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Regional differences in the coupling of cerebral blood flow and oxygen metabolism changes in response to activation: Implications for BOLD-fMRI

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Abstract

Functional magnetic resonance imaging (fMRI) based on blood oxygenation level dependent (BOLD) signal changes is a sensitive tool for mapping brain activation, but quantitative interpretation of the BOLD response is problematic. The BOLD response is primarily driven by cerebral blood flow (CBF) changes, but is moderated by M , a scaling parameter reflecting baseline deoxyhemoglobin, and n , the ratio of fractional changes in CBF to cerebral metabolic rate of oxygen consumption (CMRO₂). We compared M and n between cortical (visual cortex, VC) and subcortical (lentiform nuclei, LN) regions using a quantitative approach based on calibrating the BOLD response with a hypercapnia experiment. Although M was similar in both regions (~5.8%), differences in n (2.21 ± 0.03 in VC and 1.58 ± 0.03 in LN; Cohen $d=1.71$) produced substantially weaker (~3.7×) subcortical than cortical BOLD responses relative to CMRO₂ changes. Because of this strong sensitivity to n , BOLD response amplitudes cannot be interpreted as a quantitative reflection of underlying metabolic changes, particularly when comparing cortical and sub-cortical regions.

Keywords

Cerebral blood flow; Cerebral metabolic rate of oxygen consumption; Neurovascular coupling; Hypercapnia; Blood oxygenation level dependent (BOLD) effect; Arterial spin labeling (ASL); fMRI; Lentiform nuclei of the basal ganglia

Introduction

Local increases in brain activity are accompanied by increases in cerebral blood flow (CBF), cerebral metabolic rate of oxygen consumption (CMRO₂), and cerebral metabolic rate of glucose consumption (CMRGlc). However, a surprising aspect of this metabolic response is that fractional CBF changes are greater than fractional CMRO₂ changes, leading to increased blood oxygenation with activation (Fox and Raichle, 1986). Because of the paramagnetic nature of deoxyhemoglobin, this produces a small increase in signal measured by magnetic resonance imaging (MRI), called the blood oxygenation level dependent (BOLD) effect

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(Kwong et al., 1992; Ogawa and Lee, 1992). The BOLD effect has been widely used in functional MRI (fMRI) studies to map brain activation.

Within these mapping applications, detection of a statistically significant BOLD response is interpreted as evidence of a change in neural activity at a particular brain location (Bandettini, 2007). However, interpreting the BOLD response amplitude as a quantitative reflection of the magnitude of underlying change in neural activity or metabolism is problematic: does a larger BOLD response in one region compared to another indicate greater change in neural activity or oxygen metabolism? Two sources of physiological variability could lead to a dissociation of the magnitude of the BOLD response from the magnitude of underlying physiological responses. First, because the BOLD effect is related to local deoxyhemoglobin decreases, the ceiling for the BOLD response magnitude is set by the amount of deoxyhemoglobin present at baseline (Davis et al., 1998). Baseline conditions could vary across the brain (e.g., due to differences in cerebral blood volume (CBV), baseline oxygen extraction fraction (OEF), or local neural activity) (Buxton et al., 2004). In modeling the BOLD response, the effect of variable baseline conditions is described by the scaling factor M (Davis et al., 1998).

A second potential source of variability of the BOLD response is that the coupling of CBF and CMRO₂ could vary across the brain or potentially in the same area under different conditions (Chiarelli et al., 2007a,b; Tuunanen and Kauppinen, 2006; Tuunanen et al., 2006; Vafaee et al., 1998; Vafaee and Gjedde, 2004). Recent studies have emphasized the idea that CBF increases are driven in a feed-forward manner by mechanisms triggered by changes in neural activity, rather than feed-back responses to increased energy demands (Attwell and Iadecola, 2002; Raichle and Mintun, 2006; Uludag et al., 2004). Nevertheless, the BOLD response depends on the coupling of changes in CBF and CMRO₂, with the term ‘coupling’ simply referring to combined changes in each. If we define n as the ratio of the fractional changes in CBF and CMRO₂ with activation, then the existence of the BOLD effect is consistent with n being larger than one. However, the exact value of n plays a significant role in determining the magnitude of the BOLD signal observed for a given underlying metabolic change, particularly when $n < 3$. Hypothetical brain regions with relatively small differences in n (i.e. from $n=1.5$ to $n=2$, or from $n=2$ to $n=3$) but similar CMRO₂ changes could lead to BOLD responses that differ in magnitude by ~100% (Fig. 1). Accurate interpretation of the magnitude of BOLD responses therefore requires knowledge of whether n varies across the healthy brain.

CBF and CMRO₂ measurements using positron emission tomography (PET) have yielded a range of n values. Some PET studies found significant increases in CBF with little or no CMRO₂ increases accompanying brain activation, leading to relatively large n values (Fox and Raichle, 1986; Kuwabara et al., 1992). Other studies have observed larger CMRO₂ changes with $n \sim 1$ (Roland et al., 1987) or $n \sim 2-4$ (Marrett and Gjedde, 1997; Seitz and Roland, 1992; Vafaee et al., 1998; Vafaee and Gjedde, 2004). It remains unknown if observed differences in n reflect variability in measurement technique, true physiological differences, or both.

Davis and colleagues introduced an fMRI method for estimating CMRO₂ changes by “calibrating” the BOLD signal, a technique requiring combined measurements of BOLD and CBF responses under separate conditions of functional activation and mild hypercapnia (Davis et al., 1998). The CBF response is measured with an arterial spin labeling (ASL) technique (Detre et al., 1992). The calibrated-BOLD approach exploits the fact that BOLD signals depend on changes in CBF and CMRO₂, while the ASL signal depends only on changes in CBF. By analyzing these data within the context of a mathematical model for the BOLD signal, estimates of CMRO₂ changes, and thus n , can be calculated for a functional activation paradigm. Calibration with mild hypercapnia is necessary for determining the local BOLD scaling parameter, M .

Several groups have adopted this fMRI approach and reported large CMRO₂ changes with subsequent values of n lying within the range 2–4.5 for cortical regions including the motor and visual areas (Chiarelli et al., 2007a,b; Davis et al., 1998; Hoge et al., 1999; Kastrup et al., 2002; Kim et al., 1999; Leontiev and Buxton, 2007; Leontiev et al., 2007; St Lawrence et al., 2002; Stefanovic et al., 2004; Stefanovic et al., 2006; Uludag et al., 2004). As the calibrated BOLD fMRI method becomes more widespread, it remains critical to assess whether reported variations in n are due to physiological differences, population variability, or intrinsic biases in the methods used (Leontiev et al., 2007). Although the intra-subject reproducibility of n is quite good (<10%), inter-subject variation of n is several times larger (Leontiev and Buxton, 2007), suggesting that population variability may be an important factor.

In a recent study Chiarelli and colleagues reported differences in n between three cortical regions, finding n values ranging from 2.3 to 4.2 (Chiarelli et al., 2007a). Here, we report the first use of a calibrated BOLD approach to test for differences in n and M between cortical and sub-cortical structures. Two distinct brain areas were imaged simultaneously, using a stimulus consisting of a flashing checkerboard to activate the visual cortex (VC), and a complex motor task to stimulate the putamen and globus pallidus, referred to collectively as the lentiform nuclei (LN) of the basal ganglia. In this way potential confounds of baseline state changes or methodological differences were minimized. Our primary finding was that M values were quite similar in the two regions, but n values were significantly greater in the VC compared to the LN. Although the CMRO₂ responses in the two regions differed by only a factor of two, there was over a seven-fold difference in the magnitude of the BOLD signal. The implications of this finding for the interpretation of the BOLD response between different cortical and subcortical brain regions are discussed.

Methods

Subjects

Thirteen healthy subjects were recruited (7 males, 6 females) (age range: 21–56 years of age (mean 38±4)) and imaged according to guidelines approved by the Institutional Review Board (IRB) of the University of California San Diego (UCSD).

Experimental design

Each subject underwent two experiments within the same scanning session, one measuring CBF and BOLD responses to mild hypercapnia, and one measuring the CBF and BOLD responses to functional activation (Fig. 2). In the first experiment, subjects breathed a gas mixture containing 5% CO₂ through a mask with continuous sampling of end-tidal CO₂. In the second experiment, a combined motor and visual stimulus was used to simultaneously stimulate the LN and VC. The stimulus consisted of a black and white radial checkerboard flickering at 8 Hz while numbers, presented at 2 Hz, appeared in the center to cue finger tapping with the right hand (Fig. 2). The presented number indicated which finger to press on the appropriate key of a 4-button response box, and a fixed motor sequence (2-4-3-5) was used for all subjects. Stimulus frequencies were chosen to maximize activation within each of these ROIs (Allison et al., 2000; Kastrup et al., 2002). Common rest periods for both tasks consisted of an isoluminant gray screen with a white fixation square in the middle. A block-design sequence was utilized to maximize signal to noise ratio and consisted of 20 s of activation and 60 s of rest in one cycle. The full experimental run consisted of 60 s of rest, followed by four cycles of task/rest, followed by an additional 30 s of rest, for a total run duration of 410 s.

In order to characterize baseline CBF, a scan (3 min 20 s) was performed during which subjects fixated on a gray screen with a white fixation square in the center (i.e. the same as the stimulus rest condition described above). For conversion to absolute units, a cerebral spinal fluid (CSF)

reference scan and a minimum contrast scan were also acquired. The CSF scan consisted of a single-echo, single repetition scan acquired at full relaxation and TE=9.4 ms. The same in-plane parameters as the resting CBF scan were used, but the number of slices was increased to ensure coverage of the lateral ventricles. The minimum contrast scan was acquired with TR=2 s, TE=11 ms to ensure little contrast between gray matter, white matter and CSF. Two 8-interleave repetitions were acquired using the same slice prescription as the CSF scan.

CO₂ administration

Hypercapnia was induced by breathing a gas mixture consisting of 5% CO₂, 21% O₂ and 74% N₂ delivered through a non-rebreathing face mask (Hans Rudolph, 2700 Series, St. Louis, MI) worn by all subjects. Two runs lasting 7 min in duration were performed and consisted of breathing room air for 2 min followed by 3 min of hypercapnia, and then room air for 2 min. Throughout these runs subjects were directed to fixate on a white square centered on a uniform gray background, the same baseline condition as in the activation experiment. During all scans, including hypercapnia, subjects had constant physiological monitoring including pulse oximetry and respiratory excursions.

MRI parameters

Imaging data were acquired on a 3 T whole body system (3-T GE Excite, Milwaukee, WI) with an eight-channel receive head coil. Quantitative ASL images were acquired with a single-shot PICORE QUIPSS II (Wong et al., 1998) pulse sequence (TR=2.5 s, TI₁=700 ms, TI₂=1500 ms, 20-cm tag width, and a 1-cm tag-slice gap) with a dual-echo gradient echo readout and spiral acquisition of *k*-space (TE₁=9.4 ms, TE₂=30 ms, flip angle=90°, field of view (FOV)=24 cm, 64×64 matrix). The ASL acquisition alternates 'tag' images, in which the magnetization of arterial blood is inverted before it flows into the selected slice, and 'control' images in which the arterial magnetization is not inverted. The difference of the tag and control images from the first echo provides a CBF response time series, while the average of the tag and control images from the second echo yields a BOLD response time series. Four 7-mm-thick axial slices, which included both the LN and VC were acquired in a linear fashion from bottom to top. A high-resolution structural scan was acquired with an inversion recovery prepared 3D fast spoiled GRASS (IR-FSPGR) pulse sequence (TI=450 ms, TR=7.9 ms, TE=3.1 ms, flip angle=12°, FOV=25 × 25 × 16 cm, matrix 256 × 256 × 124). The latter images were collected after the hypercapnia experiment and before the activation experiment, and thus provided additional delay between the two experiments to minimize any possible lingering effects of CO₂ administration on the functional activation experiment.

Data analysis

In these studies, the signal to noise ratio (SNR) typically was not sufficient to perform individual voxel calculations, so regions of interest (ROI) for averaging were required. The first step of ROI selection consisted of manually delineating relevant areas on high-resolution images in order to create an anatomical mask. The LN consists of the putamen and the globus pallidus and is part of the basal ganglia. It is a large cone shaped mass of gray matter located between the caudate nucleus and the island of Reil. The VC mask for these experiments was defined by the parietal-occipital sulci, and contains not only the primary visual cortex (V1) but also supplementary regions (Fig. 2).

A general linear model (GLM) approach for the analysis of ASL data (Mumford et al., 2006; Restom et al., 2006) was used to identify activated voxels within the defined masks for the two anatomical regions in order to define an activated ROI for the LN and VC regions. The stimulus-related regressor was obtained by convolving the block design stimulus pattern with a gamma density function (Boynton et al., 1996). In addition, measured cardiac and respiratory fluctuation data were included in the GLM as regressors to model physiological modulation

of the ASL signal, and both constant and linear terms were included as nuisance regressors. Pre-whitening was performed using an autoregressive model (Burock and Dale, 2000; Woolrich et al., 2004). Data from functional runs were concatenated for the GLM analysis, with separate physiological and nuisance regressors applied for each run (Restom et al., 2006).

Clusters of voxels exhibiting CBF activation within the defined LN and VC boundaries were detected using an overall significance threshold of $p=0.05$ applied to the first echo data. Correction for multiple comparisons was performed using the AFNI AlphaSim program (Cox, 1996). In addition to this primary ROI based on CBF activation alone, we also located BOLD activated voxels with an overall significance threshold of $p=0.05$ applied to the second echo data. As has been performed in some previous studies (Chiarelli et al., 2007a,b; Davis et al., 1998; Kastrup et al., 2002), a second set of ROIs consisting of the intersection of CBF and BOLD responses was generated.

For each subject, average CBF and BOLD responses from each of the functional ROI's were generated, for both hypercapnia and activation. For each voxel, a CBF time-series was first computed by taking a running subtraction of the control and tag image series from the first echo data (TE=9.4 ms). Each data point was calculated from the difference between that value and the average of the two nearest neighbors in time with adjustments made in the sign so that each point represents a subtraction of tag from control images. A BOLD-weighted time series was also computed from the running average of the second echo (TE =30 ms), taking the average of each image with the mean of its two nearest neighbors (Liu and Wong, 2005). The mean CBF and BOLD responses were then obtained by averaging voxel time courses over the LN and VC ROIs after removal of the physiological noise components estimated from the GLM. For the functional runs, all runs were averaged together and both linear and quadratic drifts were removed from the average CBF and BOLD time series. For the hypercapnia runs, both runs were averaged together and only linear drifts were removed from the average CBF and BOLD time series in order to avoid removing part of the single-block response with a quadratic term.

For each subject, the average CBF and BOLD responses were normalized to their respective baseline values, calculated as the average of the first minute of the acquisition. The amplitude of the response to mild hypercapnia was calculated as the average over the last 2 min of CO₂ inhalation, in order to reduce the influence of the transition regions. For the functional runs, the fractional CBF and BOLD responses were calculated as the average over a 15 second period starting at the midpoint of each stimulus presentation, approximating the plateau portion of the response. Group averages for all variables were determined by averaging the responses over subjects.

The CBF time series from the baseline scan were corrected for inhomogeneities in the coil sensitivity profile using the smoothed minimum contrast images (Wang et al., 2005), and converted to physiological units (ml/100 ml/min) using the CSF image as a reference signal to determine the fully relaxed magnetization of blood (Chalela et al., 2000). The mean resting CBF for each subject was calculated by averaging the CBF time series over all time points and over all voxels within the ROI. No correction was performed for partial volume effects.

Determination of CMRO₂, n, and CRC

Calculation of CMRO₂ changes with functional stimulation was determined by methods previously described by Davis and coworkers (Davis et al., 1998). In this model, the fractional BOLD signal change ($\Delta S/S_0$) is related to the underlying fractional changes in CBF and CMRO₂ from a defined baseline state. If we define f as the ratio of CBF to its baseline value, and r as the ratio of CMRO₂ to its baseline value, the BOLD signal change is modeled as:

$$\frac{\Delta S}{S_0} = M(1 - f^{\alpha-\beta} r^\beta) \quad (1)$$

where the parameter M represents a scaling factor specific to that brain region in the defined baseline state. That is, the parameter M reflects the deoxyhemoglobin content in the baseline state, and defines the maximum possible BOLD signal change or ceiling effect for a particular region. This parameter depends on the baseline CBV and OEF, and also on experimental parameters such as field strength and echo time. The parameter α is the exponent of the empirical power law relationship between blood volume and blood flow, and is assumed to be equal to 0.38 (Grubb et al., 1974), while β is taken to be 1.5 based on numerical simulations (Boxerman et al., 1995b; Davis et al., 1998). Although the Davis model (Eq. (1)) is a relatively simple expression for a complex phenomenon, the applicability of the model may be more general than one would expect given the assumptions from which it was derived (Leontiev et al., 2007).

The hypercapnia experiment is required in order to accurately estimate M . With the assumption that mild hypercapnia does not alter CMRO₂ (Hafkenschiel et al., 1954; Kastrup et al., 1999; Kety and Schmidt, 1948; Kim and Ugurbil, 1997; Kliefoth et al., 1979; Novack et al., 1953), the parameter r in Eq. (1) is assumed to be equal to one, and from the measured CBF and BOLD responses to hypercapnia M is calculated. This estimate of M is then combined with the measured CBF and BOLD responses to activation to calculate r for the activation experiment. From this estimate of the fractional CMRO₂ change with activation, the CBF/CMRO₂ coupling ratio n is defined as the ratio of the fractional change in CBF to the fractional change in CMRO₂.

The mild hypercapnia experiment also yields a measure of vascular responsiveness, which we express in two ways. The cerebrovascular response to CO₂ (CRC) is defined as the percentage change in CBF divided by the change in end-tidal CO₂ (in mm Hg) (Olesen et al., 1971). We can also express this as a dimensionless ratio defined analogously to the CBF/CMRO₂ coupling index n : the ratio n_{CO_2} is the fractional change in CBF divided by the fractional change in end-tidal CO₂.

Statistical analysis

Simple paired t -tests were performed between the two regions using a Sidak correction with a p -value significant if $p < 0.05$. In addition, post-hoc Cohen d tests were performed to determine the size of observed effects.

Results

For hypercapnia, the ratio of the BOLD response to the CBF response was similar in VC and LN

Across subjects, breathing CO₂-enriched air increased end-tidal CO₂ by 7.2 ± 1.0 mm Hg and is similar to previous studies using similar concentrations (Chiarelli et al., 2007a,b). No changes in respiratory rate or oxygen saturation were observed. Robust CBF and BOLD changes to hypercapnia were present in both regions (Figs. 3A and B; Table 1) with significantly larger CBF responses to CO₂ (CRC) observed for the VC compared to LN. However, the ratios of the BOLD response to the CBF response for mild hypercapnia were comparable for the LN and VC (Fig. 4), leading to similar estimates of M for the two regions (Table 2).

For functional activation, the ratio of the BOLD response to the CBF response was higher in VC than LN

Robust functionally modulated changes in CBF and BOLD were observed in both brain regions (Figs. 3C and D) with responses significantly greater in the VC compared to LN (Table 1). The calculated fractional CMRO₂ changes were significantly greater in the VC compared to LN. In addition, the calculated CBF/CMRO₂ coupling ratio, n , was significantly larger for the VC compared to LN (Cohen $d=1.71$) (Table 2). Similar results were observed when a group average M value, rather than individually determined M values, was used to calculate n for each subject (data not shown).

Because calculations of M and n involve significant nonlinearities (Eq. (1) of Methods), it is important to distinguish between regional differences seen in measured data and differences in model-estimated parameters. The calibrated BOLD approach contains four measured quantities: CBF and BOLD responses to hypercapnia and functional activation. Although all four numbers are necessary for accurate calculations of model parameters, most of the information is captured by just two numbers: the ratios of the BOLD and CBF responses to hypercapnia and to functional activation. Ratios were calculated for each subject within the two brain regions (Fig. 4A), with the BOLD/CBF hypercapnia ratio similar in both brain regions, leading to similar values of M . A lower BOLD/CBF activation ratio is observed in the LN, consistent with a smaller value of n found for the LN compared to the VC. Approximate contours of constant M (horizontal) and n (angled vertically) are also plotted to graphically illustrate the transformation from measured data to calculated values (Fig. 4B). While the BOLD/CBF ratio for hypercapnia is similar in the two regions, as reflected above by similar M values, a significantly greater BOLD/CBF ratio for functional activation translates into a significantly larger n within the VC compared to the LN. Across subjects, no significant correlations existed between estimates of n and M , nor between estimates of n or M and absolute baseline CBF (Figs. 5A–C). In addition, we show a scatter plot of CBF and CMRO₂ changes for both regions (Fig. 5D). From individual calculations of n , there was a significant difference in n between the two regions as assessed using a pairwise t -test ($p<0.03$).

Higher average values of n were seen in the VC compared to LN

A scatter plot comparing n values in the VC and LN demonstrates a higher average n in the VC compared to LN, but no systematic correlation existed between n values for the two regions (Fig. 6A). A scatter plot of n against n_{CO_2} compares the vascular responsiveness in each region to two different stimuli: (1) increases in CMRO₂, and (2) systemic arterial pCO₂ changes (Fig. 6B). Both indices are expressed in dimensionless ratios of fractional responses. Normalized CBF changes are significantly larger in the VC for both stimuli, suggesting that the vascular responsiveness in general is more dynamic in the VC compared to LN.

Calculated n values are greater for VC than LN using various ROI analyses

Finally, all data presented were determined from within an ROI based solely on CBF activation. To determine if observed values are affected by ROI selection criteria we re-analyzed the data using an ROI consisting of the overlap of CBF and BOLD activation maps (Tables 3 and 4). Selection of an ROI based on the overlap between CBF and BOLD responses requires voxels displaying both CBF and BOLD activity for both the VC and LN. Four subjects did not meet this criterion and so the combined CBF/BOLD ROI analysis includes only nine subjects. Although the mean n is shifted to a higher value, the results still indicate that calculated n values are significantly greater for VC than LN (Cohen $d=3.5$). This second ROI (CBF/BOLD overlap) showed a trend for a higher value of M in LN than VC, although the difference did not reach statistical significance.

Discussion

Quantitative interpretation of the BOLD response

The BOLD effect has proven to be a powerful tool for mapping brain activation during performance of a wide range of sensory, motor and cognitive tasks (Buxton, 2002; Logothetis and Wandell, 2004; Toma and Nakai, 2002; Ugurbil et al., 1999). However, interpreting the magnitude of the BOLD response in a quantitative way is difficult because it is a complex phenomenon, depending on changes in CBF, CMRO₂, and CBV. That is, in comparing two BOLD responses – either across different brain regions, between a disease population and a healthy population, before and after administration of a drug, or conceivably even within the same brain region for two different stimuli – a larger magnitude does *not* necessarily reflect a greater change of neural activity or metabolism.

We can characterize the BOLD response in a particular brain region as primarily a function of the CBF changes, but strongly modulated by two physiological parameters associated with that particular brain region and the experimental conditions: M and n . Quantitatively, both M and n have a strong effect on the BOLD signal associated with a given CBF response. The parameter M defines the dynamic range of the BOLD effect, and reflects the amount of deoxyhemoglobin present in the baseline state and the echo time of the experiment. The potential influence of variations in M has long been recognized, particularly within voxels having large BOLD responses due to veins that drain activated tissue, or due to differences in the baseline state (Buxton et al., 2004). In this study we did not observe a significant effect of baseline CBF on M due to the relative lack of variability in baseline CBF measures. In previous studies in which the baseline CBF was increased by administration of CO₂ or acetazolamide, the BOLD response to a standard activation paradigm was substantially reduced despite similar changes in CBF with activation (Brown et al., 2003; Cohen et al., 2002). This is consistent with a washout of deoxyhemoglobin due to the increased baseline CBF, and thus a reduction of M (Buxton et al., 2004).

However, the potential importance of n has received less attention, perhaps in part because the BOLD response is relatively insensitive to n when $n > 4$ (Fig. 1). In this regime, the small CMRO₂ changes have little effect on the local deoxyhemoglobin content (and thus the BOLD response) compared to the much larger CBF changes. If n is large, then the BOLD response can be treated as primarily a hemodynamic response reflecting the change in CBF. However, in this study, and in others that have used the calibrated-BOLD technique, significantly larger CMRO₂ changes (~20%) have been observed, suggesting that CMRO₂ changes should not be neglected when interpreting the BOLD response (Chiarelli et al., 2007a,b; Davis et al., 1998; Hoge et al., 1999; Kastrup et al., 2002; Kim et al., 1999; Leontiev and Buxton, 2007; Leontiev et al., 2007; St Lawrence et al., 2002; Stefanovic et al., 2004; Stefanovic et al., 2006; Uludag et al., 2004). These larger CMRO₂ changes and subsequently smaller n , have also been observed in some PET studies (Marrett and Gjedde, 1997; Roland et al., 1987; Seitz and Roland, 1992). Within the regime of $n < 3$ the exact value of n has a strong effect on the BOLD response: for the same change in CBF, a region with a larger change in CMRO₂ (and thus a lower value of n) will exhibit a weaker BOLD response. In fact, for n less than about 1.4 the BOLD response estimated from Eq. (1) (Methods) would be negative (Chiarelli et al., 2007b), despite a positive CBF change. This follows from the conflicting effects on the BOLD signal from changes in CBV and OEF. Because of this sensitivity to n , the BOLD response should be viewed as a combined hemodynamic/metabolic response rather than simply a hemodynamic response.

CBF/CMRO₂ coupling variations

Previous studies with the calibrated-BOLD approach have primarily focused on cortical regions, and found values of n ranging from ~ 2 to 4.5. A unique feature of the current study was the simultaneous comparison of a cortical region with a sub-cortical region to test for meaningful variations in n or M . Simultaneous measurements of both CBF and BOLD responses within a cortical region (VC) and subcortical region (LN) during hypercapnia yielded quite similar values of M ($M \sim 5.8\%$). These results are similar to previous calibrated BOLD studies (Chiarelli et al., 2007a; Kastrup et al., 2002; Stefanovic et al., 2006). However, a degree of caution should be exercised when comparing M values across different studies as it depends on the echo time used in the data acquisition, the magnetic field strength, and the physiological baseline state, as noted above (Buxton et al., 2004).

In contrast, n for the LN ($n \sim 1.6$) was significantly lower compared to the VC ($n \sim 2.2$), and lower than previous reported values for cortical structures (Chiarelli et al., 2007a; Stefanovic et al., 2006; Vafaei and Gjedde, 2004). This difference in n was sufficient to have a strong effect on the magnitude of the BOLD responses. Although the stimuli activating the two areas produced CMRO₂ changes that differed by only a factor ~ 2 between the two regions, the magnitude of the BOLD response in the VC was greater by a factor of ~ 7 compared to the LN.

In addition, the CBF response to hypercapnia was significantly greater in the VC. This combined finding of a weaker cerebrovascular response to CO₂ and a smaller n in the LN compared to VC suggests the possibility that local vasculature in the LN, whether challenged by mild hypercapnia or functional activation, is less responsive than within the VC. Whether this is a result of inherent differences in biomechanical properties of vessels (Behzadi and Liu, 2005) or varying degrees of sympathetic tone (Sheth et al., 1995) within the posterior circulation has yet to be determined and is the subject of future studies.

An earlier PET study reported larger changes in CMRO₂ compared to CBF in putamen with a motor task, a result that is qualitatively consistent with our finding of a lower value of n in LN compared with VC (Vafaei and Gjedde, 2000). It is perhaps relevant to note that n is expected to be closely related to changes in tissue oxygen tension (pO₂): for large n pO₂ likely increases with activation, while for lower n pO₂ may decrease. Our observations of a low value of n potentially may be related to the increased susceptibility of the LN to diseases of metabolic dysregulation such as hypoxic injury from carbon monoxide poisoning and toxic injury (Yoshii et al., 1998) and mitochondrial disorders such as Leigh's disease (Zhu et al., 1998). Additional studies of other susceptible brain regions, and in particular disease states that predominantly affect these areas are necessary to investigate whether physiological measures such as n or CRC can be used as clinical biomarkers of pathology.

Implications for fMRI studies

The implication for fMRI studies of a lower value of n within the LN is that similar changes in energy metabolism, and presumably similar associated changes in neural activity, produce a substantially weaker BOLD response compared to VC. Further studies are needed to test whether this is a general difference in the neurovascular coupling between cortical and sub-cortical regions, a difference between motor and sensory stimuli, or a peculiarity of the VC or LN regions. We note also that a recent preliminary report by our group also suggests a lower value of n in the hippocampus (Restom et al., 2007), consistent with a trend for lower n in subcortical structures. The potential for substantial variation of the BOLD response due to differences in n should be considered when comparing BOLD responses across brain regions or subject groups.

For brain mapping studies, where statistical analysis of data depends upon a detectable level of SNR for the BOLD signal, brain regions characterized by smaller values of n will have weaker BOLD-signals and lower SNR for a given CBF change than regions with larger values of n (assuming similar values of M). The important implication of this phenomenon is that areas with significant underlying changes in neural activity could fail to demonstrate BOLD activation. For this reason, a strict interpretation of the statistical analysis is critical: identifying a statistically significant BOLD response can be interpreted as evidence for an underlying change in neural activity, but equally large changes in neural activity could be present in areas that fail to show a detectable BOLD response.

Calibrated-BOLD methodological issues

In contrast to standard BOLD-fMRI alone, a calibrated-BOLD approach provides quantitative measures of local physiological changes. However, several potential sources of systematic error can introduce bias, including: (1) assumptions within the Davis model, including the values of the two model parameters (α and β) and the assumption that CMRO₂ does not change with mild hypercapnia (Davis et al., 1998); and (2) the method used for choosing an ROI for averaging. Recently, we reviewed the assumptions of the Davis model and demonstrated that it is likely to be more robust than the original assumptions would suggest (Leontiev et al., 2007). While the model does not explicitly include a role for intravascular signal changes, which are thought to be important at 1.5 and 3T (Boxerman et al., 1995a), it still appropriately captures the behavior of the signal. Much of the complexity of more realistic modeling is included in the parameter M , so it is important to measure M , rather than use an assumed value, as has recently been stressed by Chiarelli and colleagues (Chiarelli et al., 2007a,b). In fact, accurate determination of M is critical because estimates of M and n strongly co-vary, in the sense that the same activation data will yield a lower value of n if M is overestimated. For this reason, systematic errors in the hypercapnia calibration will lead to an inaccurate estimate of n .

An important assumption of the Davis model (Eq. (1)) is that mild hypercapnia does not alter CMRO₂. A number of studies in humans (Hafkenschiel et al., 1954; Kastrup et al., 1999; Kety and Schmidt, 1948; Kim and Ugurbil, 1997; Kliefoth et al., 1979; Novack et al., 1953) have shown no significant CMRO₂ changes for small CO₂ changes (<15 mm Hg) due to mild hypercapnia. However, other studies with larger changes in pCO₂ have found reduced CMRO₂ (Jones et al., 2005; Sicard and Duong, 2005) and a recent report looking at electrophysiological activity in anesthetized monkeys found significant reductions with modest levels of inhaled CO₂ (Zappe et al., 2005). These studies suggest the possibility that CMRO₂ may decrease on inhalation of CO₂, and further work is needed to resolve this question. We investigated the effect of non-zero CMRO₂ changes with hypercapnia on our estimates of n by recalculating M and n from the average measured responses, with different assumptions for the CMRO₂ change with hypercapnia. We defined the CMRO₂ under hypercapnia, normalized to normocapnic baseline, as a parameter k . In the calculation of M from the hypercapnia data with Eq. (1), we then assumed $r=k$. For a reasonable range ($\pm 10\%$) of possible CMRO₂ changes, specific n values were altered but our conclusion that n is substantially larger in the VC compared to LN, while the M values are similar, was not affected (Fig. 7A).

In calculations based on Eq. (1), previous studies have concluded that the derived estimate of n is relatively insensitive to the exact values of the parameters α and β (Davis et al., 1998; Uludag et al., 2004). However, the vascular composition in VC and LN may be different, and so conceivably both α and β could be region-specific. We tested the sensitivity of M and n measurements to a 50% variation in α (Figs. 7C and D — β held constant at 1.5) and a 33% variation in β (Figs. 7E and F — α held constant at 0.38). We observed that n is relatively insensitive to assumptions about α and β , as originally argued by Davis et al. (Davis et al.,

1998). A clear difference in n remains between the two brain regions despite rather large variation in α and β , while values of M for the two regions remain similar.

Both the LN and VC are in close proximity to confluences of sinuses, with possible inclusion of draining veins occurring in each ROI. Choosing voxels according to an intersection between BOLD and CBF activation, rather than CBF activation alone, is therefore more likely to be dominated by draining venous artifacts compared to only using CBF activated voxels. The inclusion of draining veins in the ROI can lead to larger estimates of M , but systematic errors in the estimate of n primarily depend on whether the added contribution to the BOLD signal differs between hypercapnia and functional activation experiments. In previous work by our group (Leontiev et al., 2007), we observed that M is biased to large values when voxels are selected according to BOLD activation and even larger values when anatomical ROIs are used. The essential problem with an anatomical ROI is that all venous structures within the ROI act as draining veins during the hypercapnia experiment because of the global increase in CBF, but during the activation experiment only a focal subset of the venous structures might contribute a draining vein signal. An activation-based ROI reduces this effect, although any criterion using BOLD activation is still likely to include draining veins.

Both of the choices of ROI criteria that we used here may yield biased results. Using CBF activation as the criterion could overestimate the average CBF response to activation, and lead to a lower value of n for the same M . This could potentially lead to an underestimate of n in a smaller, more weakly activated tissue, which is what we observed for the LN. However, for our data to be consistent with this potential effect, with equal values of n in the two regions, the CBF response in LN would need to be overestimated by a factor of three, which seems unlikely.

The alternative of including an ROI based on BOLD activation introduces another potentially important bias, one that would preclude detection of 'BOLD-silent' activations. If the value of n is sufficiently low ($n \sim 1.4$ based on Eq. (1)), it is possible that there could be a CBF change without an accompanying BOLD response. By forcing an ROI to include only voxels that show a significant BOLD change, such brain regions would be excluded from the analysis with the estimate of n biased toward higher values. This is in fact what we (Tables 3 and 4) and others (Chiarelli et al., 2007a; Stefanovic et al., 2004, 2006) have typically observed when an overlap of CBF and BOLD activated voxels is used. For all of these reasons our recommendation is to use CBF activation as the criterion for choosing an ROI, but further work is needed to assess the degree of bias this may introduce.

A large difference in the number of activated voxels was observed between the VC and LN. An activation volume dependency of CBF/CMRO₂ coupling was found in a previous study (Leontiev et al., 2007) with smaller activation volumes giving rise to larger estimates of n . Therefore, in order to minimize this type of bias, either increasing the activation threshold in VC or lowering the activation threshold in the LN would be required in order to lessen the volume discrepancy between activated regions across the two brain regions. However, this would lead to an even greater difference in n than reported in this study with lower estimates of n in the LN and greater estimates of n in the VC.

The estimate of n for VC in this study is less than we found in a previous study from our group (Leontiev and Buxton, 2007). Differences could be attributed to voxel selection paradigms: a larger ROI was used in this analysis compared to a smaller retinotopic region previously defined. In the current study, active voxels were identified as those satisfying $p < 0.05$ after correcting for multiple comparisons, which typically yields a greater number of voxels than our previous approach of thresholding on $r > 0.5$ ($p < 0.0001$) combined with nearest neighbor

clustering. In both instances a larger ROI will be generated with a subsequently smaller n value resulting (Leontiev et al., 2007).

Conclusions

In conclusion, our primary finding is that there is a significant variation of CBF/CMRO₂ coupling across brain regions, as suggested earlier by Tuunanen and colleagues (Tuunanen and Kauppinen, 2006; Tuunanen et al., 2006). Specifically, the coupling ratio is lower within the lentiform nuclei of the basal ganglia, a subcortical region, than in parts of the visual cortex, a cortical region. If this is a general feature of differences between cortical and subcortical structures, then BOLD responses for similar changes in CBF may be substantially weaker in the sub-cortical structures. Further studies are required to test whether n varies with healthy development, with progression of disease, or in response to different stimuli. Although the variability of n makes quantitative interpretation of the BOLD response data alone problematic, the combination of BOLD and ASL acquisitions in a calibrated-BOLD approach provides a powerful tool for untangling some of the ambiguities of the BOLD response. Specifically, it provides a way for fMRI to move from being simply a mapping tool to becoming a quantitative probe of brain physiology. The measurement of the BOLD and CBF responses to a hypercapnic challenge in addition to an activation task makes it possible to estimate M (reflecting conditions in the baseline state), n (CBF/CMRO₂ coupling), and CRC (a measure of vascular responsiveness). A calibrated-BOLD approach therefore provides a quantitative assessment of brain function, a kind of ‘stress test’ for the brain. This may have clinical significance for the early detection of altered function in disease or for evaluating the effectiveness of pharmaceutical agents.

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Fig. 1. Relationship between fractional blood oxygenation level dependent (BOLD) and fractional cerebral blood flow (CBF) increases in response to brain activation for different ratios (n) of the fractional changes in CBF and cerebral metabolic rate of oxygen (CMRO₂). Curves were calculated for different values of n using the BOLD signal equation (Eq. (1)) with $M=0.06$, $\alpha=0.4$, and $\beta=1.5$. Filled circles indicate the BOLD and CBF responses for a fixed 20% increase in CMRO₂, illustrating that, for the same underlying change in metabolism, the magnitude of the BOLD response is strongly dependent on n . Relatively small differences in n from 1.5 to 2 or from 2 to 3 can lead to over 100% changes in the BOLD response for the same CMRO₂ change. The curve for $n=\infty$ is the response for no change in CMRO₂.

Fig. 2. Experimental design for quantitative fMRI: mild hypercapnia calibration experiment and a functional activation experiment with simultaneous measurements of BOLD and CBF responses. (A) The two hypercapnia runs consisted of 2 min breathing room air, 3 min breathing a 5% CO₂ mixture, and then 2 min breathing room air. Following these experiments, a high-resolution anatomical scan was performed for structural correlation. (B) The three functional activation block design runs were approximately 7 min each in duration. ROIs for a single representative subject based on CBF activation (C) or the overlap of CBF and BOLD activation (D) are shown.

Fig. 3. BOLD and CBF changes in the visual cortex (VC) compared to lentiform nuclei (LN) of the basal ganglia for both hypercapnia and functional activation. The panels show average response curves and standard deviations (13 subjects) for the LN and VC ROIs based on CBF activated voxels. (A) CBF responses to hypercapnia, (B) BOLD responses to hypercapnia, (C) CBF responses to activation, and (D) BOLD responses to activation. The overall larger response in VC compared to LN may reflect differences in effective amplitude of the separate visual and motor stimuli driving the two regions, but the relative response amplitudes reflect differences in the coupling of CBF and CMRO₂ changes between the two regions. The solid black lines indicate stimulus presentation.

Fig. 4.

Individual responses expressed as BOLD/CBF response ratios for hypercapnia and activation, illustrating the conversion of measured data into calculated model parameters. The essential information in calibrated BOLD data can be approximately captured by comparing the ratio of BOLD to CBF responses under hypercapnia and activation. Individual subject data for the two regions are plotted in panel A. The BOLD/CBF ratio measured under hypercapnia (y ordinate) reflects the local M value and is similar for both brain regions. However, the activation-induced BOLD/CBF ratio (x-axis) is sensitive to both local M and n values. A lower BOLD/CBF ratio associated with activation is observed in the LN, consistent with a smaller value of n in this region. Nonlinearities of the BOLD-CBF relation are shown by plotting lines of constant M and n for an assumed 50% change in CBF from the hypercapnia and activation experiments (B). The mean and standard deviations for VC and LN are also shown. The $n = \infty$ line reflects the assumption that there is no change in CMRO₂ with hypercapnia. Note that the M (horizontal lines) and n (angled vertical lines) contours are only approximate, because the full calculation of M and n requires all four measured quantities, and not just the two ratios. As seen in panel B significant differences in n but not M were seen for the two regions.

Fig. 5. Covariance of calculated and measured parameters. (A) Comparison of the calculated values of M and n for each subject, showing no significant correlation. (B) Comparison of n with baseline CBF, showing no significant correlation. (C) Comparison of M with baseline CBF, showing no significant correlation. (D) Changes in CBF as a function of $CMRO_2$.

Fig. 6. Correlation of different coupling ratios. (A) Data for individual subjects showing the CBF/CMRO₂ coupling ratio in the VC and LN (dashed line indicates equal values). The data show that n is higher in VC, but there is no correlation between the two regions. (B) For each region the response to activation, characterized by a CBF/CMRO₂ coupling ratio n , is compared to an analogous coupling ratio for hypercapnia, n_{CO_2} , defined as the ratio of the fractional change in CBF to the fractional change in end-tidal CO₂ with hypercapnia (mean values with standard error bars). The two values n and n_{CO_2} can be thought of as characterizing the CBF responses to two quite different physiological stimuli: a change in neural activity and a change in arterial pCO₂. For both measures, higher values were observed within the VC compared to the LN, suggesting a greater vascular responsiveness in the VC.

Fig. 7. Sensitivity of the estimates of M and n to the assumed values of the model parameters. Using the average data in Table 1, values of M (left column) and n (right column) were calculated for different values of the model parameters. To test the effect of the assumption that CMRO_2 does not change with mild hypercapnia, a parameter k , defined as the CMRO_2 under hypercapnia normalized to normocapnic baseline, was varied and used in the calculation of M and n (A and B). The effect of varying α and β are shown in panels C–E, respectively. Although the absolute values vary, the basic finding of similar M values and different n values in the two brain regions remains. Standard parameter values are $k=1.0$, $\alpha=0.38$, and $\beta=1.5$.

Measured values within lentiform nuclei (LN) and visual cortex (VC) for a region of interest based on CBF activated voxels (mean± standard error for 13 subjects)

Table 1

	% CBF hypercapnia	% BOLD hypercapnia	Cerebral reactivity to CO ₂ (CRC)	% CBF activation	% BOLD activation	Baseline CBF (ml/100 ml/min)
Visual cortex	71.3±3.8	2.08±0.05	9.46±0.41	77.9±2.1	0.89±0.03	52.3±2.6
Lentiform nuclei	**42.4±1.9	*1.52±0.06	**5.64±0.47	**31.6±1.7	**0.13±0.02	**74.4±3.7

* Significant difference between VC and LN values with $p<0.05$.

** Significant difference between VC and LN values with $p<0.01$.

Table 2

Calculated values within lentiform nuclei (LN) and visual cortex (VC) for a region of interest based on CBF activated voxels (mean± standard error for 13 subjects)

	% CMRO ₂	<i>n</i>	<i>M</i> (%)
Visual cortex	36.7±1.3	2.21±0.3	5.7±0.2
Lentiform nuclei	**20.1±1.0	**1.58±0.3	5.8±0.2

** Significant difference between VC and LN values with $p < 0.01$.

Table 3

Measured values within lentiform nuclei (LN) and visual cortex (VC) for a region of interest based on the overlap of CBF and BOLD activated voxels (mean $\pm$ standard error for 9 subjects)

	% CBF hypercapnia	% BOLD hypercapnia	Cerebral reactivity to CO ₂	% CBF activation	% BOLD activation	Baseline CBF (ml/100 ml/min)
Visual cortex	74.1 $\pm$ 19.4	2.13 $\pm$ 0.24	10.02 $\pm$ 2.02	76.9 $\pm$ 10.9	1.15 $\pm$ 0.09	58.3 $\pm$ 13.3
Lentiform nuclei	**35.7 $\pm$ 7.8	1.96 $\pm$ 0.28	**4.74 $\pm$ 0.68	**28.5 $\pm$ 6.2	**0.45 $\pm$ 0.07	**89.3 $\pm$ 20.7

** Significant difference between VC and LN values with $p < 0.01$.

Table 4

Calculated values within lentiform nuclei (LN) and visual cortex (VC) for a region of interest based on the overlap of CBF and BOLD activated voxels (mean± standard error for 9 subjects)

	% CMRO ₂	<i>n</i>	<i>M</i> (%)
Visual cortex	30.1±5.6	2.7± 0.2	5.7±0.5
Lentiform nuclei	**15.2±3.6	**2.0± 0.2	8.3±1.3

** Significant difference between VC and LN values with $p<0.01$.