Mapping the Tonotopy of the Mouse Brain at UHF MRI: Design and application of an auditory stimulation setup for functional studies

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Abstract

Background: The rodent auditory system has been a popular research subject for electrophysiological studies for its complexity, fine tuning and adaptability. Also, and more recently, some studies on auditory Functional Magnetic Resonance Imaging (fMRI) in rats have surfaced, hoping to unravel the intricacies of this system by relying on its larger field of view (FOV). In mice models, fewer studies have been made using MRI, and a real-time characterization of the auditory response on a global scale is yet to be made
New method: An auditory stimulation system, capable to delivering sounds up to 65 kHz directly to a mouse undergoing a MRI scan was created and validated with two studies.
Results: The auditory stimulation system proved highly reliable for providing high quality pure tones to anesthetized mice in a MR scanner albeit some limitations. Tonotopy maps were computed for low frequency signals (5,12 and 20 kHz) as well as for ultrasonic frequencies (35-39 kHz) and ROI analysis for low frequencies confirmed the validity of the mapping obtained.
Comparison with existing method(s): fMRI using auditory stimulation had been performed previously, but not in a mouse model
Conclusion: The system can be used to study auditory function in mice, hoping to shed some light on this system in humans.
Keywords: fMRI, Auditory Stimulation, Tonotopy

1. Introduction

Evolutionarily, the auditory system has been proven as an essential tool for survival across a wide range of species despite having evolved in distinct ways for each of them. Its complexity - it can deconstruct a specific sound into its basic characteristics: *e.g* frequency and amplitude; fine tuning - its ability to distinguish two or more very similar sounds; and flexibility - it adapts to extreme situations; makes it an enticing study subject.

These features of the auditory system have different manifestations for distinct species. For example, in rodent models, neuroplasticity in the system is evident in complete recovery following deep injury [1] or adaptation to adverse or adaptation to adverse conditions,[2].

Historically, researchers have focused heavily on the rodent auditory system resorting to electrophysiological and microscopical techniques. Some, motivated by the thought of getting to the root of the capabilities briefly described above[1], and others, focused in specific morphologies in the system that make exquisite synaptic models.[3] These invasive studies have provided the knowledge about how the auditory system operates. In synthesis, sound pressure waves enter the ear and vibrate the ear drum. These vibrations are transmitted to the basilar membrane, sending neuronal signals to the cochlear nucleus complex (CNC). Following this event, axons carry this signal from the CNC to the superior olivary complex (SOC), then to the lateral lemniscus (NLL), inferior colliculus (IC), medial geniculate body (MGB) and the auditory cortex (AC). A descending pathway also exists mainly associated with feedback processes and modulation.[4]
Across all structures of the identified auditory pathway, cells responding specifically to a narrow frequency spectra have been identified. Furthermore, cells that respond to similar frequencies tend to be packed closer together creating what is called a tonotopic distribution.[5]
fMRI has shown potential for tonotopic mapping studies. It is noninvasive and applicable to longi-

tudinal human and animal studies. Its signal originates from the fact that deoxyhemoglobin is paramagnetic and oxyhemoglobin in diamagnetic. This implies that deoxygenation of hemoglobin leads to a local reduction in MR signal that can be detected. This modality of imaging is called Blood Oxygen Level Dependent fMRI and is typically implemented with echo planar imaging (EPI) sequences, which provide adequately high spatial resolution for most applications. This technique has proven useful, but it is highly time inefficient and suffers from susceptibility induced signal loss which hampers studies in many fine structures in the auditory system. These limitations become more salient at high magnetic fields and have restrained the growth of high resolution auditory fMRI. Using balanced steady state free precession (bSSFP), a fast MRI acquisition sequence that provides T2/T1 contrast without sparse temporal sampling, image distortion, susceptibility-induced signal loss and sporadic noise, avoids the time inefficiencies and image artifacts of EPI and is ideally suited for auditory fMRI studies. In this study a MR-safe auditory stimulation system was built, tested and validated by performing two auditory experiments in C57BL/6 mice. These experiments may pave the way for future auditory fMRI mice studies.

2. Materials and Methods

All animal experiments were approved by the local animal research ethics committee. Two different fMRI experiments were performed in this study: (1) mapping lower frequency inferior colliculus tonotopy in normal animals; (2) lower frequency inferior colliculus tonotopy in normal animals.

Animal Preparation Normal male C57BL/6 mice (n=6, 21 to 25 g) for experiment (1) and female C57BL/6 mice (n=7, 17 to 21 g) for experiment (2) were anesthetized using isoflurane (about 4% for induction) and progressively weaned out for a medetomidine drip of a bolus of 0.1 mg/kg , followed 2 minutes after by a constant infusion of 0.2 mg/kg/h. They were kept warm with circulating water throughout the experiment. Respiration rate and rectal temperature were monitored (SAII, United States of America). **Auditory Stimulation System:** The monaural sound stimuli presented to all animals were produced using an ultrasonic loudspeaker (L010, Kemo) driven by a power amplifier (Custom made, Hardware Platform - Champalimaud Foundation) and a soundboard (AG-03, Yamaha). Sound waves were delivered to the mouse via a 88 cm long, 14 mm inner diameter (tapered to 1.6 mm in diameter over the last 10mm) nylon sound delivery tube. The narrow end of the tube was connected to a 6 cm long, 1.6 mm inner diameter semi-rigid tygon tube that fitted into the left ear canal. All sound spectra were measured prior to experiments using an omnidirectional condenser microphone (4939-A-011, Brüel and Kjær) and sampled using the Yamaha soundboard. Both ears were then occluded so that the mouse would only hear the stimulation given.

MRI Acquisition: Experiments were performed on a Bruker BioSpec 9.4T AVANCE3HD scanner, equipped with a gradient system capable of producing up to 660 mT/m in all directions. An 86mm resonator volume coil was used for transmittance, and a 4-channel array cryocoil was used for reception. In particular, the cryoprobe serves to enhance the signal-to-noise ratio by a factor typically between 2 and 3. Once the animal was properly positioned in the scanner, scout images were acquired to determine the coronal and sagital planes of the brain. A B_0 map was acquired to allow for a proper shimming. Six parallel 0.65 mm thick slices, separated by 0.15 mm (Bregma -5,80mm to -1.40mm), were oriented orthogonal to the sagital plane and used for anatomical reference. The parameters for the anatomical scans were: Turbo RARE sequence, RARE factor=8, Number of averages: 6, TR/TE=2000/10ms, FOV=15x15 mm2, data matrix=200x200. Slice acquisition order was interleaved with the order being [1 3 5 2 4 6]. After the anatomical image was acquired the fMRI acquisitions could begin. All fMRI acquisitions were made with the following parameters: True FISP sequence,TR/TE=2.8/1.4ms, Effective repetition time:1.307s, FOV=15x15 mm2, data matrix=100x100, flip angle: 30^o. Slice acquisition order was interleaved with the order being [1 3 5 2 4 6]. The slices were acquired in the same position as in the anatomical scans and the total number of volumes acquired was always equal to 334 which made all runs last 7m16s736ms. **Auditory Stimulation Paradigm:** In the Low-frequency experiment, three pure tones were used (5 kHz, 12 kHz and 20 kHz). Each mouse had one run listening to one of the three frequencies in a random fashion with a total of three runs per mouse. In the High-frequency experiment, five pure tones were used (35 kHz, 36 kHz, 37 kHz, 38 kHz and 39 kHz). In this experiment, each mouse received five different sounds in one run (presented in a random order), with an unbalance of runs across the 8 mice due to some of them waking up mid-experiment. In total 26 runs were acquired for the 7 mice, with two mice having 1 run, one mouse with 2 runs, 3 mice with 6 runs and 1 mouse with 5 runs. In both experiments, the experimental paradigm

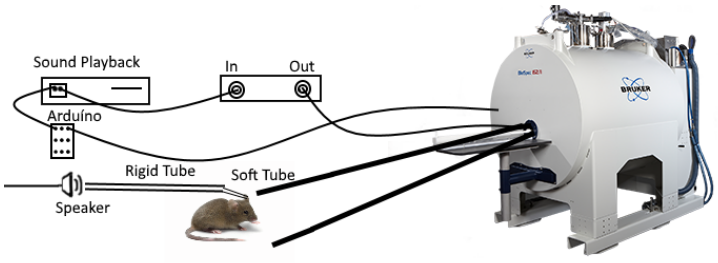

Figure 1: Schematic of the Auditory Stimulation system used

was as follows: Cycles of 50 volumes in which sound was off for 34 volumes and on in 16 (20.912s) with a total of 334 scans. In the low frequency experiment, the mice always heard the same frequency in one run. In the high frequency experiment, the mice heard all frequencies (presented in a random order) in one run. The first 50 volumes acquired were always discarded since it was hypothesized that the magnetization still hadn't reached a steady state by then. This made each run have 5 presentations of sounds. All sounds were presented to the left ear of the mouse. **fMRI data Analysis:** All data was analysed using the SPM package for MATLAB and a Grafical User Interface designed for simplifying the connection between MATLAB and SPM, dubbed fMRAT.[6] The data was first realigned to the mean image of each run and the realignment parameters were kept to be later used as regressors in the GLM model. The images were then spatially smoothed using a Gaussian Filter with a FWHM of 0.3 mm. Then the Design matrix was built using an HRF peaking at 3 seconds [7], the parameters were estimated voxel-wise and the contrasts were built. When building global maps of activation all the images were coregistered to one representative animal. ROI analysis was also performed for the low frequency experiment. To this end, the realigned images were used (unsmoothed) to reduce interference by motion. ROI's were drawn manually and all runs of each frequency were detrended, temporally filtered (notch at 0.2 Hz) averaged across mice. Then each representative run was also averaged so that one representative cycle could be shown. ROIs were only drawn for regions shown to be activated by the activation maps.

3. Results

The performance of the Auditory stimulation system was measured and is displayed in Table 1

At the end of the SPM pipeline, SPMt maps are obtained. These are basically 3-D matrices with the t-values of each voxel, resulting from the voxel by voxel t-tests performed by SPM and according to the GLM. From now on, these t-maps are shown with a threshold of $p<0.001$ and a minimum cluster of 12 voxels passing the threshold. **Low Frequency Experiment:** For the Low Frequency experiment, it was possible to compute individual maps for the frequencies 5, 12 and 20 khz as well as global maps of activation for each mouse and each frequency and a global activation map for all mice (shown in Figure 2). Global maps of activation refer to voxels activated in all frequencies considered. Tonotopy maps were also computed. These consist of the the voxels maximally activated for each frequency overlayed in the same anatomical image. In Figure 2 we can observe the global map of activation for all mice. This map indicates which voxels were consistently activated regardless of the stimulus chosen. The CNC can be observed, as well as the SOC, NLL, IC and AC. The maximally activated structure in the mouse brain was the IC, with a maximum t-value of 22.49. In Figure 3, the maximaly activated voxels for each frequency are depicted, showing a clear tonotopic pattern. The SPMt maps have 5 slices instead of 6 due to coregistration imprecisions.

After obtaining the SPMt maps, it became interesting to plot some ROI's to find out more about the evolution of the signal along time in different regions. The individual SPMt maps were used to plot the ROI A control region inside of the sample but out of expected activated regions was drawn as well. These plots can be seen in Figures 4 (where two ROI's were drawn in the IC. One more ventromedially and one more dorsolaterally) and 5 (where ROI's were ploted for the remaining structures in the auditory pathway and a control region)

High Frequency Experiment: The fact that the number of runs in each mouse in the High Frequency experiment was not uniform, made it impossible to build individual SPMt maps since the statistics were not powerful enough. As such, only global SPMt maps and tonotopy maps were built for this experiment. The spatial normalization of the 7 mice also produced global maps of only five slices in this case.

Frequency (kHz)	Peak SPL (dB)	Total Harmonic Distortion (%)
5	118	18
10	90	28.4
12	114	7.23
15	103.5	22.6
20	117.6	22.4
25	105.6	2.25
30	111.1	2.24
35	100.3	1.13
36	106.4	0.72
37	109.3	0.35
38	105.5	0.25
39	101.2	0.35
40	108.9	1.13
45	95.1	0.21
50	84	0
55	83.6	0
60	86.7	0
65	84.4	0

Table 1: Output of speaker at the level of the mice ear - measured at the tip of the semi-rigid tube

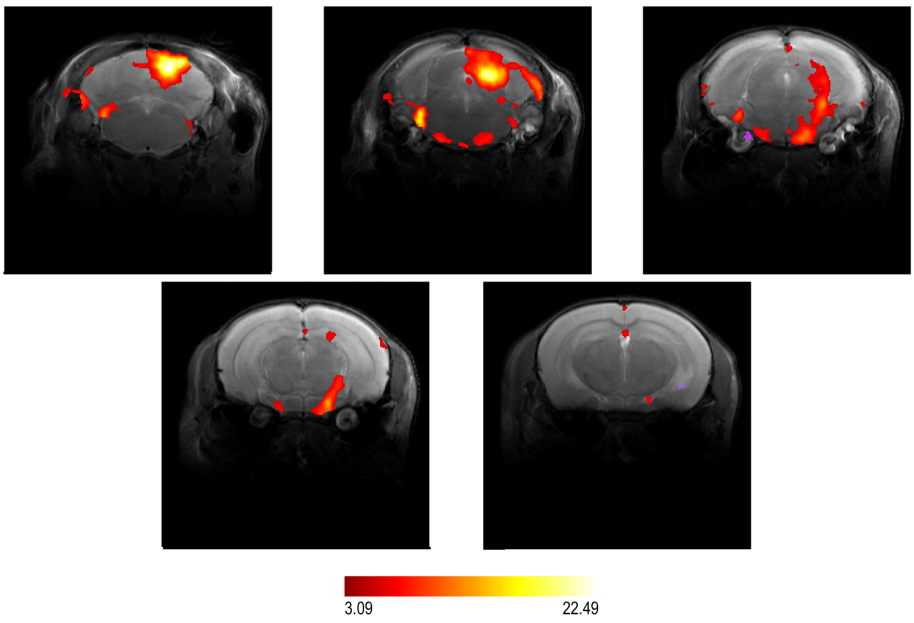

Figure 2: Map of activation across all frequencies overlaid on an anatomical image. Colorbar in t-values

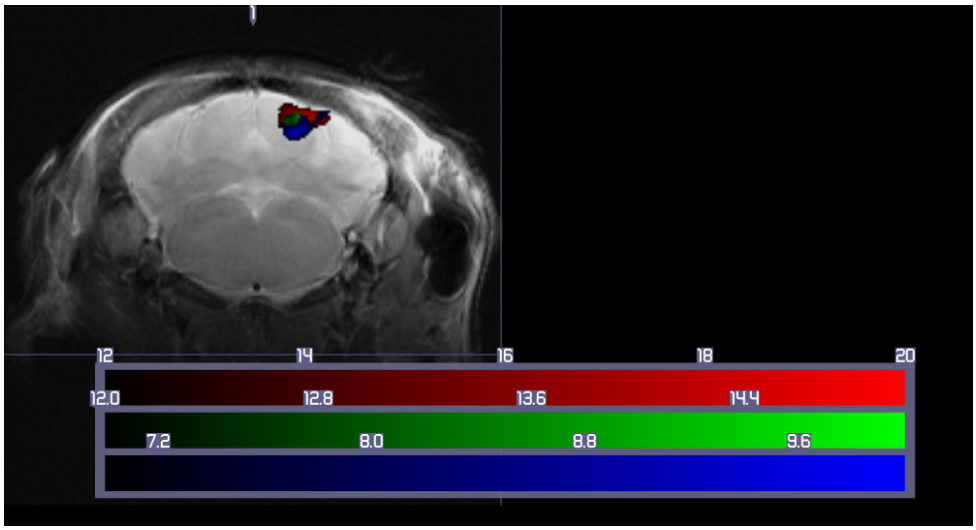

Figure 3: Tonotopy map. Colorbars in t-values and ordered so that Red - 6 kHz , Green - 12 khz and Blue - 20 kHz

4. Discussion

The auditory stimulation system is highly capable of producing pure tones with minimal distortion. It should be stated that the amplitude of the signal starts dropping at around 25 kHz and is accentuated after 45 kHz. This happens because the board is sold for ”human” applications, meaning its high sample rate is meant for high quality sampling, rather than ultrasonic playback. This difficulty was partially overturned by including an amplification stage in the circuit but since the amplificator used was linear for all frequencies, the roll off still happened, albeit at a slower rate. A reasonable alternative would have been to measure a response function of the board, and used a non linear amplificator to match the inverse of that response function so that the output would be constant across all the frequencies of study. Another alternative, would be to use a signal generator, which would increase the price of the system greatly.

The use of a soundboard, coupled with an amplifier isn't usual in such applications (ultrasonic auditory stimulation systems) and also constitutes an innovation. In essence, a low-cost waveform generator was built. Altough reliable and reproducible, the sound output of the auditory stimulation system was found to be unstable, with very short variations in frequency, resulting in very large variations in characteristics of the sound measured such as peak SPL and total harmonic distortion (especially for lower frequencies). A sensibility study should be made, so that future versions of the system have a more complete description of the input-ouput relationship.

Two experiments were made to test the capabilities of the Auditory Stimulation System. In both of them, mice between 7-8 weeks old were used since it has been proven that C57BL/6 mice start loosing earing acuity around that age.[8] Additionally, since the Hemodynamic response wildly differs for different anesthesia and brain region [7], and given the especial susceptibility of mice to isofluorane the choice was made to anaesthetise all mice used with medetomidine. The high frequency experiment used females because the initial idea was to play pup vocalizations as the auditory stimulus, which eventually didn't happen.

The frequencies of choice for the low frequency experiment were picked since they placed in a low threshold well of the mice audiogram, meaning that mice detect frequencies in this range even for very low SPL. [9] For the high frequency experiment, the frequencies used lied in a stable range of frequencies of the auditory stimulation system used, meaning that between these frequencies, harmonic distortion was low and SPL difference was lower than the lowest observed SPL difference in rats that showed difference in BOLD signal. [10]
Low Frequency Experiment: Looking at Figure 2, we see that when considering all frequencies as activation, regardless of their value, the auditory pathway structures can be mapped. The CNC, SOC, NLL, IC, MG and AC can be seen with t-values corresponding to $p<0.001$.

The tonotopy map shown in Figure 3 shows a very distinct tonotopic pattern with higher frequency specific voxels being located ventromedialy and lower frequency voxels being more dorsolaterally. This is excelent agreement with the existing literature for rodents, both with electrophysiological studies and with fMRI[11],[12], [4].

In the IC, two ROI's were drawn, one more dorsolaterally (dubbed dorsal) and one more ventromedially (dubbed ventral). These ROI's showed the tonotopy in the IC yet again since the medial ROI had higher signal than that the dorsal ROI for

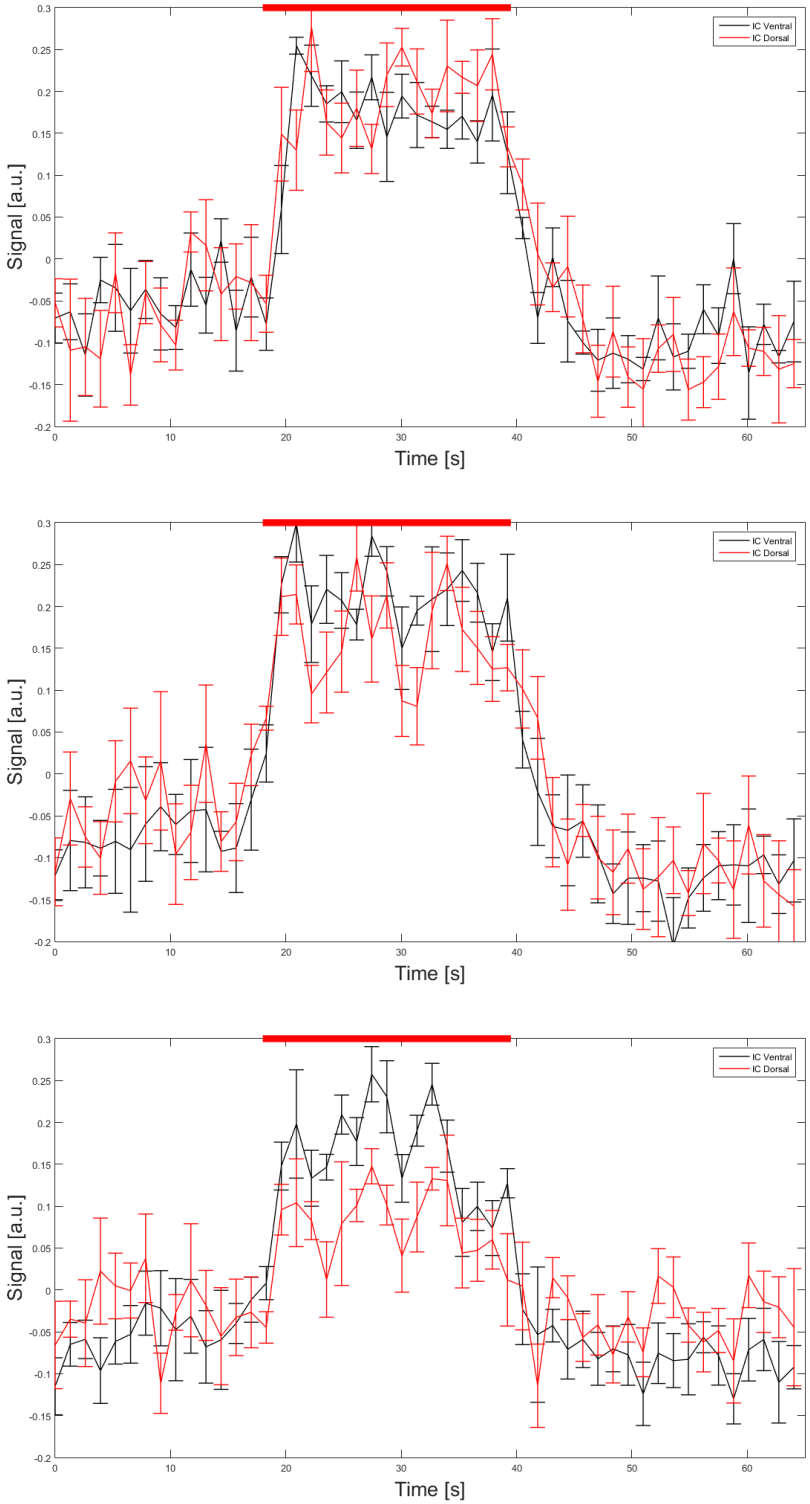

Figure 4: Averaged data across mice and across cycles for the IC for 5 kHz (Up), 12 kHz (Center) and 20 kHz (Down). Two regions of the IC are shown: Dorsal and Ventral, error bars are in standard error of the mean and the activation is shown in red

high frequencies, while the opposite happened for lower frequencies. The plateaus observed during activation also showed several peaks, possibly giving insights on the the activity of the descending auditory pathway. Further analysis should be done by modeling these several peaks and comparing them to electrophysiological studies [13].

Other structures also showed these several peaks were the NLL and SOC, structures also implicated in the descending auditory pathway. A ROI

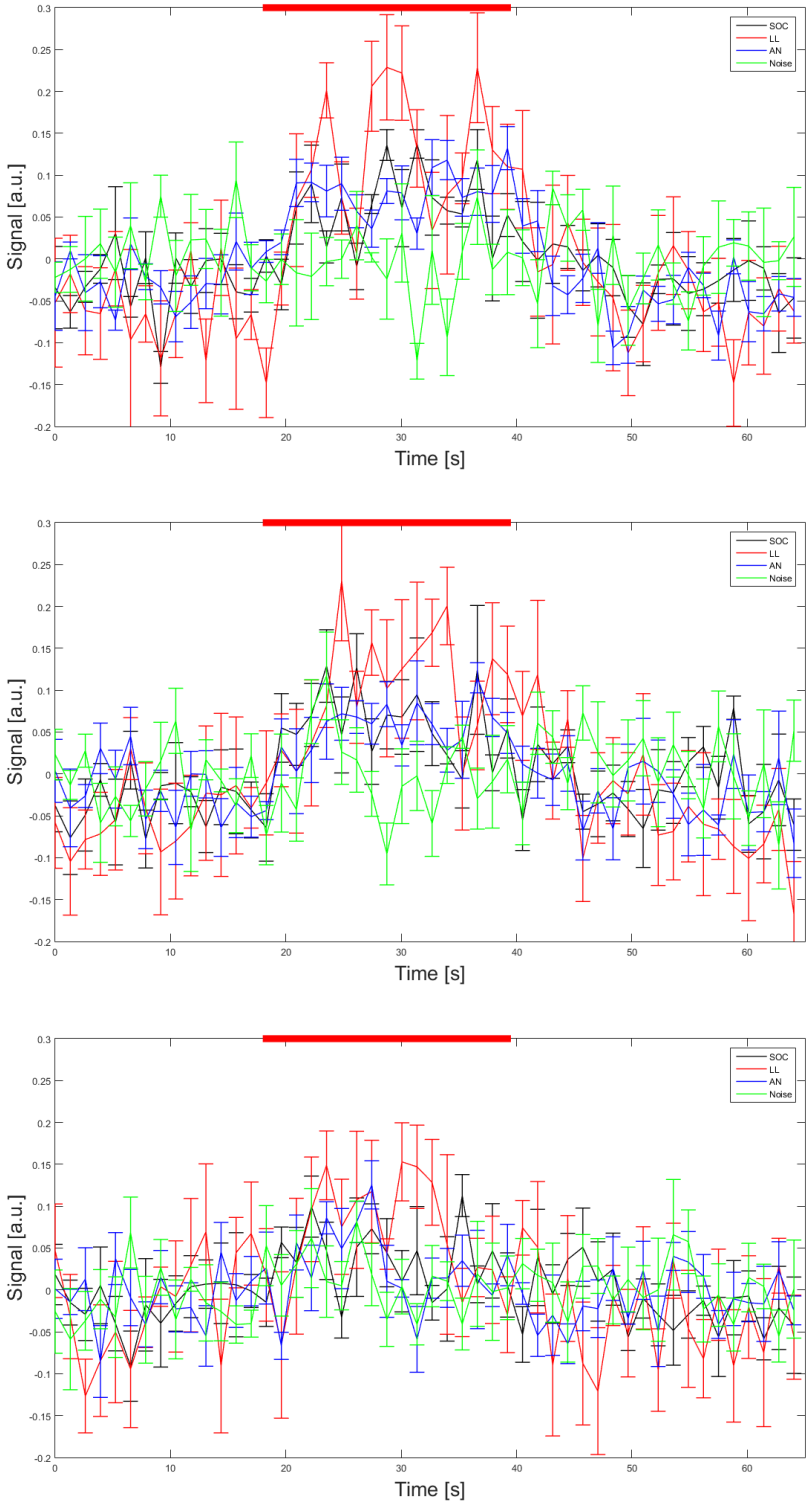

Figure 5: Averaged data across mice and across cycles for the CN, SOC, LL and a non task related brain area for 5 kHz (Up), 12 kHz (Center) and 20 kHz (Down) . Error bars are in standard error of the mean and the activation is shown in red

was also drawn in a region not believed to be involved with auditory processing and did not show significant increase in signal during activation. All this analysis will be validated in further studies resorting to t-tests. The CNC showed higher activation for lower frequencies than for higher ones which can be a consequence of its tonotopic distribuition or the higher harmonic distortion of lower frequency sounds. But the results agree with the SPMt mapping.

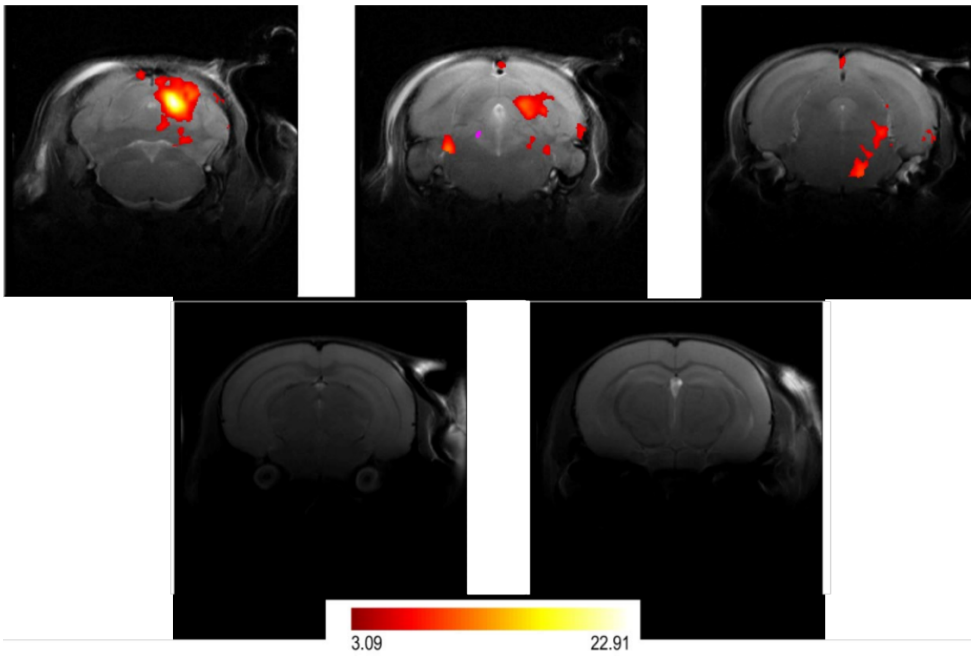

Figure 6: SPMt map for all frequencies. Colorbar indicates t-values

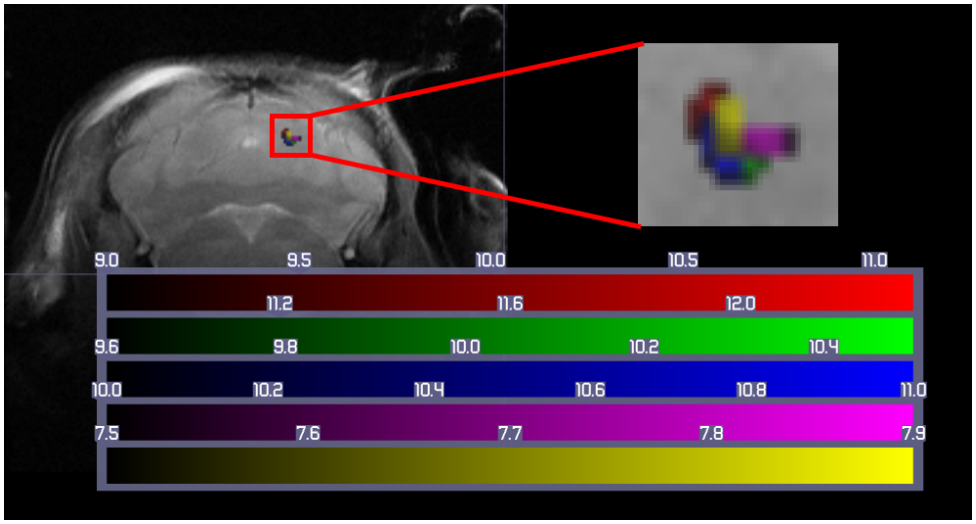

Figure 7: Global Tonotopy in the IC for the High Frequency experiment. Colorbars indicates t-values and are ordered so that Red - 35 kHz, Green - 36 kHz and so forth

The temporal resolution used (1.307s) didn't allow for proper separation of rise times for different structures but it is evident that the behaviour pattern across structures is different and rises at stimulation onset, going back to baseline when the stimulation ends. **High Frequency Experiment:** Looking at figure 6 it can be seen that the higher t-values concentrate in a very specific region of the IC - ventromedially with lower t-values existing in the dorsolateral region. When then only using the highest t-values for each frequency to plot a tonotopy map in Figure 7, a surprising result emerges with the highes t-values for each frequency occupying slightly different positions in the frequency specific area. This may be only by chance or slight errors on the spacial normalization but similar microtonotopy has been shown in AC using electrophysiology [14].

This experiment suffered from some logistical difficulties as many mice woke up midexperiment. This led to the use of more mice than expected (n=7) and to an unbalance of runs across mice with some mice having 6 times more runs than others which can introduce bias in the results. This also made impossible drawing individual SPMt maps for each mouse since the statistics were not powerful enough. For this reason, ROI analysis should be performed in further analysis by coregistering all animals to the same template so that variabil-

ity across subjects is also covered and signal from ascending pathways and descending pathways is discerned.

5. Conclusions

The present study shows BOLD activations upon auditory stimulation in the mouse auditory pathway. It also demonstrates the first in vivo tonotopic mapping in the mouse IC using pure tones. These in vivo fMRI findings agree well with the previousv electrophysiology and immunohistochemistry findings, indicating the feasibility of auditory fMRI in mouse models.

. The auditory pathway of the mouse was mapped showing activation in the CNC, SOC, NLL, IC and AC. Only MG was not found to be significantly activated for the predicted activated structures. Subsequent ROI analysis showed activation of all these structures starting less than a second after the stimulation started and returning to baseline about 3s after the stimulation ended. The activation patterns, in conjunture with the ROI analysis allow for the conclusion that both the ascending and descending auditory pathways were observed, with future analysis focusing in modeling the the response times of each structure so that a chronological order can be established between structure activations.

The IC showed, as in previous studies, macroscopic tonotopy with very distinct regions activating for different frequencies at high significance levels ($p<0.001$) despite interference of the harmonic distortion of the sounds used and the FISP accoustic noise. Additionally, more fine and precise tonotopy was observed, with maximally activated voxels for frequencies between 35 and 39 kHz being shown (with spectral resolution of 1 kHz). This was possible due to the spatial resolution of 150 μm employed, together with the multiplicative factor in SNR provided by the use of a Cryoprobe. [15] [16] [17]

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