Non-specific effects in ofMRI: Characterising the fMRI signal responses to 445 nm light delivered via optic fibers

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Introduction: The combination of optogenetics and magnetic resonance imaging (MRI) was coined ofMRI (Lee et al. 2010). Optogenetics utilises genetic engineering to introduce light sensitive actuator proteins into cells (Boyden et al. 2005). Light is applied to stimulate or inhibit neurones, allowing unrivalled cell specificity and millisecond temporal resolution (Yizhar et al. 2011). Optical stimulation of cortical excitatory neurones can generate positive BOLD responses (Lee et al. 2010, Kahn et al. 2011). Light at ~450nm wavelength (required to activate most of the currently used optogenetic actuators), is attenuated within 1mm of brain tissue due to scatter and absorption (Aravanis et al. 2007). Therefore the fibre optic tip must be in close proximity to the cells transduced to express light-sensitive channels (Lee et al. 2010, Zhang et al. 2010). However, delivering laser light without inducing confounding physiological effects such as heating is challenging. Erroneous perturbation of normal physiology could mask or confound real optogenetic activation. This study demonstrates that optical stimulation in a naïve brain causes a significant fMRI signal change. Laser synchronised signal intensity change in naïve rats has been previously mentioned (Lee et al. 2010) but not fully recognised. The aim of this study was to investigate the fMRI response to direct cortical laser stimulation in naïve rats over a range of laser power values in order to guide future ofMRI experiments.

Methods: **Animals:** Three male Sprague-Dawley rats (~200g) were anaesthetised with a mixture of ketamine 60mg/kg and medetomidine 250mg/kg (i.m). An external guide cannula and dummy cap (Plastic One Inc, USA) were implanted into the somatosensory cortex (AP -1.00, ML -2.50, DV-1.00) five days prior to imaging. **Light delivery:** The internal cannula was used to secure the optic fibre (200µm diameter) flush with the end of the guide cannula (-1.00 DV) (Zhang et al. 2010). A laser (Omicron, Rodgau, Germany) was used to deliver blue light (445 nm wavelength) at the following powers: 1.2mW, 3.9mW, 8mW, 12mw, 16mW (pulsed at 20ms on, 20ms off). Light power was measured from the tip of the optic fibre, before and after each experiment using a Licomix power meter (Model 55PM). **MRI:** Images were acquired using a 9.4 T Agilent horizontal bore scanner with a single loop surface coil (Agilent Inc., Palo Alto, CA). For imaging rats were anaesthetised with α-chloralose (I.V. bolus 75mg/kg, constant infusion of 20mg/kg/hour) following isoflurane induction and placed in an MRI cradle; the head was secured with tooth and ear bars. Core body temperature, breathing and ECG were monitored throughout the experiment. fMRI images were acquired using a two shot segmented GE EPI sequence (TE/TR = 12ms/1500ms, matrix size = 64x64, FOV = 35x35mm, 3 slices, slice thickness =2mm Acquisition consisted of 60s rest followed by 30s optical stimulation repeated 4 times. This paradigm was applied twice to each animal for each laser power. Images were analysed using SPM 8 ($p<0.05$, FWE, nv =0).

Results: Both positive and negative fMRI responses were demonstrated in all laser stimulations except 1.2mW, in which there is only a small negative response (Fig1.a-e). The maximum responses were observed at 16mW (Fig.1a) and subsequently decreased in a proportionate fashion. The external cannula terminates in the cortex in the first slice (see figure1 (e) asterix). Laser light induced negative responses close to the optic fibre and positive responses further away from the fibre; this distribution of positive and negative responses was consistent across the range of laser powers. The combined positive and negative responses extended over a region greater than 4mm.

Figure 1: Anatomical reference images with statistical activation maps superimposed to show positive (red) and negative (blue) laser synchronised fMRI signal changes at decreasing laser power (from 16mW to 1.2mW) a) 16mW, b) 12mW, c) 8mW, d) 3.9mW, e)1.2mW

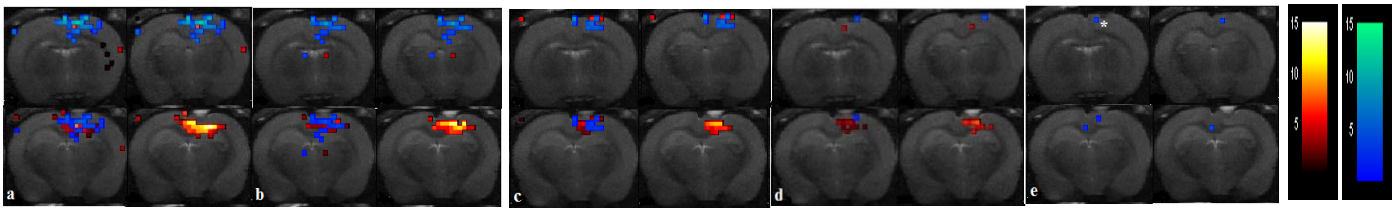
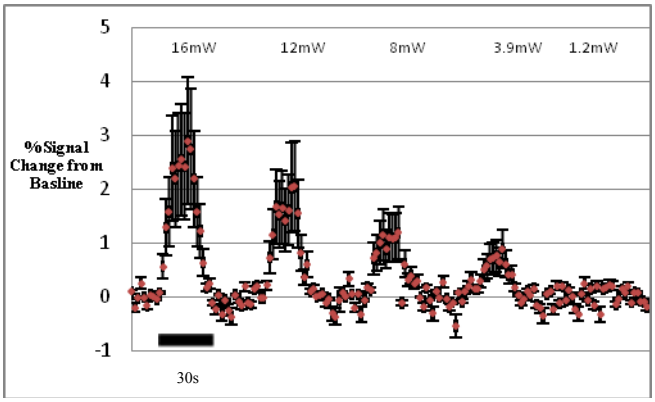
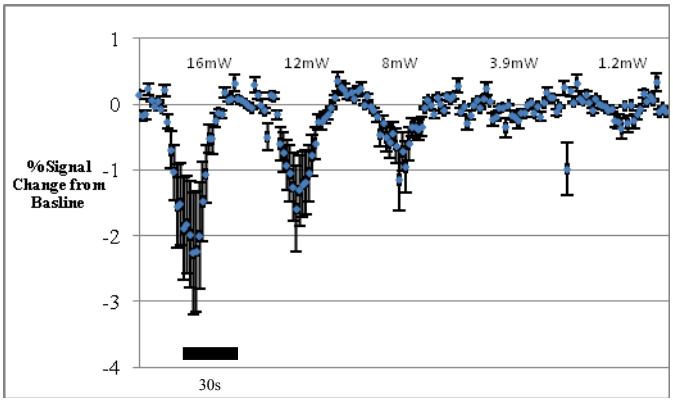


Figure 2: Graphs to show mean percentage change in signal intensity from baseline (within the "activated" voxels at 16mW laser power) at decreasing laser power. Error bars represent standard error of the mean across 8 repeated laser stimulations in the same animal.

a) Positive fMRI signal change



b) Negative fMRI signal change



Discussion and Conclusion: : OfMRI is likely to make a significant contribution to understanding the relationship between neural activity and the hemodynamic response measured in functional MRI. Therefore it is crucial that optogenetically-induced hemodynamic changes are not confounded by artefacts produced by light delivery. This study highlights the existence of profound non-specific effects of 445nm light readily detected by fMRI and reiterates the importance of control experiments in opMRI for careful calibration of laser stimulation parameters. This effect is probably due to heating and further work is required to determine the mechanisms underpinning these observations. In conclusion, both positive and negative functional MRI responses can be observed using established light delivery protocols, which has profound implications for the design and interpretation of combined optogenetic and MRI experiments.

References: Aravanis et al. (2007) J. Neural Eng 4, 143-156, Boyden et al. (2005) Nat. Neurosci. 8, 1263-1268, Kahn et al. (2011) J. Neurosci.31,15086-15091, Lee et al. (2010) Nature 465, 788-792, Yizhar et al. (2011) Neuron 71, 9-34 , Zhang et al. (2010) Nat. Protoc. 5, 439-456

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