Statistical mapping of auditory activity by Manganese-enhanced MRI

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Introduction and Significance

Our previous studies have established the utility of Mn-Enhanced MRI (MEMRI) to detect the pattern of sound-evoked activity within the mouse auditory midbrain, inferior colliculus (IC) [1]. The present goal was to establish an unbiased, three dimensional (3D) statistical image analysis protocol to map the neuronal coding of acoustic signals in the mouse IC. This protocol has enabled quantification of 3D IC activity via voxel-by-voxel comparison of sound-evoked MEMRI activity patterns. By using analysis of variance (ANOVA), we reconstructed 3D iso-frequency bands of different pure tones (4, 16, 40 kHz) in the IC. ANOVA analysis of MEMRI data also revealed the spatial distribution of the amplitude sensitive neurons, which play important role in acoustic perception. These results form the basis for future analyses of altered auditory signal processing in the mouse brain following genetic or environmental manipulation.

Methods

The MEMRI protocol is similar to previously described [1]. Briefly, mice were injected IP with 0.4 mmole/kg body weight of $MnCl_2$ in saline at postnatal 19 to 21 days, exposed to 24 hr of defined sound stimulation, and then anesthetized with isoflurane (1-1.5% in air) during MRI. Three pure tones (4, 16 and 40 kHz) were used with amplitude modulated between 65 and 89 dB. To detect the amplitude sensitive neurons, we also exposed mice with 40 kHz pure tone at different amplitudes (65, 77 and 89dB Peak). Normally behaving mice were maintained in a free field inside an acoustic isolation chamber during sound exposure (Mac-1; Industrial Acoustics). MRI was performed on a SMIS console interfaced to a 7T horizontal bore magnet with 250-mT/m actively shielded gradients (Magnex), using a custom mouse head holder and volume coil. MR images were acquired using 3D T1-weighted gradient echo sequence (TE/TR=4/50ms) with 100-µm isotropic spatial resolution and an acquisition time of 2 hours. 3D images of the IC were analyzed with Amira (Mercury Computer Systems). The volumetric MRI data from each mouse brain were extracted, co-registered and normalized to establish a uniform ground mean of the whole brain. The IC was then segmented from 3D brain images using an interactive threshold-based region-growing algorithm. Voxel-by-voxel 3D T-test and ANOVA statistical analysis protocols (Matlab) were used to analyze the activated IC neuronal populations.

Results

Three groups of mice were exposed to pure tones: 4 kHz, 16 kHz or 40 kHz. There were obvious differences in IC-enhancement (Fig 1): a finger-like pattern of enhancement through the mid-dorsal axis of IC after 4 kHz pure tone stimulation (N=8; Fig 1B); a central enhanced region of IC after 16 kHz pure tone stimulation (N=8; Fig 1C); and a clear ventral-caudal band after 40 kHz pure tone stimulation (N=8; Fig 1D). ANOVA p-maps showed three frequency bands corresponding to the three pure tones (Fig 1F), consistent with previously published tonotopic maps in the mouse (Fig 1E), demonstrating similar spatial sensitivity as *in vivo* electrophysiology and providing verification of MEMRI 3D brain mapping [2]. The amplitude-sensitive neurons can be detected by comparing MEMRI enhancement patterns after stimulation with variable amplitudes (65, 77 and 89 dB at 40 kHz). ANOVA p-maps reveal that this important population of neurons (Fig 2D, yellow) surrounds the high activity 40 kHz region (Fig 2G, red), which was localized by T-test comparison between control and 40 kHz (89dB) stimulated mice (Fig 2E). A similar distribution of amplitude-sensitive neurons surrounding the high activity 16 kHz region has also been observed (data not shown). At the amplitudes used in these experiments, we expect most of the monotonic IC neurons (spike rate increases with sound level) to be saturated, suggesting that the ANOVA maps are showing, for the first time, the spatial distribution of non-monotonic IC neurons (spike rate decreases with sound level after reaching peak value), which are believed to play a critical role in acoustic perception [3] [4].

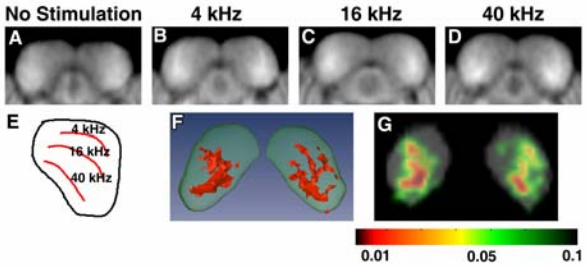

Fig1. Activity patterns of three different pure tones are shown in 2D images (top row, A-D). ANOVA analysis p-maps show three frequency bands corresponding to 4, 16, 40 kHz pure tones (bottom panel: E, electrophysiological tonotopic map; F, 3D p-map p<0.05, red contour; G, 2D-slice with p-value colorbar).

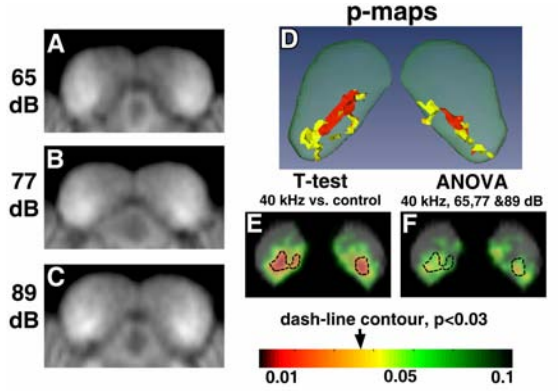

Fig2. Activity patterns were acquired for three different amplitudes (65, 77 & 89 dB) of 40 kHz pure tone (A-C). ANOVA analysis reveals the amplitude-sensitive neuronal population (D, yellow, p<0.05) surrounding the iso-frequency activity 40 kHz band (D, red, T-test, p<0.01). This distribution of amplitude-sensitive neurons is confirmed in 2D p-maps (E, F). A contour of the iso-frequency activity 40 kHz band has been defined in figure E and overlaid in figure F .

Conclusions

Statistical analysis of MEMRI enhancement patterns provides a quantitative, 3D method for mapping sound-evoked activity in the mouse IC. Unbiased MEMRI statistical maps are consistent with previous studies using electrophysiology and reveal a clear coding of sound frequency and amplitude in IC neurons. These results further our understanding of signal processing in the auditory system and provide an efficient method for mapping sound frequency and amplitude in the mouse brain. Future studies of defined mouse mutant will enable analysis of the genetic basis for sound processing in the mammalian brain.

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Reference

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