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Coupling of cerebral blood flow and oxygen metabolism is conserved for chromatic and luminance stimuli in human visual cortex

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Abstract

The ratio of the changes in cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO₂) during brain activation is a critical determinant of the magnitude of the blood oxygenation level dependent (BOLD) response measured with functional magnetic resonance imaging (fMRI). Cytochrome oxidase (CO), a key component of oxidative metabolism in the mitochondria, is non-uniformly distributed in visual area V1 in distinct blob and interblob regions, suggesting significant spatial variation in the capacity for oxygen metabolism. The goal of this study was to test whether CBF/CMRO₂ coupling differed when these subpopulations of neurons were preferentially stimulated, using chromatic and luminance stimuli to preferentially stimulate either the blob or interblob regions. A dual-echo spiral arterial spin labeling (ASL) technique was used to measure CBF and BOLD responses simultaneously in 7 healthy human subjects. When the stimulus contrast levels were adjusted to evoke similar CBF responses (mean 65.4%±19.0% and 64.6%±19.9%, respectively for chromatic and luminance contrast), the BOLD responses were remarkably similar (1.57%±0.39% and 1.59%±0.35%) for both types of stimuli. We conclude that CBF-CMRO₂ coupling is conserved for the chromatic and luminance stimuli used, suggesting a consistent coupling for blob and inter-blob neuronal populations despite the difference in CO concentration.

Keywords

cerebral blood flow (CBF); cerebral metabolic rate of oxygen (CMRO₂); blood oxygenation level dependent (BOLD); functional magnetic resonance imaging (fMRI); cytochrome-oxidase blobs

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Introduction

The physiological connections between neural activity, cerebral blood flow and energy metabolism are fundamental for understanding and interpreting current functional brain imaging studies. Functional magnetic resonance imaging (fMRI) exploits the sensitivity of the local MR signal to shifts in deoxyhemoglobin content associated with altered brain activity. The central phenomenon behind blood oxygenation level dependent (BOLD) imaging is that cerebral blood flow (CBF) increases much more than cerebral metabolic rate of oxygen (CMRO₂) with brain activation such that blood oxygenation increases with brain activity (Fox & Raichle 1986). The physiological function of this mismatch is still poorly understood (Buxton 2010), but it nevertheless provides a detectable signal for mapping patterns of brain activity. However, the magnitude of the BOLD response depends strongly on the exact degree of mismatch between the CBF and CMRO₂ responses to a stimulus (Ances et al 2008). The coupling of CBF and CMRO₂ can be described empirically by an index n , the ratio of the fractional change in CBF to the fractional change in CMRO₂ for a particular experiment. A number of studies in human subjects with positron emission tomography (PET) and calibrated-BOLD fMRI methods have found a wide range of coupling ratios, although most fall in the approximate range of $n \sim 1.6\text{--}4$ (Ances et al 2008, Ances et al 2009, Chiarelli et al 2007a, Chiarelli et al 2007b, Davis et al 1998, Hoge et al 1999b, Kastrup et al 2002, Kim et al 1999, Leontiev & Buxton 2007, Leontiev et al 2007, Perthen et al 2008, Pike 2012, Stefanovic et al 2005, Stefanovic et al 2004).

The sources of the variability of CBF/CMRO₂ coupling are still unclear. A number of studies suggest that a simple feedback mechanism in which neural activity drives energy metabolism changes, and these in turn drive blood flow changes, is likely to be incomplete (Attwell & Iadecola 2002). Instead, a number of mechanisms have been identified in which aspects of neural signaling itself drive CBF changes, either through direct signaling effects or through activation of astrocytes (Hamel 2006, Iadecola & Nedergaard 2007, Koehler et al 2009). While feedback mechanisms from energy metabolism to CBF exist (e.g., vascular reactivity to pH changes that result from accumulation of carbon dioxide or lactate), current evidence suggests that the acute CBF response to a stimulus is driven by aspects of neural signaling, so that effectively CBF and CMRO₂ are driven in parallel by neural activation (Attwell & Iadecola 2002, Bonvento et al 2002, Buxton et al 2004, Raichle & Mintun 2006, Uludag et al 2004). Variability of the CBF/CMRO₂ coupling ratio could then result if particular aspects of the stimulus-evoked neural activity drive CBF and CMRO₂ to different degrees.

Human primary visual cortex (area V1) is potentially an important test-bed for probing the connections between neural activity, CBF and CMRO₂ because of the nonuniform distribution of cytochrome oxidase (CO) (Wong-Riley 1989). In addition to variations across cortical layers, tangential sections of supragranular cortical layers show a pattern of cytochrome oxidase (CO)-rich blobs (also called “puffs” or “patches”) roughly 200 microns across surrounded by interblob regions with lower CO concentration (Horton 1984, Horton & Hubel 1981, Wong-Riley 1979). In the mitochondria, CO is the last enzyme of oxidative metabolism, catalyzing electron transfer to oxygen, and so the CO concentration reflects the local capacity for oxygen metabolism. Importantly, though, the CO concentration is dynamically controlled, changing over weeks in response to shifts in neural activity, such as suppressing input from one eye (Wong-Riley 1989). In addition, a recent study demonstrated that the nuclear transcription factor that regulates CO concentration also is involved in regulation of a subunit of the NMDA receptor (Dhar & Wong-Riley 2009), suggesting a close link between CO concentration and average level of excitatory neural activity.

Since the discovery of the blob structure in V1 there have been numerous studies investigating the functional significance of these regions in terms of neural processing (Horton 1984, Horton & Hubel 1981, Tootell et al 1988a, Tootell et al 1988b, Wong-Riley 1979). Although electrophysiology studies of the responses of neurons in the blob and interblob regions to different types of visual stimulus have produced mixed results (see (Horton & Adams 2005) for a review), there is substantial evidence for a preferential response of blob neurons to color stimuli. Using optical imaging techniques sensitive to reflectance changes related to oxy- and deoxy-hemoglobin, Lu and Roe (Lu & Roe 2008) compared responses in macaque V1 to gratings alternating color (red/green) and luminance (black/white). Subtracting maps of these two responses showed “blob-like” patterns of color response that were strongly associated with CO blobs in stained tissue.

Other studies have investigated capillary density differences between blob and interblob regions as an indirect test of whether CBF was matched to the capacity for CMRO₂ as reflected in CO concentration. In an early study in the squirrel monkey, Zheng and colleagues (Zheng et al 1991) found a 42% higher capillary density in the blob regions compared to the interblob regions. In a more recent study in macaque visual cortex, Weber and colleagues found a good correlation between laminar differences in CO concentration and capillary density (Weber et al 2008) but a much weaker (although statistically significant) difference in capillary density of only about 4% between blob and interblob regions (Keller et al 2011). Both capillary density and CO concentration, however, likely reflect the capacity for CBF and CMRO₂ in a region, as modified by the average level of neural activity, and it is still an open question whether such anatomical differences translate into a different coupling of CBF and CMRO₂ responses to acute changes in neural activity.

In human fMRI experiments a typical imaging voxel in area V1 contains both blob and interblob regions. The ability to alter the balance of activation between blob and interblob neurons with color or luminance stimuli provides an opportunity to test whether the specific subpopulations of stimulated neurons alters the macroscopic coupling of CBF and CMRO₂ responses. In a pioneering study, Hoge and colleagues (Hoge et al 1999b) used a calibrated BOLD methodology to test for differences in the coupling ratio n for different visual stimuli. The calibrated BOLD approach, introduced by Davis and co-workers, involves measuring the CBF response with an arterial spin labeling (ASL) technique in addition to the BOLD changes accompanying separate periods of brain activation and mild hypercapnia (Davis et al 1998). Assuming that mild hypercapnia increases CBF while producing a negligible change in CMRO₂ (Jones et al 2005, Sicard & Duong 2005), this allows determination of a BOLD scaling factor M , which reflects the maximum BOLD signal change upon washout of all deoxyhemoglobin. By analyzing the data in the context of a biophysical model for the BOLD signal, activation-induced CMRO₂ changes, and thus n , can be calculated. Hoge et al (Hoge et al 1999b) used a FAIR acquisition for CBF measurement, and a retinotopically determined V1 for region of interest (ROI) selection, and found that a wide range of graded visual stimuli were consistent with constant $n \sim 2$. However, FAIR is a qualitative measure of CBF (Buxton et al 1998) that might underestimate the global CBF change with hypercapnia and thus overestimate M . In addition, we recently found that the use of a V1 localizer also systematically overestimates M compared to a CBF localizer (Leontiev et al 2007), and Chiarelli et al (Chiarelli et al 2007b) have shown that an overestimate of M tends to drive all calculations from activation data to a similar low value of n . Finally, other recent studies have challenged the assumption that CMRO₂ is not altered by the levels of CO₂ administered for the calculation of M (Zappe et al 2008), and if CMRO₂ decreases with hypercapnia M will be overestimated. A recent study comparing hypercapnia calibration with an alternative hyperoxia calibration method found somewhat higher values for M with the hypercapnia method (Mark et al 2011).

Therefore, in light of these recent findings, we revisited these experiments with a different experimental design that exploits the sensitivity of the BOLD response to the exact ratio of the CBF and CMRO₂ changes. To remove the dependence on the estimation of M , we adjusted the chromatic and luminance stimuli to yield the same CBF responses in human subjects, and then compared the BOLD responses in the same region of interest for the two stimuli to test for a difference that would indicate a difference in the CMRO₂ response. Our primary finding was that the BOLD and CBF responses for the two stimuli were virtually identical.

Methods

Subjects

Seven healthy subjects (4 males, 3 females, ages 22–42) were recruited and scanned in a 3 Tesla MR imaging system after obtaining informed consent according to the guidelines set by the University of California San Diego (UCSD) Institutional Review Board (IRB). All subjects underwent a preliminary scan session on a different day in which retinotopic mapping was performed. A pilot study on four additional subjects was performed beforehand in order to determine the necessary contrast level of the luminance stimulus to approximately match the CBF change elicited by the chromatic stimulus.

Retinotopic Mapping

In a preliminary scan session on a different day, subjects were presented with standard visual stimuli for retinotopic mapping (Engel et al 1997, Press et al 2001). During presentation of visual stimuli, images were acquired with an EPI sequence with the following parameters: TR = 2 s, TE = 30 ms, flip angle 90°, FOV 19cm, matrix 64 × 64, 3-mm isotropic resolution, 20 interleaved slices. The entire set of stimuli (meridian, ring and wedge) yielded a single representation of V1. A high-resolution whole brain structural scan (3D FSPGR with 1-mm isotropic resolution) was acquired for each subject. The segmented cortical gray matter of occipital cortices was flattened using surface rendering methods described in (Wandell et al 2000). Linear trends were removed from the datasets. Activation was assessed by correlating detrended data with the first harmonic of the stimulus variation frequency (Press et al 2001). Representations of the primary visual area V1 of each subject were delineated on the computationally flattened visual cortex to define the retinotopic ROI for each subject. A combination of in-house Matlab (www.mathworks.com) code and the *mrLoadRet-1.0* (<http://white.stanford.edu/software/>) software package was used for retinotopic mapping.

Visual Stimulus

All stimulus presentations were created on a MacIntosh Power Book G3 and projected on a screen in the scanner bore. Psychophysics toolbox version 2 (<http://www.psychtoolbox.org>) was used to present visual stimuli. For all combined ASL/BOLD studies, subjects viewed a simultaneously rotating (both clockwise and counter-clockwise) and expanding/contracting radial checkerboard alternating between a chromatic (red/green) and luminance (black/white) pattern in block-design sequence for 6 consecutive runs lasting 228s each. Each stimulus block was comprised of sub-blocks of the same stimulus type (chromatic or luminance) but switching direction every 1.0 – 2.5 s in a random fashion in order to reduce the effect of adaptation. The switching pattern, however, was identical within in each run between the chromatic and luminance stimuli. The spatial and temporal parameters were identical for color and luminance stimuli, with spatial frequency changing linearly from 2.5 cycles/degree in the fovea to 1.0 cycles/degree at 10 degrees eccentricity, and with drifting temporal frequency of 2 Hz. A linearly varying spatial frequency was used in order to ensure a homogenous activation pattern because the spatial frequency relative to receptive field size

is greater at peripheral eccentricities. All image frames presented to subjects had the same average luminance. Individual runs were comprised of an initial 60s second period where subjects viewed a gray screen, one chromatically-driven (red/green) stimulus block lasting 24s, a 60s rest period where subjects viewed a gray screen, one luminance-driven (black/white) stimulus block lasting 24s, and a 60s tail period where subjects viewed the gray screen. The first stimulus type (luminance or chromatic) in each run alternated between runs and from subject-to-subject to reduce experimental and data normalization biases.

The RGB value for the red color was held constant for all subjects (255), and the RGB value of the green color was determined for each subject to match the luminance of the red color in a separate flicker photometry experiment carried-out at the beginning of each scan session (Lee et al 1988). Heterochromatic flicker photometry is a method for determining the luminance of two colors at which they appear of equal intensity (perceptual equiluminance) and presumably correspond to equal neuronal population activations in visual cortex. Perceptual equiluminance differs from isoluminant values measured photometrically and varies across subjects. Two lights of different color are alternated at typically 10–20 Hz and their relative intensities are adjusted by the observer until the sensation of flicker is minimized. Subjects were instructed on how to perform the flicker-fusion experiments and were allowed to practice in the MR console room for approximately 10 minutes immediately prior to scanning. Once inside the magnet bore, subjects performed three flicker-fusion trials and the mean green RGB value from the three trials was used for the chromatic stimulus. The tuning of the luminance stimulus was performed in a set of preliminary experiments whereby the contrast between adjacent gratings was adjusted in a symmetric fashion around the mean luminance of the chromatic stimulus. For each subject in these preliminary studies (4 subjects), the contrast of the luminance stimulus was increased in a graded fashion until the CBF change induced by both color and luminance stimuli were approximately equal.

MRI parameters

Imaging data were acquired on a 3 Tesla 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 ($TR=2.0$ s, $TI_1=600$ ms, $TI_2=1500$ ms, 10-cm tag width, and a 1-cm tag-slice gap) with a dual-echo gradient echo (GRE) readout and spiral acquisition of k -space ($TE_1=9.4$ ms, $TE_2=30$ ms, flip angle= 90° , field of view (FOV)=24 cm, 64×64 matrix). Small bipolar crusher gradients ($b=2$ s/mm²) were applied to remove signal from large vessels supplying blood to more distal slices. 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 oblique slices, centered around the calcarine sulcus, were acquired in a linear fashion from bottom to top. A high-resolution structural scan was acquired with a magnetization-prepared 3D fast spoiled GRASS (FSPGR) pulse sequence ($TI=450$ ms, $TR=7.9$ ms, $TE=3.1$ ms, flip angle= 12° , FOV= $25 \times 25 \times 16$ cm, matrix $256 \times 256 \times 124$). The latter images were collected after functional runs and were used to perform registration of retinotopic maps acquired in a prior scan session.

Data Analysis

For each subject, data were analyzed to generate average CBF and BOLD responses separately for runs beginning with the chromatic stimulus and those beginning with the luminance stimulus. First, the dynamic ASL data were co-registered to correct for subject movement during the study using AFNI software (Cox 1996). For each voxel, a CBF time-

series was computed by taking a running subtraction of the control and tag image series from the first echo data. Each data point was calculated from the difference between that value and the average of the two nearest neighbors with adjustments made in the sign so that each point represents a subtraction of tag from control images. A BOLD-weighted time series was computed from the running average of the second echo, taking the average of each image with the mean of its two nearest neighbors (Liu & Wong 2005).

In these studies, the signal to noise (SNR) ratio typically was not sufficient to perform individual voxel calculations, so regions of interest (ROI) were required. The first step of ROI selection consisted of rendering the flattened representation of the boundaries of V1 on a high-resolution anatomical volume. This volume was registered to the anatomical volume acquired in the ASL scan session using AFNI software with displacement and rotation parameters applied to the V1 representation to create a high resolution ROI. A general linear model analysis was performed to identify CBF-activated voxels within the defined mask for V1 (Mumford et al 2006, Restom et al 2006). The first four images in each run were discarded in order to achieve steady-state conditions. The stimulus-related regressor was obtained by convolving the block designed 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 & 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 boundaries of V1 were detected using a correlation threshold of $r = 0.5$ (Bonferroni-corrected p value of 3.18×10^{-7}) applied to the perfusion data.

Chromatic and luminance responses from all six runs were averaged together and both linear and quadratic drifts were removed from the average CBF and BOLD time series. For each subject, both average CBF and BOLD responses were normalized to their respective baseline values, calculated as the average of the first minute of the acquisition following the exclusion of the first four images (e.g. 8s) due to steady-state considerations. For the functional runs, the fractional CBF and BOLD responses were calculated as the average over a 16 s period beginning 8 seconds after the onset of the stimulus, approximating the plateau portion of the response and thus avoiding the initial transient period at the beginning of the stimulus. Group averages for BOLD and CBF responses were determined by averaging the responses over subjects. In addition to the primary measurement of the positive CBF and BOLD responses during the stimulus, we also examined the post-stimulus undershoot. Both CBF and BOLD signals were averaged over a 16s window starting 12s after the end of the stimulus. The averaging range was chosen to match that used for the positive BOLD response, and the delay chosen so that this range was approximately centered on the typical maximum of the undershoot. A paired t-test was performed comparing the positive and undershoot responses between the chromatic and luminance stimuli with significance defined as $p < 0.05$.

Results

The average number of voxels in V1 was 78 ± 15 from the retinotopic mapping, and the average number of CBF activated voxels within V1 was 53 ± 9 . The result of the preliminary study was that a luminance contrast level of about 77% produced a similar CBF change to the chromatic stimulus in the tested subjects, and this value was used for the subsequent study. Figure 1 shows the average across subjects of the CBF and BOLD response curves, showing no effect of the order of the luminance and chromatic stimuli.

Figure 2 shows the single-block average responses across all runs for the BOLD and CBF responses, showing remarkably little difference between the average curves for luminance and chromatic stimuli.

For each subject the BOLD and CBF temporal responses were averaged over the two time windows shown in Figure 2, corresponding to the primary positive response period and to the post-stimulus undershoot period. Table 1 lists the positive responses for each subject and Table 2 lists the undershoot values. The average positive CBF response generated from the chromatic stimulus was nearly identical to that generated from the luminance stimulus (62.3 ± 16.9 and $62.8 \pm 19.4\%$, respectively). The associated BOLD-responses also were nearly identical for both types of stimuli (1.55 ± 0.39 and $1.54 \pm 0.33\%$ for chromatic and luminance stimuli, respectively). During the post-stimulus period the BOLD response showed a significant undershoot ($-0.37 \pm 0.12\%$, $p=0.0002$ for the chromatic stimulus; $-0.45 \pm 0.20\%$, $p=0.0009$ for the luminance stimulus). However, the trend for a larger undershoot to the luminance stimulus did not reach statistical significance ($p=0.11$). Post-stimulus undershoots in the CBF response were much weaker, although the undershoot for the luminance stimulus was significantly different from zero ($-2.0 \pm 3.7\%$, $p=0.20$ for the chromatic stimulus; $-3.5 \pm 3.3\%$, $p=0.031$ for the luminance stimulus). However, the difference between the CBF undershoots for the chromatic and luminance stimuli did not reach statistical significance ($p=0.16$).

Figure 3 shows comparisons of the individual data for each response, including both the primary positive responses and the post-stimulus undershoot. Despite a greater than two-fold range of response magnitudes across subjects, the responses within subjects were tightly correlated. For the positive BOLD responses to chromatic and luminance stimuli the correlation coefficient was 0.957, and for the positive CBF responses the correlation coefficient was 0.946.

The primary finding was a null result, that there was little difference between the CBF and BOLD positive responses for chromatic and luminance stimuli. We further tested this result to identify the limits of variation that would have been detected by our experiments. As a simple first test, we scaled each of the responses to luminance contrast by a fixed amount, and increased this scaling until a paired t-test yielded a significant difference of the means ($p<0.05$). Based on this test, an 11% difference in the mean CBF response or an 8% difference in the BOLD response would have been detected in our data. A second test, using an offset rather than a scaling to preserve the original variance, yielded similar results. In a third test we used the pooled variance and the observed correlation between responses to calculate the effect size that would have been detectable with a power of 0.8, and this test yielded similar levels of change.

Using these statistical power limits we determined the range of variation in $CMRO_2$ and n that would have been detectable in this experiment. For these calculations it is necessary to invoke the formalism of the calibrated BOLD methodology to estimate the $CMRO_2$ change. The Davis model (Davis et al 1998) for the BOLD signal is:

$$\delta s = M(1 - f^\alpha (r/f)^\beta) \quad [1]$$

where δs is the BOLD response expressed as a percentage signal change, f is the activated CBF divided by the baseline CBF, and r is the activated $CMRO_2$ divided by the baseline $CMRO_2$. The two parameters α and β are dimensionless and usually taken to be $\alpha=0.38$ and $\beta=1.5$ (Davis et al 1998), although $\alpha=0.2$ and $\beta=1.3$ have become more common for 3T studies (Chen & Pike 2009, Mark et al 2011, Mark & Pike 2012), and in a recent modeling study we found that $\alpha=0.14$ and $\beta=0.91$ gave a somewhat better fit to the simulations of a

detailed BOLD signal model (Griffeth & Buxton 2011). The scaling parameter M , expressed as a percentage, is normally determined from a hypercapnia experiment or, more recently, a hyperoxia experiment (Chiarelli et al 2007c, Mark et al 2011). In the current study M was not measured, because the design was to use the sensitivity of the BOLD response to n directly. That is, from Eq [1] the ratio of the BOLD responses to the two types of stimulus cancels out the factor M . Our goal now, however, is to test the range of CMRO₂ variation that could have escaped detection in our experiment. In a previous calibrated BOLD study of visual responses using similar acquisition and analysis methods (with the same mean ratio of CBF to BOLD response to the stimulus as the current study), the hypercapnia experiment showed a mean CBF change to hypercapnia of 47% with a BOLD signal change of 2.6% (Perthen et al 2008). Using these values for the calibration experiment with Eq [1] with the three pairings of (α, β) values noted above, the estimated range of the average fractional CMRO₂ response to the stimulus for the current data is 23–27%. The estimated detectable difference of the BOLD response between chromatic and luminance stimuli of 8% corresponds to a difference in the CMRO₂ response amplitude of less than 2% of baseline CMRO₂ for all three pairings of α and β values.

Discussion

The BOLD response has proven to be a sensitive mapping signal for detecting changes in local deoxyhemoglobin concentration accompanying changes in neural activity. However, the magnitude of the BOLD response depends strongly on the exact balance of the CBF and CMRO₂ changes with activation, which we can describe as the ratio n of the fractional changes. For example, theoretical estimates indicate that for the same CMRO₂ change, the BOLD response for $n=2$ and $n=3$ differs by more than 100% (Ances et al 2008). For this reason, it is important to develop a better understanding of how much n varies both in health and disease. In this study we tested whether CBF/CMRO₂ coupling in human primary visual cortex differed for stimuli varying in luminance but not color and stimuli varying in color but not luminance. The motivation was to probe different populations of neurons, based on the association of neurons responding to color with the blobs of higher concentration of cytochrome oxidase (CO) (Lu & Roe 2008). By choosing stimuli that evoked similar CBF responses, we tested whether there was any difference in the BOLD response that would reflect a difference in the CMRO₂ response. We found virtually identical CBF and BOLD responses for the two stimuli, and so no evidence for a difference in the CMRO₂ response. Based on post-hoc testing of the data, we estimate that a CMRO₂ change of 2% of baseline CMRO₂ (compared with an estimated 23–27% CMRO₂ response to the stimulus) would have been detected. The finding of consistent CBF/CMRO₂ coupling for color and luminance stimuli is in good agreement with the original finding of Hoge and colleagues (Hoge et al 1999a).

This study specifically exploited the sensitivity of the BOLD signal to n in order to investigate differences in CBF-CMRO₂ coupling between two populations of neurons with potentially different metabolic requirements. The advantage of this design is that the results have minimal dependence on exactly how the BOLD signal is modeled. That is, the basic idea is that for the same defined brain region, two stimuli evoking the same CBF response but different CMRO₂ responses will produce different BOLD responses regardless of the details of the BOLD model. To achieve similar CBF responses to the two types of stimuli we conducted a pilot experiment varying the contrast of the luminance stimulus to match the CBF response of the chromatic stimulus. This contrast level was then used for the luminance stimuli for all seven subjects in the study. Matching luminance between the red and green colors of the chromatic stimulus could be done either by physical measurement with a photometer gun or specifically-tailored to an individual's own spectral sensitivity with a

flicker-fusion experiment, and we used the latter approach to minimize luminance variations within the chromatic stimuli.

The spatial and temporal properties were identical for the luminance and chromatic stimuli. It is known that CO-rich blobs are more sensitive to relatively low spatial frequency (1–1.5 cycles/degree), whereas interblob regions respond better to higher frequencies (3–7 cycles/degree). We chose intermediate spatial frequencies based on findings by Tootell et al (Tootell et al 1988c), which show similar sensitivity profiles to color and luminance stimuli in the 1.5–3 cycles/degree range. The switching of stimulus direction in a random fashion, occurring in periods of 1–2.5s was done in order to avoid stimulus adaptation. The degree to which the switching component of the stimulus contributed to the total CBF change is unknown and it is possible that the significance of this effect was great enough to impair detection of differences in response magnitude based purely on effects of color or luminance. The switching pattern, however, was identical for both types of stimuli within in each run. Further experiments are needed to characterize the magnitude of this potential effect.

In our experimental design we did not directly measure the coupling ratio n . This would require a calibrated-BOLD experiment (Davis et al 1998), based on Eq [1] with a separate measurement with hypercapnia or hyperoxia (Chiarelli et al 2007c, Mark et al 2011) to estimate the scaling parameter M . For this reason, we cannot conclude anything directly about the variability of n across the population for these stimuli. However, in a previous calibrated BOLD study (Leontiev & Buxton 2007), we used a stimulus similar to the luminance stimulus of the current study, and found that n varied across the population but was highly reproducible in repeated studies on individual subjects. The current study supports this observation with highly conserved coupling for the two types of stimuli within individuals despite a considerable variation of the CBF and BOLD responses across subjects (Figure 3).

Several previous studies have found evidence for variability of CBF/CMRO₂ coupling across brain regions (Ances et al 2008, Chiarelli et al 2007a, Vafae & Gjedde 2004). In a recent study from our group we found that n for a visual stimulus can be modulated by a drug, decreasing following administration of caffeine due to a larger evoked CMRO₂ response to the same stimulus (Griffeth et al 2011). Also, in another recent study from our group we found that the coupling ratio for a visual stimulus was modulated by attention, with lower n when the stimulus was attended (Moradi et al 2012). There have been fewer studies comparing n for different stimuli within the same brain region. In pioneering studies Hoge and colleagues (Hoge et al 1999a, Hoge et al 1999b) compared responses between luminance and color stimuli and found no evidence for a difference in n . In particular, they found a consistent relationship between BOLD and CBF responses for color and luminance stimuli that also demonstrated in a model-independent way that CBF/CMRO₂ coupling was not significantly different (Figure 6 in (Hoge et al 1999a)). The current study corroborates their result with somewhat different stimuli. However, other studies have found evidence for varying n as other aspects of the stimulus were varied. Studies with both a calibrated BOLD methodology and with PET have found evidence for varying n as the frequency of a flickering stimulus is changed, with larger n for the highest frequencies (Lin et al 2008, Vafae et al 1999). In a recent preliminary study from our group varying the contrast of a visual stimulus we found an increase of n with increasing contrast (Liang & Buxton 2009).

Taken together, the studies above indicate that CBF/CMRO₂ coupling can vary significantly across subjects, across different brain regions within a subject, with administration of a drug, and even within the same brain region for different stimuli or differences in attention. For this reason, it is somewhat surprising that the coupling is so strongly conserved for color and

luminance stimuli, and this was our motivation for revisiting the original study of Hoge and colleagues. In particular, the nonuniform distribution of cytochrome oxidase in the brain suggests the possibility that some brain regions are better equipped for oxidative metabolism, and by preferentially activating neurons in blob regions with chromatic stimuli or neurons in interblob regions with luminance stimuli we could alter the balance of activated neurons relative to the CO distribution. Our simple prediction was that CMRO₂ would respond to a larger degree for the color stimuli, but there was no evidence of this in our data.

In an early study designed to separately stimulate blob and interblob regions, Bandettini and colleagues (Bandettini et al 1997) found a larger post-stimulus undershoot of the BOLD response for luminance stimuli. As part of an extensive evaluation of visual cortex response modulations, Hoge and colleagues (Hoge et al 1999c) also found larger post-stimulus undershoots for luminance stimuli compared to color stimuli. In our study an undershoot was present for both chromatic and luminance stimuli, with a trend for a larger undershoot for the luminance stimulus in agreement with the earlier studies, but this difference did not reach statistical significance in our data.

In conclusion, we tested whether the coupling ratio of CBF and CMRO₂ responses in primary visual cortex differed for chromatic and luminance stimuli, predicting that differences in cytochrome oxidase concentrations in the brain regions responding to these stimuli would translate to a difference in that ratio. Although CBF responses to a stimulus varied by more than a factor of two across subjects, there was a remarkably conserved CBF/CMRO₂ coupling ratio for the two stimuli within subjects. Based on our data, the difference of the CMRO₂ responses to the two stimuli is less than 2% of baseline CMRO₂ when the stimuli are adjusted so that the CBF responses are matched. These data indicate that, although cytochrome oxidase concentrations adjust based on the average level of activity within a brain region (Wong-Riley 1989), these differences do not translate to a significant difference of the CBF/CMRO₂ coupling ratio when the different populations of neurons are stimulated.

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Highlights

BOLD response is sensitive to blood flow/oxygen metabolism coupling

Variability of flow/metabolism coupling in human primary visual cortex was tested

Chromatic and luminance stimuli to excite cytochrome oxidase blob and interblob areas

For stimuli tuned for similar flow response, no detected difference in BOLD response

Figure 1.

Average (±SD) BOLD and CBF responses for runs beginning with the chromatic stimulus (A and B) and runs beginning with the luminance stimulus (C and D) for 7 subjects, with stimulus periods indicated by horizontal bars. Stimuli were chosen to produce similar CBF responses, and the resulting BOLD responses are also similar, consistent with uniform CBF-CMRO₂ coupling for luminance and chromatic stimuli.

Figure 2.

Average ($\pm$ SD) single block responses to the chromatic stimulus (solid) and luminance stimulus (dashed) for: **A)** BOLD response, and **B)** CBF response. The stimulus duration (24s) is indicated by a gray bar, and the 16s ranges for averaging are indicated for the positive response and the undershoot response. The response curves for the two types of stimulus are similar. The post-stimulus undershoot is much more pronounced in the BOLD response compared to the CBF response.

Figure 3.

CBF and BOLD positive responses and undershoot responses for chromatic and luminance stimuli for 7 subjects. **A)** Comparison of BOLD and CBF responses, with the cluster in the negative quadrant representing the undershoot responses. Other panels show paired comparisons of responses to chromatic and luminance stimuli for: **B)** the ratio of the CBF change (%) to the BOLD signal change (%); **C)** the BOLD response; and **D)** the CBF response. For all of the data, the paired responses to chromatic and luminance stimuli fall very close to the line of identity, shown as a solid line.

Table 1

Positive responses: average %BOLD and %CBF responses for luminance and chromatic stimuli for individual subjects.

Subject	BOLD Response (%)		CBF Response (%)	
	Luminance	Chromatic	Luminance	Chromatic
1	1.79	2.02	60.6	71.4
2	1.31	1.28	75.2	72.7
3	2.15	2.14	64.4	66.3
4	1.42	1.54	97.3	86.5
5	1.58	1.50	37.9	38.1
6	1.27	1.18	46.5	45.0
7	1.26	1.20	57.9	55.9
Mean $\pm$ SD	1.54 $\pm$ 0.33	1.55 $\pm$ 0.39	62.8 $\pm$ 19.4	62.3 $\pm$ 16.9

Table 2

Undershoot responses: average %BOLD and %CBF responses during the undershoot period for luminance and chromatic stimuli for individual subjects.

Subject	BOLD Response (%)		CBF Response (%)	
	Luminance	Chromatic	Luminance	Chromatic
1	-0.71	-0.61	-5.6	-2.7
2	-0.40	-0.27	1.7	5.6
3	-0.76	-0.44	-6.4	-3.8
4	-0.31	-0.35	0.01	-0.8
5	-0.27	-0.25	-3.1	-6.0
6	-0.37	-0.31	-3.9	-2.2
7	-0.35	-0.34	-7.1	-4.1
Mean $\pm$ SD	-0.45 $\pm$ 0.20	-0.37 $\pm$ 0.12	-3.5 $\pm$ 3.3	-2.0 $\pm$ 3.7