

Echo-Planar BOLD fMRI of Mice on a Narrow-Bore 9.4 T Magnet

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The feasibility of BOLD fMRI in association with electrical somatosensory stimulation on spontaneously breathing, isoflurane-anesthetized mice was investigated using spin-echo, echo-planar imaging (EPI) on a vertical narrow-bore 9.4 T magnet. Three experiments were performed to derive an optimal fMRI protocol. In Experiment 1 (n = 9), spin-echo BOLD responses to 10% CO₂ challenge under graded isoflurane (0.25–1.25%) ranged from 10 ± 2% to 3.5 ± 0.9%; the optimal BOLD contrast-to-noise ratio peaked at 0.75% isoflurane. In Experiment 2 (n = 6), hindpaw somatosensory stimulations using 1–7 mA under 0.75% isoflurane revealed the optimal BOLD response was at 6 mA. In Experiment 3 (n = 5), BOLD responses to 4 and 6 mA stimulation under 0.75% and 1% isoflurane were evaluated in detail, confirming the optimal conditions in Experiment 2. These results demonstrated that BOLD fMRI using single-shot, spin-echo EPI in a mouse somatosensory stimulation model could be routinely performed on high-field, vertical, narrow-bore magnets. This protocol might prove useful for fMRI studies of transgenic mice. Magn Reson Med 52: 430–434, 2004. © 2004 Wiley-Liss, Inc.

Key words: isoflurane anesthesia; spin-echo; high fields; hypercapnia; somatosensory stimulation; microimager

Mice have been widely used in research because of their genetic similarity to humans, availability of genetically altered strains, and disease models, combined with their small size, high reproductive rate, and cost effectiveness. Many of these transgenic strains and disease models are very valuable, which necessitate and/or benefit significantly with the use of noninvasive longitudinal assessments.

MRI is a powerful tool for noninvasive imaging of brain anatomy, physiology, and function. Narrow-bore, high-field, vertical magnets are well suited for imaging mice because of the potential for improved signal-to-noise ratio (SNR), spatial resolution, and functional spatial specificity (1–3), while being cost effective. However, these systems pose several challenges. The narrow bore size limits the use of physiological monitoring and animal support equipment. Maintaining stable and normal physiology is critical for eliciting robust functional MRI (fMRI) responses. For example, fMRI of electrical somatosensory stimulation in rats is generally performed under α -chloralose (an analgesic and a mild anesthetic (4)) and mechanical ventilation (5–7), where their physiological parameters are maintained within normal ranges by changing ventilation rate

and volume based on periodic blood-gas sampling. Mice are an order of magnitude smaller than rats and, thus, maintaining physiology using a similar approach is difficult. Other potential challenges are the increased eddy-current effect due to reduced spacing between the gradient coils and the cryostat, and the increased susceptibility artifacts at high fields due to the large air–tissue interface of the small mouse brain coupled with generally poor shimming capability. These effects could make certain protocols, such as diffusion MRI and echo-planar imaging (EPI), challenging.

For these reasons, the fMRI literature on narrow-bore vertical magnets is sparse. Ahrens and Dubowitz (8) investigated the hindpaw stimulation in mice under α -chloralose (i.p. administration) using conventional gradient-echo (FLASH) acquisition at 11.7 T. Similarly, Huang et al. (9) used gradient-echo (FLASH) BOLD to study visual stimulation in mice under pentobarbital anesthesia at 9.4 T. Mueggler et al. (10) reported forepaw stimulation in mice under isoflurane using an exogenous blood-volume contrast reagent and a fast-spin-echo (RARE) acquisition at 7 T. Conventional gradient-echo acquisition has the disadvantage of poor temporal resolution, poor SNR per unit time, and limited multislice capability. Fast spin-echo acquisition has poor BOLD sensitivity unless an exogenous contrast agent is used. On the contrary, EPI yields reduced motion and physiological artifacts, higher SNR per unit time, and higher BOLD sensitivity relative to other techniques and is thus the most widely used data acquisition method for BOLD fMRI (11). Grieve et al. (12) were the first to explore EPI on a 7 T. However, a fMRI study was not performed.

The goal of this study was to develop a robust somatosensory-stimulation mouse model for BOLD fMRI studies using spin-echo EPI on a vertical, narrow-bore 9.4 T magnet. BOLD fMRI was explored on spontaneously breathing mice anesthetized with isoflurane. Mice were allowed to breathe on their own because it is simple to set up and they can autoregulate their physiology. Isoflurane was explored because its anesthetic level can be maintained stable over a long time for repeated measurements. Hypercapnic (CO₂) challenge under graded isoflurane levels was used for optimizing the isoflurane condition because inhaling CO₂ induces a robust and global BOLD increase. Graded stimulation currents were evaluated to derive an optimal current for BOLD fMRI studies.

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Received 1 December 2003; revised 12 March 2004; accepted 14 March 2004.

DOI 10.1002/mrm.20158

Published online in Wiley InterScience (www.interscience.wiley.com).

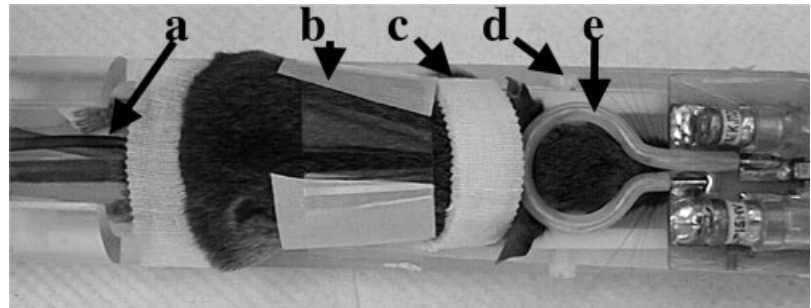
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MATERIALS AND METHODS

Animal Preparation

Three sets of experiments were performed on spontaneously breathing mice (BALB/c, 23–30 g) under isoflurane: 1) 10% CO₂ challenge under 0.25–1.25% isoflurane in

FIG. 1. Animal holder with stereotaxic headset, RF probe, and monitoring sensors. a: Rectal probe; b: respiratory belt (sensor underneath the animal); c: shoulder bar (a hollow tube); d: stereotaxic headset; e: RF probe. The tooth bar, not visible, is located below the RF probe platform. Warm water tube and air tube run below the animal's body.



steps of 0.25% ($n = 9$); 2) hindpaw stimulation using 1–7 mA currents under 0.75% isoflurane ($n = 6$); and 3) hindpaw stimulation using 4 and 6 mA under 0.75% and 1% isoflurane ($n = 5$). A stereotaxic headset consisting of ear bars and a tooth-bar was used to keep the mouse head immobile (Fig. 1). A shoulder bar was used to minimize transduction of respiratory movement to the imaging slices. Air flowed (~ 1 L/min) to the animal chamber via a tube running underneath the body. Rectal temperature was maintained at $37.5 \pm 0.5^\circ\text{C}$ via a circulating warm-water tube running underneath the body. Respiration rate, monitored via a force transducer, a differential amplifier, and an oscilloscope, was maintained within normal physiological ranges (60–220 bpm) (13). A surface coil was placed over the animal's head. Saline (0.3 cc every ~ 2 hr ip) was administered. Support was given to the legs and lower body with the mice curved up slightly. All mice were kept physiologically stable throughout the entire study, typically lasting 4–6 hr.

In some animals ($n = 4$), bench-top observations were made with the animal in the vertical position. Body movement, respiration rate, and waveform were observed during hindpaw stimulation.

Hypercapnic Challenge (Experiment 1, $n = 9$)

Ten percent CO_2 was used to determine the isoflurane dose for eliciting optimal BOLD responses while minimizing baseline signal fluctuation. A relatively high CO_2 concentration was used because of the smaller hypercapnia-induced fMRI signal changes in anesthetized, relative to awake, animals (14). Graded isoflurane levels, varied from 0.25–1.25% in steps of 0.25% (randomized), were explored. For each trial, BOLD images were acquired for 2 min during baseline (air) and 2 min during CO_2 challenge. A 15–20-min break was given between trials. Typically, two trials at each isoflurane level were repeated on each animal.

Somatosensory Stimulation (Experiments 2 and 3)

Two needle electrodes were inserted under the skin of the hindpaw. Experiments were performed using graded electrical currents with pulse duration of 0.3 ms at 3 Hz (6). In Experiment 2 ($n = 6$), 1–7 mA in steps of 1 mA (randomized) under 0.75% isoflurane were explored. In Experiment 3 ($n = 5$), only 4 and 6 mA under 0.75 and 1% isoflurane were explored. For each trial, images were acquired for 2 min during baseline, 1 min of stimulation, and 2 min of baseline. A break of 15–20 min was given between

trials. Two trials for each current were typically repeated on each animal.

MRI Experiments

MRI was performed on a 9.4 T, 89 mm vertical magnet (Oxford Instruments, Oxford, UK) equipped with a Varian^{INOVA} console (Palo Alto, CA) and a 100 G/cm gradient (45 mm ID and 100 μs risetime; Resonance Research, Billerica, MA). A surface coil (1.5 cm ID), where remote tuning and matching were achieved via long tuning rods from the top of the magnet, was optimized for imaging the entire mouse brain. Shimming was performed over an 8-mm thick slab and the linewidth ranged from 30 to 45 Hz.

Anatomical images were acquired using conventional spin-echo sequence with a TR = 2.5 sec, TE = 35 ms, four averages, FOV = 2×1 cm, matrix = 128×64 , and nine 0.6-mm slices (0.15 mm gap). BOLD fMRI was acquired using single-shot, spin-echo EPI with identical parameters except an acquisition matrix of 64×32 ($312 \times 312 \times 600 \mu\text{m}^3$) and without signal averaging. Spectral width, readout time, and readout gradient were 125 kHz, 17 ms, and 14 G/cm, respectively. The echo time was set to approximate tissue T_2 at 9.4 T (~ 38 ms in rats (1)) for optimal BOLD contrast.

Data Analysis

The first two stimulation trials on each animal were discarded. For hypercapnia, BOLD percent changes were calculated from an ROI covering essentially the entire brain. BOLD contrast-to-noise ratio (CNR) was computed by taking the ratio of the average percent change to the standard deviation of the percent changes across time during hypercapnia. For hindpaw stimulation, cross-correlation percent-change maps were calculated using the STIMULATE software (University of Minnesota) and overlaid on EPI or anatomical images. To avoid bias to a particular current or isoflurane level, cross-correlation BOLD maps were obtained and averaged across all stimulation conditions. ROI of the hindpaw primary somatosensory cortex in the contralateral hemisphere was carefully drawn with reference to the average maps, mouse brain atlas, and anatomy as guides. Percent changes for each condition were derived without using an activation-map mask.

T-test was used for statistical comparison. $P < 0.05$ was considered to be statistically significant. Data in text were mean \pm SD and error bars on graphs were SEM.

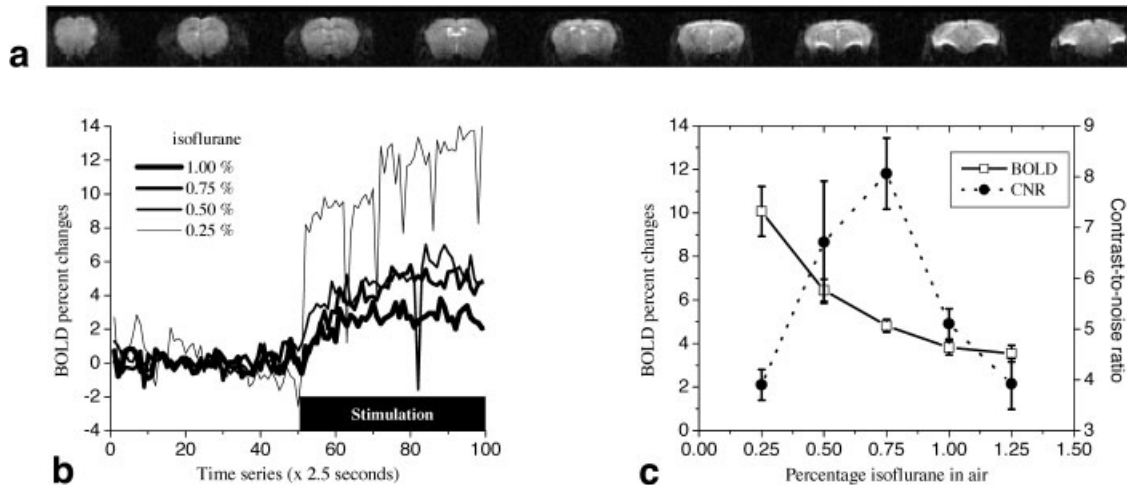


FIG. 2. **a:** Representative single-shot, spin-echo EPI images. **b:** BOLD time courses from a spontaneously breathing mouse under graded isoflurane levels. **c:** Group-average BOLD percent changes and CNR under graded isoflurane levels (mean \pm SEM, $n = 9$, Experiment 1).

RESULTS

Hypercapnic Challenge

Figure 2a shows representative spin-echo EPI images from one animal. Major brain structures could be identified on the EPI images. Hypercapnic challenge evoked a global and spatially heterogeneous BOLD increase (maps not shown). The hypercapnic BOLD time courses for graded isoflurane levels from one animal are shown in Fig. 2b. At low (0.25% and 0.5%) isoflurane levels, some motion-related spikes in the BOLD time courses are evident. At $\geq 0.75\%$ isoflurane, BOLD time courses showed no apparent movement artifacts. Group-average hypercapnic BOLD responses increased with decreasing isoflurane. However, motion-related signal fluctuation increased with decreasing isoflurane. The group-average optimal BOLD CNR peaked at $\sim 0.75\%$ isoflurane (Fig. 2c).

The respiration rates under 0.25, 0.50, 0.75, 1.0, and 1.25% isoflurane were, respectively, 155 ± 19 , 143 ± 24 , 128 ± 15 , 127 ± 10 , and 116 ± 18 bpm before hypercapnia. Respiration rates during hypercapnia increased by 33 ± 13 , 32 ± 14 , 26 ± 12 , 16 ± 6 , and $19 \pm 10\%$, respectively. Spikes in the respiratory traces were evident at low (0.25 and 0.5%) isoflurane and often manifested into spikes in the BOLD time courses.

Hindpaw Stimulation

Bench-top observations indicated little or no movement during stimulation with ≤ 6 mA under 0.75% isoflurane; no significant changes in respiration rate during stimulation were detected. At 7 mA (and rarely at 6 mA), twitching of the hindpaw was sometimes observed. There were no burn marks in any study. At 7 mA, transient irregular respiration patterns immediately following the stimulus onset were observed and the respiratory rate increased by $\sim 15\%$ ($P < 0.05$) during stimulation.

Figure 3a shows representative activation maps from an animal stimulated with 6 mA under the optimal 0.75% isoflurane. Activations were observed in the primary hind-

paw somatosensory cortex in the contralateral hemisphere. Figure 3b shows the BOLD time courses at different stimulation currents for a single animal. The group-average percent changes increased with graded stimulation currents and appeared to begin to plateau at ~ 6 mA (Fig. 3c). Since it is conceivable that the optimal isoflurane for hypercapnic challenge might not be the same for electrical somatosensory stimulation because the latter could agitate the animal, 4 and 6 mA stimulation under 0.75% and 1% isoflurane were studied in detail (Experiment 3). Relative to 0.75% isoflurane, respiration rate at 1% isoflurane was more stable. Under 1% isoflurane, 4 and 6 mA evoked negligible and $1.3 \pm 0.5\%$ BOLD increases, respectively. In contrast, under 0.75% isoflurane, 4 and 6 mA evoked $1.8 \pm 1.2\%$ and $3.2 \pm 1\%$ BOLD increases, respectively.

DISCUSSION

One major finding of this study is that isoflurane anesthesia can be used for fMRI studies of electrical somatosensory stimulation in spontaneous breathing mice. The use of isoflurane, however, has some disadvantages. Isoflurane is a potent vasodilator (15) which caused a global CBF increase in a dose-dependent manner. Basal CBF had been shown to modulate the magnitude and the dynamics of the stimulus-evoked BOLD responses (16,17). Higher basal CBF yields a smaller BOLD percent change, and a slower, broader hemodynamic response to visual stimulation. It is thus critical to minimize the isoflurane concentration. More importantly, isoflurane suppresses neuronal activity, which reduces BOLD responses and/or requires stronger stimulation. The higher currents used herein are consistent with a recent forepaw-stimulation fMRI study in spontaneously breathing isoflurane-anesthetized rats (18). In that study, blood gases, blood pressure, heart rate, and respiration rate were measured under graded stimulation currents. Optimal BOLD and CBF fMRI responses were determined to be ~ 6 mA at 1.1% isoflurane without in-

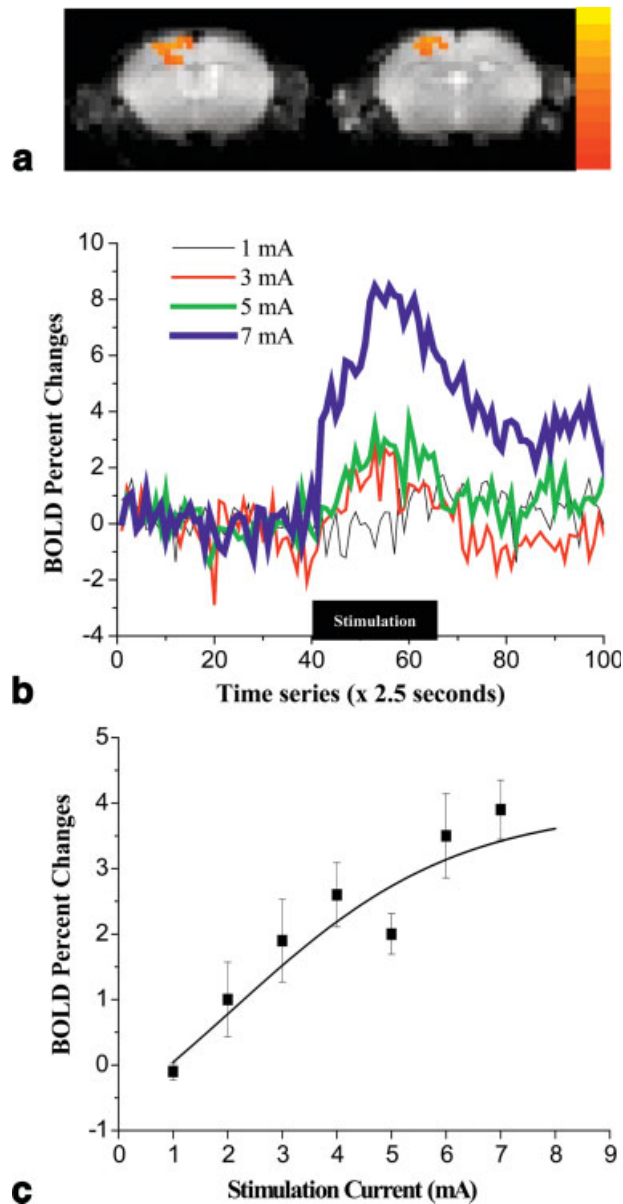


FIG. 3. **a**: Representative activation maps (overlaid on anatomy) obtained with 6 mA stimulation under 0.75% isoflurane. Activations are localized to the contralateral primary hindpaw somatosensory cortex. Scale bar indicates cross-correlation coefficient from 0.4–0.8. **b**: Representative BOLD time courses at selected graded stimulation currents under 0.75% isoflurane. **c**: Group-average BOLD percent changes at graded stimulation currents under 0.75% isoflurane (mean \pm SEM, $n = 6$, Experiment 2).

voicing changes in blood pressure, heart rate, and respiration rate.

It was noted that the spatial activation patterns in the spontaneously breathing isoflurane mice model is more variable across animals compared to the mechanically ventilated, α -chloralose rat model. This could be explained in part by the increased physiological noise due to irregular breathing. The effects of different anesthetics on neural activity and/or the stimulation current used could also be a factor.

The issue of potential adverse effects on physiology resulting from positioning mice in the vertical orientation had been raised (19). The use of anesthetic could exacerbate these effects although its dosage was minimized. Support given to the legs and lower body presumably reduced cardiac workload and minimized blood draining away from the head. Supplemental fluid prevented dehydration. Respiration rate was not different between the beginning and the end of the study. Furthermore, BOLD responses were observed throughout the entire study, suggesting CBF and other physiological functions are by and large unperurbed. All animals became conscious within 1–2 min upon removal of isoflurane. Further investigation of potential adverse effects due to vertical positioning is nonetheless warranted.

CONCLUSIONS

A robust somatosensory stimulation model in mice was established for fMRI studies. Spin-echo BOLD fMRI using single-shot EPI at reasonably high spatiotemporal resolution can be routinely performed on cost-effective, vertical-bore microimaging systems at high field. This fMRI protocol is expected to have widespread applications for studying transgenic mice. Improvement in spatial resolution (via multisegment EPI) and in BOLD contrast (via asymmetric spin-echo or gradient-echo BOLD EPI) are under investigation.

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