Does VASO contrast really allow measurement of CBV at High Field (≥7T)? *An in-vivo quantification using concurrent Optical Imaging Spectroscopy.*

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Target audience: All neurovascular coupling researchers, sequence developers, signal processors, pre-clinical scientists, & users of BOLD/CBV-weighted fMRI.

Purpose: Interpretation of the Blood Oxygenation Level Dependent (BOLD) functional magnetic resonance imaging (fMRI) signal in terms of the underlying neuronal activity is problematic. The BOLD signal only reflects changes in magnetic field homogeneity caused by varying concentrations of paramagnetic deoxyhemoglobin (Hbr) in the blood stream. Under altered baseline physiological conditions, increased neuronal activity can result in no measurable change in BOLD signal even though neurovascular coupling is maintained. In the standard gradient echo regime, BOLD is further confounded by baseline blood volume, vessel size and orientation in the external magnetic field and as such is often weighted towards the cortical surface and draining venous system away from the site of neuronal activity. However, it is known that the concentration of Hbr is determined by the cerebral blood volume (CBV) and oxygenation (Y) which in turn are driven by neuronally elicited changes in blood flow (CBF) and the metabolic rate of oxygenation consumption ($CMRO_2$). Hence direct measurement of these haemodynamic variables using fMRI can provide a 'map' of neuronal activity with better spatial specificity than BOLD fMRI.

Vascular space occupancy (VASO) MRI is a functional technique which allows non-invasive measurement of CBV [1]. Using an inversion pulse followed by an excitation pulse at the blood nulling time, the measured MR signal within a brain voxel will be from extravascular tissue. Any changes in this signal over time must reflect changes in CBV. However, due to the convergence of tissue and blood longitudinal relaxation times (T_1) at high magnetic field strengths and the inflow of non-inverted blood during the blood nulling time the VASO signal becomes unreliable. To make the technique viable at higher field strengths (≥7T) a novel Slice-Selective-Saturation (SSS) VASO pulse sequence was used (fig 1a) in the current study. Additional slice-selective and global saturation pulses were implemented to account for the different z-magnetization relaxation histories (steady-state) of stationary tissue and blood flowing into the imaging slice. Thus using the fact that the longitudinal relaxation rates are comparable to vasculature refill time the signal from stationary tissue is increased at the blood nulling time at high fields compared to slab-selective (SS) VASO [2].

The purpose of the present study was to quantify the SSS-VASO pulse sequence at High Field (≥7T) using concurrent 2D optical imaging spectroscopy (OIS) in a rodent model [3]. OIS independently measures changes in total hemoglobin (HbT) and oxygen saturation (Y). Under the assumption of constant hematocrit HbT changes are equal to changes in CBV. The high spatial and temporal resolution of OIS allowed us to use it as a 'Gold Standard' technique to validate the SSS-VASO signal mechanism and sensitivity to CBF artifacts.

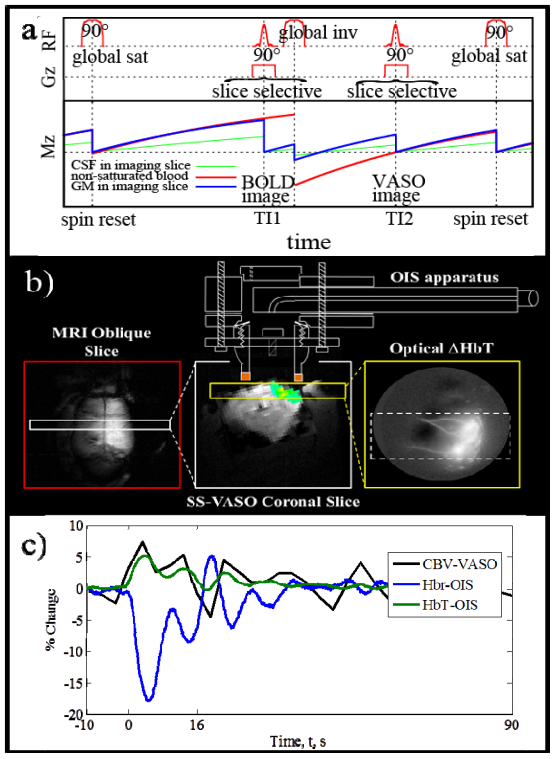

Fig. 1 Quantification of VASO with 2D-OIS; a) SSS-VASO pulse sequence, b) apparatus, c) time series comparison

Methods: MRI measurements were made at 7 Tesla in a small animal magnet facility (Bruker BioSpec, 310mm bore). Urethane anaesthetized animals were artificially ventilated and cannulated for monitoring arterial blood pressure and intravenous infusion. A thinned skull cranial window allowed direct imaging of the cortex. SSS-VASO MRI measurements of BOLD and CBV signal changes were obtained concurrently with optical measurements of HbT and Y changes using an MR compatible endoscope. The 'endoscope' assembly incorporated a 2.0cm diameter surface coil fixed to the head around the cranial window to form a well. The well was filled with deuterium oxide to avoid air-tissue susceptibility artifacts around the thinned cranial window. 2D OIS used a switching Galvanometer using 4 λ (495, 586, 559 and 575nm) and a CCD with an effective frame rate of 8Hz for each λ. The spectral analysis was based upon the path length scaling algorithm incorporating a MR based heterogeneous tissue model. The spectral analysis produced 2D images over time, of oxy-, deoxy- and total-haemoglobin changes (HbO_2, Hbr and HbT).

SSS-VASO data were acquired in the coronal reference frame with a GE-EPI readout (TR/TI1/TI2/TE = 3200/1800/2810/13.1 ms, 64*64, FOV = 30mm, sl. thk =2mm) during electrical stimulation of the whisker pad and hypercapnic (10% increased $FICO_2$) challenges. A global, adiabatic 90° spin-reset pulse was implemented to control the steady-state of flowing blood. To assess and eliminate residual BOLD effect contamination from the CBV measurement, we acquired BOLD interleaved with VASO-MRI. The slice selective 90° excitation pulse prior BOLD image acquisition saturates tissue magnetization in the imaging slice, but does not affect blood magnetization refilling the vasculature until the next VASO image is acquired. This increases tissue signal at the blood nulling time (fig 1a). Combined measurements from VASO *f*MRI and 2D-OIS were used to examine the degree of concordance between the two imaging methods (fig 1b).

Results: Using these concurrent techniques we:

(1) found agreement in terms of both magnitude and temporal dynamics between VASO-CBV and OIS-HbT measurements (fig 1c) during both electrical stimulation of the whisker pad and hypercapnic challenges confirming constant hematocrit levels during activation.

(2) Input 2D-OIS measurements of Hbr and HbT into a Monte Carlo simulation of MR signal attenuation to relate these changes to the observed BOLD signal changes [3] in the superficial layers during VASO data capture. As such the model could be inverted to estimate oxygenation changes (Y) from the SSS-VASO BOLD and CBV measurements to assess this important haemodynamic variable through depth in the coronal MR frame.

Application and quantification of the contrast source for the SSS-VASO technique in a rodent model allows future exploration and comparison of data between the species (Human and Rat). Following a visual stimulus VASO derived CBV changes of up to 30-60% have been reported in human with differing temporal dynamics [1,2] to those in the present study (5-10%). Our study discusses possible confounding sources of variation (anesthetic, different stimuli etc.).

Conclusions & Discussion:

This study used concurrent 2D-OIS and high field fMRI to quantify the signal source of a SSS-VASO pulse sequence for non-invasive assessment of CBV signal changes. We show direct evidence that during both electrical stimulation and hypercapnia the CBV derived from SSS-VASO is equivalent to HbT changes measured concurrently with 2D-OIS in rat somatosensory cortex. This pre-clinical *in-vivo* multimodal imaging data has greatly increased our understanding of signal source of this new SSS-VASO technique and verifies it can be used for more clinical based Human studies for mapping CBV changes – a better predictor of neuronal activity than BOLD fMRI.

References: [1] Lu, H. et.al. (2003) Magnetic Resonance in Medicine (50):263-74; [2] Huber, L. et.al. (2012) ISMRM Melbourne 331; [3] Kennerley, A.J. et.al. (2012) *NeuroImage* 61(1) , pp. 10-20.