BOLD fMRI investigation of rat auditory system

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INTRODUCTION Various electrophysiological and histological techniques have been extensively utilized to investigate the auditory system [1,2]. However, an in vivo and non-invasive functional imaging method is essential to understanding the complex functional organization of the auditory system. Recently, auditory BOLD was demonstrated in primates using EPI in the presence of gradient noise [3] by exploiting the fact that adverse effects brought by scanner noise largely depend on its spectral overlap with the stimulus. Acoustic stimulus with frequency close to that of gradient noise would be the most affected [4]. In this study, we aimed to investigate the auditory pathway, and various components including tonotopic mapping in inferior colliculus (IC) in rat brains using BOLD fMRI. Such study of the hemodynamic response in rat auditory system upon auditory stimulations could provide a valuable tool in hearing research of normal and diseased models. To our knowledge, this was the first BOLD study of rodent auditory system and tonotopic mapping study of IC in rodent or human fMRI studies.

METHODS ***Animal preparation*** Normal male SD rats (n=6, 230-280g) were anesthetized using isoflurane (3% for induction and 1.5% for maintenance) and kept warm with 37^{0}C water. Respiration rate, heart rate, SpO_2, end-tidal CO_2 level, and rectal temperature were monitored. ***Auditory stimuli*** The block-design auditory stimulation protocol consisted of 40-s silence and 20-s stimulation block, repeated for 4 blocks. Rats were allowed to rest for several minutes in between different stimulation sessions. The stimuli were produced using a closed-field electrostatic loudspeaker (TDT EC1) driven by an amplifier (TDT ED1), and delivered to the rat ear canal via a flexible sound delivery tube (Tygon) of diameter 2mm and length 10cm. (**1**) To examine the auditory pathway in the entire brain, broadband white noise was used and delivered [5]. (**2**) To study the BOLD response vs. acoustic power, the power of white noise was altered between -30 dB to +6 dB in 6dB steps and presented in a randomized order. (**3**) To map the frequency-specific tonotopy in IC, three pure tone sounds of 7, 20 and 40 kHz were presented in a randomly interleaved order. ***MRI protocol*** MRI experiments were performed using a Bruker 7T scanner. Eight slices of single-shot SE-EPI images were acquired with TR/TE=2000/28ms, FOV=32x32mm^2, data matrix=64x64. ***Data analysis*** Data in the first 30s of each scan were discarded. The remaining fMRI time series were co-registered, spatially and temporally low-pass filtered and then analyzed using STIMULATE software. Voxels with cc > 0.2 and cluster size > 2 voxels were considered as activated by the stimulus. (**i**) Different auditory components were identified and their temporal signals were averaged across the 4 stimulation blocks and 6 animals. Percentage changes of cochlear nucleus (CN) ipsilateral and other structures contralateral to the stimulation were measured. (**ii**) To analyze the effect of acoustic power, percentage changes of the 4 most activated voxels from CN and IC were measured. (**iii**) To analyze the tonotopic response in IC, 12- 24 blocks of the same stimuli were averaged from multiple scans per frequency. The averaged time profiles of the three frequencies were concatenated before statistical analysis. Cc thresholds for individual frequency were selected with minimal spatial overlap.

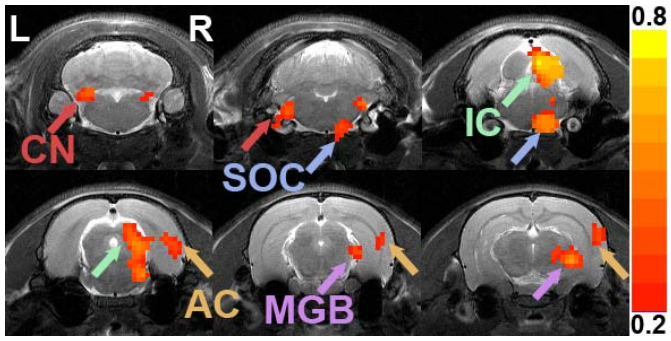

Fig.1 Typical correlation coefficient map (cc>0.2) of an animal averaged by 6 scans (~30 minutes) overlaid on anatomical scans. CN: cochlear nucleus; SOC: superior olivary complex; IC: inferior colliculus; MGB: medial geniculate body; AC: auditory cortex

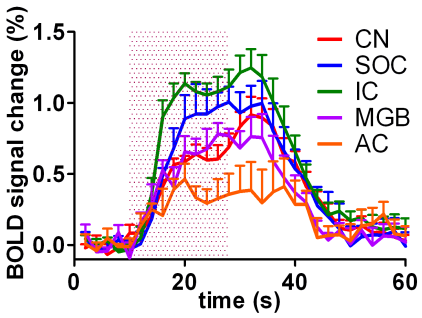

Fig.2 Time courses of different auditory component BOLD responses averaged across different animals (n=6, mean±SEM). Shaded region indicates the applied stimulus.

RESULTS Fig. 1 shows the typical activated regions observed in multiple structures along the auditory pathway, namely cochlear nucleus (CN), superior olivary complex (SOC), inferior colliculus (IC), medial geniculate body (MGB) and auditory cortex (AC). It should be noted that not all the above specified structures were activated in every animals. Amongst the 6 animals, CN (6/6), SOC (5/6), IC (6/6), MGB (5/6), and AC (4/6) were activated with the applied cc threshold. Fig. 2 shows that the BOLD amplitudes in various auditory structures were found statistically different (ANOVA, $p<0.05$). Fig. 3 shows the linear monotonic BOLD signal increase with stimulus power. Fig. 4 shows that the IC activations by the 3 pure tone sounds could be differentiated spatially. Tonotopic activations were found to shift from lateral to medial region of IC with increasing frequency.

DISCUSSIONS and CONCLUSIONS Most of the known relay centers in the rat auditory system were found to be activated in this study. Among all the listed structures, the hemodynamic response of IC was found to be the most robust. Similar findings have been obtained in primate [6] and human [7] fMRI studies using sparse temporal sampling techniques. The reason of the robustness in IC activation still requires further examination, which likely arises from its structural differences in terms of neuronal activities [8], vasculature distribution or energy consumption. A second peak after cessation of stimulation was often observed in IC and CN (Fig. 2). This may be due to the off-response that is known in auditory systems [9]. The absence of off-responses in other structures may be due to the lowered contrast-to-noise ratio. The major challenge in auditory fMRI studies is believed to be the scanner noise. Fig.3 shows that the BOLD percentage change was approximately linear in the range of stimulus power applied, which was in parallel with previous findings of neuronal activities [10]. Such linear relationship indicated that the *observed* BOLD activation can therefore be considered as the additional on/off activation that is superimposed on the elevated *baseline* activation by scanner noise. As a result, reproducible activation could be found in rat auditory system using EPI without sparse temporal sampling. Lastly, frequency-specific response could be clearly found in Fig. 4, highlighting the capability of BOLD fMRI in investigating diseases that may alter frequency topography along auditory pathway. In conclusion, this study demonstrated the BOLD activations upon auditory stimulation along rat auditory pathway and tonotopic mapping for the first time, and these in vivo fMRI findings were consistent with known rat neuroanatomy.

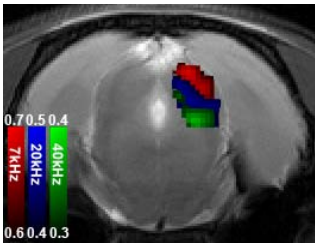

Fig.4 Typical frequency-specific tonotopic mapping of IC with cc map overlaid on an anatomical image (red: 7kHz; blue: 20kHz; green: 40kHz).

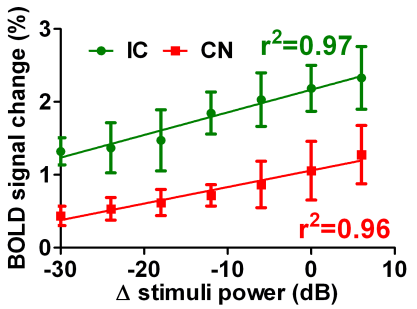

Fig.3 BOLD change (mean±SD) vs. change of power (dB) of acoustic stimulation. Both signals from CN and IC show an approximately linearly increasing trend with stimulus power.

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