Restoring susceptibility induced MRI signal loss in rat deep brain structures at 9.4T and acquiring true whole brain scale fcMRI network

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Target Audience: Researchers study rat brain fMRI/fcMRI.

Purpose: 1. Setup study method that recovers susceptibility induced EPI signal loss in deep brain areas (especially in insular cortex and amygdala). 2. Acquiring EPI based fcMRI using seed from the previously dropped out areas to show the whole brain scale fcMRI network.

Methods: Five male Sprague-Dawley rats weighing between 300-450 g were used in this study. In four out of the five rats, the ear tubing procedure was performed on the left ear, and Fomblin Y was filled into the external and middle ear. The right external ear canal of these four rats was filled with gel toothpaste, and the tympanic membrane was kept intact. In order to demonstrate the effect of ear tubes, the final rat had both external ears filled with Fomblin Y, but the ear tube procedure was only performed in the left ear. The middle ear filling procedure was performed under 2% isoflurane inhalation anesthesia. A 23-gauge silicon needle was used for the tympanostomy. The silicon needle was inserted into the external ear canal to about 10 mm deep. Once it broke through the tympanic membrane, a clear, cracking sound was heard. The silicon needle was then inserted a further 5 mm to reach the end of the middle ear. Following the silicon needle placement, Fomblin Y, with a molecular weight of 3,300 g/mol, was injected to fill the air pocket in both the middle ear and the external ear. Once the ear canal was filled with Fomblin Y, a plug made with medical grade cotton soaked in Fomblin Y was placed inside the external ear canal to seal the space and keep the fluid from leaking out. About 0.2 ml of Fomblin Y was used to fill the ear canal. Positive caloric reflex can be observed by the end of the procedure, indicating a successfully filled ear. This middle ear Fomblin filling technique was named as "**MEFF**". A 9.4 Tesla MRI system with a 31 cm horizontal bore was used for scanning. A Bruker linear transmit-coil was used with the center of the coil located 3 mm anterior to the external ear canal. Signal acquisition was achieved using our self-designed 10 mm receive coil with low-noise-amplifier (LNA) on board. The coupling circuit uses an American Technical Ceramics 800R series, high-Q, non-magnetic capacitor, a Rogers RT/duroid 5880 low-loss circuit board with 1-ounce copper trace on both sides, and a WanTCom WMA9RA LNA, with an input impedance of 1.5 Ohms and an overall gain of 28 dB. In order to conform to most of the resolution levels used in research worldwide, two sets of parameters were used. (1) Full k-space, single-shot gradient-echo EPI. TE = 18.76 ms, TR = 2 s, 10 contiguous interleaved 1 mm slices. (2) For Fig. 2. A partial k-space sequence with 20 over-scan lines was used for this high resolution EPI acquisition. TE=10.78 ms, TR=2 s, FOV=25.6 mm, slice thickness=0.2 mm, matrix size=128×128. Data analysis was performed using AFNI. The seed region was chosen from the left insular cortex, consisting of 26 voxels, and the left amygdala, consisting of 16 voxels, across two slices. These seeds cannot be found on the right side of the cortex where an ear tube procedure was not performed. A band-pass filter was used for all resting-state EPI acquisitions with a low-pass filter of 0.1 Hz and a high-pass filter of 0.01 Hz on a voxel-by-voxel basis covering the entire brain. Results were smoothed with 0.3 mm FWHM and underwent fisher-Z transformation. The p value for fcMRI results was set at 0.05.

Results: Figure 1 shows the results of EPI imaging at 0.4*0.4*1mm resolution. It is clear that the EPI signal in the insular cortex and the amygdala was restored on the left side of brain. However, on the right side, where the ear tubing procedure was not performed, EPI signal dropout remains significant. Figure 1A demonstrates the significance of the ear tube procedure. Although Fomblin Y was filled into both external ears of the rat, EPI signal was only restored in the left hemisphere, which shows that the air pocket in the middle ear is the major source of the magnetic susceptibility artifact in the deep brain area. The susceptibility artifact cannot be removed by filling the external ear canal alone. The newly acquired EPI image on the left side of the brain is comparable to the RARE image (Fig. 1B). Filled squares define seed regions used later for fcMRI analysis (red for insular cortex seeds and yellow for amygdala seeds). Open squares represent the seed regions that are lost because of magnetic susceptibility dropout. An alternative way to reduce the deep brain signal drop out is to use high resolution imaging setup, despite the reduced SNR and slice coverage. With our self-designed coil, we are able to collect EPI data at 200 micron cubic resolution. Even at this high resolution, significant loss of EPI signals in the deep brain areas occur, which can be avoided by use of the techniques of this paper. Fig. 2 is an example of an EPI image at 200 micron cubic resolution[1]. The MEFF procedure was done in the left ear and right ear was filled with Fomblin only. Fig. 3A was acquired using two seed regions from the left amygdala, one as indicated in Fig.1 and the other in an adjacent plane. Due to the incomplete coverage of the EPI signal on the non-Fomblin-filled side (right side), we were only able to acquire a small portion of the right side amygdala and insular cortex with fcMRI analysis. However, we can acquire a functional network of limbic system containing the hypothalamus, insular cortex, hippocampus, and cingulate cortex using amygdala seeds. The bilateral sensory-motor cortex is also involved in this network. Figure 3B was acquired using insular cortex seeds from the left side. fcMRI analysis reveals a complex network of insular cortex, caudate putamen, cingulate cortex, retrosplenial granular cortex (RSG), ventral lateral thalamus (VL), ventral posterolateral nucleus (VPL), periaqueductal gray matter, rost linear raphe (Rli), and basal forebrain. Note: due to the incomplete coverage of the EPI signal on the non-Fomblin-filled side (right side), the right insular cortex does not contribute to the fcMRI analysis.

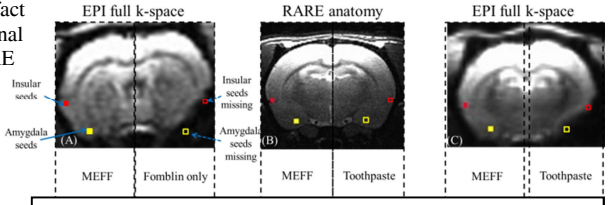

Fig. 1 Comparison of EPI signal dropout with and without the MEFF procedure. (A) Comparison of the use of MEFF, left, and of Fomblin in the external ear, right. MEFF restores the EPI signal in deep brain areas, and the result is highly comparable to the RARE image (B). (C) Comparison of MEFF, left, with toothpaste in the external ear, right. Although Fomblin Y was present in both external ear regions, the EPI signal was only restored by MEFF.

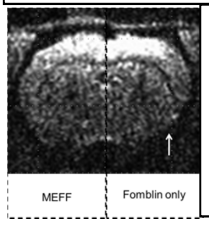

Fig. 2 EPI image at 200 micron cubic resolution showing that the magnetic susceptibility signal dropout still occurs (white arrow) and can be eliminated by MEFF. Significant EPI signal dropout in the insular cortex and amygdala areas are seen in the right side.

Discussion: Using seed from previously unavailable areas such as amygdala, we found an extensive fcMRI network of the limbic system at a truly whole brain scale. Similarly, using seed from insular cortex, we also found a complex whole brain scale functional network that connected the insular cortex to the caudate putamen, cingulate cortex, RSG, VL, VPL, periaqueductal gray matter, nuclear raphe, and basal forebrain (Fig. 3B). Although these connections have been found in previous studies, our study is the first to show them by functional connectivity based on cross-correlation of the fluctuations found in resting-state time courses. With the MEFF procedure, these networks and interactions become available for in vivo neuroscience and pharmaceutical research.

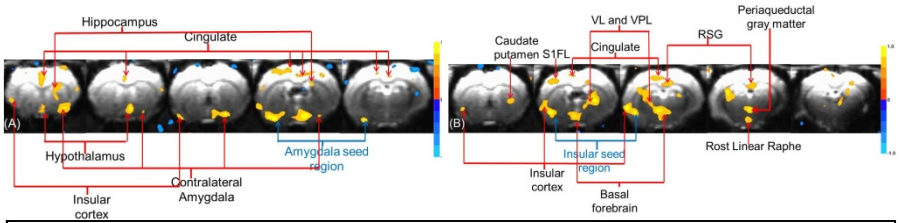

Figure 3. (A) fcMRI network using regionally averaged seeds from the amygdala and (B) the insular cortex. All seeds were chosen from the MEFF side. Large scale resting state functional connectivity networks, including multiple key components of the limbic system, were found.