Rat BOLD fMRI using Domitor for Anesthesia: Investigation of Stimulus Dependence and Negative BOLD Response in Somatosensory Network

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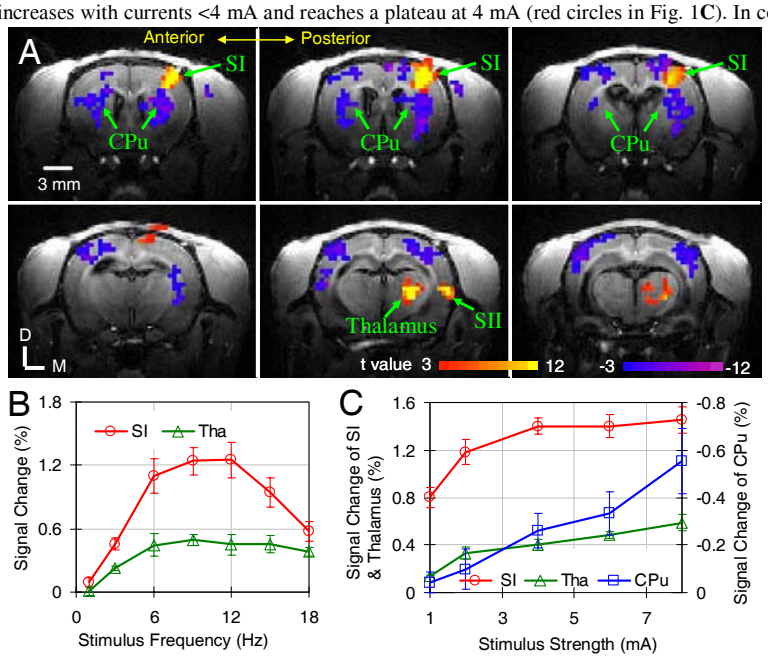
INTRODUCTION It is known that stimulus-frequency dependence of BOLD response in rat to forepaw stimulation depends on anesthesia, such as isoflurane and α-chloralose (1). Yet such dependence is unknown for domitor-anesthetized rat (2). Therefore, a frequency dependence study was performed by varying the stimulus frequency from 1 to 18 Hz. Subsequently, with the optimum frequency, rat BOLD fMRI was performed by changing the stimulus strength (electrical current) to study strength dependence of both positive and negative BOLD responses. Previously, stimulation-induced negative blood oxygenation level dependent (BOLD) signal has been observed in both human and cat (3-5). However, its physiological source remains controversial (3, 4, 6). It can be due to either a "stealing" effect, activated brain region stealing blood flow from adjacent regions (4, 6), or neuronal origin, uncoupled CMRO2 and CBF decreases caused by a local neural activation decrease (3). To further understand the negative BOLD signal and its physiological source, rat BOLD fMRI in response to electrical forepaw stimulus was obtained in domitor-anesthetized rats at 9.4T.

METHOD Eight rats (250–350 g) were anesthetized initially with 5% isoflurane in O_2:N_2 (3:7), which was reduced to 2% for maintenance. A pair of needle electrodes was inserted into one of the forepaws for electrical stimulation. A bolus of 0.05 mg/kg domitor was injected subcutaneously, and isoflurane was disconnected after 10 minutes. The air mixture O_2:N_2 (3:7) was delivered to the nosecone for spontaneous respiration. A continuous subcutaneous infusion of domitor (0.1 mg/kg/hour) was started 15 minutes after the bolus injection. Rectal temperature was maintained at ~37°C by a feedback-controlled, water-circulated heating pad and blood oxygen saturation was monitored by non-invasive oxygen saturation monitor during preparation and fMRI studies. NMR measurements were performed on a 9.4T/20cm system (Bruker) with a 2-cm diameter actively-decoupled surface coil as a receiver and a 72 mm actively-decoupled volume coil as a transmitter. Eight consecutive axial slices were acquired with TE=15 ms, thickness = 1 mm, matrix size = 64×64 and FOV = 3×3 cm^2, using single-shot GE EPI. Each fMRI run consisted of 10 – **10** – 20 image acquisitions (boldface represents stimulation on) with TR = 2 s. For frequency dependent study, stimulus with fixed pulse width of 0.3 ms, current of 2 mA and varied frequencies of 1, 3, 6, 9, 12, 15 and 18 Hz were interleaved in different fMRI runs. For stimulation strength dependent study, stimulus with fixed frequency of 9 Hz, pulse width of 0.3 ms and varied current of 1, 2, 4, 6 and 8 mA were interleaved in different runs. At each condition, 10 to 20 runs were repeated for signal averaging. Statistical *t* value maps were calculated by comparing the images acquired during control and stimulation periods on a pixel-by-pixel basis and thresholded with t > 3 and a minimum cluster size of 4 pixels ($p<0.01$). Average time courses of the 30 pixels with the highest t-value in the primary somatosensory cortex; the 20 pixels with highest t-value in the thalamus, and the 60 pixels with the largest t-value in the Caudate Putamen were used for quantitative analysis.

RESULTS With domitor as the anesthesic agent, BOLD fMRI successfully detected well-localized activation foci in the forepaw area of the primary and secondary somatosensory cortices (SI and SII) and in the thalamus contralateral to the stimulation side (Fig. 1**A**). Interestingly, robust negative BOLD responses were detected in the Caudat Putamen (CPu) and some other cortical regions of both sides by strong stimulation. The effect of stimulus frequency on the response of SI and thalamus were shown in Fig. 1**B**. With a fixed pulse width of 0.3 ms and current of 2 mA, the 9-12 Hz stimuli evoked the highest change in BOLD signal intensity in SI compared with stimuli at other frequencies (red circles in Fig. 1**B**). But for signal change in thalamus, the frequency-dependence is not strong and plateaus beyond 6 Hz (green triangles in Fig. 1**B**). The effect of stimulus strength on the response of SI, thalamus and CPu was shown in Fig. 1**C**. In SI, the signal change monotonically increases with currents <4 mA and reaches a plateau at 4 mA (red circles in Fig. 1**C**). In contrast, positive signal change in the thalamus (green triangles in Fig. 1**C**) and negative signal change in the CPu (blue squares in Fig. 1**C**) monotonically increase with the current. The slope of signal change in CPu is higher than that in thalamus.



DISCUSSION In all rats with high stimulation strength, positive BOLD response were detected in SI, SII and thalamus of the contralateral side, while negative response were detected in CPu and some other cortical regions of both sides. The BOLD contrast is indicative change in the local magnetic field inhomogeneity induced by paramagnetic deoxygenated blood. The positive BOLD is caused by venous blood deoxyhemoglobin decrease resulted from that the CBF increases exceeds the CMRO2 increase. The negative BOLD should be caused by venous blood deoxyhemoglobin increase, which can result from either a CMRO2 increase and/or a reduction in CBF or uncoupled CMRO2 and CBF decreases. In fMRI of cat visual cortex (4), negative BOLD was mainly detected in the adjacent brain area. Hence it is concluded that the negative BOLD is most likely caused by "stealing" – activated visual cortex stealing blood from less active regions. But we detected the negative BOLD in both hemispheres and far away (>5mm) from the activated site. Hence it is not likely to be caused by such "stealing" effect. By direct measurement of BOLD and CBF (3), it was found that the negative BOLD is caused by uncoupled CMRO2 and CBF decreases which maybe due to the decrease of neural activity. In this study, with negative BOLD signal detected in functional connected regions (CPu and some other cortical regions in left and right hemispheres), it is likely of a neuronal origin.

Figure 1. BOLD fMRI under anesthesia by domitor. (**A**) Activation maps (t-value maps) in 6 consecutive slices (1mm thick) induced by forepaw stimulation with a 9 Hz, 0.3 ms wide pulse overlaid on corresponding T1-weighted images. The data were averaged from results of various currents, ranging from 1 to 8 mA. D: Dorsal; M: Medial; SI & SII: primary & secondary somatosensory cortices; CPu: Caudate Putamen. (**B**) Amplitudes of BOLD response in SI and thalamus (Tha) as a function of stimulus frequency (Mean ± Standard Error Mean, SEM. n=4). (**C**) Amplitudes of BOLD signal in SI, thalamus and CPu (negative) as a function of electrical current (Mean ± SEM, n=4).

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