Somatosensory Stimulus Frequency-dependent Neural, CBF, and BOLD fMRI Responses in Isoflurane-anesthetized Rat

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Introduction

Functional magnetic resonance imaging (fMRI) during electrical stimulation of the rodent forepaw has been used to investigate the biophysical properties of fMRI signals (1,2). Most rodent fMRI studies have been performed with α-chloralose. However, α-chloralose anesthetized animals have to be euthanized after the experiment, which hamper its use for *survival* experiments (3). Therefore, inhalation anesthetics (e.g. isoflurane) are preferable. Recently, we reported the stimulation parameters (for 10 fixed numbers of stimuli) that maximize the hemodynamic response in the somatosensory cortex of isoflurane-anesthetized rat (4). However, high frequency (12 Hz) for optimal stimulation found in previous study may not applicable to longer stimulus duration which is preferable for many fMRI studies because shortening an inter-pulse interval (i.e. increasing stimulation frequency) will induce a more pronounced reduction in neural activity over time (i.e. neural adaptation). Therefore, to obtain large hemodynamic fMRI responses in the rat cortex for long stimulation periods, it is crucial to measure the stimulus frequency and duration-dependent relationships between neural activity and hemodynamic responses. In this study, we measured field potential (FP), cerebral blood flow (CBF) and blood oxygenation level dependent (BOLD) fMRI responses in the rat somatosensory cortex during 30-s forepaw stimulation with various frequencies under 1.3-1.5% isoflurane anesthesia.

Methods

Twelve male Sprague-Dawley rats weighting 350-450g were studied. Two separate animal groups were used: one with simultaneous measurements of CBF using laser Doppler flowmetry (CBF_{LDF}) and FP (n = 6), and another with concurrent recordings of CBF using arterial spin labeling (ASL) (CBF_{ASL}) and BOLD fMRI at 9.4T (n = 6). Electrical stimulation pulses (1.0 ms pulse width and ~ 1.5 mA current) were applied to forepaw with nine frequencies (1, 1.5, 3, 4, 6, 8, 12, 16, and 24 Hz) for FP and CBF_{LDF} measurements, and four frequencies (1.5, 3, 6, and 12 Hz) for fMRI studies. A tungsten microelectrode was inserted to a depth of 0.5 mm perpendicular to the cortical surface at the center of activation focus area and a reference electrode was placed on the scalp. A needle-type LDF probe was placed on the thinned skull preparation within 0.5 mm of the location of the FP electrode while avoiding large visible pial vascular area. ASL studies performed to obtain CBF_{ASL} and BOLD responses. A single 2-mm thick coronal slice was acquired with matrix size of 64 (readout) × 32 (phase-encoding) and FOV = 2.56 × 1.28 cm^2. The TR was 2.5 s consisting of 2.4 s spin-preparation period for ASL and 0.1 s for image acquisition, and the TE was 20 ms. Cumulative changes over the stimulation duration 0 – 10s, 0 – 20s and 0 – 30s were also determined from the area under hemodynamic response curves.

Results and Discussion

Bin-FP was calculated by the summation of FP over every 2 s to compare FP activity across the different frequencies in which it contains different number of pulses per a given time (the number of stimulus in 12 Hz is eight times more than that of 1.5 Hz) (n = 6, Fig.1A). The bin-FP amplitude was largest for the 12 Hz stimulus frequency over the first 4 s from the stimulus onset. However, the bin-FP amplitude for the higher stimulus frequencies decrease more rapidly over time, while the bin-FP amplitude for the lower frequencies was maintained over the entire stimulation period. To examine frequency-dependence in the FP and CBF responses over stimulation duration, the cumulative FP and CBF_{LDF} of 0 – 10 s, 0 – 20 s, 0 – 30 s after stimulation onset are plotted as a function of stimulation frequency (n = 6, Figs 1B and 1C); for the first 10 s of stimulation duration, relatively high cumulative responses were observed from higher stimulation frequencies (squares), for longer stimulation durations, the maximal response was observed by 6 – 8 Hz of stimulation frequencies (triangles for 0 – 20s and circles for 0 – 30s).

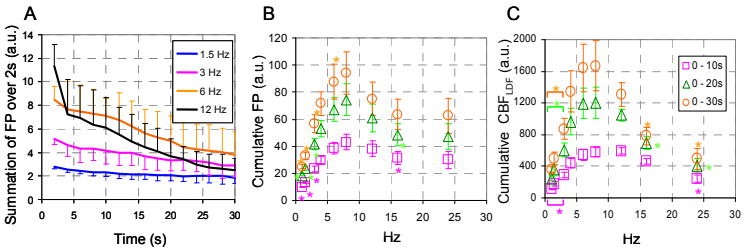

Fig. 1. (A) Averaged temporal profiles of FP response. Cumulative signal changes were determined as a function of stimulation frequency for FP (B) and CBF_{LDF} (C). * $P < 0.05$ with 12 Hz data.Error bars: SD.

The averaged BOLD time courses measured from the 16-pixel ROI over the somatosensory area are plotted in Fig. 2A (n = 6). Similar trends were observed in CBF_{ASL} measurements (data not shown). The hemodynamic responses of fMRI signals showed similar frequency-dependent trends to the bin-FP time courses across all stimulation frequencies (Fig. 1A). The peak amplitude of the averaged BOLD and CBF_{ASL} responses increased with stimulation frequency. However, over later stimulation periods, fMRI responses from higher frequency stimulation were significantly reduced compared to those from lower stimulation frequencies showing that repetitive stimulation attenuates the hemodynamic response, i.e. neural adaptation. As a result, fMRI signal changes are closely dependent on the stimulus duration. In order to evaluate the effect of stimulus duration on fMRI maps, we generated statistical maps over three different stimulation periods (see color bars underneath time courses in Fig. 2A). For the BOLD fMRI data with 12 Hz stimulation frequency, the activation area was observed to dramatically decrease over the later stimulation periods, while the activation area was roughly preserved with all of the other stimulation frequencies (Fig. 2B). Similar stimulus duration-dependent findings were observed in the CBF_{ASL} fMRI maps. In conclusion, our findings suggest that the optimal stimulation frequency is dependent on stimulation duration.

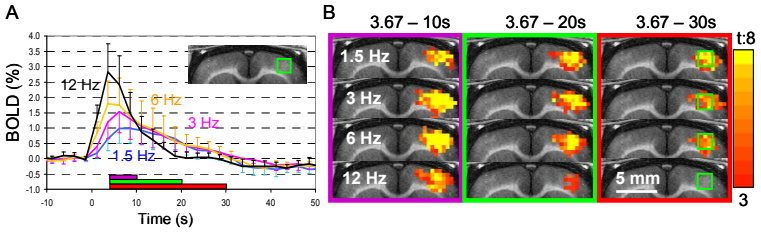

Fig. 2. (A) Averaged BOLD time courses. The green box in the inserted image shows the ROI. Error bars: SD. (B) BOLD fMRI maps were overlaid on T_1-weighted images

References 1. MRM 1999; 42(5): 919. 2. JCBFM 1999; 19(8): 871. 3. Lab Anim Sci 1993; 43(3) 210. 4. Cereb Cortex 2007; 17(4):942
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