Investigation of BOLD Signal Dependence on Cerebral Blood Flow and Oxygen Consumption: The Deoxyhemoglobin Dilution Model

Richard D. Hoge, Jeff Atkinson, Brad Gill, Gérard R. Crelier, Sean Marrett, and G. Bruce Pike

The relationship between blood oxygenation level-dependent (BOLD) MRI signals, cerebral blood flow (CBF), and oxygen consumption (CMRO2) in the physiological steady state was investigated. A quantitative model, based on flow-dependent dilution of metabolically generated deoxyhemoglobin, was validated by measuring BOLD signals and relative CBF simultaneously in the primary visual cortex (V1) of human subjects (N = 12) during graded hypercapnia at different levels of visual stimulation. BOLD and CBF responses to specific conditions were averaged across subjects and plotted as points in the BOLD-CBF plane, tracing out lines of constant CMRO2. The quantitative deoxyhemoglobin dilution model could be fit to these measured iso-CMRO2 contours without significant (P ≤ 0.05) residual error and yielded MRI-based CMRO2 measurements that were in agreement with PET results for equivalent stimuli. BOLD and CBF data acquired during graded visual stimulation were then substituted into the model with constant parameters varied over plausible ranges. Relative changes in CBF and CMRO2 appeared to be coupled in an approximate ratio of ~2:1 for all realistic parameter settings. Magn Reson Med 42:849–863, 1999. © 1999 Wiley-Liss, Inc.

Key words: BOLD contrast; aerobic metabolism; perfusion; hypercapnia

A detailed understanding of the relationship between the blood oxygenation level-dependent (BOLD) MRI signal, cerebral blood flow (CBF), and oxygen consumption (CMRO2) is critical for the rigorous physiological interpretation of BOLD fMRI activation data. It is also essential for validation of MRI-based methods for measuring CMRO2 that have recently been proposed (1,2).

The relationship between blood oxygenation level-dependent (BOLD) MRI signals, cerebral blood flow (CBF), and oxygen consumption (CMRO2) is critical for the rigorous physiological interpretation of BOLD fMRI activation data. It is also essential for validation of MRI-based methods for measuring CMRO2 that have recently been proposed (1,2).

The relationship between blood oxygenation level-dependent (BOLD) MRI signals, cerebral blood flow (CBF), and oxygen consumption (CMRO2) is critical for the rigorous physiological interpretation of BOLD fMRI activation data. It is also essential for validation of MRI-based methods for measuring CMRO2 that have recently been proposed (1,2).
tive metabolism is responsible for generating virtually all dHb in tissues, and is therefore a critical determinant of the BOLD signal. Under steady-state conditions, CMRO$_2$ depends primarily on the rate of ATP turnover in tissues and the availability of oxygen and glucose.

A number of theoretical and experimental studies have examined BOLD signal dependence on the concentration of deoxyhemoglobin in venous blood ([dHb]$_v$) and the blood volume fraction of tissue (9,10). Because these parameters directly reflect the amount and distribution of dHb in tissues, it is possible to predict their influence on the $T^*_2$-weighted MRI signal using Monte Carlo simulations and in vitro model systems.

Venous blood volume and dHb concentration, in turn, depend primarily on local rates of CBF and oxygen consumption. Although BOLD signal dependence on CBV and [dHb]$_v$ has been reasonably well characterized, there is much less data on the influence of CBF and CMRO$_2$, which are more directly linked to neuronal activity. Furthermore, the limited amount of experimental data that does exist has been difficult to integrate into a coherent framework. Kim et al. measured BOLD signal and perfusion values at a single level of visual stimulation and, using the quantitative biophysical model of Ogawa et al. (9) to interpret their data, concluded that oxygen consumption did not increase during visual stimulation (11). Davis et al. measured BOLD and perfusion data at a single level of hypercapnia and at a single level of visual stimulation and, using a different formulation of Ogawa's model, concluded that oxygen consumption increased (2). Although the latter study made the important advance of using hypercapnia to calibrate the BOLD signal, the single level of activation and hypercapnia used did not permit elucidation of variable relationships between physiological and MRI parameters. Lack of validation of the quantitative relationships used to calculate CMRO$_2$ in the studies cited above makes it difficult to assess the accuracy of such measurements, which have yet to be verified against an independent standard such as positron emission tomography (PET).

Two fundamental relationships must be better characterized to establish the exact physiological significance of activation-induced BOLD signals and validate MRI-based CMRO$_2$ measurement techniques. The first is the dependency of the BOLD signal on CBF when oxygen consumption is held constant, and the second is the association between these quantities during neuronal activation. Both of these relationships may vary between tissue samples (e.g., different MR image voxels), and the latter relationship could conceivably vary within a given tissue sample for different types of stimulation (due to differences in the biochemistry or neurovascular coupling of target neurons within the sample). In this study we focus on examining steady-state signals in a single cortical area with well defined and homogeneous characteristics (peripheral V1).

Although transient features of the BOLD response such as the initial dip (12), and poststimulus undershoot (13) have attracted considerable interest recently, clarification of the steady-state relationship between the BOLD signal, CBF, and CMRO$_2$ is essential for understanding the physiological basis of the BOLD effect.

This report presents a detailed derivation of a specific formulation of the dHb dilution model of BOLD contrast, emphasizing quantitative predictions of BOLD signal dependence on CBF and CMRO$_2$. We introduce the concept that, for a given tissue sample, different combinations of BOLD and CBF responses represent specific levels of CMRO$_2$ (the CBV within a tissue sample is assumed to be a simple correlate of CBF). Plots of simultaneously measured BOLD and perfusion-sensitive fMRI responses on orthogonal axes can therefore be viewed as maps of CMRO$_2$ on the BOLD-CBF plane. We demonstrate that by introducing vasoactive substances such as CO$_2$ into the arterial blood to manipulate CBF independently of tissue metabolism during different (but respectively constant) levels of neuronal activation, it is possible to trace out lines of constant CMRO$_2$ (iso-CMRO$_2$ contours) in such maps.

In contrast, BOLD-CBF responses measured during graded visual stimulation are shown to travel between (rather than along) iso-CMRO$_2$ contours. By adjusting the contrast of a visual stimulus to match hypercapnia-induced perfusion increases, we show that activation-induced BOLD responses include significant attenuation due to increases in metabolic dHb production. A modified version of the method of Davis et al. (2) is used for explicit calculation of relative CMRO$_2$ changes, and the region of the BOLD-CBF plane corresponding to a $\Delta$%CBF: $\Delta$%CMRO$_2$ coupling ratio of $\sim$2:1 is shown to encompass the BOLD and CBF responses observed over a broad range of activation states ($\Delta$% denotes the percent change in a quantity).

An activation paradigm employed in previous PET studies of CBF and CMRO$_2$ (14), was included among these conditions, providing an independently determined reference point for our quantitative results. Finally, the V1 flow-metabolism coupling relationship was re-computed with assumed model constants varied over plausible ranges, to estimate the possible magnitude of error associated with uncertainty of these parameters. This exercise indicated that the true relationship is not likely to deviate greatly from the $\sim$2:1 linear function computed using best estimates of model parameters.

Portions of this work, including preliminary studies performed using different pulse sequences and additional visual stimuli, have been published previously (15–17). The objective of the present report is to present a detailed analysis of the BOLD signal model underlying these CMRO$_2$ measurements. We also discuss factors affecting the accuracy of our MRI-based flow measurements. In a separate paper (18) we investigate the temporal dynamics of BOLD and perfusion signals, including some of the responses recorded in the present study.

**THEORY**

In this study we present a quantitative model predicting BOLD signal changes during increased CBF and CMRO$_2$, based on expected changes in venous dHb concentration and CBV. The expressions derived constitute an alternate formulation of previous biophysical models (1,2,9), with an emphasis on testable predictions of BOLD-CBF interdependence. We introduce a novel formalism for interpreting simultaneously acquired BOLD and CBF data in terms of iso-CMRO$_2$ contours in the BOLD-CBF plane, and an extension of the calibration method described in (2) that incorporates graded hypercapnia.
We first consider the effect of the amount and distribution of dHb in tissues. The net rate constant \( R^*_2 \) for transverse relaxation can be viewed as the sum of a component \( R^*_{2\text{,dHb}} \) caused by dHb and a contribution, \( R^*_{2\text{,other}} \), due to other sources:

\[
R^*_2 = R^*_{2\text{,dHb}} + R^*_{2\text{,other}}.
\]

Boxerman et al. determined that \( R^*_{2\text{,dHb}} \) was related to CBV and \([dHb]_v\), according to the expression

\[
R^*_{2\text{,dHb}} = A \cdot CBV \cdot [dHb]_v^\beta, \tag{2}
\]

where \( A \) is a field strength and sample-specific proportionality constant and \( \beta \) is a constant, in the range \( 1 \leq \beta \leq 2 \), depending on the average blood volume within a tissue sample. The accuracy of this semiempirical expression was thoroughly validated using in vitro models (10).

The magnitude of \( R^*_{2\text{,dHb}} \) is reduced by increases in CBF, due to dilution of venous dHb, although resultant CBV increases partially counteract this effect. The change in transverse relaxation rate (\( \Delta R^*_{2\text{,dHb}} \)) at nonbaseline values of CBV and \([dHb]_v\), can be expressed as follows, based on Eq. (2):

\[
\Delta R^*_{2\text{,dHb}} = A(CBV[dHb]^\beta_v - CBV_0[dHb]^\beta_v), \tag{3}
\]

where the subscript ‘0’ is used here and elsewhere in the text to refer to the baseline steady-state value of a variable.

Reductions in \( R^*_{2\text{,dHb}} \) lead to increases in the \( T^*_2 \)-weighted image intensity observed at a given echo time (TE), which can be expressed in fractional form as

\[
\Delta \text{BOLD} = e^{-TE\Delta R^*_{2\text{,dHb}}} - 1. \tag{4}
\]

For the small changes in \( R^*_{2\text{,dHb}} \) that occur during fMRI experiments, the exponential function in Eq. (4) can be linearized, resulting in the following simplified expression:

\[
\frac{\Delta \text{BOLD}}{\text{BOLD}_0} = -TE\Delta R^*_{2\text{,dHb}}. \tag{5}
\]

Substitution of Eq. (3) for \( \Delta R^*_{2\text{,dHb}} \) leads to

\[
\frac{\Delta \text{BOLD}}{\text{BOLD}_0} = TE \cdot A(CBV_0[dHb]^\beta_v - CBV[dHb]^\beta_v)
\]

\[
= TE \cdot A \cdot CBV_0 \cdot [dHb]^\beta_v \left(1 - \frac{CBV}{CBV_0} \cdot \frac{[dHb]^\beta_v}{[dHb]^\beta_v} \right). \tag{6}
\]

The term \( TE \cdot A \cdot CBV_0 \cdot [dHb]^\beta_v = TE \cdot R^*_{2\text{,dHb}} \) is approximately equal to the fractional BOLD signal attenuation that occurs due to dHb at baseline, and therefore represents the maximum possible BOLD response that would be observed upon elimination of all dHb from a tissue sample (as pointed out by Davis in Ref. 2). For clarity, we use the constant \( M \) to denote this quantity:

\[
M = TE \cdot A \cdot CBV_0 \cdot [dHb]^\beta_v. \tag{7}
\]

We assume that there is a consistent functional relationship between blood flow and volume, in which CBV depends passively on CBF. Several investigators have determined this to be

\[
\frac{CBV}{CBV_0} = \left(\frac{CBF}{CBF_0}\right)^\alpha, \tag{8}
\]

where \( \alpha \) is a constant with an approximate value of 0.38 (4). The baseline-normalized CBV term of Eq. (6) can thus be determined from MRI-based measurements of relative CBF (defined here as the volume influx of blood per unit time through a tissue volume element, normalized to baseline).

Assuming that the concentration of dHb in arterial blood is negligible, the steady-state dHb concentration within the venous compartment of a constant unit volume element of tissue depends on CMRO2 and CBF, from basic mass conservation (Fick’s principle):

\[
[dHb]_v = \frac{1}{4} \frac{\text{CMRO}_2}{\text{CBF}}. \tag{9}
\]

where simplified units of mol/ml, mol/sec, and ml/sec are used respectively for \([dHb]_v\), CMRO2, and CBF (the factor 1/4 reflects the fact that each dHb molecule delivers 4O2). If CMRO2 is held constant, the baseline-normalized \([dHb]_v\) term in Eq. (6) is therefore inversely proportional to normalized CBF, reflecting simple dilution:

\[
\frac{[dHb]_v}{[dHb]_v^0} = \frac{\text{CBF}_0}{\text{CBF}}. \tag{10}
\]

Substitution of Eqs. (8) and (10) into Eq. (6) gives the following expression predicting the fractional BOLD signal change for a given change in relative CBF at constant CMRO2:

\[
\frac{\Delta \text{BOLD}}{\text{BOLD}_0} = M \left(1 - \frac{\text{CBF}}{\text{CBF}_0} \right)^{\alpha - \beta}. \tag{11}
\]

Because \( \beta \) is larger than \( \alpha \) by at least a factor of two (4,10), the exponent \( \alpha - \beta \) is negative and the term \((\text{CBF}/\text{CBF}_0)^{\alpha - \beta}\) decreases with increasing CBF, leading to monotonic BOLD increases with perfusion. Figure 1a shows Eq. (11) plotted over a large range of CBF values with \( \alpha = 0.38 \) and \( \beta = 1.5 \). The predicted BOLD-CBF relationship for constant CMRO2 contains a linear region for moderate CBF increases (up to ~50%), but over a larger range the function becomes increasingly nonlinear and eventually plateaus to an asymptotic value of \( M \). This picture is intuitively correct, since we would expect monotonic increases in the BOLD signal that must be close to linear for small changes but plateau to some maximal level when capillary transit occurs so rapidly that venous blood is almost completely oxygenated.
The curve in Fig. 1a can be viewed as the baseline iso-CMRO\textsubscript{2} contour in the BOLD-CBF plane. The shape of this function, and hence the extent of the linear domain, depends entirely on the difference $a_2 b$; only the vertical scale factor $M$ is unknown. Inspection of Eq. [7] reveals that $M$ depends on the pulse sequence echo time, the static magnetic field strength, and MRI-relevant structural properties of the tissue sample (via the constant $A$) as well as its baseline blood volume and dHb concentration.

In the more general case, where CMR\textsubscript{O2} is not constant, Eq. [10] must be replaced with the following expression for normalized $[\text{dHb}]_v$, based on Eq. [9]:

$$[\text{dHb}]_v = \frac{\text{CMR}_0}{\text{CMR}_{0,0}} \frac{\text{CBF}}{\text{CBF}_0}$$

This results in a modified form of Eq. [11]:

$$\frac{\Delta \text{BOLD}}{\text{BOLD}_0} = M \left(1 - \frac{\text{CMR}_0}{\text{CMR}_{0,0}} \frac{\text{CBF}}{\text{CBF}_0}^\beta \right)^{a - \beta}$$

The above expression can be used to generate a set of iso-CMRO\textsubscript{2} contours by plotting the BOLD signal as a function of CBF at different levels of oxygen consumption. This is illustrated in Fig. 1b, which shows iso-CMRO\textsubscript{2} curves plotted over a region of the BOLD-CBF plane using Eq. [13].

Figure 1 shows the expected shape of the BOLD-CBF-CMR\textsubscript{O2} relationship given the values of $\alpha$ and $\beta$ believed to apply in typical fMRI experiments. Because the horizontal scaling of the plot is based on experimentally determined parameter estimates, the BOLD and CBF increases observed in most fMRI experiments should be restricted to the linear regime of the relationship, in which the iso-CMRO\textsubscript{2} contours are approximately straight and parallel. One of the goals of this study was to confirm these predictions experimentally by mapping out iso-CMRO\textsubscript{2} contours using graded hypercapnia and visual stimulation.

If the positions of one or more iso-CMRO\textsubscript{2} contours are known, then pairs of BOLD and CBF measurements can be translated into relative CMRO\textsubscript{2} increases by examining their position with respect to the contours. It is also possible to compute relative CMRO\textsubscript{2} increases from BOLD-CBF measurement pairs by solving Eq. [13] for the oxygen consumption term, as in the method of Davis et al. (2):

$$\text{CMR}_0 = \left(1 - \frac{\Delta \text{BOLD}}{\text{BOLD}_0} \frac{\text{CBF}}{\text{CBF}_0} M \right)^{1/\beta} \frac{\text{CBF}_0^{1-\alpha/\beta}}{\text{CMR}_{0,0}}$$

Davis’ approach is equivalent to solving Eq. [11] for $M$ using BOLD and CBF measurements at a single level of hypercapnia and using Eq. [14] to calculate CMRO\textsubscript{2} during activation. In the present study, $M$ was estimated using an optimization procedure to fit Eq. [11] to BOLD and CBF data acquired during graded hypercapnia. The symbol CMR\textsubscript{O2} = CMR\textsubscript{O2}/CMR\textsubscript{O2,0} will be used as a concise notation for relative (baseline-normalized) CMR\textsubscript{O2} in the remainder of the text.

Measurement of relative CMR\textsubscript{O2} from MRI-based BOLD and CBF measurements can thus be summarized as a two-part process. First, the positions of iso-CMRO\textsubscript{2} contours in the BOLD-CBF plane must be determined. Then the BOLD signal and perfusion increases during activation must be measured. It is sufficient to map out the baseline iso-CMRO\textsubscript{2} contour, because once $M$ is known the other contours can be computed using Eq. [13]. Ideally, the baseline contour would be measured over a wide range of CBF values spanning both the linear region and the asymptotic portion at high perfusion levels. Unfortunately, this is not possible using hypercapnia in human subjects, because
comfortably tolerable levels of CO₂ produce relatively small (≤20%) CBF increases.

Although direct measurements of $M$ are not feasible in humans, this quantity can be estimated by fitting Eq. [11] to graded hypercapnia data in the linear regime of the model. Furthermore, it can be shown that for small excursions in the BOLD-CBF plane, the spacing between iso-CMR$_{O_2}$ contours depends primarily on the slope of the baseline iso-CMR$_{O_2}$ contour near $\Delta$CBF = 0. Because CMR$_{O_2}$ increases as an approximately linear function of position over small patches of the BOLD-CBF plane, the spacing $D$ between iso-CMR$_{O_2}$ contours for a given step size $\Delta$CMR$_{O_2}$ is equal to $\Delta$CMR$_{O_2}$/∇CMR$_{O_2}$, where $\nabla$CMR$_{O_2}$ is used to denote the magnitude of the gradient vector of CMR$_{O_2}$ in the BOLD-CBF plane. It can be shown that the gradient magnitude of Eq. [14] for small BOLD and CBF changes is given by

$$\lim_{\Delta \text{BOLD} \to 0} \frac{\nabla \text{CMR}_{O_2}}{D} = \left(1 - \frac{\alpha}{\beta}\right)^{1 + \frac{m^2}{1 + m^2}}$$

$$= \left[1 - \frac{\alpha}{\beta}\right]^{1 + \frac{m^2}{1 + m^2}}$$ for $m \ll 1$, [15]

where $m$ is the slope, in the linear region, of the BOLD versus CBF relationship at constant baseline CMR$_{O_2}$ (e.g., during a hypercapnia experiment). The spacing between iso-CMR$_{O_2}$ contours for step size $\Delta$CMR$_{O_2}$ is therefore given by

$$D = \frac{m}{(1 - \alpha/\beta) \Delta \text{CMR}_{O_2}}.$$ [16]

Because $\beta$ is greater than $\alpha$ by a factor of at least two, the denominator $(1 - \alpha/\beta)$ in the above expression is only weakly sensitive to changes in $\beta$ because the relationship lies on the asymptotic tail of the reciprocal function $\alpha/\beta$. This means that if BOLD and CBF measurements during graded hypercapnia confirm a linear relationship between the two quantities for BOLD increases of up to a few percent, moderate uncertainty in the model parameters $\alpha$ and $\beta$ is not likely to have a large effect on the accuracy of CMR$_{O_2}$ calculations. However, when data are only available in the linear regime, the consequence of inaccurate values for $\alpha$ and $\beta$ would be a large error in estimates of the asymptomatic fractional BOLD signal increase $M$.

METHODS

The main experimental objectives of this study were: 1) to verify that BOLD and CBF responses during graded hypercapnia (GHC) at different levels of visual stimulation (VS) followed iso-CMR$_{O_2}$ contours consistent with Eq. [13]; 2) to determine whether a fixed relationship between CBF and CMR$_{O_2}$ applies during brain activation; and 3) investigate the sensitivity of the measured relationship to various assumptions implicit in the model. In this section we describe the experiments used to meet these objectives and give details regarding region of interest definition, hypercapnic modulation of CBF, visual stimulation, and MRI data acquisition.

Experiments

Each experiment consisted of a controlled comparison of the BOLD-CBF trajectories produced by two graded stimulus types, at four potency levels for each. A standardized experimental protocol was used in which BOLD and CBF responses were simultaneously measured and the different stimulation conditions interleaved in random order. Data were averaged over many subjects with different randomization orders, and baseline signal levels were sampled before and after each stimulation interval. This approach ensured that any sources of systematic bias were distributed uniformly across all baseline and activation conditions, permitting accurate isolation of signal modulation due to deliberate changes in experimental conditions. All relative signal changes in all experiments were referenced to the same baseline condition. To ensure that only physiological steady-state responses were included in percent change calculations, data acquired less than 1 min after changes in stimulation state were excluded. The experimental protocol used for all experiments is outlined in Fig. 2, and the different experiments performed are summarized in Table 1. The main experiments are described in detail below, numbered as in Table 1. Stimulus types in each experiment are labelled A and B, corresponding to the notation used in Table 1 and Fig. 2a. Subjects (healthy volunteers) gave informed consent and the experimental protocol was approved by the Research Ethics Committee at the Montreal Neurological Institute.

Experiment #1: Iso-CMR$_{O_2}$ Contour Mapping

In this experiment, the two stimulus types were: A) graded hypercapnia at visual baseline (GHC) and B) graded hypercapnia at a constant level of visual stimulation (GHC + VS). To compute iso-CMR$_{O_2}$ contours, we fit Eq. [13] to the BOLD-CBF data acquired during GHC and GHC + VS by adjusting $M$ and the average percent change in CMR$_{O_2}$ evoked by the visual stimulus. The quality of fit between the computed iso-CMR$_{O_2}$ contours and the experimentally determined BOLD-CBF points was assessed based on a generalized $\chi^2$ function (19) of the residual error.

The visual stimulus used in the GHC + VS condition was a radial checkerboard pattern containing both color (yellow/blue) and luminance contrast, with 30 spokes and 6.5 rings (counting from 0.5–10° eccentricity; defined as the angle from the center of the visual field) of equal radial thickness, modulated in a temporal squarewave at 4 Hz (all frequencies in this paper are specified as squarewave modulation frequencies; 4 Hz is equivalent to 8 contrast reversals per second). A relatively low color saturation of $\sim$25% ($\sim$8% luminance contrast) was used, to ensure closely spaced iso-CMR$_{O_2}$ contours. A uniform gray field at the mean luminance of the checkerboard was presented throughout GHC scanning runs and used as the baseline in the GHC + VS runs. Twelve subjects participated in this experiment (subsequent experiments used a different group of volunteers). In this study only, subjects were instructed to fast for 2 hr prior to the experiment.
Experiment #2: Comparison of Graded Visual and Hypercapnic Stimulation at Matched Perfusion Levels

Here the stimulus types were: A) graded hypercapnia at visual baseline (GHC); and B) graded visual stimulation (GVS) at normocapnia. Contrast levels of the visual stimulus were adjusted (based on pilot studies) to match, approximately, the GHC-induced perfusion increases. The visual stimulus used in this experiment was a 4 cycle per degree (cpd) black and white squarewave grating drifting across the visual field at 1°/sec at systematically varied orientations. The baseline display was again a uniform gray field at the mean luminance of the stimulus. To visualize any potential graded CMRO₂ increase during increasingly intense visual stimulation, the BOLD-CBF trajectory produced by GVS was compared to iso-CMRO₂ contours derived from the GHC data as described above. This experiment was performed on 12 subjects (this group or a subset of it was used in all subsequent experiments).

Experiment #3: Stimulus Specificity Experiment

In this experiment, the stimulus types were: A) GVS with the 4 cpd squarewave grating used in experiment #2, and B) GVS using a red uniform field changed to isoluminant gray and back at 3 Hz. Stimulus potency was graded by varying color saturation of the red phase without changing the luminance. Psychophysical isoluminance between the red and gray phases was achieved by having subjects adjust the luminance of the gray phase to minimize apparent flashing of a 5–10° red annulus in a flicker photometry procedure (20) conducted in the scanner. The BOLD-CBF trajectories produced by the two stimulus types were then compared for evidence of stimulus-specific CBF/CMRO₂ coupling. Twelve subjects were included in this experiment.

Experiments #4–6: Graded Visual Stimulation Over Extended Perfusion Range

In the preceding experiments, the visual stimuli used were adjusted to produce CBF responses that overlapped with the levels of perfusion observed during hypercapnia. To investigate CBF/CMRO₂ coupling at higher CBF levels, we conducted experiments with the radial checkerboard stimulus at higher contrasts and different reversal rates (contrast levels and frequencies used are summarized in Table 1). The weaker 4 cpd grating stimulus was included as a reference condition in Experiments 4 and 5, to determine whether iso-CMRO₂ contours derived from GHC in Experiment 2 could be applied to the other experiments. Relative CMRO₂ changes during GVS were computed using Eq. [14] with the $M$ value derived from the GHC experiment.

Model Sensitivity Analysis

To determine the extent to which inaccuracies in our assumed model parameters might skew our conclusions, we reanalyzed our data with the constants $M$, $\alpha$, and $\beta$ varied over plausible ranges.

Region of Interest Definition

To satisfy the assumption of a homogeneous and uniformly responding tissue sample made in the model derivations given above, all measurements in this study included only tissue in primary visual cortex from 5–10° eccentricity. At the spatial resolution of our MRI measurements, V1 is likely to be relatively homogeneous in terms of its neuronal, metabolic, and MRI-relevant structural properties. The only significant difference between distinct volume elements in V1 is the proportion of visual field represented. Blood volume fraction may vary somewhat between voxels, but the average CBV within V1 is likely to resemble whole-brain values for perfused cortex. There is some functional heterogeneity due to cortical magnification of central visual field, but restriction of our measurements to peripheral eccentricities minimized the significance of this effect.
Maps of visual field eccentricity and polar angle representation within V1 were generated for each subject in separate preliminary scanning sessions, using methods adapted from Ref. 21. The BOLD acquisition used for retinotopic mapping was identical to the one used in interleaved BOLD and CBF measurements (described below), except that a 16-slice acquisition with isotropic 4 mm resolution was used. The V1 visual field maps were resampled onto the voxel grids used in subsequent experiments according to the computed transformation required to align high resolution anatomic images acquired at the beginning of each session (22). Primary visual cortex was defined as being the retinotopically organized region within the left or right calcarine sulcus containing a mirror-image representation of the contralateral visual hemifield.

The 5–10° eccentricity range was chosen because it lay within the region stimulated by the test patterns used in our experiments, while avoiding sagittal sinus interference and confluence of multiple visual areas that can occur near the fovea. Because all stimuli used in the present study encompassed this portion of the visual field, every voxel within the retinotopically defined region of interest (ROI) was guaranteed to contain activated neurons during stimulation. Hypercapnia can also be assumed to increase perfusion uniformly in all ROI voxels.

The average ROI volume was 1.7 cc.

**Hypercapnic Modulation of CBF**

Hypercapnic modulation of CBF was used in this study due to the relative rapidity with which perfusion can be manipulated, compared with infused agents such as L-arginine and acetazolamide. We induced graded hypercapnia by administering different concentrations of a CO2/air mixture through a nonrebreathing face mask (Hudson RCI Model 1069, Temecula, CA) worn by subjects. The baseline condition was always inhalation of atmospheric composition medical air ([CO2], 300 ppm) delivered at 16 L/min while attending to a standard baseline visual display (uniform gray field with attention/fixation task). Mild hypercapnic episodes were initiated during scanning runs by switching the breathing gas to a mixture of 5:21:74% CO2:O2:N2 (BOC Canada Ltd., Montreal, Quebec, Canada) and medical air. Different levels of hypercapnia (inhaled CO2 concentrations of 1.25%, 2.5%, 3.75%, and 5%) were achieved by combining the premixed CO2/air preparation with medical air in a Y-connector and adjusting respective flow rates to achieve the desired proportions while maintaining a total flow rate of 16 L/min.

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Condition A</th>
<th>Condition B</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stimulus</td>
<td>Parameter varied</td>
<td>Stimulus</td>
</tr>
<tr>
<td>1</td>
<td>graded hypercapnia (GHC)</td>
<td>% CO2: 1.25 2.50 3.75 5.00</td>
<td>graded hypercapnia + 4-Hz/25% radial checkerboard (GHC + VS)</td>
</tr>
<tr>
<td>2</td>
<td>graded hypercapnia (GHC)</td>
<td>% CO2: 1.25 2.50 3.75 5.00</td>
<td>graded 4 cpd squarewave grating (GVS)</td>
</tr>
<tr>
<td>3</td>
<td>graded uniform red field (GVS)</td>
<td>% saturation: 25 50 75 100</td>
<td>graded 4 cpd squarewave grating (GVS)</td>
</tr>
<tr>
<td>4</td>
<td>graded 4 Hz radial checkerboard (GVS)</td>
<td>% saturation:* 6.25 12.50 18.75 25.00</td>
<td>graded 4 cpd squarewave grating (GVS)</td>
</tr>
<tr>
<td>5</td>
<td>graded 4 Hz radial checkerboard (GVS)</td>
<td>% saturation:* 25 50 75 100</td>
<td>graded 4 cpd squarewave grating (GVS)</td>
</tr>
<tr>
<td>6</td>
<td>graded 8 Hz radial checkerboard (GVS)</td>
<td>% saturation:* 12.50 25.00 37.50 50.00</td>
<td>variable frequency 50% radial checkerboard (GVS)</td>
</tr>
</tbody>
</table>

* luminance modulation amplitude = color saturation ÷ 3 for yellow and blue radial checkerboard.
End-tidal CO₂, measured via a nasal cannula with monitoring aspirator (Normocap 200, Datex Inc., Plymouth, Maine), increased by 5 ± 1 mmHg on average (a 12% increase) during inhalation of the highest concentration CO₂ mixture. Subjects were instructed to breathe at a constant rate, which was easily maintained to within ± one breath per minute (tidal volume was not controlled, however). Pulse rate and arterial oxygen saturation were also monitored (Oxygen/Pulse Monitor, Nonin Medical Inc., Oakville, Ontario, Canada), and both remained constant throughout hypercapnia experiments. Sensations of respiratory stimulation were minimal in all subjects, even at the highest concentration of CO₂ (5%), and no undue discomfort was reported.

Visual Stimulation

Visual stimuli were generated in real time using a Silicon Graphics O₂ computer with locally developed OpenGL-based software. The RGB output was used to drive an LCD projector (NEC MT820) operating in 640 × 480 mode at 60 Hz. Subjects viewed stimuli projected onto a screen mounted above their heads via a mirror while lying prone in the scanner. Stimulus presentation was automatically synchronized to data acquisition, and alertness and fixation were continually verified and logged in all subjects by requiring them to report, at 3-sec intervals throughout all experiments, the orientation of a small, low-contrast triangular fixation marker presented at the center of the display in a left-right orientation (< or >) that was varied at random intervals. Feedback was given via an MRI-compatible two-button mouse.

All stimuli filled the entire rectangular area of the 640 × 480 pixel display (radical checkerboard stimuli were not restricted to a circular region), subtending 27° of visual field. The marker used in the attention/fixation task performed by subjects during all baseline and activation periods occupied the central ±0.5° of the display, but this region did not encroach on the 5–10° eccentricity range of V1 used for quantitative analysis.

The same baseline condition, consisting of a uniform gray field at the mean luminance of the stimulus patterns, was presented at the beginning and end of all scanning runs in this study, as indicated in Fig. 2b. Potency of the various stimuli was varied by changing their luminance contrast and, where applicable, chromatic saturation (by dilution with variable amounts of white). Luminance contrast was defined as the temporal luminance modulation amplitude expressed as a percent of the mean luminance, while chromatic saturation was defined to be the percent contribution of a pure color (red, blue, or yellow) to the total luminance of a pixel. All stimuli converged in appearance to the uniform gray field as contrast/saturation approached zero.

MRI Data Acquisition

The BOLD signal and relative CBF were simultaneously recorded using an interleaved MRI pulse sequence consisting of a standard FAIR acquisition (23) with a T₂*-weighted (BOLD) EPI acquisition added after each of the two inversion-recovery (IR) acquisitions used in the basic FAIR technique (see Fig. 2c). The inversion time (TI) used in the FAIR acquisitions was 900 msec, with an TE of 20 msec. A longer TE of 50 msec was used in the T₂*-weighted BOLD acquisitions. Single-slice images were acquired on a 64 × 64 matrix with 5 × 5 mm² in-plane voxel dimensions and 7-mm slice thickness, along an oblique axial plane parallel to the calcarine sulcus. Thickness of the selective inversion slab in the FAIR acquisition was 15 mm, chosen to null difference signals in tissue-equivalent static phantoms (24). Nominal thickness of the nonselective slab was approximately 60 cm, the length of the system body coil used for inversion. Both FAIR and BOLD images used identical EPI readouts, resulting in exact spatial correspon-
idence between voxels in the two modalities. Excitation pulses were separated by a 3-sec repetition time (TR), and audible gradient activity associated with the inversion prepulses of the FAIR acquisition was duplicated before the BOLD acquisitions, making the different phases of the sequence indistinguishable to the subjects. BOLD contamination of FAIR data and inflow effects in BOLD images, assessed by examination of nonselective IR images and comparison of BOLD images following selective versus nonselective IR acquisitions, were found to be negligible (18).

Subjects were immobilized using a head-holder assembly incorporating a bite bar (not used in sessions including GHC), rigidly mounted ear cups that could be tightly clamped against the head, and a small saddle-shaped fixture pressed firmly into the subject’s nose bridge. Subject motion was negligible using this apparatus. A receive-only circularly polarized surface coil was built into the head immobilization assembly, providing high signal-to-noise ratio MRI signals from the occipital lobe. Excitation and inversion were performed using the system body coil. All experiments were performed on a 1.5 T Siemens Magnetom Vision MRI system.

RESULTS

Experimental results are described here, numbered according to the order used in the Methods section and Table 1.

Experiment #1: Iso-CMR O2 Contour Mapping

Average perfusion and BOLD signal changes in V1 during GHC and GHC1 VS are shown in Fig. 4a and b. Addition of CO2 to the subjects breathing air produced CBF and BOLD signal increases that were linearly proportional to concentration, and additive with visually evoked responses.

Figure 5a shows average responses during GHC and GHC + VS plotted in the BOLD-CBF plane. The values of M and D%CMRO2 yielding the best fit between Eq. (11) and the measured data were 0.15 ± 0.06 and 11.7 ± 0.5%, respectively. A generalized x² value of 0.17 was computed, indicating that the level of residual error was not statisti-
cally significant at the $P = 0.05$ level for six degrees of freedom.

Experiment #2: Comparison of Graded Visual and Hypercapnic Stimulation at Matched Perfusion Levels

Figure 4c shows perfusion as a function of time during GHC and GVS at contrast levels adjusted to match the GHC-induced perfusion increases. The corresponding BOLD signals (Fig. 4d) reveal significant attenuation of the visually evoked responses compared with those produced by hypercapnia. The degree of attenuation increased with perfusion, indicating graded CMRO$_2$ increases. Figure 5b shows the above data plotted on the BOLD-CBF plane. The visual stimulation data diverge from the baseline iso-CMR$_O_2$ contour, and climb the CMR$_O_2$ gradient. The $M$ value providing the best fit of Eq. [11] to the GHC data was $0.22 \pm 0.03$. Because the quality of this model fit (as indicated by the confidence interval for $M$) was considerably better and also because the same group of subjects was used in subsequent experiments, this $M$ value was employed for CMR$_O_2$ computation in the remainder of the study.

Experiment #3: Stimulus Specificity Experiment

Figure 6 shows BOLD-CBF trajectories measured during GVS with the red uniform field and 4 cpd grating. These were precisely colinear, indicating that steady-state CBF: CMR$_O_2$ coupling averaged over V1 does not vary for these stimuli.

Experiments #4–6: Graded Visual Stimulation Over Extended Perfusion Range

Slopes of the BOLD-CBF relationship were found to be similar for different visual stimuli and reproducible across sessions. All visual stimulation data fell in a single linear cluster in the BOLD-CBF plane, as shown in Fig. 7a, and responses observed by averaging multiple sessions in a single subject (Experiment 4 in Table 1) were colinear with group average data.

Regions of the BOLD-CBF and CMR$_O_2$-CBF planes corresponding to relative CMR$_O_2$/CBF response ratios in the range $0.5 \pm 0.1$, shown as darker gray areas in each plot, include virtually all visual stimulation responses observed in the present study. Slopes of the relative $\Delta$%CMR$_O_2$ versus $\Delta$%CBF relationships are summarized in Fig. 7.
Model Sensitivity Analysis

To investigate the effect of possible variations in $M$, the $\Delta \% \text{CMRO}_2$ versus $\Delta \% \text{CBF}$ plot shown in Fig. 7b was recalculated using $M$ values 2 standard errors above and below the best-fit value of 0.22 from Experiment 2 (0.29 and 0.15). The results of this exercise, shown in Fig. 8a, indicate that the overall linearity and slope observed are not changed drastically by this substitution of calibration parameters (the recalculated slopes were $0.56 \pm 0.01$ and $0.43 \pm 0.03$, respectively). Biophysical model uncertainties can alternately be viewed as possible variation in the value of $\beta$. Figure 8b shows the effects of variations in this parameter on the calculated $\Delta \% \text{CMRO}_2$ versus $\Delta \% \text{CBF}$ relationship, which is shown for $\beta$ values of 1.0, 1.5, and 2.0 [considered to be a physically realistic range (10)]. The relationship obtained is linear and stimulus-independent for all three $\beta$ values. Varying $\beta$ (or $\alpha$) changes the $M$ value producing the best fit with the graded hypercapnia data in a manner that counteracts the influence of the new parameter on the equation used to compute $\text{CMRO}_2$. The change in the slope of the $\Delta \% \text{CMRO}_2$ versus $\Delta \% \text{CBF}$ relationship is therefore modest (0.42 to 0.57). Note that because $\beta$ and $M$ are not independent, the changes associated with $M$ and $\beta$ would not be cumulative. Varying $\alpha$ from 0.3 to 0.5 resulted in slightly smaller changes (slopes ranged from 0.45 to 0.55; plots not shown).

DISCUSSION

Measured iso-$\text{CMRO}_2$ contours were approximately linear and parallel, as predicted by the quantitative dHb dilution model, over the range of CBF changes expected during brain activation. Under such conditions, BOLD-CBF coordinates translate to $\text{CMRO}_2$ levels as a simple linear function of position in the BOLD-CBF plane, whose gradient can be determined from the slope of the baseline iso-$\text{CMRO}_2$ contour. Robust measurements of relative $\text{CMRO}_2$ can thus be obtained from pairs of BOLD and CBF measurements, without excessive sensitivity to uncertainty in the model parameters $\alpha$ and $\beta$. Figure 9a shows a map of relative $\text{CMRO}_2$ over an extensive range of the BOLD-CBF plane, fit to data acquired during graded hypercapnia. It is clear from this illustration that brain activation studies occur in a domain in which the BOLD signal, CBF, and $\text{CMRO}_2$ are related in an approximately linear fashion (for clarity, the remainder of the discussion is arranged by heading).

Alternate BOLD Signal Models

Fits of the BOLD signal formula used by Kim et al. (11) to the data from Experiment 1 led to a statistically significant level of residual error ($\chi^2$ of 5.2 as opposed to 0.17 for the dHb dilution model described here). This is probably due to the inclusion of dHb concentration and CBV effects as

![Figure 7](image7.png)

**FIG. 7.** Coupling relationships between CBF and $\text{CMRO}_2$ during visual stimulation. Regions in which $(\Delta \text{CMR}O_2/\text{CMR}O_2)_b(\Delta \text{CBF/CMR}O_2) = 0.5 \pm 0.1$ are shown as the darker gray areas in both plots. a: CMRO$_2$ map, derived from graded hypercapnia data (black circles), on BOLD-CBF axes. BOLD and CBF measurements during graded visual activation with different stimuli form a well-defined linear cluster within the darkened region. b: Relative CMRO$_2$, calculated using the BOLD-CBF data in a and Eq. [14]. The data reveal a strict $\Delta \% \text{CBF}:\Delta \% \text{CMRO}_2$ ratio of $\approx 2:1$. 

![Figure 8](image8.png)

**FIG. 8.** Model sensitivity analysis showing effects of varying constant parameters over plausible ranges. a: Flow/metabolism coupling relationship calculated with $M$ values two standard errors above and below the best-fit value of 0.22 from Experiment 2. b: Flow/metabolism coupling relationship computed over a physically extreme range of $\beta$ values around the central estimate of 1.5 used in Fig. 7. Varying $\alpha$ from 0.3 to 0.5 resulted in slightly smaller changes (not shown).
independent additive terms, which is not physically realistic.

The BOLD signal model described by Buxton et al. (25) (a revision of their earlier model presented in (26)) produced good fits ($\chi^2 = 0.17$) and resulted in a computed flow/metabolism coupling relationship that was identical to that determined using the dHb dilution model. An advantage of the approach described in this paper is that the numerous constant parameters that must be assumed in the Buxton model (including physiological quantities such as baseline oxygen extraction fraction) are lumped into the parameter $M$, which can be measured.

Comments on Prior Gas Inhalation Studies

The BOLD-CBF-CMR$_{O_2}$ relationship derived in this paper and depicted in Fig. 9a is consistent with reports of increased BOLD sensitivity to activation-induced CBF changes during hypocapnia in rats (27). This behavior can be attributed to the increase in the slope, in the BOLD-CBF plane, of iso-CMR$_{O_2}$ contours at lower CBF levels. During hypocapnia, baseline CBF is decreased, but activation-induced CBF increases combine additively with this effect in such a way that the relative CBF responses to stimuli are not affected (27,28). This results in larger fractional BOLD signal changes during activation due to the increased partial derivative of the BOLD signal with respect to CBF at lower perfusion levels. Conversely, increased baseline CBF (due to hypercapnia and acetazolamide infusion) has been associated with reductions in the amplitude of activation-induced BOLD signal changes measured in human visual and motor cortices (5,29). Although the experimental conditions of the present study produced responses within the linear domain of the quantitative dHb dilution model, the level of hypocapnia induced in the rat study of Hsu et al. (27) was fairly intense (a $\sim$30% decrease in end-tidal CO$_2$) and the human studies of Bruhn and Bandettini (5,29) combined maximal levels of vasodilatory and neuronal stimulation. It is therefore possible that the above trends are due to the decreasing slope of iso-CMR$_{O_2}$ contours with increasing relative CBF depicted in Fig. 9a.

Metabolic BOLD Signal Attenuation

By matching perfusion levels during graded hypercapnia and graded visual stimulation, we obtained direct demonstrations of the effect of increased metabolic dHb production during brain activation. This constitutes truly model-independent evidence for graded increases in CMR$_{O_2}$, because experimental comparisons were controlled for CBF (and its correlate CBV) with no reliance on extrapolated relationships. Acceleration of oxidative metabolism reduced the visually evoked BOLD response to about one half of the value observed during equal perfusion increases produced by direct vasodilatory stimulation, equivalent to a reduction in the BOLD signal by $\sim$1.5% of its average baseline value. Comparison of visually evoked steady-state BOLD responses during stronger stimulation with the extrapolated GHC-derived baseline iso-CMR$_{O_2}$ contour reveals even greater attenuation of up to 5.7% (of baseline). This is a significantly larger effect than the $\sim$0.5% initial BOLD dip reported by Menon et al. using a 4 T MRI system (12), indicating that the transient dip does not reflect steady-state CMR$_{O_2}$ levels but, more likely, a slight lag of the CBF response with respect to onset of CMR$_{O_2}$ increases.

Absence of Stimulus-Specific Flow/Metabolism Coupling

Our comparison of BOLD-CBF responses during visual activation with highly dissimilar stimuli revealed virtually indistinguishable BOLD:CBF ratios. The two stimuli used have been found, in previous autoradiographic studies (30,31), to selectively activate either the blob or inter-blob systems of primate V1. Due to disparate levels of the aerobic metabolic enzyme cytochrome oxidase, it has been suggested that these tissues might exhibit different coupling between CBF and CMR$_{O_2}$ (14). Although this idea has led to speculation that the BOLD-CBF relationships pro-

![FIG. 9. Extrapolation of experimentally determined iso-CMR$_{O_2}$ contours.](image-url)
duced by the two types of stimulus may be characteristically different (32–34), our measurements (which presumably are variably weighted superpositions of blob and inter-blob responses, since they were restricted to V1 tissue) indicate that they are not.

Visually evoked BOLD-CBF relationships averaged across sufficient numbers of sessions were found to be reproducible, as shown by the well-defined cluster in Fig. 7a. Although previous reports have described large frequency-dependent variations in CBF-CMRO$_2$ coupling (35,36), our data showed no evidence of such an effect.

Estimation of $M$ and Model Sensitivity

For unknown reasons, the $M$ values determined in Experiments 1 and 2 were somewhat different ($0.15 \pm 0.06$ and $0.22 \pm 0.03$, respectively), although the 95% confidence intervals given do overlap. One possible explanation for this relates to the use of a different group of subjects for the first experiment, under slightly modified conditions. Notably distinct attributes of the first experiment include fasting of subjects, the use of individuals with no prior fMRI experience, a statistically significant superiority in performance of the attention task in this group (standing out from all other experiments that were conducted), and a higher level of residual error between the data and model. Because the $M$ value obtained in Experiment 2 was more typical of those obtained in pilot studies that we conducted using the same pulse sequence, and because the grating stimulus that was also included in Experiment 2 produced reproducible responses in subsequent experiments, we feel the use of the second $M$ value in our GVS experiments was justified. It should be noted that estimates of $M$ are quite sensitive to small variations in the model parameters $\alpha$ and $\beta$, as illustrated in Fig. 9b, so these should be interpreted with some caution. Nevertheless, the positions of iso-CMRO$_2$ contours in the BOLD-CBF domain relevant to our experiments (the white patch in Fig. 9b) were relatively insensitive to these effects and the overall flow/metabolism coupling relationship did not vary radically when recomputed with the two values of $M$ that we measured (as shown in Fig. 8).

In general, the $M$ values noted in our study were considerably larger than those reported by Davis et al. (2). This is probably due to the fact that they computed $M$ values for single voxels prior to averaging. Under such conditions, the extremely high variance of single-voxel FAIR measurements leads to underestimation of the asymptotic BOLD increase $M$, since many voxels exhibit very high perfusion changes (due simply to noise) with relatively small BOLD increases. This is why spatial smoothing (which increases the signal-to-noise ratio) was found to shift their calculated $M$ to higher values (Fig. 4 in Ref. 2). Pooling the raw BOLD and CBF data over homogeneous regions of interest and multiple subjects, as done in the present study, is likely to produce more robust CMRO$_2$ estimates, given the nonlinearity of the model and the extremely high variance of single-voxel MRI-based perfusion measurements. Systematic underestimation of $M$ is also likely to account for the lower level of BOLD signal attenuation attributed to aerobic metabolism during neuronal activation (2).

Additional improvements in the accuracy of $M$ could be achieved by driving CBF to higher levels during hypercapnic calibration. This would also allow the model to be further tested by verifying that the predicted proportionality between the slope of the baseline iso-CMRO$_2$ contour in the linear regime and the asymptotic $M$ value is observed. Perfusion responses to hypercapnia in the present study were relatively weak due to the use of low CO$_2$ concentrations, a nonrebreathing ventilation apparatus, respiratory dead space, and the allowance of increased tidal volume during CO$_2$ inhalation. Because the levels of hypercapnia thus achieved were barely perceptible to the subjects, there would appear to be scope for significantly larger CBF responses.

Quantitative Accuracy of MRI-Based Flow Measurements

In the present study, the FAIR technique was employed for measurement of relative CBF changes in a specific tissue sample. Although previous reports (37,38) have correctly pointed out inaccuracies that can occur with FAIR, we feel that the implementation used here was minimally affected by such errors. In order for any arterial spin labelling (ASL) method to be a quantitative index of fractional changes in the CBF through a tissue volume, the ASL signal must be a linear/zero-intercept function of CBF. Inaccuracies arise when excessive arrival-time delay of the near side of the tagging slab causes the ASL signal intercept to be significantly different from zero or when the far side of the tagging slab arrives during the inflow period, causing the ASL signal to saturate during large local flow increases. Although the first effect (arrival-time delay) can indeed be significant in multi-slice studies, it is likely to be very small in our acquisitions, which were single slice with a gap of only 3 mm between the image slice and the near side of the tagging slab. The second effect (running out of tag at high flow rates) is also readily observed when tagging slabs of limited thickness are used, such as with EPI-STAR and inverting with a head transmit/receive coil (e.g., a 9-cm slab was used in Ref. 38). In our experiments, however, we implemented FAIR with a completely nonelective inversion prepulse generated using the system body coil (capable of imaging a whole spine). It is highly unlikely that the far end of this tagging slab, which generally extended well into the torso, reached the imaging slice used during the weak hypercapnic episodes induced in our subjects. This is confirmed by inspection of the perfusion signals shown in Fig. 4a, which were recorded during inhalation of CO$_2$ with (blue) and without (black) visual stimulation. If the FAIR signal was saturated (due to limited tag width) during inhalation of 5% CO$_2$, then it would not be possible for the signal to increase further with the addition of visual stimulation. However, it is clear that the visually evoked component of the FAIR signal is as large with 5% CO$_2$ as it is when there is almost no CO$_2$. It will of course be desirable to perform future experiments with multi-slice CBF measurements and in such cases (or when a body transmit coil is not available) it will be necessary to turn to more advanced ASL methods such as QUIPPS II (quantitative imaging of perfusion using a single subtraction—second version; 37).
Flow/Metabolism Coupling

Analysis of our graded visual stimulation data revealed a consistent coupling ratio of approximately 2:1 for fractional changes in CBF and CMRO₂ during brain activation. Our MRI-based measurements of relative CBF and CMRO₂ changes during 4 Hz yellow/blue radial checkerboard stimulation at maximal contrast (48 ± 5% and 25 ± 4%, respectively) are in reasonable agreement with values obtained by one of the authors (S.M.) using PET (68 ± 5% and 25 ± 5%) during an identical activation protocol (14). In the same PET study, a (high-contrast) luminance varying uniform field stimulus flashing at 4 Hz was found to produce CBF and CMRO₂ increases of 50 ± 5% and 22 ± 5%, which also corresponds well with our findings. In a separate study, Davis et al. reported visually evoked CBF and CMRO₂ increases of 45 ± 4% and 16 ± 1%, respectively, at a single level of visual stimulation (2). These values are also in rough agreement with our data at maximal stimulation.

Although the reports cited above yielded evidence of activation-induced CMRO₂ increases, the present study validates a specific BOLD signal model and supports our conclusion that an invariant, linear CBF/CMRO₂ coupling relationship is maintained in human visual cortex. Theoretical analyses of oxygen delivery (39,40) have supported the assertion that relative CBF increases exceed fractional rises in CMRO₂ to accelerate diffusion limited delivery of oxygen across the blood-brain barrier. Although the Δ%CMRO₂:Δ%CBF ratio observed in our experiments is higher than that generally predicted by Buxton et al. (39), a recent extension of that model to include variations in capillary diffusion area is consistent with larger ratios (40).

Another discrepancy relating to the GH + VS data of Experiment 1 is that, on average, the percent change in CMRO₂ was coupled to the visually evoked component of the CBF response in a ratio of 0.34 instead of the value of 0.5 observed repeatedly in the GVS experiments. The implied increase in visually evoked perfusion at a given level of CMRO₂ could be due to an interaction between elevated blood CO₂ and the mechanisms regulating blood flow in response to activation, although the visually evoked component of the CBF increase did not appear to change significantly with CO₂ level (see Fig. 4a). It is also possible that other peculiarities of this experiment (noted above) contributed. We intend to conduct additional experiments using combinations of neuronal activation and hypercapnia to resolve these questions.

Although we did not endeavor to map CMRO₂ changes in multiple cortical areas, several general conclusions about flow/metabolism coupling in the brain can be speculated from our data. First, the amplitudes of both perfusion and BOLD responses to CO₂ inhalation were relatively similar throughout the regions of cortex sampled (although large differences between grey and white matter were consistently observed). This implies that cortical M values may be relatively homogeneous at a gross spatial scale and that the CMRO₂-BOLD-CBF mapping implicit in Fig. 7a is therefore likely to apply (approximately) in other cortical regions. Because stereotypical BOLD responses (measured using comparable fMRI methods) and CBF responses (measured using FAIR or PET) tend to fall within the darker gray area of this plot (Fig. 7a), the coupling relationship maintained in other regions would appear to resemble that observed in V1 in the present study. This must be verified experimentally, however, by repeating the protocols described in this paper in other cortical systems (e.g., sensorimotor and frontal cortex as well as basal ganglia). Another point worth noting is that if we plot the BOLD response in V1 as a function of CMRO₂, a highly linear relationship is revealed. This is a reassuring finding, as it means that the mapping signal used so widely in fMRI applications is likely to be a meaningful index of the synaptic workload evoked by a particular stimulus.

CONCLUSION

Since the emergence of fMRI, the BOLD effect has been attributed to the constancy of CMRO₂ across different activation states. Our results show that the BOLD phenomenon is more accurately described as the outcome of strong competition between CMRO₂ and flow-related dHb dilution during activation. Inspection of experimentally derived CMRO₂ maps on the BOLD-CBF plane reveals that the balance is a delicate one, and that the BOLD signal is highly sensitive to shifts in CMRO₂ at a given level of CBF. This sensitivity makes the BOLD signal a surprisingly powerful tool for studying flow/metabolism coupling.

ACKNOWLEDGMENTS

We thank Pamela Rabbitz for technical assistance. We are also grateful to Curtis Baker, Gareth Barnes, Joe Mandeville, and Bruce Rosen for discussion and critical reading of the manuscript.

REFERENCES

Investigation of BOLD Signal Dependence on CBF and CMRO₂


