation paradigm, SSI could probe the tuning curve characteristics of neurons across the auditory system. Note that changes in hemodynamic response measured by BOLD fMRI have been related to corresponding changes in neuronal firing by earlier multimodality studies (Mukamel et al., 2005). However, the exact relationship between BOLD signals, spiking activity of neurons, and local field potentials is still under investigation (Nir et al., 2008).

Effect of acoustic scanner noise

Scanner noise has been a significant limiting factor in auditory fMRI studies. EPI sequences used by conventional auditory fMRI techniques emit sporadic acoustic noise that confounds fMRI results (Seifritz et al., 2006). Sparse temporal sampling EPI sequences reduce this problem, but they are prohibitively time consuming for tonotopic mapping. In this study, acoustic stimuli were presented simultaneously with the continuous and relatively smooth scanner noise emitted by bSSFP. Note that the level of interference in auditory fMRI by scanner noise depends on both the temporal and spectral overlap of the scanner noise with the acoustic stimulus. Given the sporadic nature of EPI scanner noise, the intended stimulus is more likely to be contaminated by EPI based fMRI. As shown in Fig. 8, the BOLD signal amplitude increased in an approximately linear manner with SPL within the range studied. Such a trend was consistent with electrophysiological findings (Ehret and Schreiner, 2005). The rate level functions of most IC neurons increase monotonically with SPL, indicating the neuronal firing rate and the number of active neurons increase with SPL. This linear relationship provided the basis for the robust detection of auditory activation by continuous bSSFP scanning demonstrated in this study. It indicated that the BOLD signal detected by SSI can be viewed as the additional activation to the baseline activation caused by the continuous and relatively smooth bSSFP scanner noise.

In conclusion, we developed magnetic resonance swept source imaging integrating bSSFP acquisition and acoustic frequency sweeping stimulation for efficient and high fidelity tonotopic mapping. We demonstrated SSI in the rat inferior colliculus and acquired tonotopic maps with approximately 2 kHz resolution and 40 kHz bandwidth, vastly superior to conventional fMRI maps and in excellent agreement with invasive findings. Changing SPL allowed SSI to detect tonotopic shifts and potentially characterize the auditory neuronal responses. We further studied tonotopic injury following developmental noise exposure, facilitating future longitudinal studies. The results of this study were the first noninvasive mapping of tonotopic organization in rodents. SSI can be readily applied to study tonotopy in all auditory structures and in humans and other animal models. This in vivo and noninvasive technique will greatly facilitate future investigation of auditory information processing and tonotopic changes due to disorders, plasticity, development and aging. SSI can also be adapted to study topographic organization in other sensory systems such as retinotopy and somatotopy.
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