# In-vivo Optogenetic Activation of Cortical Astrocytes with fMRI at 9.4T: OptoMRI

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## Introduction

Functional magnetic resonance imaging (fMRI), with blood oxygen level dependent (BOLD) contrast is an established method for making inferences about regionally specific activations in the brain (1). However, the relative contribution of the neuronal and glial activation to the BOLD signals is not fully established (2). Optogenetic techniques, in which particular brain cells are engineered to express light-sensitive ion channels, offer minimally invasive and temporally precise control of the activities of distinct cellular populations (3). Targeted cells can be activated or inhibited by exposure to light of different wavelengths. Such methods have enormous potential to increase our understanding of the mechanisms underlying generation of BOLD signals.

Several studies have provided evidence of the prominent function of astrocytes in neurovascular coupling (e.g. 2, 4), however, their precise role in generation of the BOLD signals is still unknown. To address this issue we have developed a cell-type-specific adenoviral vector for selective expression of light-sensitive ChR2 in brain astrocytes *in-vivo*. In this study we performed simultaneous optogenetic activation of cortical astrocytes with high field fMRI - *OptoMRI*. Astrocytes in the cortex of the anaesthetised rat brain were stimulated during continuous imaging using gradient echo EPI at 9.4T. Here we present our preliminary data.

## Methods

The animal was kept under isofluorine (2% in oxygen) anaesthesia during the course of the experiment. fMRI was performed using a Varian 9.4T VNMRS 20 cm horizontal-bore system (Varian Inc. Palo Alto, CA, USA). Four shot gradient echo EPI images were acquired with the following parameters: 5 slices; 40 × 40mm FOV; 64 × 64 pixels; TR = 500ms; TE = 4ms; temporal resolution =2s; slice thickness =1mm. The cortex was exposed to 445 nm light (20/20 ms duty cycle) at four time points during continuous EPI acquisition. The total imaging time was 75 minutes to allow sufficient time for the activated areas of the brain to return to baseline following laser stimulation.

## Results

Figure 1 shows typical gradient echo EPI images acquired on the 9.4T system (temporal resolution =2s). Figure 2 (b-e) shows the integral of the measured MR signal over 2 minutes directly proceeding laser stimulation at four time points during the experiment (data from 1 of 5 slices is shown). The integral is reported here as a measure of the systematic increase in the MR signal following optical activation. Visual inspection provides initial evidence for a heightened BOLD response in the transduced cortex in comparison to the mid- and deep brain area (Fig.2f).

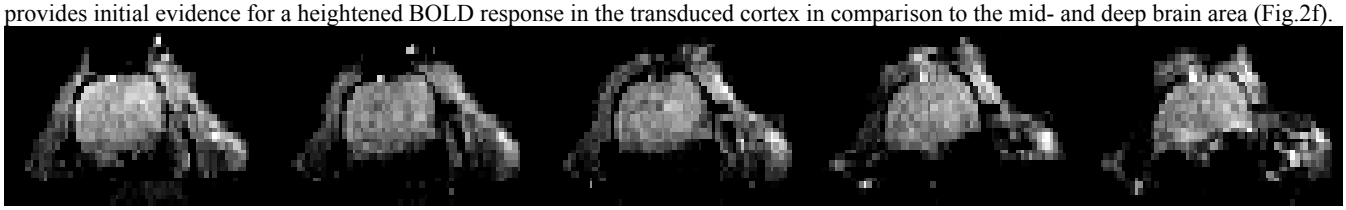
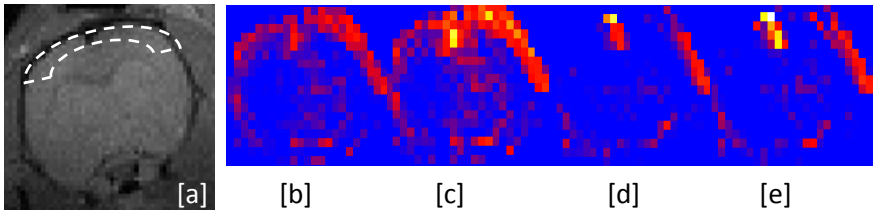


**Figure 1**
*Gradient Echo EPI images at 9.4T (5 slices).*



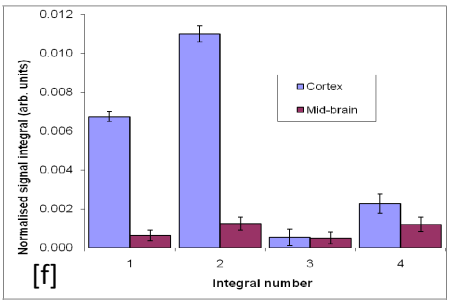




**Figure 2**
*Figure 2(a) shows an anatomical reference with the cortex highlighted (a schematic representation of the approximate area of light exposure). Figure 2 (b-e) shows the integral of the measured signal over 2 minutes following astrocyte excitation at four time points (slice 2 of 5; masked brain). Figure 2(f) shows the mean integral of the measured signal over 2 minutes following astrocyte excitation in a cortical and deep brain ROI across the 5 slices. Error bars represent the standard deviation of the pixel values within the ROI.*

## Conclusion

Here we present the first demonstration of simultaneous optogenetic activation of CNS astrocytes and fMRI *in-vivo*. These data provide evidence for a positive BOLD response following selective activation of brain astroglia and may suggest that astrocyte activation plays a prominent role in the generation of the fMRI signal. Further experiments are necessary to verify and characterise possible haemodynamic changes following selective astrocyte, as well as neuronal stimulation.

**References1** Frackowiak R. Human Brain Function. Academic Press; 2nd edition**. 2** Schummers J, Yu H, Sur M. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. Science 2008. 20;320 (5883);1597-9. **3.** Han X, Qian X, Bernstein JG Zhou HH, Franzesi GT, Stern P, Bronson RT, Graybiel AM, Desimone R, Boyden ES. Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. Neuron 1009 30 62 191-8. **4** Fields RD Stevens-Graham B. New insights into neuron-glia communication. Science 2002; 298;5593; 556-562.