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Technical Note

fMRI response to blue light delivery in the naïve brain: Implications for combined optogenetic fMRI studies

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ABSTRACT

The combination of optogenetics and functional magnetic resonance imaging (fMRI) is referred to as opto-fMRI. Optogenetics utilises genetic engineering to introduce light sensitive actuator proteins into cells. Functional MRI (fMRI) is a specialist form of magnetic resonance imaging concerned with imaging changes in blood flow and oxygenation, linked to regional variation in metabolic activity, in the brain. This study describes a methodological concern regarding the effects of light delivery into the brain for the purposes of opto-fMRI. We show that blue light delivery to the naïve rat brain causes profound fMRI responses, despite the absence of optogenetic activation. We demonstrate that these fMRI responses are dependent upon laser power and show that the laser causes significant heating. We identify how heating impacts upon the MR signal causing NMR frequency shifts, and T1 and T2* changes. This study brings attention to a possible confounder which must be taken into account when opto-fMRI experiments are designed.

Introduction

Optogenetics is an emerging technique which utilises genetic engineering to introduce light sensitive actuator proteins into cells (Boyden et al., 2005). Light at specific wavelengths can activate ion channels, G-protein coupled receptors and other biochemical pathways, allowing targeted cell specificity and millisecond temporal control of distinct cellular populations (Yizhar et al., 2011). Recently, optogenetics has been combined with fMRI (opto-MRI) to measure the BOLD response to light induced activation of specific cellular populations (Desai et al., 2011; Kahn et al., 2011; Lee et al., 2010). The popularity of the opto-fMRI methodology has since escalated with many reported applications of the technique to answer key questions in neuroscience (Becerra et al., 2011; Downey et al., 2011; Lebhardt et al., 2012; Li et al., 2011; Weber-Fahr et al., 2011).

During these studies light is typically delivered via an optic fibre inserted directly into the brain, via a chronically implanted cannula (Lee et al., 2010). Alternatively the fibre can be positioned resting upon the surface of the brain after an acute craniotomy (Desai et al., 2011; Kahn et al., 2011). The optic fibre tip must be positioned in close proximity to the transduced cells since ~90% of light at ~450 nm wavelength is attenuated within 1 mm of brain tissue (Aravanis et al., 2007). In our preliminary validation opto-fMRI experiments (Wells et al., 2010) we noted an unrecognised fMRI signal during laser light delivery in the naïve brain. Another study has reported a small negative fMRI response in control animals (Desai et al., 2011), while other publications have mentioned heating confounds without presenting data (Lee et al., 2010). These findings have prompted a detailed investigation into the nature of this phenomenon.

This is the first study to demonstrate positive and negative fMRI responses during blue light delivery to the brain in naïve rats, together with an investigation of the cause of the pseudo BOLD activations. In this study we observe marked positive and negative fMRI responses in the naïve rat cortex, temporally synchronised with laser stimulation at 445 nm wavelength. We report robust fMRI signal changes dependent on laser power, together with an associated brain temperature rise. Further investigation suggests that the fMRI response is not due to a BOLD haemodynamic response, but rather a heat-induced NMR effect leading to the observed fMRI signal change. Finally we discuss the broad implications of this confounding factor for the methodological design and interpretation of combined optogenetic and fMRI studies.

Abbreviations: ASL, arterial spin labelling; BOLD, blood oxygenation level dependent (imaging); CBF, cerebral blood flow; ChR2, channelrhodopsin; fMRI, functional magnetic resonance imaging; GE-EPI, gradient echo echo planar imaging; NMR, nuclear magnetic resonance; PRF, proton resonant frequency; SE, spin echo; SPM, statistical parametric mapping; ROI, region of interest.

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Materials and methods

All experiments were performed on Sprague–Dawley rats in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986.

Surgical procedures

Cannula implantation

Plastic external guide cannulas were purchased from Plastic One (Plastic One, Inc. USA). The internal cannula was inserted and both were trimmed to allow ~1 mm cortical depth penetration. Male Sprague–Dawley rats (~200 g) were anaesthetised with a mixture of ketamine 60 mg $kg^{-1}$and meditomidine 250 mg kg^{-1} i.m. The head was shaved and cleaned with chlorhexidine. The head was positioned in a stereotaxic frame and protective ophthalmic gel was applied. A midline incision was made and a hole was drilled (~1 mm^2) on the right parietal bone until the brain was exposed. A smaller hole was drilled more caudally at approximately −8.00 AP and −2.00 ML. A plastic screw (Plastic One, USA) was positioned in the caudal hole and turned until it rested on the skull. In the larger rostral hole the dura mater was punctured with a 26-gauge needle and the external guide cannula (Plastic One) was inserted into the cortex. Once in position the skull was dried thoroughly before applying cement around the screw and guide cannula. The dummy cap (Plastic One, Inc. USA) was inserted and secured within the guide cannula. The skin was sutured around the guide cannula. Buprenorphine was administered (0.05 mg kg^{-1}, s.c.) and anaesthesia was reversed with atipemazole (1 mg kg^{-1}, i.m.). Post-operative care was given and animals' appearance and body weight were monitored during recovery.

Craniotomy for thermal imaging

A Sprague–Dawley rat (200–300 g) was anaesthetised with urethane (1.5 g kg^{-1}, i.p.). The head was positioned in a stereotaxic frame. A midline incision was made and the right parietal bone was drilled to expose the cortex without damaging the dura mater.

MRI methods

Images were acquired using a 9.4 T Agilent horizontal bore scanner with a single loop surface coil to transmit and receive RF signals (Agilent Inc., Palo Alto, CA). Rats were anaesthetised with α-chloralose (I.V. bolus 100 mg kg^{-1}, constant infusion of 20 mg kg^{-1} h^{-1}) following isoflurane induction and placed in an MRI cradle; the head was secured with tooth and ear bars. Core body temperature and breathing were kept within physiological ranges throughout the experiment. A high resolution anatomical reference scan was acquired using a fast spin echo sequence (TR/TE_{eff} = 3100/48 ms, ETL = 8, matrix size = 256 × 256, FOV = 35 mm × 35 mm, 30 slices, 1 mm slice thickness). fMRI images were acquired using a two shot segmented GE EPI sequence (TE/TR = 12 ms/1500 ms, matrix size = 64 × 64, FOV = 35 × 35 mm, 3 slices, slice thickness = 2 mm, echo spacing = 0.472 ms, spectral width = 156.2 kHz). The effective bandwidth in the phase encoding direction was 70 Hz (Schmitt et al., 2000). The position of the fMRI slices was centred at the location of the optic fibre tip from visual inspection of the high resolution anatomical reference scan. The fMRI paradigm for each experiment was 60 s rest followed by 30 s optical stimulation repeated 4 times. For each experiment a different laser power was tested; the experiments were performed in ascending order with respect to laser power. Two different duty cycles were tested 50% (20 ms on, 20 ms off) and 20% (20 ms on, 80 ms off). Time averaged light power was measured from the tip of the optic fibre for both duty cycles (200 μm), using a Licomix power metre (Model55PM). Light power was measured during pulsed protocols in order to provide accurate measurement of time averaged laser power per second directly delivered to the cortex. Light density (time averaged) was calculated based on Yizhar et al. (2011) (see Table 1).

Table 1
Table to show time averaged light power values and corresponding light power density.

mW (50%)	mW/mm^2 (50%)	mW (20%)	mW/mm^2 (20%)
1.2	39	0.6	19
3.9	126	2	65
8	258	3.5	113
12	387	5	161
16	516	7.5	242

In vivo/deceased comparison

In order to investigate the extent of haemodynamic contributions to light-induced MRI signal changes we performed the same fMRI paradigm at all the laser powers and duty cycles in the deceased rat. The animal was sacrificed with an isoflurane overdose and remained in the same position between the alive and deceased tests. Deceased experiments were performed approximately 1 h after all physiological variables had ceased.

Based on the findings of the in vivo/deceased comparison (see Results) further experiments were designed to investigate the cause of the artefact. These experiments aimed to ascertain if temperature induced PRF shifts were causing errors in spatial encoding or changes in relaxation times (T1 and T2*). We established that a PRF shift was present at the location of light delivery by positioning a spectroscopy voxel (2 mm × 2 mm × 2 mm), centred on the tip of the optic fibre in the cortex of the dead brain. Two interleaved spectroscopy measurements at baseline and during light delivery (16 mW, 50%) were performed. In order to modulate the sensitivity of the fMRI measurements to direct errors in spatial encoding of signal due to the observed PRF shift, fMRI acquisitions were performed using GE-EPI with a variable number of shots. fMRI acquisitions were performed in the dead brain using 1, 2, 4 and 8 shot GE-EPI giving an effective phase encoding bandwidth of 35, 70, 140 and 280 Hz (each image was made up of 8, 4, 2 and 1 averages respectively for an identical temporal resolution of 3 s). Other sequence parameters were identical to those described above but with a TR of 375 ms. In order to investigate possible changes in T1 and T2* during light delivery, T1 and T2* maps were acquired at baseline and during 16 mW light delivery (50% duty cycle). T2* maps were generated from a 4 shot GE-EPI sequence (TR = 1000 ms, matrix size = 64 × 64, FOV = 35 × 35 mm, 3 slices, slice thickness = 2 mm, TE = 6, 8, 10, 12, 16, and 20 ms, number of averages = 5). 5 interleaved measurements were performed at baseline and during 16 mW blue light delivery. T1 maps were generated from a single shot GE-EPI inversion recovery sequence (TE = 10 ms; TR = 20000 ms, matrix size = 64 × 64, FOV = 35 × 35 mm, 3 slices, slice thickness = 2 mm, TI = 14, 25, 44, 78, 138, 240, 431, 763, 1350, 2400, 4200 and 7500 ms, number of averages = 1). 3 interleaved T1 measurements were performed at baseline and during light delivery.

MRI data analysis

Statistical analysis was performed using the general linear model SPM8 approach (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). fMRI data for each animal at each laser power and duty cycle were analysed separately. Times series fMRI images were first realigned with a 6 parameter (rigid body) spatial transformation. Possible pseudo activations were modelled as delta functions convolved with the canonical haemodynamic response function as commonly implemented in SPM for both positive and negative pseudo-BOLD changes. A critical t value for each voxel was calculated for the significance level of $p<0.05$ with family wise error correction for multiple comparisons, resulting in a statistical parametric map that was then overlaid onto the high-resolution anatomical reference scan. For each animal, the mean fMRI signal was taken within the voxels that showed statistically significant (on the parametric map) response to 16 mW laser stimulation before and during the 4 periods of laser stimulation for each of the 5 laser power values and 2 duty cycles. T2* and T1