Spatial Correlations of Neurovascular Coupling Studied using Single Pulse Opto-fMRI

Jack A Wells[1], Isabel N Christie[1], Sergey Kasparov[2], Alexander Gourine[3], and Mark F Lythgoe[1]

[1]*Centre for Advanced Biomedical Imaging, University College London, London, London, United Kingdom,* [2]*Department of Physiology and Pharmacology, University of Bristol, Bristol, United Kingdom,* [3]*Neuroscience, Physiology & Pharmacology, University College London, London, United Kingdom*

• **TARGET AUDIENCE –** Researchers interested in neurovascular coupling and fMRI.

• **PURPOSE** – Many studies have sought to characterize the temporal link between neuronal activity and the BOLD response [e.g. (1-4)]. However, spatial correlations have been relatively unexplored due in part to methodological limitations. We developed a method to investigate the spatial correlations of neurovascular coupling using a single 10 ms pulse of light to elicit optogenetic stimulation with simultaneous fMRI. Single pulse opto-fMRI, minimizes neuronal and vascular adaptation, heating artefacts and enables confined neuronal recruitment. We used this method to examine spatial correlations between the estimated volume of optogenetically induced action potentials and the volume of the BOLD response in the anaesthetised rat.

• **METHODS –** Viral injections were performed in the somatosensory cortex (AAV2-CaMKIIa-hChR2(H134R)-eYFP UNC Vector Core, diluted 1:1 with saline) to transfect cortical glutamatergic neurons to express the light sensitive channelrhodopsin protein (n=8). Imaging was performed with a 9.4T small animal scanner (Agilent) with a single loop transmit/receive surface coil (Imgenious). Functional images were centered around the location of light delivery and were acquired using single shot gradient echo EPI sequence with the following parameters: TR/TE = 1500/15 ms, matrix size = 64 × 64, FOV = 35 mm × 35 mm, 8 slices, 1 mm slice thickness. A single pulse of light was delivered directly to the cortex using an optic fibre for 10ms with an inter-stimulus interval (ISI) of 20s, repeated 15 times for each scan giving a total acquisition time of approximately 5 minutes. Data was acquired at variable light intensities (7, 14, 23, 60, 140 and 302 mW/mm^2) and each fMRI paradigm was repeated twice at each intensity in an interleaved manner. Image analysis was performed using the standard SPM pipeline (without spatial smoothing). To estimate the volume of activated tissue by a single 10ms pulse at variable light intensity, we used the model described in Foutz *et al*., ((6) [Eqs 11-20]). An action potential threshold of 2mW/mm^2 using a 10ms pulse was chosen based on the work of Ishizuka *et al*.,(7).

• **RESULTS –** We detected robust localized BOLD signal changes following activation of cortical ChR2- expressing glutamatergic neurons in response to a single 10 ms pulse of light *in-vivo* (Figure 1). Light intensity was gradually increased to modulate the spatial extent of the stimulus (Figure 1). The estimated volume of tissue exceeding the intensity threshold required for action potential generation (2 mW/mm^2) was dependent on the applied laser power (Figure 1). As the light intensity increased, so did both the magnitude (Figure 2) and spatial extent (Figure 1, 3) of the detected BOLD signal. A directly proportional relationship was observed between the volume of BOLD response and the estimated volume of light-activated brain tissue (Figure 3).

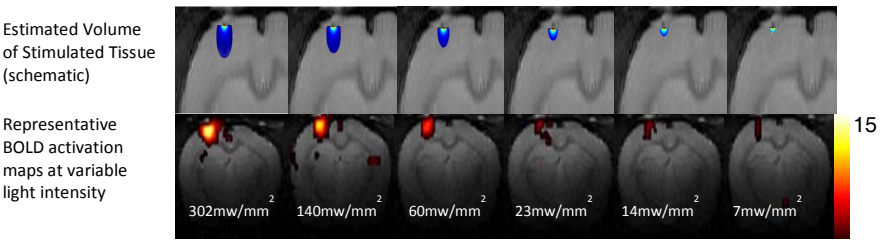

Figure 1 – The estimated volume of stimulated tissue and the spatial extent of the BOLD response for a representative subject

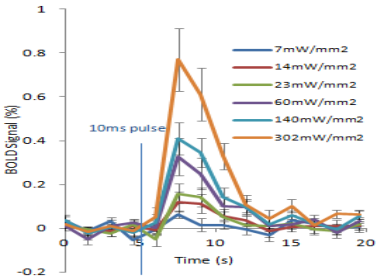

Figure 2 – The mean BOLD timecourse at different stimulus intensities (n=8)

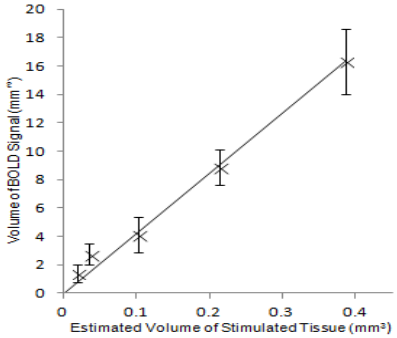

Figure 3 – The estimated volume of stimulated tissue vs volume of BOLD response together with a fit of direct proportionality (n=8)

• **DISCUSSION -** Here we present evidence for direct proportionality between the predicted volume of neuronal activation and the size of the fMRI response in the single pulse paradigm (Figure 1, E). Despite the well-recognized spatial mismatch between neural activity and BOLD (5) (with the volume of increased CBF markedly exceeding the predicted volume of increased neuronal activity), this finding suggests that the cortex scales the size of the hemodynamic response in direct proportion to the volume of increased neural activity. Thus, the spatial extent of the "over-generosity" of BOLD is a fundamental linear multiplying factor determined by the volume of activated neural tissue (ultimately numbers of activated neurons) across the range of spatial scales tested here.

• **CONCLUSION-** In this work we describe a new method to investigate neurovascular coupling and report a novel discovery pertaining to a directly proportional spatial dependence underlying neurovascular coupling in the rat cortex.

1. Kahn I, *et al.* (2011). *The Journal of neuroscience* 31(42):15086-15091. 2. Ogawa S, *et al.* (2000). *Proceedings of the National Academy of Sciences of the United States of America* 97(20):11026-11031. 3. Logothetis , *et al.* (2001) *Nature* 412(6843):150-157. 4. Sanganahalli , *et al.* (2009) *The Journal of neuroscience* 29(6):1707-1718. 5. Vazquez AL, *et al.* (2013). *Cerebral cortex (New York, N.Y. : 1991)*. 6. Foutz TJ, , *et al.* (2012) *Journal of neurophysiology* 107(12):3235-3245. 7. Ishizuka T, *et al.* (2006) *Neuroscience research* 54(2):85-94.