ATP signaling plays a key role in the BOLD Response

Jack A Wells^[1], Isabel N Christie^[1], Nephtali Marina[2], Melina F Figueiredo[3], Anya G Teschemacher[3], Sergey Kasparov[3], Alexander V Gourine*[2], and Mark F Lythgoe*[1]

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

^joint first author;*joint senior author

Introduction: Understanding the mechanisms of neurovascular coupling is essential to the interpretation of fMRI BOLD signals. There are many vasoactive chemicals known to be released by neurons and astrocytes and debate continues about their precise contribution to the vascular response(s) (Attwell et al. 2010). Non-excitable cells, such as astrocytes communicate via release of purine nucleotide ATP, however the role of the latter in neurovascular coupling has not been considered. To explore the contribution of ATP-mediated signalling in generation of BOLD responses, we developed a lentiviral vector (LVV) to express on cellular membranes a potent ectonucleotidase - transmembrane prostatic acid phosphatase (TMPAP) - with a catalytic domain facing the extracellular space, which degrades ATP, effectively preventing its action. We have previously demonstrated membrane localization of TMPAP and its efficacy in breaking down released ATP (Marina et al. 2013). In this study we implemented high field fMRI to test our hypothesis that astroglial ATP signalling plays a role in generation of the BOLD signal.

Methods: Viral microinjections were performed in the forepaw region of the somatosensory cortex (SSFP) in male Sprague-Dawley rats (~160g, n= 11), 14 ±3days prior to imaging. One hemisphere was injected with TMPAP/GFP-expressing LVV and the other hemisphere was injected with a GFP-expressing LVV. Rats were anaesthetised with α-chloralose (I.V. bolus 60 mg/kg) following isoflurane induction and placed in an MRI cradle; Body temperature, respiratory activity and blood pressure were monitored throughout the experiment. Rats were artificially ventilated with oxygen enriched air and arterial blood samples were taken to ensure blood gases remained within physiological ranges: pH 7.4±0.5; O_2 120±20 mmHg; CO_2 40±5 mmHg. All imaging was performed using a 9.4T VNMRS horizontal bore scanner (Agilent Inc., Palo Alto, CA). A 72mm inner diameter volume coil was used for RF transmission (Rapid Biomedical) and signal was received using a 4 channel array head coil (Rapid Biomedical). fMRI images were acquired using a one or two shot GE EPI sequence (TE/TR = 15ms/1500ms, matrix size = 64x64, FOV = 35x35mm, 8 slices, slice thickness =1mm). The fMRI paradigm for each subject was 120s rest followed by 20s bilateral forepaw stimulation (1.5mA, 3Hz), repeated 18 times for each animal. fMRI images were re-aligned, spatially and temporally filtered and registered to a standard rat brain atlas. The general liner model SPM8 approach (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) was used to generate a statistical parametric map ($p<0.01$) for each subject. Second level random-effects group analysis was then performed to estimate regions of statistically significant BOLD increases across all subjects. The BOLD signal in bilateral SSFP and thalamic ROIs was taken from the raw fMRI data using an automated atlas registration approach.

Results:

During bilateral forepaw stimulation, a smaller BOLD response was observed in the SSFP region of the hemisphere transduced to express TMPAP (asterisk), relative to the control hemisphere, transduced to express GFP. The BOLD response in the thalamus was symmetrical (Fig 1B), indicating that the effect upon neurovascular coupling is confined to the TMPAP expressing region of the cortex and indicates that the afferent stimulations generated in the forepaw were of equal magnitude. In the SSFP region the mean BOLD response was significantly greater in control conditions (Fig 1C, Wilcoxon Sign Rank p=0.01) with no TMPAP expression. In the thalamus no significant differences between responses were observed bilaterally (Fig 1D,p=0.8)

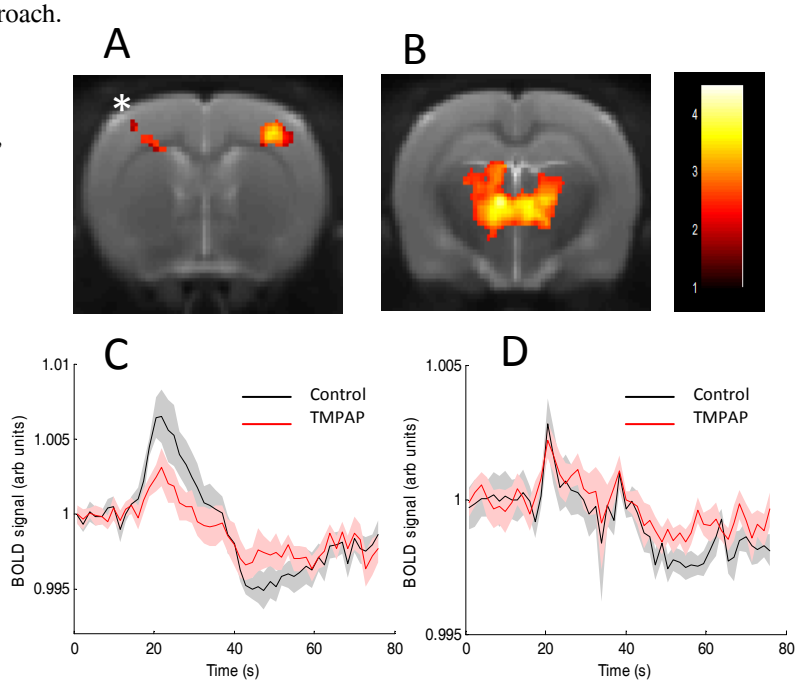

Figure 1 A/B: Activation maps based on 2nd level random-effects group analysis (n=11, p<0.02) demonstrating a reduced BOLD response to forepaw stimulation in the SSFP (A) due to TMPAP expression (*). In contrast the thalamus presents with relatively symmetrical bi-lateral BOLD activation (B). C/D the mean BOLD response within bi-lateral SSFP (C) and thalamic (D) ROIS (n=11).

Discussion:

Astrocytes are widely believed to mediate neurovascular coupling and in this study we used a novel approach to interfere with astroglial communication by promoting rapid degradation of the major astroglial signalling molecule - ATP. The result of TMPAP expression is a significant suppression of the BOLD fMRI response indicating that astroglial ATP-signalling is important for neurovascular coupling in the intact brain.

References: Attwell et al. 2010 Nature Nov; 468(7321):232 Marina et al. 2013 Basic Res Cardiol Jan; 108(1):317

We are grateful for the funding support of the Medical Research Council (Grant Ref. MR/J013110/1 and a MRC Capacity Building Studentship) and the British Heart Foundation.