# Simultaneous Recordings of BOLD fMRI and Electrophysiological Responses to Whisker Stimulation in Conscious Rabbits

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## INTRODUCTION

We report preliminary results of simultaneous recordings of BOLD fMRI and electrophysiological responses from conscious rabbits during whisker stimulation at frequencies of 10 – 75Hz. The results show that our physiological recording does not affect MR image quality and that recorded physiological signals can be analyzed unambiguously after proper removal of the interfering signals induced from strong gradient pulses during MRI. Consistent results for across-subject data suggest that the awake, habituated rabbit is a suitable subject for combined fMRI and neurophysiological studies.

## METHODS

Animal preparation Five female rabbits (Dutch Belted) were used in this study. A head-piece with four nylon screws was stereotaxically implanted into the rabbit skull with sterile technique as described elsewhere (1), and a single, 100μm, PtIr electrode (1.5MOhm) was implanted into each side of whisker cortex at 6.3mm lateral from midline and 2mm posterior to Bregma. After recovery from the surgery, the rabbits were habituated to the MRI environment (1). The whiskers that activated neurons near the electrode tip were identified by listening for a maximum response while manually actuating each of the whiskers. All other whiskers were cut to 5mm or less in length to prevent nonintentional stimulation during experiments.

MRI experiments All MRI experiments were performed on a Bruker Biospec 4.7T imaging spectrometer. The spectrometer is equipped with actively-shielded gradient coils (a maximum gradient field of 20 G/cm). A single-turn, 40mm-dia., circular RF surface coil was used for both transmission and reception. A multi-slice gradient-echo imaging sequence was used to locate the whisker barrel cortex. The same sequence was used to obtain anatomical brain images. For fMRI data acquisitions, a single-shot, gradient-echo multi-slice EPI sequence was used. MR signal was detected from four consecutive, 1mm-thick slices of brain in the axial plane, with FOV of 48mm×48mm. For the high-resolution anatomical images, imaging matrix=128×128, TR=1500ms and TE=10ms. For EPI, the imaging matrix was 64×80, which yields a voxel volume of 450 $\mu m^3$, TR=2s, and TE = 20ms. One session of an fMRI experiment consisted of blocks of ten trials with 32 images acquired in each trial.

Whisker stimulation The whisker vibration was generated by a 15mm-dia., six-turn, circular coil that was placed in the magnet bore, and was driven by an alternating current. The coil was positioned 30cm away from the imaging coil and secured on a straight fiber band. One edge of the fiber band was fixed and the other end was adjustable and secured to the front of the cradle. Selected whiskers in rows C and D on the left side of rabbit face were then attached to one end of the fiber band. The vibration amplitude was controlled by adjusting the strength of the driving current. To ensure that the fiber band was able to accurately follow the preset frequency and amplitude, an optical monitoring system was designed and built based on our previous eyeblink detection system (2). An output signal induced by the vibration was monitored in real time and recorded in a computer for later reference in data analyses. The stimulus paradigm consisted of a baseline period (12 images), a stimulation period (10 images), and a rest period (10 images). The vibration amplitude of the stimulator was kept constant, while the vibration frequency was varied from 10 to 75Hz from block to block within each session. The driving current was controlled such that the whisker deflection was ±1.5mm or ±0.7mm.

Neuronal activity recordings Neuronal activity was recorded through the electrodes and was differentially amplified and filtered to collect action potentials (band pass 300-3000Hz) and local field potentials (LFP band pass 40-150Hz). Electrophysiological signals from the neuronal activity were analyzed after removal of blocks of strong interfere signals induced from gradient pulses. The neuronal signals were half-wave rectified and integrated. The portions of the signal surrounding the stimulation were analyzed across trials.

Detection of BOLD responses All images were processed off-line using in-house developed software with IDL (Research Systems, Boulder, CO). The first two images were removed from each trial to ensure stable image intensities. The images were examined for global motion. Any trial with images showing significant global motion was excluded from further analyses. The remaining trials were averaged to generate a series of 30 images. Cross-correlation coefficients (CC) were calculated on a pixel-by-pixel basis by correlating the 30-point time course of a pixel with a boxcar reference function. All pixels with CC higher than 0.5 were further evaluated with a *t*-test. Only pixels with a *P*-value < 0.001 were considered to represent significant activation. An activation map was generated by superimposing color-coded CC's of the activated pixels on an EPI anatomical image.

## RESULTS

Figure 1 shows typical BOLD activation maps in the whisker barrel cortex as a function of increasing stimulation frequency at constant whisker deflection of ±0.7mm. These maps show an increase in the average CC and size of activation with increasing stimulation frequency. Activated pixels surround the electrode tip on the right side of the brain. The bar at the bottom displays a color-coded scale of 0.0-1.0 of CC values. Figure 2 shows segments of the LFP, multiunit spike activity (MUA), and

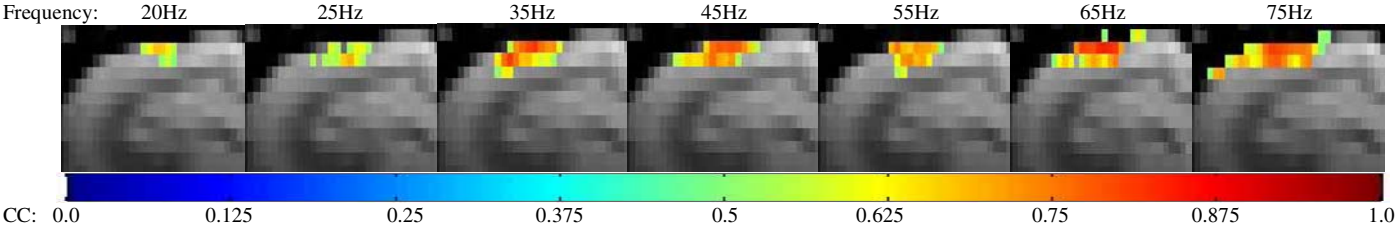


Figure 1

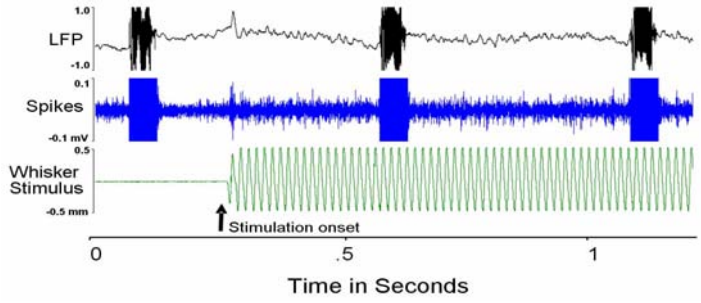


Figure 2

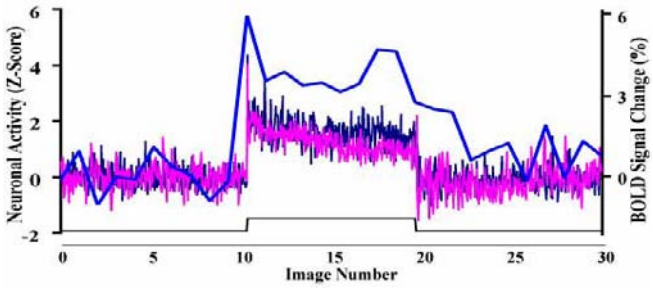


Figure 3

the vibration monitoring signal. The segments surround a typical onset of whisker stimulation, providing smooth, sinusoidal movement of the whiskers. The gradient pulses induced artifacts in the MUA and LFP traces. The artifacts were removed before electrophysiological analysis. The onset of whisker stimulation did not coincide with image acquisition, allowing us to analyze the neurophysiological data from stimulation onset. Figure 3 shows changes in BOLD signal (blue), LFP (purple) and spike activity (black) simultaneously recorded from a rabbit during 75Hz whisker stimulation. All these figures and other data demonstrate strong correlation of BOLD fMRI responses with neuronal activity. Therefore this work suggests that the whisker barrel system in the awake rabbit provide a useful model system to study the neurophysiological base of fMRI responses.

**REFERENCES:** 1. Wrywicz, A.M., *et. al.*, MRM 2000; **44:** 474-478. 2. Miller, M. *et. al.*, J. of Neuroscience Methods, 2005; **141:** 83-87.

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