Tonotopic mapping of the mouse auditory midbrain with Mn-enhanced MRI

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Introduction and Significance

Our previous studies have established the utility of Mn-enhanced MRI (MEMRI) for detecting accumulative sound-evoked neuronal activity in the mouse auditory midbrain, the inferior colliculus (IC) [1]. Numerous previous investigations using electrophysiology have established that neuronal populations in the IC display distinct sensitivity and response patterns to sound-stimuli with different frequency components (tonotopicity) [2] and sound pressure levels [3]. For each given sound-stimulus with defined frequency and amplitude components, a unique pattern of active neurons will encode the acoustic information in the IC. The goal of the current studies was to establish the feasibility of detecting and mapping the three-dimensional, frequency-dependent patterns of activity in the mouse IC using MEMRI.

Methods

Mn administration and sound stimulation were performed as previously described [1]. Briefly, mice were injected IP with 0.4 mM/kg body weight of $MnCl_2$ in saline at postnatal 19 to 21 days, exposed to sound stimulation for 24 hr, and then anesthetized with isoflurane (1-1.5% in air) during MRI. Sound stimulation consisted of calibrated exposure levels (88 dB peak SPL) covering frequency ranges audible to mice: Broadband (1-59 kHz or 20-50 kHz) signals, simultaneously frequency (4Hz) and amplitude (5 Hz) modulated; and pure-tone (16 kHz or 40 kHz) amplitude modulated signals. Normally behaving mice were maintained inside an acoustic isolation chamber during sound exposure (Mac-1; Industrial Acoustics, Bronx NY). 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 with a three-dimensional (3-D) T1-weighted gradient echo sequence (TE/TR=4/50ms) with 100-µm isotropic spatial resolution and an acquisition time of 2 hours. 3-D images of the IC were generated with Amira (Mercury Computer Systems, San Diego CA). In each experimental group, the volumetric MRI data from each mouse brain were extracted, co-registered and averaged using the 3-D image registration tools in Amira. The IC was then segmented from the averaged 3-D brain images using an interactive threshold-based region-growing algorithm. Within each averaged 3-D IC dataset, histograms were analyzed and enhanced regions were defined to contain all voxels in the highest 6% of the grayscale range. These voxels were then reassigned an arbitrary color (red), and displayed in surface renderings representing the volumetric regions of activity.

Results

Three groups of mice were exposed to defined sound stimuli: 1) broadband, 1-59 kHz; 2) high frequency (HF)-broadband, 20-50 kHz; and 3) pure tone, 40 kHz. There were obvious differences in IC-enhancement (Fig. 1), with a diffuse pattern of enhancement covering most of the central nucleus after broadband stimulation (N=8; Fig. 1B, F), which was more confined to the ventral-caudal IC after HF-broadband stimulation (N=8; Fig. 1C, G), and resolved into a clear ventral-caudal band after 40-kHz pure-tone stimulation (N=8; Fig. 1D, H). These results are in excellent agreement with previously published tonotopic maps in the mouse [4], demonstrating similar spatial sensitivity as *in vivo* electrophysiology and providing verification of MEMRI brain mapping. Comparing 16 kHz and 40 kHz pure tone evoked activity pattern in IC (Fig. 2), we observed maximum 16 kHz enhancement in the central part of IC (N=4; Fig. 2A), while 40 kHz stimulation resulted in highest enhancement in ventral-caudal band, with a secondary extension to the lateral-central IC (N=4; Fig. 2B). This result shows that additional neuronal elements, outside the electrophysiological 40 kHz iso-frequency band, are also activated by the 40 kHz, 88 dB SPL sound stimulus, and detected by MEMRI.

Conclusions

Consistent with the known tonotopic organization of the IC, MEMRI revealed a dorsal-to-ventral pattern of enhancement corresponding to low-to-high frequency sound exposure. Significantly, our MEMRI results indicate a more elaborate IC neuronal activity representation for pure-tone stimuli than has been previously appreciated from electrophysiological recordings. These results will form the basis for future analyses of altered tonotopicity in mouse mutants with abnormal midbrain development.

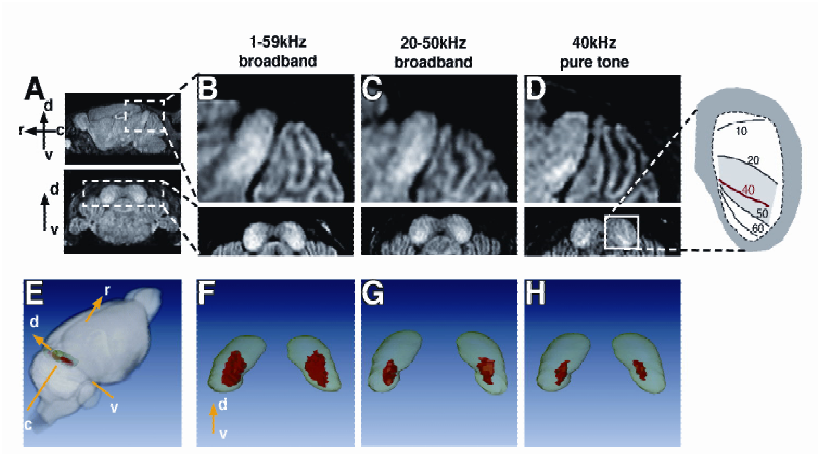

Figure 1. MEMRI can be used to map the tonotopic organization of the mouse IC. (A-D) Sagittal and coronal images of the P21-IC showed obvious differences in mice exposed to defined stimuli. (D) After 40 kHz pure-tone stimulation, enhancement was restricted to an iso-frequency band in excellent agreement with electrophysiological maps (inset) (N=8). Averaged images (E) were used to generate 3-D maps of MEMRI IC-enhancement (red) after stimulation with 1-59 kHz (F), 20-50 kHz (G) and 40 kHz (H).

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References

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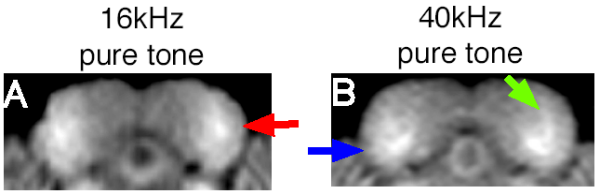

Figure 2. Averaged neuronal activity pattern in coronal inferior colliculus images. (A) 16 kHz sound stimulus evoked a patch-like Mn-enhanced neuronal area in the central part of IC (red arrow). (B) 40 kHz sound stimulated signal enhancement pattern is similar to the 40 kHz iso-frequency contour of tonotopicity (blue arrow). It also covers part of the central area of IC (green arrow).