# CBV-fMRI Signal in Rat Increases Non-Linearly with Whisker Stimulus Frequency but Approximately Linearly with Amplitude

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**Introduction:** The rat whisker barrel cortex is a special region where each of the whiskers is topographically mapped to a barrel-shaped column. Substantial anatomical and electrophysiological data about the barrel cortex have accumulated (1). In several studies fMRI was applied to this model (2,3). However, the characteristics of fMRI response to different stimulus conditions have not been established. In a large part, this has been due to the lack of an MRI-compatible stimulator. We have designed a new whisker stimulator that can output sinusoidal waveform in the range of 4~24 Hz in deflection frequency and 2~6 mm in amplitude. CBV-fMRI experiments employing iron oxide contrast agent were conducted in rat barrel cortex (n = 8). Results show that the signal increases with stimulus frequency up to 12 Hz and plateaus at higher frequencies. In addition, it increases approximately linearly with stimulus amplitude from 2 to 6 mm deflection amplitude. Our results also reveal that the plateau of CBV response at higher frequency (> 12 Hz) is not due to the "ceiling effect."

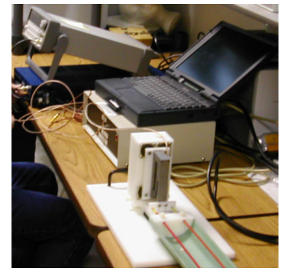

FIG. 1. The Stimulator Setup.

**Materials and Methods:** Figure 1 shows the whisker stimulator designed in this laboratory. A laptop computer is used to generate pulses of the desired frequency and timing. The pulses are amplified and used to drive a custom-made piezoelectric actuator. Two whisker combs are connected to long plastic rods which provide isolation of the piezoelectric actuator from the center of the magnetic and RF fields. Rat whiskers are loosely constrained within the teeth of the comb, which displaces them passively as the comb moves while allowing sufficient freedom for the whiskers to slide in and out of the teeth during large deflection amplitude. The distance between the comb and rat face is about 1 cm such that only long whiskers on both sides of rat face (no facial hairs) are moved.

FMRI experiments were carried out on a Bruker Biospec 30/60 3 Tesla scanner equipped with local gradient coil and RF coils. Eight α-chloralose anesthetized rats were scanned under artificial ventilation (n = 4 for graded frequency only, n = 2 for graded amplitude only, n = 2 for both). Animal preparation procedures are described in Ref. 3. Scan parameters: TR = 1 s, TE = 27.2 ms, FOV = 3.5 cm, three coronal slices, slice thickness = 2 mm, matrix size = 64 × 64. MION was injected at an iron dose of 12 mg/kg. *For the graded frequency exp.*: The displacement amplitude of the comb was keep at 4 mm peak-to-peak, whereas the displacement frequency was pseudo-randomly changed between 4, 8, 12, 16, 20 and 24 Hz. The scan paradigm was a block design consisting of seven cycles of 30 s on and 120 s off plus 30 s initial baseline scan. *For the graded amplitude exp.*: The displacement frequency was fixed at 12 Hz and the amplitude was changed between 2, 4 and 6 mm (peak-to-peak) randomly.

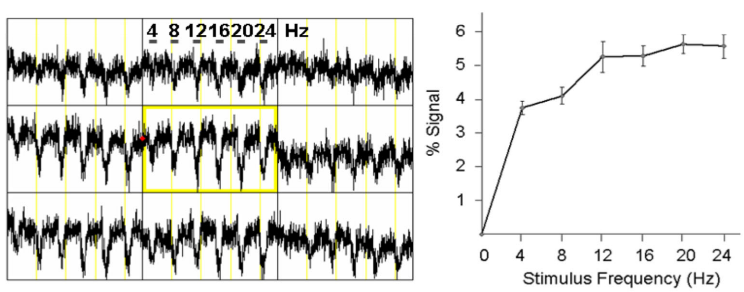


FIG.2. **a**: Raw CBV-fMRI time courses during stimuli of variable frequency from one rat.

**b**: Averaged percent fMRI signal changes (n = 6).

**Results:** Figure 2a shows representative raw CBV-fMRI time courses in nine neighboring pixels during whisker deflection. The stimulus frequency was 4 to 24 Hz as shown in the figure. Figure 2b shows the averaged fractional fMRI signal (n = 6). It appears that the fMRI response increases with stimulus frequency up to 12 Hz and plateaus at higher frequency. Percent fMRI signal change at 12 Hz was 5.2 ± 0.5% (mean ± SEM). Figure 3 shows averaged CBV response (n = 4) at stimulus amplitude of 2, 4 and 6 mm. FMRI response increases approximately linearly with the amplitude. Percent fMRI signal change at 6 mm deflection was 7.6 ± 1.2 % (mean ± SD).

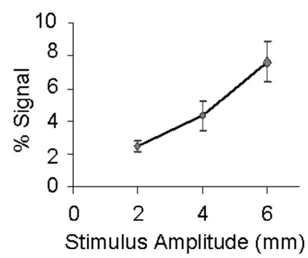


FIG. 3. Averaged percent fMRI changes during stimuli of different deflection amplitude (n = 4).

**Discussion:** The fMRI data shown in Fig. 3 are consistent with an optical imaging study by Devor et al. (4). By deflecting a single whisker with different amplitude, Devor et al. showed that optical signals of different spectra, including oxygenated hemoglobin (HbO), hemoglobin (Hb) and total hemoglobin (HbT), increase approximate linearly with the deflection amplitude. Figure 2 shows that the fMRI response plateaus at a frequency higher than 12 Hz. Considering the maximum % signals of Figs. 2 and 3, it is unlikely that this plateau is due to a "ceiling effect", i.e., a vascular response that reaches a maximum. Instead, the vasculature still has reserve capacity in the plateau region of Fig. 2.

**References:** 1. Simons DJ. J Neurophys 1978;**41**:798-820. 2. Yang et al. PNAS 1996;**93**:475-8. 3. Lu et al. MRM 2004;**52**:1060-68. 4. Devor A. et al. Neuron 2003;**39**:353-359.