High-temporal-resolution BOLD responses to visual stimuli measured in the mouse superior colliculus

Arun Niranjan, Bernard Siow, Mark Lythgoe, Jack Wells
UCL Centre for Advanced Biomedical Imaging, University College London; The Francis Crick Institute

Abstract
Line-scanning fMRI offers the opportunity to measure blood-oxygen-level-dependent (BOLD) signals at sub-second temporal resolution, by sacrificing the acquisition of a 3D image. This work demonstrates its first application to the mouse brain, measuring BOLD responses to a visual stimulus in the superior colliculus with a repetition time of 200 ms. This work is an important step in matching mouse BOLD signal data with 2D optical imaging spectroscopy studies measuring high-temporal-resolution haemodynamics and may prove useful for haemodynamic modelling.

Introduction
Mouse fMRI techniques are of value for three reasons. One, mice are one of the most commonly used in vivo animal models and are heavily used for invasive neuroscience experimentation. Transgenic mouse models are increasingly available, allowing the genetic effects on brain function to be studied [1]. Two, fMRI is one of the most common functional neuroimaging techniques used in humans, and therefore developments in mouse fMRI will potentially bridge the gap between invasive mouse neuroimaging studies and non-invasive human studies. Three, there is an increasing body of work applying optical imaging techniques [2] [3] to understand mouse brain haemodynamics. Measurements of the BOLD signal in the mouse brain complement these studies and will potentially lead to better validation of models which map tissue oxygenation and haemoglobin concentration to the BOLD signal.

Mouse fMRI is particularly difficult due to the small size of the brain, requiring magnetic field strengths >7 Tesla, which in turn amplifies imaging artefacts (spatial distortion) caused by discontinuities in magnetic susceptibility (brain-bone-air). Multiple studies have been conducted using task-based fMRI block designs with electrical stimulation of the paw [4] [5] [6] [7] [8] [9] [10] [11], and two using visual stimuli [12] [13]. A typical fMRI data from a GE-EPI sequence has three spatial dimensions and one temporal dimension and is most easily thought of as a BOLD signal time course located at each point (voxel) in a 3D Cartesian space. In all reported mouse fMRI studies, spatial resolution is typically of the order of hundreds of microns and temporal resolution in the range of 1–3 s. Published results often take the form of statistical maps highlighting voxels that respond to the stimuli, shown with BOLD signal time courses from a priori defined regions.

However, if only one ROI is of interest, then the typical fMRI approach hugely oversamples the BOLD signal in the spatial domain. By sacrificing the generation of a 3D map, the BOLD signal at the ROI can be sampled more heavily in the temporal domain. This was first suggested by Yu et al. [14] for looking at layer-specific BOLD signal responses to optogenetic stimulation in the rat brain. Briefly, the method works by using a gradient echo pulse sequence without phase encoding, but in combination with saturation bands, to define a line of tissue from which to sample the BOLD signal.

In this work, we demonstrate the feasibility of applying the line-scanning technique to measure high-temporal-resolution BOLD responses to visual stimuli in the superior colliculus of the mouse brain, a region strongly associated with visual processing [15].

Objective
To demonstrate the implementation of line-scanning fMRI in the mouse brain.
Figure Legend

Figure 1.

(A) Spatial localisation. Top panel shows the position of saturation bands (white rectangles) and FOV (red rectangle). The read direction (frequency encoded) is rotated in line...
with the line-of-interest defined by the 2 mm space between saturation bands. Lower panel shows the corresponding line profile for the first time point and the definition of two regions-of-interest (ROIs) with grey boxes: the superficial layers of the superior colliculus (SCs) and a grey matter control region (Ctrl).

**B** Extracted BOLD responses for both ROIs, averaged over stimulus blocks for each run. The grand mean BOLD response for each region is plotted in black. The onset and duration of stimulus (20 s) is shown by the grey box in each plot.

**C** Voxel time plot illustrating the change in normalised BOLD signal as a function of depth and time.

**Animals**

A single female C57BL6/J mouse of mass 21.6 g was used. Anaesthesia was induced with isoflurane (2%) and maintained with medetomidine (0.4 mg/kg bolus, 0.8 mg/kg/h infusion) through a subcutaneous injection to the flank. A gas mixture of 0.1 L/min O$_2$ and 0.4 L/min medical air (BOC Healthcare (Linde AG)) was continuously supplied during imaging. Respiratory rate was measured using a pressure-sensitive pad, and core body temperature was measured using a rectal thermometer (SA Instruments). Core body temperature was maintained using a warm water pipe system. For the duration of imaging, respiratory rate was in the range of 130–160 breaths per minute and temperature in the range 36.6–37.0°C.

**MRI methods**

MRI was performed using a 9.4 T VNMRS horizontal bore MRI scanner (Agilent Inc., Palo Alto, CA) with an Agilent 205/120HD gradient set, in conjunction with a 72 mm inner diameter volume coil for RF transmission (Rapid Biomedical) and a room-temperature 2 channel array surface coil (Rapid Biomedical) for RF reception. Shimming was performed using a GE 3D protocol [18] [19] with first and second-order shims optimised for the mouse brain with a user-defined shim voxel. The measured linewidth (FWHM) within this voxel was 62 Hz. To localise the line of interest, a single-slice Gradient Echo (GEMS) sequence was used (TR/TE = 200/18 ms, 1 average, matrix size 128×128, FOV = 35 mm$^2$, 1 slice (1 mm thick), flip angle = 25°). Saturation bands and line-scanning FOV was orientated as shown in figure 1A. Once the line-scanning acquisition space was determined, phase encoding was turned off. The same sequence parameters were used as in the GEMS localiser, except the FOV in the read direction was set to 10 mm and a matrix size of 128×1. The effective FOV perpendicular to the read direction was 2 mm, with a final voxel dimension of 0.078×1 mm$^3$. The line was acquired for a total of 605 s per fMRI run, and the initial 5 s of data per run were discarded to allow for net magnetisation to reach equilibrium.

**Visual stimulation**

Stimulation timings were triggered from the beginning of the pulse sequence using a POWER1401 control system (CED Ltd., UK) with Spike2 software. The stimulus was generated using a cold white LED (Thor Labs), transmitted into the scanner bore using a fibre optic cable. The cable was placed above the mouse head, secured to the top of the surface coil and aimed into the bore in order that light reflected off the surface of the coil interior for binocular stimulation. The stimulus design was as follows: a dim but non-zero baseline intensity (20 mA) with bright flashes (1000 mA) and dark intervals (0 mA). Pulse duration was 10 ms, and a 2 Hz pulse flashing frequency was used during periods of activation. Each run began with 10 s of baseline (of which the first 5 s was discarded to allow net magnetisation to reach equilibrium), and then 20 s of activation followed by 40 s of baseline were repeated 10 times. Eight line-scanning fMRI scans were conducted, for a total line-scanning imaging time of approximately 80 min.

**Data analysis**

fMRI data were reconstructed using the average magnitude of the Fourier-transformed signal from each channel of the RF coil. The resultant line profile for a single time point is shown in figure 1A. Using the Allen Mouse Brain Atlas [16], the thickness of the superficial layers of the superior colliculus was estimated to be approximately 0.4 mm. For a pixel width of 0.078125 mm, this corresponded to a ROI of approximately five pixels. Based on the positioning of the FOV and with reference to the 2D GEMS localiser scans, it was inferred that pixels 24–28 corresponded to the superior colliculus.
A control region-of-interest (Ctrl) of the same dimensions was placed in the grey matter (pixels 64–68). These ROIs are shown in figure 1A (lower panel). In the temporal domain, each voxel time course was high-pass-filtered with a cut-off period of 128 s to account for signal drift and then normalised to the baseline signal before averaging. In figure 1B, the mean BOLD responses for each run are plotted in colour, with the grand mean BOLD response across all stimulus block trials plotted in black. **Results & Discussion**

BOLD responses to visual stimuli (binocular, 2 Hz flashing light) were recorded at 200 ms temporal resolution in a single mouse using line-scanning fMRI. Using structural information in reference to the Allen Mouse Brain Atlas [16], the superficial layers of the superior colliculus and a control region in the grey matter were located (Fig. 1A) and BOLD responses extracted and averaged (Fig. 1B). Upon stimulation, the BOLD signal in the superior colliculus rapidly peaks to 3% before levelling at approximately 2% and then returning to baseline with a post-stimulus undershoot. Figure 1C shows the line-scanning data in a voxel time plot. A clear change in signal in the superior colliculus can be seen in response to the stimulus, along with signs of the post-stimulus undershoot. An 'initial dip' in the BOLD signal at the beginning of the stimulus block is not present in this data. The shape of this response closely matches those reported by Niranjan et al. [13] measured with a GE-EPI pulse sequence at 2.5 s temporal resolution. Future work will focus on modelling of the BOLD response, in order to make inferences on parameters which define the haemodynamic response function in the mouse superior colliculus.

**Conclusions**

This study has demonstrated the feasibility of line-scanning fMRI in the mouse brain, with clear BOLD responses to visual stimuli recorded in the superior colliculus at 200 ms temporal resolution, an order of magnitude faster than the average temporal resolution reported in the literature. This is the first BOLD fMRI signal measurement in the highest temporal resolution ever recorded in the mouse brain.

**Limitations**

1. This study demonstrates the feasibility of line-scanning fMRI in the mouse brain in a single animal, averaging over 80 trails acquired in approximately 80 min of imaging time. It is likely that further pulse sequence and hardware optimisation is possible to improve temporal contrast-to-noise, which would result in a shorter imaging time in future studies.

2. Variability of the BOLD response across subjects cannot be inferred from this data.

3. BOLD responses in the mouse brain have previously been shown to be modulated by anaesthesia [8] [17], and care must be taken when inferring underlying neuronal activity directly from the measured BOLD signal in anaesthetised mice. However, the comparison of the superior colliculus BOLD response with a control region unrelated to visual processing does suggest that neurovascular coupling is preserved.

**Additional Information**

**Methods**

**Animals**

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Supplementary Material

Please see https://sciencematters.io/articles/201701000001.

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Citations


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Ethics Statement

All experiments were performed in accordance with the European Commission Directive 86/609/EEC (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes) and the United Kingdom Home Office (Scientific Procedures) Act (2013) with project approval from the Institutional Animal Care and Use Committee.