Laminar Profile of Intracortical Resting-state Functional Connectivity

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INTRODUCTION: Spontaneous BOLD fluctuations in resting-state fMRI (rsfMRI) have been used increasingly to understand different brain networks in normal and pathological conditions [1]. The cortical networks revealed by rsfMRI usually lack laminar specificity. However, based on neuroanatomy and neurophysiology, the cortical laminae are conceived as stack of distinct but loosely interconnected neuronal networks [2, 3]. Besides, each lamina has different specific inputs, projection targets, and feedback connections. The primary visual cortex (V1) is best suited for studying the laminar rsfMRI connectivity, as its laminar inputs, outputs and local connections are well-known [2, 3]. In this study, high spatial resolution rsfMRI was applied to investigate the laminar interconnections in the rat V1 and manganese-enhanced MRI (MEMRI) was used to visualize the layer specific neuroanatomy.

MATERIALS AND METHOD: Animal Preparation: rsfMRI, Mn^{2+} injection (100nl, 100mM, pH=7.4, location=right-V1), and MEMRI (24hrs after injection) were applied to male Sprague-Dawley rats (350 – 400g, 10 weeks, N=5). Animals were under mechanical ventilation during MRI experiments with 1.4% (rsfMRI) and 2.0% (MEMRI) isoflurane. **MRI Protocols:** Imaging was performed using a 7T Bruker (PharmaScan) scanner, a custom-made single-loop surface coil (rsfMRI) and a Bruker surface coil (MEMRI). rsfMRI data were acquired using a single-shot GE-EPI sequence with TR/TE=750/17ms, flip angle=50°, FOV=25×25mm^2, MTX=100×100 and 560 data points. MEMRI data were acquired using T_1-weighted MDEFT sequence with RI/TR/TE=1100/12/4ms, FOV=32×32mm^2 and MTX=256×256. **Data Analysis:** Standard pre-processing steps were applied to rsfMRI data, and connectivity was derived using seed-based analysis (SBA) and independent component analysis (ICA). Three 3×3 seed-voxels were defined along the V1 cortical depth (Fig. 1). The power spectral density was calculated for the seeds, and the correlation coefficient maps were obtained. Z-score maps of the laminar network were obtained using GIFT v1.3h (Group ICA Toolbox). For MEMRI, images were registered and normalized using the right posterior cortex. Finally, ROIs were defined to examine the laminar connectivity and Mn^{2+} enhanced profiles along the cortical depth, and the profiles were plotted.

RESULTS: Fig. 1 shows the averaged power spectral density in the seed locations. Fig. 2 shows the mean correlation coefficient maps, the mean z-score map, and a typical MEMRI image. It also shows the ROIs and respective profiles. These results indicate the presence of laminar rsfMRI connectivity and layer specific neuroanatomy in the left V1.

DISCUSSION AND CONCLUSION: The neuroanatomy and neurophysiology in the V1 of rat is arranged in a lamina manner, with layers IV and VI receive input signals from the lateral geniculate nucleus (LGN) [2, 3]. After intracortical processing in layers II, III, V and VI [2], neuronal signals are routed to the contralateral cortical areas in the superficial layers II and III [2]. Subsequently, subcortical targets receive cortical feedback signals from layers V and VI. Our results indicate that layers II/III and layers V/VI are more functionally connected during resting-state (Fig. 2a, c & d), which may be associated with the intracortical processing in V1 layers II, III, V and VI upon visual stimulation [2]. It could be observed that layer IV was slightly more functionally connected to layers V/VI than that of layers II/III (Fig. 2b), which might correspond to similar functionality in these layers as they receive inputs from LGN. Other than laminar interactions, our results showed that the power spectral density were distinct in different layers. Seed-1 lying on layer II/III had significantly higher power in the relatively low frequency when compared to seed-2 and seed-3, which lies on layers IV and V/VI, respectively. This spectral difference may relate to the slower long range interhemispheric communication [4], as layers II/III are connected to the contralateral cortical areas [2]. MEMRI results showed layer specific enhancement in the left V1, revealing the neuroanatomy with a profile similar to that of intracortical laminar functional connectivity (Fig.2). This suggests that the layer specific neuroanatomy might be correlated to the laminar functional connectivity. In conclusion, we have demonstrated for the first time that intracortical laminar functional connectivity in the V1 with layers II/III and layers V/VI is more functionally connected using rsfMRI. We have also showed layer specific Mn^{2+} enhancement in the rat V1 to reveal layer specific neuroanatomical structure. The intracortical laminar functional connectivity may provide further insights in intracortical and intrahemispheric neural communication.

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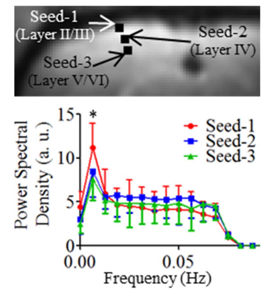
Fig. 1: Seed placement at different cortical depths (top) for seed-based analysis (SBA) to study laminar connectivity. The averaged power spectrum (bottom) extracted in the seed locations show that Seed-1 had significantly higher power in low frequency compared to Seed-2 & Seed-3. Paired t-test was performed, where * denote p<0.05.



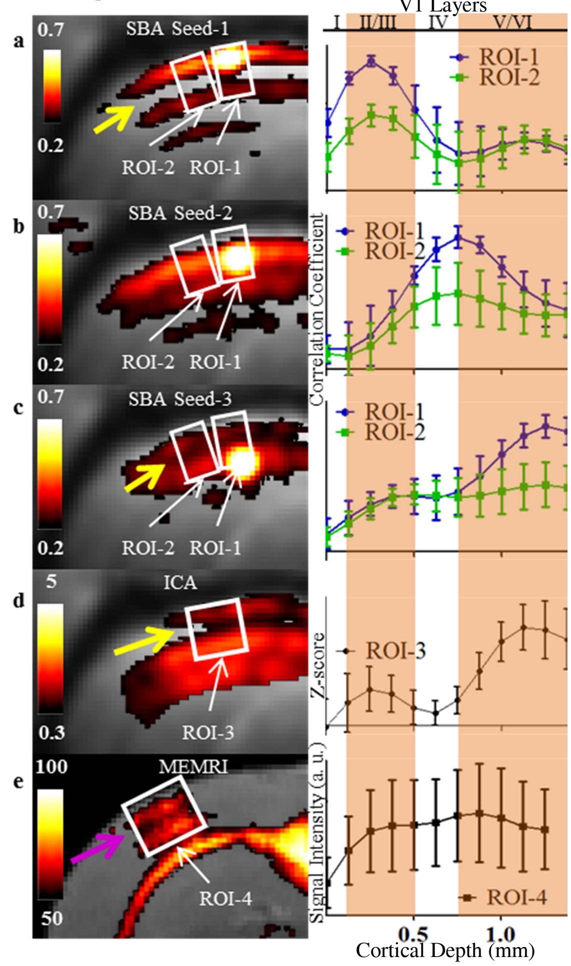


Fig. 2: The left shows the mean correlation coefficient maps obtained with seed-1 (a), seed-2 (b) and seed-3 (c), the mean z-score map obtained using ICA (d), and a representative MEMRI image (e). Laminar connectivity in the left V1 was observed (yellow arrows), and the layer specific neuroanatomy was also observed (pink arrow). Four ROIs were defined as a stripe along the cortical depth direction to extract respective profiles (plotted on the right). The plots are shown in mean ± standard deviation. Our results indicate that layers II/III and V/VI are more functionally connected during resting-state (a, c & d; shaded regions). MEMRI reveals the layer specific neuroanatomy which have a similar pattern to the laminar connectivity (d; shaded regions).