Echo-Planar Imaging BOLD fMRI in Mice on a 9.4 T Vertical Bore Microimager

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INTRODUCTION High-field, small vertical-bore microimaging systems are cost effective. However, functional MRI using echo-planar imaging (EPI) on these microimagers is relatively more challenging. One major challenge is the limited use of physiological monitoring and other supporting equipment. Maintaining stable and normal physiology, for example, is critical for eliciting robust fMRI responses. In rats, fMRI of electrical stimulation had been generally performed under α-chloralose and mechanical ventilation (1-4), in which rats' physiological parameters are carefully maintained within normal physiological ranges by changing ventilation rate/volume and anesthetic dose based on periodic blood-gas sampling. Mice are an order of magnitude smaller than rats and, thus, maintaining physiology using similar approach is difficult. Another potential challenge is the increased eddy current effect due to the reduced spacing between the gradient coils and the cryostat, and the increased static magnetic susceptibility at high fields. These difficulties could explain the lack of published fMRI studies using EPI on high-field microimagers. The availability of unique transgenic and disease models in mice could benefit significantly with the use of non-invasive longitudinal fMRI assessments.

The goal of this study was to establish a robust mouse model for electrical somatosensory stimulation fMRI studies on a 9.4 T vertical-bore microimaging system using echo planner imaging. BOLD fMRI was explored on spontaneously breathing mice anesthetized with isoflurane. We chose to have mice breathe on their own (as opposed to tracheotomy and mechanical ventilation) because it is simple to set up and they can maintain their own physiology. We chose isoflurane because the anesthetic level can be readily maintained stable over a relatively long time for repeated fMRI measurements. The stimuli used were hypercapnic challenge and electrical hindpaw stimulation. Graded isoflurane levels and stimulation currents were evaluated to determine the optimal conditions for BOLD fMRI studies on mice.

METHODS Mice were secured in a stereotaxic head restrainer with tooth-, ear- and shoulder-bars. Rectal temperature and respiration rate were maintained within normal physiological range. Three groups of mice were studied. In Group I (n=9), hypercapnic (10% CO_2) challenges under graded isoflurane levels (from 0.25 to 1.25% in steps of 0.25%) were studied. In Group II (n=6), electrical hindpaw stimulations (electrodes under the skins) with graded current (1-7 mA) under 0.75% isoflurane were studied. In Group III (n=5), studies were performed under 0.75% and 1% isoflurane and only 4 and 6 mA stimulation currents were explored.

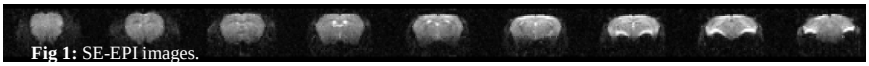


Fig 1: SE-EPI images.

MRI was performed on a 9.4T, 89mm vertical bore magnet with a 100G/cm gradient insert (100 μs rise time) and a small RF transceiver surface coil optimized for imaging mouse brain. BOLD fMRI was acquired using single-shot, spin-echo (SE) EPI with TR = 2.5 s, TE = 35 ms, 90° flip angle, matrix of 64x32, FOV = 2 cm x 1 cm, 9 slices of 0.6-mm thickness with interslice gap of 0.15 mm, readout gradient of 14 G/cm, and a spectral width of 120 kHz. The echo time, approximating tissue T_2 at 9.4 T (~38 ms) (5), was used to achieve optimal BOLD responses. Anatomical images were acquired using conventional spin-echo sequence with similar parameters, but double the resolution.

For the hypercapnic study (Group I), BOLD changes and contrast-to-noise ratio (CNR) were computed using ROI's covering essentially the entire brain. For the hindpaw stimulation (Group II and III), cross-correlation analysis was performed to obtain activation maps. ROI of the hindpaw primary somatosensory cortex was carefully drawn using anatomical images. BOLD percent changes were derived without using an activation mask to avoid any bias to particular current or isoflurane level. All values reported in text are in mean ± SD and on plots are mean ± SEM.

Fig 2: Group I.

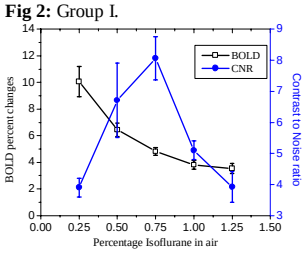


Fig 3: Group II.

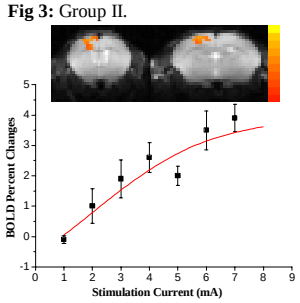
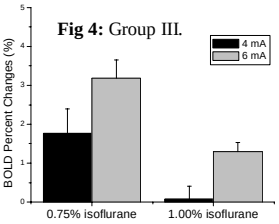


Fig 4: Group III.



RESULTS All mice survived at the end of ~6 hours of imaging. **Fig. 1** shows representative spin-echo EPI images with TE approxitely equal to the tissue T_2 for optimal BOLD contrast. Robust hypercapnia-induced BOLD changes were observed (Group I). BOLD percent changes decreased with increasing level of isoflurane. At lower levels of isoflurane (0.25% and 0.5%) some movement-related spikes was observed in the BOLD timecourses. The optimal CNR for hypercapnic challenge was determined to be ~ 0.75% isoflurane (**Fig. 2)**, which was used for subsequent electrical somatosensory stimulation.

Robust BOLD activation was observed in the primary somatosensory cortex following hindpaw electrical stimulation (**Fig. 3 inset, Group II**). Group-average BOLD percent changes increased with graded stimulation currents and appeared to level off at ~6 mA (**Fig. 3**). Of the current explored (1-7 mA), 7 mA and occationally 6 mA stimulations under 0.75% isoflurane caused a significant increase in respiration rate and occasional twitching of the hindleg. Thus, fMRI studies were performed under 0.75% and 1% isoflurane in details (Group III). Relative to the 0.75% isoflurane, respiration rates and waveforms at 1% isoflurane was more stable during stimulations. However, no consistent SE BOLD fMRI responses were observed with 4 mA stimulation under 1% isoflurane. With 6 mA stimulation under 1% isoflurane, robust BOLD responses were observed with an average SE BOLD percent changes of 1.3 ± 0.51% (mean ± SD, n=5). In comparison to 0.75% isoflurane, 4 and 6 mA stimulation yielded 1.75 ± 1.2 % and 3.2 ± 1.0%, respectively (**Fig. 4**).

DISCUSSIONS Relatively high stimulation currents (compared to ~2mA under α-chloralose in rats) were needed to evoke robust BOLD responses. This is likely due to isoflurane being a potent anesthetic, which suppresses neural activity. Our study is consistent with a forepaw stimulation study in rats (6) in which isoflurane anesthesia was used. In that study, blood gases, blood pressure, heart and respiration rate were carefully monitored under graded stimulation currents. An optimal stimulation current of ~6 mA under 1% isoflurane (without evoking substantial changes in blood pressure and heart rate) was reported.

Literatures on BOLD fMRI using EPI or other imaging techniques on microimagers are sparse. Ahrens *et al.* (7) used conventional gradient-echo (GE) acquisition on an 11.7 T microimager to investigate the hindpaw electrical stimulation in mice under α-chloralose anesthetic (i.p.). Conventional (GE) BOLD yielded poor temporal resolution, more sensitive to movement and physiological artifacts, and the single-slice activation map showed activation localized to draining veins and cortical surfaces (7). Grieve *et al.* (8) was the first to report the use of EPI on microimager. Despite minimal echo time used, the GE and SE EPI appeared to have limited applications and no fMRI were performed.

CONCLUSION This study demonstrated a BOLD fMRI study on a 9.4 T microimager using single-shot SE EPI with TE ~ tissue T_2 for optimal BOLD contrast. A model for somatosensory stimulation was also established in mice under spontaneously breathing and isoflurane anesthesia, which yielded stable physiology and robust BOLD responses. The optimal stimulation current was between 4-6 mA for 0.75% isoflurane and ~6 mA for 1% isoflurane. Further improvement in spatial resolution is under investigation. This technique and model are expected to have widespread applications in longitudinal fMRI studies of unique transgenic mouse models.

References 1) Hyder et al., JCBFM 1994; 14: 649. 2) Mandeville et al., MRM 1998; 39: 615. 3) Silva et al., JCBFM 2000; 20: 201. 4) Duong et al., MRM 2000; 43: 338. 5) Lee et al., MRM 1999; 42: 919. 6) Liu et al., MRM 2003, submitted. 7) Ahrens et al, NMR Biomed 2001, 14:318. 8) Grieve et al. MRM 2000, 43:747.