Ultra high resolution functional MRI and electrophysiology of the rat primary somatosensory cortex

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INTRODUCTION Extracellular field potential (FP) reflects summated synaptic potentials of a recording region, in contrast to spiking activity that primarily from the soma. FP (synaptic input) has been shown to better correlate with the BOLD response [1], but if this theory still valid at very high resolution across different cortical laminae remains largely unexplored. The present study aimed to investigate the coupling and uncoupling of FPs and CBV responses in the rat primary somatosensory cortex (S1) with laminar specificity. The hypothesis was that intracortical changes in FPs were accompanied by corresponding CBV response. We employed graded forepaw stimulation to evoke S1 responses. CBV fMRI was performed at 11.7 T with unambiguous 40x40 μm in-plane resolution, while layer-specific FP recording was performed by 16-channel microelectrode array. Apparent FP-CBV uncoupling was observed at laminar resolution.

METHODS Monocrystalline iron oxide nanoparticle (MION, 30 mg Fe/kg, i.v.) CBV fMRI was performed on six adult Sprague Dawley rats under α-chloralose anesthesia (60 mg/kg first dose, followed with 25 mg/kg/hr for maintenance, i.v.), mechanical ventilation, and paralysis. MRI was performed on Bruker 11.7 T Biospec with a small surface coil (ID~0.7 cm) placed on the contralateral S1. CBV fMRI was acquired with FLASH sequence using spectral width = 14.8 kHz, TR/TE = 26/10ms, FOV = 0.768x0.768 cm, slice thickness = 2 mm, matrix = 192x192, yielding in-plane resolution = 40x40 μm and temporal resolution = 5 s. Four stimulus intensities (1, 2, 6, and 10 mA) were applied, with fixed 3 Hz and 0.3 ms pulse width at the right forepaw. Stimulation paradigm was OFF-ON-OFF-ON-OFF, where OFF = 100 s and ON = 50 s. FP recording was performed with identical animal preparation using a 16-channel polyimide-based microelectrode array [2]. Stimulation paradigm for FP recording was OFF-ON-OFF. fMRI data were analyzed using a custom-built image processing interface [3]. Summated FP (ΣFP) was calculated by integrating the *magnitude* FP responses from 0 to 75 ms. Statistical analysis employed ANOVA followed by Fisher's post-hoc test. Significant level was set at $p<0.05$. Error bars were SEM.

RESULT & DISCUSSION This study demonstrates ultra-high resolution CBV fMRI and multichannel FP recording of the rat S1. The major findings are: (i) Column-like activations were observed using CBV fMRI at 40x40 μm resolution. Those column-like activations may result from vasodilation of small arterioles nearby the cortical microcolumns. (ii) 10 mA stimulation showed stronger CBV fMRI response than 1 mA in layer IV−VI, but not in layer I−III, whereas strongest CBV responses were observed in layer IV of all stimulus intensities. (iii) Weakest ΣFP was observed in layer IV at 1 and 2 mA, but not at 6 and 10 mA. Layer IV−VI were more sensitive to changes in stimulus intensities, while layer I was difficult to be modulated. Strongest CBV activation was observed in the layer IV, this is because layer IV primarily receives synaptic input from the thalamus and then spreads the current laterally to the adjacent layer IV neurons [4,5]. Our FP data followed by current source density analysis revealed earliest current sink at layer IV for all stimulus intensities, with higher stimulus amplitude elicit stronger current sink at deeper layers (data not shown). The FP responses in layer I and II were generated by polysynaptic activities and occurred later than that in layer IV. These responses were recruited and reverberated by intracortical circuits at low intensity and saturated. In contrast, those in layer IV were incremented by the increase of synaptic inputs. In addition, he current cascade occurred at layer IV also relayed transynaptically to more superficial and deeper cortical layers [6]. These intracortical synaptic activities, however, are not apparent in high resolution CBV fMRI. This was evident in ΣFP, but not in CBV, showing deeper cortical layers were more responsive to noxious stimuli (i.e. 6 or 10 mA). Our results suggest that a regional uncoupling occurred within cortical layers. It is likely that the dilation of microvessels at the deeper layers follows arterial volume increase at the cortical surface and/or middle layers [7,8], making those column-like microvessels showed less CBV laminar dependency to different stimulus intensities.

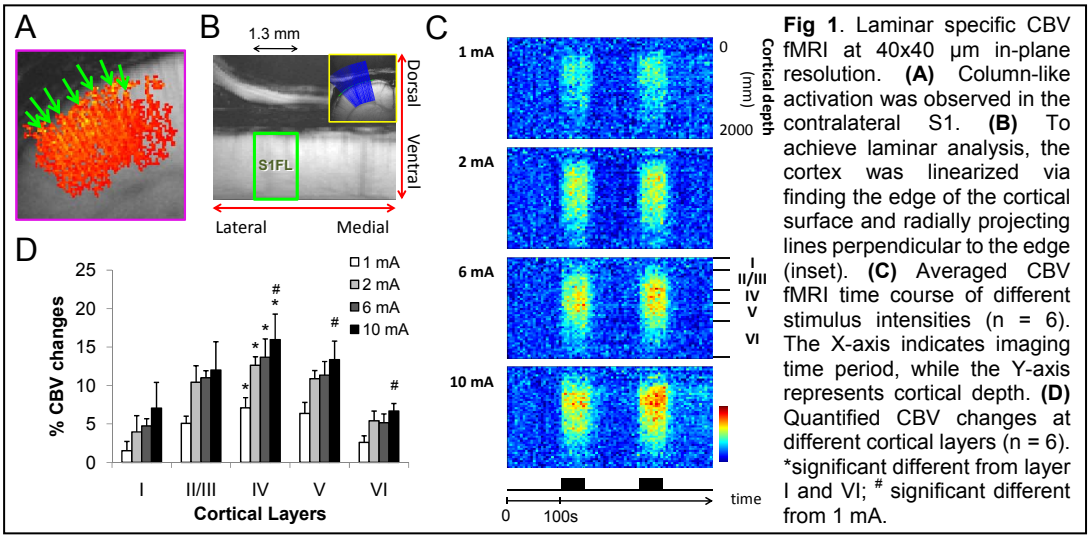

Fig 1. Laminar specific CBV fMRI at 40x40 μm in-plane resolution. **(A)** Column-like activation was observed in the contralateral S1. **(B)** To achieve laminar analysis, the cortex was linearized via finding the edge of the cortical surface and radially projecting lines perpendicular to the edge (inset). **(C)** Averaged CBV fMRI time course of different stimulus intensities (n = 6). The X-axis indicates imaging time period, while the Y-axis represents cortical depth. **(D)** Quantified CBV changes at different cortical layers (n = 6). *significant different from layer I and VI; # significant different from 1 mA.

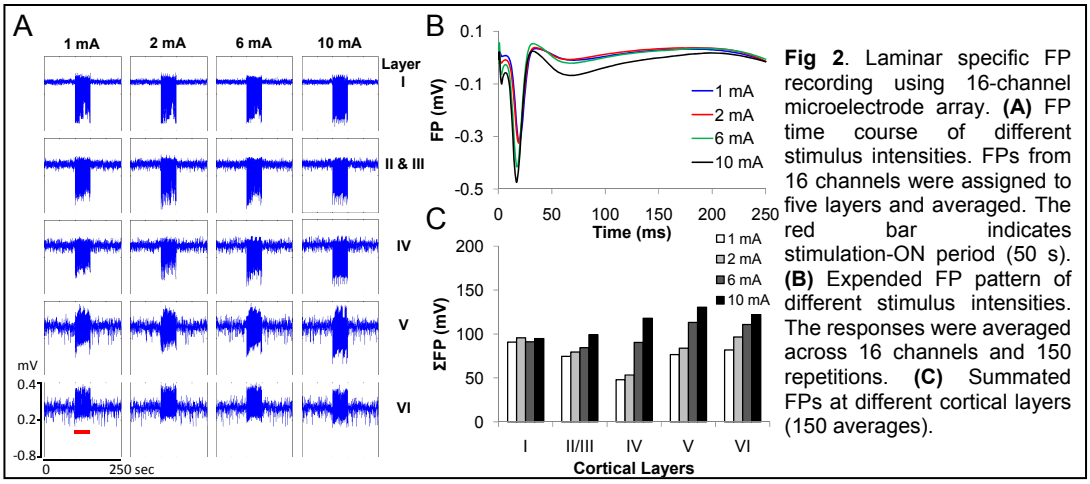

Fig 2. Laminar specific FP recording using 16-channel microelectrode array. **(A)** FP time course of different stimulus intensities. FPs from 16 channels were assigned to five layers and averaged. The red bar indicates stimulation-ON period (50 s). **(B)** Expended FP pattern of different stimulus intensities. The responses were averaged across 16 channels and 150 repetitions. **(C)** Summated FPs at different cortical layers (150 averages).

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