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The BOLD response and vascular reactivity during visual stimulation in the presence of hypoxic hypoxia

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A disproportionate increase in cerebral blood flow (CBF) relative to the cerebral metabolic rate of oxygen ($CMRO_2$), in response to neuronal activation, results in a decreased oxygen extraction fraction (OEF) and hence local 'hyperoxygenation'. The mismatch is the key 'physiological substrate' for blood oxygenation level dependent (BOLD) fMRI. The mismatch may reflect inefficient O_2 diffusion in the brain tissue, a factor requiring maintenance of a steep [O_2] gradient between capillary bed and neural cell mitochondria. The aim of this study was to assess vascular responsiveness to reduced blood oxygen saturation, using both BOLD fMRI and the CBV-weighted vascular space occupancy (VASO)-dependent fMRI technique, during visual activation in hypoxic hypoxia. Our fMRI results show decreased amplitude and absence of initial sharp overshoot in the BOLD response, while VASO signal was not influenced by decreasing oxygen saturation down to 0.85. The results suggest that the OEF during visual activation may be different in hypoxia relative to normoxia, due to a more efficient oxygen extraction under compromised oxygen availability. The data also indicate that vascular reactivity to brain activation is not affected by mild hypoxia.

Keywords: fMRI; BOLD; VASO; Vascular reactivity; Hypoxia; Visual; Oxygen limitation

Introduction

The blood oxygen level dependent (BOLD) response is one of the most widely used functional imaging contrasts for brain activation studies. The BOLD effect provides a natural contrast for functional magnetic resonance imaging (fMRI) due to the paramagnetic effect of deoxyhaemoglobin (Hb) on T_2 (Brindle et al., 1979; Thulborn et al., 1982) and T_2^* (Ogawa et al., 1990). As a result of venous 'hyperoxygenation' (i.e. decrease in Hb/HbO_2 ratio) due to a decrease in the oxygen extraction fraction (OEF), the T_2(*)-weighted MR signal increases locally. Several fMRI studies have shown that the increase in CBF-to-$CMRO_2$ ratios during brain activation ranges from 2 to 6 (Davis et al., 1998; Fox and Raichle, 1986; Hoge et al., 1999; Kim et al., 1999; Mandeville et al., 1999; Marrett and Gjedde, 1997). Physiological underpinnings linking the BOLD signal to brain activation are not fully understood, and the fundamental physiological mechanism underlying the mismatch between CBF and $CMRO_2$ remains to be elucidated.

According to the O_2 limitation model (Buxton and Frank, 1997), a large CBF to $CMRO_2$ ratio during brain activation is required to maintain a steep O_2 gradient between the capillary space and site of tissue mitochondria, facilitating oxygen diffusion in the tissue. The results showing a consistent CBF-to-$CMRO_2$ ratio, derived from additive BOLD responses to graded visual stimulation during elevated CBF baseline, have been used to support this key claim of the O_2 limitation theory (Corfield et al., 2001; Hoge et al., 1999). Conversely, the O_2 limitation model implies that a drop in arterial O_2 tension should result in augmented CBF response and thus vasodilation in order to sustain a low OEF during brain activation. Assuming unidirectional O_2 transport from capillaries and close to zero tissue O_2 tension at mitochondrial sites, the physiological parameters can be related to each other by:

$$CMRO_2 = \mathrm{OEF} \cdot \mathrm{CBF} \cdot C_a \quad (1)$$

where $CMRO_2$ is the cerebral metabolic rate of oxygen, C_a is the arterial oxygen content (Buxton and Frank, 1997).

Despite the crucial need for O_2 for brain function, however, mild to moderate hypoxic hypoxia appears to be well tolerated. Studies have shown that haemodynamics, metabolism, and higher brain functions

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are not disrupted even down to Y_a of 0.8 (Mintun et al., 2001; Rostrup et al., 2005; Shimojyo et al., 1968; Siesjo, 1978; Tuunanen et al., 2006a). Stimulus-evoked CBF, as measured by PET and MRI techniques in conscious humans (Mintun et al., 2001; Rostrup et al., 2005; Tuunanen et al., 2006a; Tuunanen and Kauppinen, 2006) and anaesthetized animals (Sicard and Duong, 2005), proceeds at the same level at Y_a of 0.8 as at 1 (full saturation). Several studies have reported that baseline $CMRO_2$ does not change during transient moderate hypoxia (Emoto et al., 1988; Kety and Schmidt, 1948; Sicard and Duong, 2005). Tuunanen et al. (2006a) reported that visual evoked potentials were not affected by hypoxia down to Y_a of 0.8, indicating that visual processing in the primary visual cortex is sustained. On the other hand, laser-doppler flowmetry has indicated increase in blood velocity in large arteries by 13% upon a decrease of Y_a by 10% in humans (Meadows et al., 2004). Earlier papers on animals have shown increased CBF at oxygen saturation below 0.6 (Ekstrom-Jodal et al., 1979; Koehler et al., 1984; Siesjo, 1978).

The effects of limited O_2 availability on metabolic and vascular responses during brain activation are still unclear, and in this present study our aim was to determine the effects of hypoxic hypoxia, during visual stimulation, on the BOLD response and vascular responsiveness, as assessed using the CBV-weighted (Donahue et al., 2006) vascular space occupancy (VASO)-dependent technique (Lu et al., 2003). The benefit of the VASO technique is a high contrast-to-noise ratio that affords spatial and temporal resolution comparable to that obtained with BOLD fMRI. If O_2 limitation at the tissue level applies, increased vascular reactivity, i.e. larger signal change is expected to be revealed by the VASO technique during visual stimulation in hypoxic hypoxia than for normoxia.

Materials and methods

Subjects and experimental design

The protocol was approved by the Committee on the Ethics of Research on Human Beings of the University of Birmingham. Eight healthy subjects (5 males, 3 females; ages ranging from 24 to 51 years) gave written, informed consent prior to participation. One subject was subsequently excluded from the study due to discomfort during hypoxic exposure. The design of the study was to present visual stimulation in each of the two oxygenation states, with two sets of functional MRI scans (VASO and BOLD) for each of these conditions.

Inspired oxygen tension (FIO_2) was either 21% (room air) or 12% (O_2 balanced with N_2 in a non-rebreathing circuitry, delivered through a valve with a mouth piece by a device from Hans Rudolph Inc., Kansas City, KS, USA). Arterial oxygen saturation (Y used for saturation values from pulse oximeter) and pulse rate were monitored from a finger on the left hand with a Pulse Oximeter (System 4500 MRI, In Vivo Research, Inc., Orlando, USA). After an adaptation of about 5–7 min to 12% FIO_2, during which a stable (variation ± 0.02) hypoxic Y level was reached, the MRI protocol was started.

For visual stimulation, subjects were asked to fixate on the centre of a screen on which a black and white contrast-reversing checkerboard at 8 Hz frequency was displayed. Each run consisted of 5 blocks that alternated between baseline and stimulation (starting and ending with baseline) of 45 s per block.

MR parameters

MRI was performed using a Philips Achieva 3.0 T clinical imager (Philips Medical Systems, Best, The Netherlands) using standard body coil transmission and SENSE head coil reception. Using a sagittal localiser, a coronal 5 mm slice was aligned to the calcarine sulcus to cover the visual cortex. T_2^*-weighted, dual-echo BOLD images were acquired with single shot, gradient echo (GRE), echo-planar imaging (EPI): TR = 3 s, TEs = 5 and 40 ms, flip angle = 90°, matrix = 128 × 128, FOV = 224 × 224 mm with partial Fourier = 0.67 and SENSE factor = 2.5. VASO images were acquired with an inversion-recovery pulse for nulling blood (Lu et al., 2003) at TI = 889 ms; other parameters were similar to those of BOLD fMRI, except the pair of TEs = 10.3 and 56 ms. Each BOLD and VASO time series consisted of 225 volumes.

Data analyses

Processing and statistical analyses of the fMRI scans were performed using in-house software written using IDL (ITT Visual Information Solutions, Boulder, CO, USA). Realignment of the data was done to correct for motion (AIR algorithm). T_2^* was calculated from the double echoes in the GRE EPI images according to:

$$T_2^* = (TE_2 - TE_1)/(\ln S_1/S_2) \quad (2)$$

where TE_1 and TE_2 are the echo times, and S_1, and S_2 are the respective signal intensities at those echoes. The calculated T_2^* data was then used for all subsequent analyses of the BOLD effect. All BOLD results shown refer to the absolute T_2^* analyses, and the absolute $\Delta R_2^*(=1/\Delta T_2^*)$ values shown in conjunction with the BOLD results.

T-tests were used to detect task-related T_2^* (and R_2^*) changes in the fMRI time series on a voxel-by-voxel basis. Areas of significant activation were delineated by using a voxel threshold of $t > 3.24$, $p < 0.001$ (uncorrected) plus a cluster threshold of three voxels. The first five volumes of the baseline blocks and first volume of the stimulation blocks were not included in the t-tests to ensure steady-state signal intensity. Using the thresholded areas, fractional signal time courses were obtained from each scan and averaged across all stimulation cycles and subjects. This group of analyses was termed "All Voxels".

In addition to assessing thresholded BOLD and VASO responses specific to normoxia and hypoxia, we also determined the response amplitude in the thresholded areas *common* in both normoxia and hypoxia, i.e. in this case only voxels that were activated during both normoxia *and* hypoxia for each particular scan set were considered. This enables direct comparison of BOLD and VASO signal changes in the same parenchymal structures by excluding responses from areas that would be subthresholded in either of the two conditions. This data analysis procedure was termed "Common Voxels".

Using the baseline corrected data sets for "All Voxels" and "Common Voxels", the differences in the shapes of the signal response curves in normoxia and hypoxia were analysed using the slopes of the response curves from the peak of T_2^* response to the end of the stimulation block. The data were linearly regressed to determine estimates for the slopes. For analyses of mean response amplitudes, undershoots were analyzed for amplitudes by comparing the corresponding sections of time courses after the onset and termination of visual stimulation, respectively. The steady-state signal responses for baseline and stimulation conditions were calculated by averaging across each condition, but excluding the first five volumes from baseline data sets and first three volumes for the stimulation conditions, in order for steady-state comparisons. In addition, the BOLD and VASO signal changes in hypoxia