Quantitative fMRI using hyperoxia calibration: Reproducibility during a cognitive Stroop task

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A B S T R A C T

Arterial spin labelling allows simultaneous measurement of both the blood-oxygenation-level-dependent (BOLD) and the cerebral blood flow (CBF) response to changes in neural activity. The addition of a hypercapnia or hyperoxia calibration allows additional quantification of changes in the cerebral metabolic rate of oxygen (CMRO2). In this study we test the reproducibility of measurements derived using the hyperoxia approach, during a cognitive Stroop task. A QUIPSSII sequence is used at 3 T to collect simultaneous CBF and BOLD signal during two 3 min periods of hyperoxia and an 8 min Stroop task. Hyperoxia was administered via an open system and end-tidal values were sampled via a nasal cannula; average end-tidal values of 60% were reached. This procedure is repeated to allow the reproducibility of the estimated parameters to be tested. The use of a cognitive Stroop task allows testing of the measurements in frontal and parietal regions as well as sensorimotor areas in which previous studies have been focussed. We find reduced reproducibility of the calculated parameters compared to the hypercapnia approach, thought to be attributable to lower absolute BOLD and CBF responses. In particular we do not find n’ to have improved reproducibility compared to other parameters, as has been found in previous work using the hypercapnia approach. Across all brain areas we report a value of ΔCMRO2 of 12% and neurovascular coupling constant n of 2.5. Interestingly we find n to be higher in parietal and frontal areas in comparison to the primary motor cortex.

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Introduction

The Blood-Oxygenation-Level-Dependent (BOLD) signal is widely used in studies of normal brain function, but clinical applications are problematic due to possible alterations in neurovascular coupling, making signal interpretation difficult (Pineiro et al., 2002; D’Esposito et al., 2003; Detre, 2006; Jezzard and Buxton, 2006). The BOLD signal is sensitive to the deoxyhaemoglobin content of the blood, which is altered during changes in neural activity due to concomitant changes in cerebral blood flow (CBF), cerebral blood volume (CBV) and cerebral metabolic rate of oxygen (CMRO2) (Buxton et al., 2004). It is possible to separate these physiological contributions by using an arterial spin labelled (ASL) pulse sequence, and including an iso-metabolic calibration scan, where it is assumed that there is no change in oxygen metabolism. The most common approach is to administer approximately 5% CO2 in order to induce hypercapnia (Davis et al., 1998), however some concern remains as to whether this approach is truly iso-metabolic (Kliefoth et al., 1979; Jones et al., 2005; Sicard and Duong, 2005; Zappe et al., 2008). Recently, a new approach to calibration using hyperoxia has been suggested which offers a number of possible benefits, including increased comfort for the participant, and no reliance on the noisy ASL signal for calibration (Chiarelli et al., 2007a).

Most previous work using a calibration approach for quantitative fMRI has considered activation of primary visual and motor cortices (Davis et al., 1998; Chiarelli et al., 2007b; Leontiev and Buxton, 2007) with only very recent work concerning cognitive tasks (Restom et al., 2008, 2007). If this approach is to become used more widely for clinical applications it is important to test the reproducibility of the measurements for cognitive fMRI paradigms, often involving smaller BOLD activations than in visual or motor cortices. The aim of our work is to test the reproducibility of the measurements derived from the hyperoxia calibration approach for a cognitive Stroop task (Zysset et al., 2001). We use a reduced hyperoxia protocol taking only 13 min which also makes this approach more clinically feasible than previous work (Chiarelli et al., 2007a). In this study, we also compare quantitative fMRI measures across activated brain regions.
Materials and methods

Subjects took part in a single scanning session consisting of a hyperoxia calibration scan (13 min), a cognitive Stroop task (8 min) a structural scan (7 min) and then a repeat of both the hyperoxia scan and Stroop task, giving a total imaging time of approximately 50 min.

Subjects

Approval for this study was obtained from the University of Liverpool Ethics Committee and all volunteers provided signed informed consent prior to their participation. 10 subjects (4 male, 6 female; age range 19–36) participated in the study.

Stimulus paradigm

A color–word Stroop task was used, similar to that described in (Zysset et al., 2001), programmed in Presentation software (http://www.neurobs.com/). Subjects had to decide if the meaning of a word presented in white at the bottom of the screen matched the color of the top word (Fig. 1). They were asked to respond as quickly and accurately as possible with a press of the index or middle finger on a button box held in the right hand as to whether the words matched or not. Only incongruent conditions were presented, where the meaning and color of the top word did not match. There were 20 self-paced stimuli in each block, with a minimum time of 1.5 s between stimuli, giving a total block length of approximately 30 s. Following each response, a fixation cross was displayed before the presentation of the next stimulus. Eight active blocks of 30 s were interspersed with 30 s fixation cross on a black screen, giving a run time of 8 min. The task was first practiced for 4 min outside of the scanner prior to scanning so that the subjects became familiar with the task, and to prevent learning effects across the two runs.

Previous work using the Stroop task has focussed on the differential activity between congruent and incongruent conditions (Zysset et al., 2001; Langenecker et al., 2004), and often an event-related fMRI design is used. For our study, using arterial spin labelling which is inherently noisy, we would be unable to measure this subtle interference effect and so we used the incongruent condition in comparison to fixation. This produces robust activation over a large number of brain regions. We chose a block design, as the analysis assumes that a steady-state is reached, which would not occur for an event-related design.

Hyperoxia paradigm

For the BOLD calibration scan, 100% oxygen was delivered via an open mask covering the mouth and nose at a flow-rate of 15 L/min. This was interspersed with periods of breathing normal air through the holes in the mask. We hypothesized that the level of oxygen reached did not need to be precisely controlled as it is the change in BOLD signal for a given change in oxygen that is important which should be independent of the oxygen level reached. The fan in the scanner bore was turned on to maximum to allow fast dispersal of the excess oxygen to prevent any build up. The protocol was composed of 2 min of air, 3 min of oxygen, 5 min of air and 3 min of oxygen. The gas composition inside the subject’s nose was continuously sampled via a nasal cannula. The cannula was connected via a vacuum pump to both a carbon dioxide and oxygen analyser supplied by Applied Electrochemistry Inc, Pittsburgh USA (S-3A/I O2 oxygen analyser, CD-3A CO2 analyzer, and Flowcontrol R-2 vacuum pump). The oxygen analyser was calibrated prior to each scan against an oxygen depletion monitor (GasMonitor4) situated in the scanner room that continually monitors the oxygen concentration of room air. Respiratory data logging was performed at intervals of 1 ms using Powerlab software (ADInstruments, Colorado Springs, USA).

Image acquisition

MRI measurements were performed using a 3 T Trio whole-body scanner (Siemens, Erlangen, Germany). An 8–channel RF coil was used for signal collection and the body coil for signal transmission. Images were acquired using a QUIPSSII Arterial Spin Labeling sequence with PICORE labelling scheme (Wong et al., 1998) in order to collect simultaneous BOLD and CBF signal. Acquisition parameters were: TR 2.13 s, T1i 0.7 s, T1 1.4 s, TE 25 ms, labelling slab thickness = 10 cm with a 10 mm gap between labelling and imaging volumes. Crusher gradients with b = 5 mm s⁻¹ were used in order to remove signal from the larger vessels. Twelve slices of 3.5 mm thickness and 0.35 mm gap were collected, covering frontal, motor and parietal cortices as shown in Fig. 2. The pulse sequence included prospective motion correction (PACE (Thesen et al., 2000)). Two ‘dummy’ scans were discarded at the start of each functional run in order to avoid T1 relaxation effects. At the end of each functional run an M0 calibration image was also collected for use in producing quantitative CBF maps (see fMRI data analysis). The QUIPSSII sequence with parameters as above was used with the labelling switched off and a TR of 10 s. A 3D MPRAGE (Mugler and Brookeman, 1990) sequence with 1 mm isotropic resolution was used for the anatomical scan.

fMRI data analysis

Calculating CBF

The ASL data from all 4 functional runs (2 Stroop and 2 hyperoxia) was first processed in Matlab in order to extract BOLD and CBF time-courses. Pairs of control and labelled images were subtracted to produce a time-course of perfusion-weighted images, and also...
averaged to produce a time-course of BOLD images. The perfusion-weighted images were converted into quantitative CBF maps through the use of a model that assumes a single blood compartment model, i.e. assuming that the labelled water does not cross the capillary wall or leave the voxel during the inversion time (Parkes and Tofts, 2002). The equilibrium magnetization of arterial blood was estimated from the average signal of the $M_0$ calibration image, assuming a whole-brain value of 0.9 for the brain:blood partition coefficient ($L$) (Roberts et al., 1996). Correcting for the different T2* relaxation times of blood and tissue (Buxton et al., 1998), perfusion maps were produced using this equation:

$$f = \frac{S \times e^{-T_1/T_1}}{2M_0 T_1}$$

where $f$ is perfusion, $S$ is the signal in the difference image, $T_1$ is the $T_1$ of blood (Lu et al., 2004), $T_2^*$ is 100 ms and $T_2^*$ is 50 ms are the approximate T2* of blood and tissue respectively, and $S_0$ is the whole-brain equilibrium tissue magnetisation.

Identifying the active regions of interest

The CBF and BOLD time-course data were then analysed on an individual basis using BrainVoyager. Pre-processing consisted of spatial (FWHM 6 mm) and temporal (10 s FWHM) smoothing and linear trend removal. The BOLD and CBF images were co-registered to the structural T1-weighted image and were then transformed into Talairach space. Activated regions of interest were found on each individual for each Stroop run. The general linear model consisted of the block design (taken from the timings in the individual Presentation logfiles) convolved with a haemodynamic response function. Both the CBF and BOLD data were modelled simultaneously and regions where the model accounted for significant variance in both of the time-courses were inspected at a threshold of $p<0.05$ (corrected for false-discovery rate). Six regions of interest (ROI) were identified, representing combined regions where both BOLD and CBF show significant signal change. BOLD and CBF signal time-courses were recorded in each ROI for the Stroop and hyperoxia runs. The six regions were left and right middle frontal gyrus (MFG), primary motor cortex (M1), left and right parietal lobule (PL), and supplementary motor area (SMA). Regions of approximate volume of 1 cm$^3$ were chosen centrally on the maximum activity for each region. All analysis was repeated independently for the second set of Stroop and hyperoxia data.

Quantification of neural and vascular parameters

First, the calibration constant $M$ was calculated for each region of interest. The respiratory data was analysed in Matlab in order to extract the end-tidal oxygen values. Average values were extracted for 4 time-periods: two rest periods of 1 min prior to hyperoxia, and the last minute of each hyperoxia period. The BOLD signal percentage change was then found averaged over the last minute of each hyperoxia period compared to 1 min prior to hyperoxia. (Chiarelli et al., 2007a), demonstrated that for simplification of analysis, it is sufficient to use an assumed value of CBF change in response to hyperoxia rather than measured values, which are inherently noisy, and need to be corrected for changes in the $T_1$ of arterial blood during hyperoxia. For each period of hyperoxia the calibration constant $'M'$ was found using the Chiarelli and Bulth model:

$$\Delta \text{BOLD}_{\text{HO}} = M \left( 1 - \left( \frac{\text{CBF}_{\text{HO}}}{\text{CBF}_0} \right)^{\alpha} \left( \frac{[d\text{Hb}]_{\text{HO}}}{[d\text{Hb}]_0} + \frac{\text{CBF}_{\text{HO}}}{\text{CBF}_0} - 1 \right)^{\beta} \right)$$

where $\Delta \text{BOLD}_{\text{HO}}$ is the change in the BOLD signal during hyperoxia, CBF$_{\text{HO}}$ is the CBF during hyperoxia, and $[d\text{Hb}]_{\text{HO}}$ is the concentration of deoxygenated haemoglobin in the venous vasculature during hyperoxia. The subscript ‘0’ refers to these parameters at baseline. The model assumes that the system is in equilibrium and hence that CBV changes can be calculated from CBF changes using the Grubb relation (Grubb et al., 1974), giving a value of $\alpha$ of 0.38. $\beta$ is a field-dependent parameter describing the influence of deoxyhaemoglobin on $R_2^*$. We assume a value for $\beta$ of 1.5 as in previous work (Buxton et al., 2004; Chiarelli et al., 2007a). Values for $[d\text{Hb}]_{\text{HO}}/[d\text{Hb}]_0$ were calculated from the end-tidal measurements following the procedure described in (Chiarelli et al., 2007a) assuming a baseline oxygen extraction fraction of 0.4. We assume a reduction in CBF of 5% during hyperoxia. Values for $M$ over individual hyperoxia periods and a mean value of $M$ over both hyperoxia periods were calculated in each ROI for each subject.

The average BOLD and CBF time-courses in each region were found by averaging over the 8 activation blocks. The changing BOLD response produces some contamination of the CBF response as it introduces differences between the label and control images that are not due to CBF change. This effect was corrected for each subject on a region-by-region basis by calculating the change in BOLD signal at each time point and, using Eq. (1), subtracting the respective contribution to the CBF curve. The percentage change in BOLD and CBF in each region was then found from the maximum signal change over the plateau region (between 12 and 36 s post-stimulus). Values for change in CMRO$_2$, and the coupling constant ($n = \Delta \text{CBF}/\Delta \text{CMRO}_2$) were then calculated using the model described in (Buxton et al., 2004) in each region of interest:

$$\text{RESULTS AND DISCUSSION}

Hyperoxia

All subjects tolerated the mask and oxygen delivery well. Fig. 3 shows the end-tidal O$_2$ and CO$_2$ measurements for a typical subject. It
can be seen that the periods of oxygen and air are of sufficient duration to reach equilibrium in end-tidal oxygen (Fig. 3a) and that the effect of oxygen on the CO2 values are small (Fig. 3b). The oxygen measurements were carefully calibrated, but the CO2 measures were not as they were not central to this study. However, based on normal end-tidal CO2 values of around 5%, the values shown in Fig. 3b can be approximately equated to percent, and hence we find that inspired CO2 changes from 0.1% during breathing oxygen to approximately 0.3% during breathing air. This would lead to a CBF increase of less than 1% (Hoge et al., 1999; Leontiev and Buxton, 2007) which is insignificant with regards to this study.

Subjects reached a mean end-tidal oxygen concentration of 61% with a standard deviation across subjects of 11%. The coefficient of variation (calculated using the same approach as described below for the imaging measures) for the end-tidal oxygen values was 2.4% across the two periods of oxygen within one run and 5.3% across the two runs, demonstrating the high reproducibility of the end-tidal oxygen values.

Our approach of using an open circuit and delivering an imprecise level of oxygen to the subjects assumes that the exact amount of oxygen delivered is unimportant. \( M \) depends on the change in BOLD signal for a given change in end-tidal oxygen and so as long as these are accurately measured the absolute values are not important. As shown in Fig. 4 we found no significant correlation between \( M \) and end-tidal oxygen (either within or across subjects), supporting our assumption that these values are independent. An open mask offers improved comfort for subjects and is easy to implement with non-specialized equipment.

### Behavioural data

All participants performed the Stroop task correctly with mean accuracy of 97% and standard deviation of 4%. Mean reaction time was 1021 ms with a standard deviation of 175 ms. A paired two-tailed \( t \)-test revealed no significant difference in either accuracy or reaction time across the two runs.

### Activated regions

We found consistent activation of regions for the Stroop task across the two runs for each subject, but less consistency across subjects with some subjects showing activation in only a few regions. Fig. 5 shows a typical activation map from one subject for one functional Stroop run. Our acquisition parameters are optimised for quantification rather than sensitivity which may be why not all regions were identified in all subjects. In order to improve the identification of active regions, future work could consider a functional localiser using an optimised BOLD sequence. However, while a BOLD localiser will identify regions more robustly, this may be to the detriment of precision in the estimated parameters. Previous work shows that use of a CBF localiser produces the most precise measurements (Leontiev and Buxton, 2007).

Group analysis over all subjects revealed the central Talairach coordinates for the 6 regions to be: M1 \((-35 -23 54)\); SMA \((-3 -5 53)\); Left Parietal Lobule \((-24 -61 45)\); Right Parietal Lobule \((-31 -57 45)\); Left MFG \((-47 0 37)\) and Right MFG \((46 0 38)\), as confirmed with the Talairach daemon (www.talairach.org). Previous work using the Stroop task found similar regions of activation (Zysset et al., 2007), with the exception of the primary motor cortex, which we obtained through button box presses, in response to the task. This allowed us to compare quantitatively, fMRI measures in cognitive and non-cognitive related brain regions.

### Signal time-course

The signal time-course for BOLD and CBF averaged across all regions and all subjects for both Stroop and hyperoxia runs is shown in Fig. 6. It can be seen that both the amplitude and the shape of the

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**Fig. 4.** Effect of end-tidal oxygen on the calibration constant \( M \). Each color represents a different subject, with values of all four periods of hyperoxia shown (i.e. 2 periods in run 1 and 2 periods in run 2). Average values for \( M \) are shown over all of the consistently activated regions for each particular subject.

**Fig. 5.** Activated regions. The figure shows the activated regions for a single run in a single subject at a statistical threshold of \( p < 0.05 \) corrected for false-discovery rate. The white circles indicate the regions of activation from which data was recorded. M1 = primary motor cortex, SMA = supplementary motor area, MFG = middle frontal gyrus, PL = parietal lobe.
signal time-course for the Stroop task (Figs. 6a and b) are similar across the two runs, shown in red and blue. Interestingly, the shape of the BOLD signal and CBF time-courses are different. The BOLD signal shows a later and slower onset than the CBF signal and the CBF appears to respond more strongly to the onset and offset of activity with a peak in the response to both the stimulus onset and offset. While the CBF response reaches somewhat of a plateau, the peaks seen at onset and offset suggest a dynamic system which may invalidate the Grubb relation, and thus affect the results. The shorter lag time and faster rise time of the CBF response in comparison to the BOLD response has been previously reported (Liu et al., 2000; Yang et al., 2000, 2004; Huppert et al., 2006), and is thought to reflect the arterial origin of the ASL signal in comparison to the venous origin of the BOLD signal with added delay and dispersion. The BOLD response during hyperoxia (Fig. 6c) is less consistent than the response to the cognitive task (Fig. 6a) over the two runs, reflecting the known drift in BOLD baseline measures. In addition, due to the open mask system the exact concentration of oxygen delivered on each run is not controlled, causing differences in the level of hyperoxia and therefore the BOLD response between the two runs. We find no significant reduction in CBF during hyperoxia (Fig. 6d). This is despite the fact that our CBF measurements are likely underestimated during hyperoxia due to the additional effect of the increased $T_1$ of arterial blood (Bulte et al., 2007). The standard deviation of CBF measurements (from 1 min of data) across subjects was found to be 17%, hence 24 subjects would be required for the confidence interval on the mean to be less than 5%. Therefore with only 10 subjects we were underpowered to detect a 5% change. Previous studies do report a reduction in CBF during hyperoxia ranging from 5% to 20% (Bergofsky and Bertun, 1966; Watson et al., 2000; Bulte et al., 2007; Zaharchuk et al., 2008). The degree of reduction depends on the duration of hyperoxia, and for a brief period of 3 min there is reasonable agreement in the literature that this corresponds to approximately 5% reduction in CBF. This, combined with the inherently low signal to noise of our measured CBF

![Fig. 6. Average signal time-courses over the two runs. The figure shows the signal change for both BOLD and perfusion averaged across all regions and all subjects for run 1 (red) and run 2 (blue). a and b show the BOLD signal and CBF response respectively during the Stroop task, c and d show the BOLD signal and CBF response respectively during hyperoxia.](image)

Fig. 6. Average signal time-courses over the two runs. The figure shows the signal change for both BOLD and perfusion averaged across all regions and all subjects for run 1 (red) and run 2 (blue). a and b show the BOLD signal and CBF response respectively during the Stroop task, c and d show the BOLD signal and CBF response respectively during hyperoxia.

![Fig. 7. Effect of level of assumed CBF reduction on estimated parameters. The figure shows the importance of the level of assumed CBF reduction on the estimated parameters M, CMRO2 and n. These were calculated using data from a typical subject with end-tidal O2 of 61%, BOLD change during hyperoxia of 1.22%, BOLD change of 1.0% and CBF change 42% during the Stroop task.](image)

![Fig. 8. Reproducibility of neurovascular coupling parameters. The figure shows percentage difference between measurements from the two scans, for each subject in each region, and the coefficient of variation (standard deviation between these differences divided by the square root of 2). ‘M 6 mins’ refers to the value of M calculated from only 6 min of the hyperoxia data (i.e., the first period of 3 min off and 3 min on). ’M 13 mins’ is the value for M calculated from the full hyperoxia scan. CVLeontiev refers to the equivalent values reported in (Leontiev and Buxton, 2007) for a hypercapnic calibration using a V1 localiser.](image)

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data suggests that the use of an assumed CBF reduction of -5% is a reasonable compromise, at least for an age matched cohort. There is evidence that CBF reduction changes with age (Watson et al., 2000), hence the application of an assumed CBF value rather than a measured one may not be ideal over a large cohort age range. Fig. 7 shows the simulated effect of the assumed reduction of CBF on the value of M, CMRO₂ and n. It can be seen that all three of the calculated parameters are highly sensitive to the level of assumed CBF reduction. M passes through a discontinuity with increasing CBF reduction, but the effects on CMRO₂ and n remain approximately linear, which follows from inspection of Eqs. (2) and (3). Further work is needed to establish the level of CBF reduction during brief periods of hyperoxia, and the variability across subjects, and hence the relative importance of this for the hyperoxia calibration technique.

Due to the nonresponsiveness of CBF during hyperoxia, this gave us an opportunity to measure ‘resting state’ CBF as the average value during the 13 min hyperoxia scan in each region.

Reproducibility

We found that we had to discard approximately 8% of the data due to negative values for ΔCMRO₂ which were deemed to be biologically implausible. The source of these errors appears to be low values of M in comparison to relatively large values of ΔBOLD during the Stroop task. This may occur for regions of interest close to the ventricles or the edge of the brain where M may be particularly susceptible to movement artefacts.

In addition, not all subjects showed significant activation in all regions on both runs, leaving a total of 37 regions from which to calculate the reproducibility of each parameter. The percentage differences between measurements acquired on different scans were calculated for each region according to:

\[ \Delta = 100 \times \frac{x_1 - x_2}{x_{\text{avg}}} \]  

Fig. 8 shows a ‘Bland–Altman’ plot of these percentage differences. The coefficient of variation for each parameter is also given, which is equal to the standard deviation of the differences (retaining the sign) divided by the square root of 2 (Leontiev and Buxton, 2007).

The coefficients of variation of the measurements using hyperoxia calibration are generally higher than those found during hypercapnia calibration (Leontiev and Buxton, 2007) as shown in Fig. 8. Leontiev and Buxton reported improved reproducibility on use of a CBF localiser; however this was not possible for our data due to the poor activation seen in cognitive areas using the CBF data alone. The absolute values of BOLD and CBF change we report are approximately half that in the previous study (0.8% and 26% respectively, compared to 1.9% and 73%), which is likely due to the use of a cognitive rather than a visual task. The response in the motor cortex is also low due to the slow rate of button presses associated with the task (approximately 0.7 Hz). Reproducibility for a visual task using hyperoxia calibration would likely be improved compared to our results. The source of variability in ΔCMRO₂ and n was investigated further by simulation using Eq. (3) with fixed parameters: \( \Delta\text{BOLD}=0.81 \), \( \Delta\text{CBF}=26.4 \), \( M=9.4\% \), taken from average values over all subjects and regions (Table 1). Each of these 3 parameters was then varied independently from zero to twice the average value to determine the effect on CMRO₂ and n. Fig. 9 shows that ΔCBF has the largest effect on ΔCMRO₂, which could explain why the ΔCMRO₂ values show poor reproducibility as ΔCBF is the least reproducible of the measured parameters (Fig. 8). Away from the discontinuity, which occurs as ΔCMRO₂ passes through zero, n is most strongly affected by ΔBOLD which is the most reproducible of the measured parameters, and could explain why the reproducibility for n is better than for ΔCMRO₂.

We find that our reproducibility values are improved if values close to the discontinuity are avoided.

Differences in neurovascular coupling across the brain

For comparisons of values across different brain regions, measurements were averaged over both runs in order to improve the precision. Table 1 shows the mean and standard deviation (across subjects) of the measured parameters. An average value for M of 9.4% is similar to previous studies (Table 2). However, as M depends on field strength and on the particular scanning parameters used, these values would not necessarily be expected to be in close agreement as some are measured at 1.5 T and some at 3 T. We find an average increase in oxygen metabolism of 12% and neurovascular coupling parameter of

Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>M (%)</th>
<th>(Δ)CBF (%)</th>
<th>(Δ)BOLD (%)</th>
<th>(Δ)CMRO₂ (%)</th>
<th>n</th>
<th>Resting CBF (ml/min/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary motor cortex</td>
<td>9.1±2.0</td>
<td>29.7±14.5</td>
<td>0.6±0.22</td>
<td>15.4±8.1</td>
<td>2.0±0.25</td>
<td>48.5±9.8</td>
</tr>
<tr>
<td>Supplementary motor area</td>
<td>10.8±4.2</td>
<td>25.1±6.2</td>
<td>0.85±0.21</td>
<td>11.3±4.8</td>
<td>2.51±0.25</td>
<td>43.0±7.3</td>
</tr>
<tr>
<td>L parietal lobe</td>
<td>10.2±4.9</td>
<td>27.4±9.1</td>
<td>0.91±0.2</td>
<td>11.9±3.9</td>
<td>2.57±0.7</td>
<td>60.0±22.4</td>
</tr>
<tr>
<td>R parietal lobe</td>
<td>7.58±4.4</td>
<td>33.3±11.6</td>
<td>0.90±0.19</td>
<td>12.9±6.6</td>
<td>3.14±1.1</td>
<td>57.3±17.7</td>
</tr>
<tr>
<td>L middle frontal gyrus</td>
<td>9.0±5.8</td>
<td>28.1±8.0</td>
<td>0.82±0.25</td>
<td>10.8±4.0</td>
<td>2.83±1.0</td>
<td>43.3±7.6</td>
</tr>
<tr>
<td>R middle frontal gyrus</td>
<td>7.42±4.0</td>
<td>25.5±7.9</td>
<td>0.73±0.21</td>
<td>9.4±4.2</td>
<td>2.98±0.92</td>
<td>43.2±7.6</td>
</tr>
<tr>
<td>Average</td>
<td>9.4</td>
<td>26.4</td>
<td>0.81</td>
<td>11.6</td>
<td>2.52</td>
<td>51.3</td>
</tr>
</tbody>
</table>

Fig. 9. Source of variability in estimations of CMRO₂ and n. The graph shows the effect of BOLD (a) CBF (b) and M (c) on the estimates of CMRO₂ (red dotted line) and n (blue solid line). n passes through a discontinuity as shown by the vertical dotted line.
which are again in good agreement with literature values (Table 2). Reported values for ‘n’ also depend on analysis details, such as whether individual or group data is fitted, and on how the regions of interest are chosen, which must contribute to the variability seen in Table 2.

Inspecting the data in Table 1 it can be seen that both BOLD and ‘n’ appear lower in primary motor cortex compared to other regions, whereas other parameters such as M do not. This was tested formally. First, in order to reduce the total number of comparisons, values from homologous left and right regions were averaged, then paired two-tailed t-test were performed comparing the values in M1 with all 3 other regions. We find that the BOLD response and ‘n’ are indeed significantly lower in primary motor cortex in comparison to both the parietal and frontal regions, but are not significantly different than values in the SMA (Table 3). This suggests a greater vascular responsiveness in frontal and parietal areas in response to a neural demand in comparison to motor regions.

Previous work has shown regional variations in coupling parameters, with higher values of M in the visual cortex compared to the motor cortex (Stefanovic et al., 2007; Chiarelli et al., 2007), although no conclusive difference in ‘n’ between these regions. The study by (Ances et al., 2008) showed reduced coupling ‘n’ in the lentiform nuclei of the basal ganglia in comparison to the visual cortex, suggesting a reduced vascular responsiveness in the basal ganglia. One further study has suggested reduced coupling ‘n’ in the medial temporal lobe compared to the motor cortex (Restom et al., 2008), although a direct comparison of regions was not made.

We expected there to be increased BOLD activation on the left side of the brain compared to the right in frontal and parietal regions, as previously reported (Zysset et al., 2007) for the Stroop task. Indeed, inspection of the values in Table 1 shows a slightly increased BOLD response on the left side. Significance testing of all parameters between homologous left and right regions with a paired two-tailed t-test found however no significant differences.

In summary we report reasonable reproducibility for the measurements derived from the hyperoxia-calibrated fMRI technique and values for the neurovascular coupling parameter are consistent with those reported using a hypercapnia technique. We find different neurovascular coupling between the motor cortex and frontal and parietal regions.

Table 3

<table>
<thead>
<tr>
<th>Region</th>
<th>M</th>
<th>ΔCBF</th>
<th>ΔBOLD</th>
<th>ΔCMRO2</th>
<th>n</th>
<th>Resting CBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplementary motor area</td>
<td>0.2</td>
<td>0.39</td>
<td>0.24</td>
<td>0.31</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>0.60</td>
<td>0.93</td>
<td>0.01</td>
<td>0.26</td>
<td>0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>0.74</td>
<td>0.31</td>
<td>0.18</td>
<td>0.04</td>
<td>0.002</td>
<td>0.14</td>
</tr>
</tbody>
</table>

More generally, this approach demonstrates the ability to measure quantitative neurophysiological parameters underlying the fMRI signal.

Acknowledgments

Thanks to Unilever Food and Health Research Institute and the Medical Research Council for funding this work.

References


Table 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Calibration</th>
<th>M (%)</th>
<th>ΔCMRO2 (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Chiarelli et al., 2007b)</td>
<td>Visual cortex</td>
<td>HO</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Leontiev and Buxton, 2007)</td>
<td>Visual cortex</td>
<td>HC</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Davis et al., 1998)</td>
<td>Visual cortex</td>
<td>HC</td>
<td>11.1</td>
<td>27</td>
<td>2.6</td>
</tr>
<tr>
<td>(Hoge et al., 1999)</td>
<td>Visual cortex</td>
<td>HC</td>
<td>7.9</td>
<td>16</td>
<td>2.8</td>
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<tr>
<td>(Hyder, 2004)</td>
<td>Sensory cortex</td>
<td>PET</td>
<td>18</td>
<td>2.9</td>
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<tr>
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<td>HC</td>
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<td>16</td>
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<td>HC</td>
<td>25</td>
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<td>HC</td>
<td>7.6</td>
<td>4.5</td>
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<td>HC</td>
<td>7.2</td>
<td>3.3</td>
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<td>HC</td>
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<td>2.5</td>
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<tr>
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<td>HC</td>
<td>4.3</td>
<td>3.2</td>
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<td>HC</td>
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<td>10</td>
<td>2.9</td>
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</table>

* This is the average of the 14 PET values given in the table in this paper, where values for ΔCMRO2 are greater than zero. In general, values from regions of CBF/BOLD overlap are taken where possible.