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Luminance contrast of a visual stimulus modulates the BOLD response more than the cerebral blood flow response in the human brain

Christine L. Liang[1], **Beau M. Ances**[2], **Joanna E. Perthen**[1], **Farshad Moradi**[1], **Joy Liau**[3], **Giedrius T. Buracas**[1], **Susan R. Hopkins**[1,4], and **Richard B. Buxton**[1,*]

[1]Department of Radiology, University of California, San Diego, CA, USA

[2]Department of Neurology, Washington University in St. Louis, MO, USA

[3]Department of Bioengineering, University of California, San Diego, CA, USA

[4]Department of Medicine, University of California, San Diego, CA, USA

Abstract

The blood oxygenation level dependent (BOLD) response measured with functional magnetic resonance imaging (fMRI) depends on the evoked changes in cerebral blood flow (CBF) and cerebral metabolic rate of oxygen ($CMRO_2$) in response to changes in neural activity. This response is strongly modulated by the CBF/$CMRO_2$ coupling relationship with activation, defined as n, the ratio of the fractional changes. The reliability of the BOLD signal as a quantitative reflection of underlying physiological changes depends on the stability of n in response to different stimuli. The effect of visual stimulus contrast on this coupling ratio was tested in 9 healthy human subjects, measuring CBF and BOLD responses to a flickering checkerboard at four visual contrast levels. The theory of the BOLD effect makes a robust prediction—independent of details of the model—that if the CBF/$CMRO_2$ coupling ratio n remains constant, then the response ratio between the lowest and highest contrast levels should be higher for the BOLD response than the CBF response because of the ceiling effect on the BOLD response. Instead, this response ratio was significantly lower for the BOLD response (BOLD response: 0.23 ± 0.13, mean ± SD; CBF response: 0.42 ± 0.18; p=0.0054). This data is consistent with a reduced dynamic range (strongest/weakest response ratio) of the $CMRO_2$ response (~1.7-fold) compared to the CBF response (~2.4-fold) as luminance contrast increases, corresponding to an increase of n from 1.7 at the lowest contrast level to 2.3 at the highest contrast level. The implication of these results for fMRI studies is that the magnitude of the BOLD response does not accurately reflect the magnitude of underlying physiological processes.

Keywords

visual contrast; functional magnetic resonance imaging (fMRI); cerebral blood flow (CBF); cerebral metabolic rate of oxygen consumption ($CMRO_2$); blood oxygen level dependent (BOLD) effect; arterial spin labeling (ASL); visual cortex

*Correspondence: Richard B. Buxton, Ph.D., University of California, San Diego, Center for Functional MRI, W. M. Keck Building, 0677, 9500 Gilman Drive, La Jolla, California, CA 92093-0677, USA, rbuxton@ucsd.edu.

Introduction

The blood oxygenation level dependent (BOLD) response measured with functional magnetic resonance imaging (fMRI) provides a useful mapping tool to identify brain areas that respond to a particular stimulus. However, the magnitude of the BOLD response is not a simple quantitative reflection of the magnitude of the underlying physiological changes because of the complexity of the BOLD response. A positive BOLD response primarily reflects a decrease of blood deoxyhemoglobin within the activated brain region due to a larger increase of cerebral blood flow (CBF) than cerebral metabolic rate of oxygen ($CMRO_2$). Although both CBF and $CMRO_2$ increase with activation, increased CBF tends to increase the BOLD signal, while increased $CMRO_2$ tends to decrease the BOLD signal. The overall magnitude of the BOLD response thus depends on the coupling ratio n, defined as the fractional change in CBF divided by the fractional change in $CMRO_2$ in response to a stimulus (Buxton et al., 2004). A basic question for interpreting the BOLD response is then: if two stimuli activate the same brain area, can we take the ratio of the BOLD responses as a quantitative estimate of the relative magnitudes of evoked physiological activity? The answer depends on whether the CBF/$CMRO_2$ coupling ratio n varies with the stimulus.

Although there are relatively robust methods for measuring CBF with positron emission tomography (PET) and MRI methods, measurement of $CMRO_2$ is much more difficult. The PET methods require multiple tracers to account for recirculating labeled water of metabolism and blood volume effects, and the MRI methods based on a calibrated BOLD approach require combined imaging of the BOLD and CBF responses and an individual calibration with hypercapnia or hyperoxia (Buxton, 2010). For this reason, there have been relatively few systematic studies of the coupling of CBF and $CMRO_2$ responses as the stimulus is varied.

The calibrated BOLD approach introduced by Davis and colleagues (Davis et al., 1998) has been used by several investigators to measure the CBF/$CMRO_2$ coupling ratio n in different brain regions for different stimuli. A wide range of values of n have been reported, but most fall in the range n~2–4 (Davis et al., 1998; Hoge et al., 1999a; Kim et al., 1999; Kastrup et al., 2002; Stefanovic et al., 2004; Stefanovic et al., 2005; Chiarelli et al., 2007a; Chiarelli et al., 2007b; Leontiev and Buxton, 2007; Leontiev et al., 2007; Ances et al., 2008; Perthen et al., 2008; Ances et al., 2009). Model calculations based on the original calibrated BOLD equation (Davis et al., 1998) suggest that the magnitude of the BOLD response is particularly sensitive to the exact value of n when n~2–3. In a recent study comparing activation in visual cortex with basal ganglia structures, we found BOLD responses that differed by a factor of 7, while the CBF responses differed by only a factor of 2.5 (Ances et al., 2008). This large discrepancy was due to a seemingly small difference in n of 2.2 in visual cortex and 1.6 in basal ganglia.

While our previous study and others (Chiarelli et al., 2007a) have suggested regional variations in n across the brain, n also may vary with aspects of the stimulus. Using PET methods and varying the flicker frequency of a blue/yellow checkerboard, Vafaee and Gjedde found similar values of n~3 for 1Hz and 4Hz flicker rates, but a strong jump to ~7 for an 8Hz stimulus (Vafaee and Gjedde, 2000). In more recent studies with a modified calibrated-BOLD technique, Lin and colleagues used a black and white flickering checkerboard and found variable CBF/$CMRO_2$ coupling when the frequency was varied, consistent with the PET results (Lin et al., 2008).

Our goal in the current study was to test whether CBF/$CMRO_2$ coupling varies with graded changes in the luminance contrast of a visual stimulus. Our primary empirical finding was that the dynamic range of the BOLD response, taken as the ratio of the responses to the

highest and lowest contrast levels, was larger than the dynamic range of the CBF response. Because of the ceiling effect on the BOLD response, the expected relationship for a fixed coupling ratio n is that the BOLD response should approach a saturation level as the CBF continues to increase, so that the dynamic range of the BOLD response is expected to be less than the dynamic range of the CBF response. The opposite pattern we observed is consistent with a significant increase of the $CBF/CMRO_2$ coupling ratio from n~1.7 to n~2.3 as stimulus contrast increased from the lowest to highest contrast level.

Materials and Methods

Subjects

Ten healthy adults (4 males, 21– 32 years of age, mean age 26) participated in this study after giving written informed consent. This study was approved by the Institutional Review Board at the University of California at San Diego (UCSD). One subject was excluded due to excessive head motion. The remaining 9 subjects reported low daily caffeine intake (0 – 100mg) and had not consumed caffeine for at least 3 hours prior to scanning.

Visual stimulus

The stimuli were presented using a Macintosh G3 (Cupertino, CA) computer and an NEC VT575 LCD projector (Itasca, IL), onto a small screen attached to the scanner bore approximately at the subject's waist. A block design was utilized. The functional activation task consisted of a grayscale radial checkerboard flickering at 8Hz with the rest condition consisting of an isoluminant gray background. Each experimental run was 410s in duration, starting with a 60s rest period and followed by 4 cycles of activation/rest (a 20s activation block followed by a 60s rest condition), with an additional 30s of rest at the end. Within each activation block one of four fixed contrast levels was presented such that a single run included all contrast levels across the 4 blocks (Figure 1A). To control for adaptation and order effects we performed 4 functional runs in which each run contained a block of each contrast level but with different permutations of the order of the blocks. The 4 stimulus runs were acquired for each subject, with the order of the runs randomized between subjects. Throughout both the rest and stimulus conditions a small white square containing a left or right-pointing arrow (< or >) was visible in the center of the screen. Orientation of the arrow reversed at random intervals throughout the scan. Subjects were instructed to respond via a button box when the orientation changed in order to ensure attention during scanning.

Stimulus calibration

Stimulus scripts were written in-house in Matlab (Natick, MA) with the Psychophysics Toolbox version 2 extensions (Brainard, 1997; Pelli, 1997). Since precise contrast levels were required, machine RGB values (i.e. laptop display settings) were calibrated against the luminance displayed on the screen. A Tektronix J-Series photometer (Richardson, TX) recorded luminance measurements and a gamma table was created for scaling RGB values.

The grayscale mid-point between pure black and white on the displayed image was determined to be a luminance of 518cd/m^2. This value was used for the isoluminant gray screen in the rest condition, and equidistant grayscale values were chosen above and below this value for the flickering checkerboard. For each contrast level, the differences in luminance between the two gray values of the flickering radial checkerboard were 11 cd/m^2 (contrast 1), 52 cd/m^2 (contrast 2), 94 cd/m^2 (contrast 3), and 1036 cd /m^2 (contrast 4). Expressed as a ratio of the difference in luminance of the two gray levels to the sum of the two luminance values (twice the baseline luminance), the corresponding contrast values were 0.0106, 0.0502, 0.0907, and 1.0.

Imaging protocol

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 arterial spin labeling (ASL) and BOLD-weighted images were acquired with a single-shot PICORE QUIPSS II (Wong et al., 1998) pulse sequence (TR=2.5 s, TI_1=600 ms, TI_2=1500 ms, 20-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=9.1 ms, TE_2=30 ms, flip angle=90°, field of view (FOV)=24 cm, 64 x 64 matrix). Four 7 mm-thick axial slices covering the visual cortex were acquired in a linear fashion from bottom to top. A high-resolution structural scan was acquired with an inversion recovery prepared 3D fast spoiled GRASS (IR-FSPGR) pulse sequence (124 axial slices, 1.3 mm slice thickness, TI=450 ms, TR=7.9 ms, TE=3.1 ms, flip angle=15°, FOV=25 x 25 x 16 cm, matrix 256 x 256 x 124). The anatomical images were collected immediately before the functional activation scans.

In addition to these scans, a resting-state CBF scan (200 seconds) was acquired at the scanning session for quantifying the baseline state. During the baseline scan, subjects viewed the same isoluminant gray screen with a stationary central white fixation point as in the functional runs. For conversion of the ASL data to absolute CBF units, a cerebrospinal fluid (CSF) reference scan and a minimum contrast scan were also acquired, as described previously (Perthen et al., 2008).

Throughout the imaging session physiological monitoring of the subjects' heart rate and respiratory rate was performed, using a pulse oximeter (INVIVO Research Inc, Orlando, FL) and a respiratory effort transducer (BIOPAC Systems, Goleta, CA). TTL pulses generated by the scanner were also recorded in order to align the physiological data to the image acquisition.

CBF and BOLD time series: Preprocessing and GLM analysis for ROI selection

The first four images (10 seconds) of the functional scans were excluded from data analysis to allow the MRI signal to reach a steady state. All functional runs were motion corrected and registered to the first functional run using AFNI software (Cox, 1996). The ASL data consists of alternating tag and control images, with two echoes acquired for each, and this data was processed to yield separate CBF and BOLD-weighted time series. For each voxel in the functional scans, a CBF time-series was computed by taking a running surround 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 represented a subtraction of tag from control images. A BOLD-weighted time series for the functional scans was computed from the running average of the second echo data, taking the average of each image with the mean of its two nearest neighbors (Liu and Wong, 2005).

Statistical analysis of the functional data was performed using a GLM approach for all functional activation data (Mumford et al., 2006; Restom et al., 2006). The stimulus-related regressor in the GLM was obtained by convolving the block design stimulus pattern with a gamma density function (Boynton et al., 1996). The block design stimulus pattern did not account for differences between the amplitudes of the responses to each contrast level (i.e. all blocks had equal amplitude). The measured cardiac and respiratory data were included in the GLM as regressors to account for the modulation of the ASL signal caused by physiological fluctuations (Glover et al., 2000; Restom et al., 2006). A constant and a linear term were used as nuisance regressors. Pre-whitening was performed using an autoregressive model (Burock and Dale, 2000; Woolrich et al., 2001). The data from the

four functional runs were concatenated for the GLM analysis as previously described (Restom et al., 2006).

An anatomical mask that included the visual cortex was drawn for each subject, and further analysis was restricted to this area. The visual cortex mask was defined by the parietal-occipital sulci and contained the primary visual cortex as well as supplementary regions. This anatomical mask was subsequently resampled to match the resolution of the functional images. Clusters of voxels exhibiting CBF activation within the mask were detected using an overall significance threshold of p=0.05 applied to the first echo data. Correction for multiple comparisons was performed using AFNI AlphaSim program (Cox, 1996). The set of activated voxels was then treated as the ROI for constructing individual average BOLD and CBF response curves for each of the contrast levels. To test for effects of potential bias due to voxel selection, a second ROI was produced by applying the same analysis to the BOLD time series, and taking the ROI as the intersection of the CBF and BOLD activation maps.

CBF and BOLD responses to visual stimulation

For each of the contrasts, the CBF and BOLD time series were cropped into segments 80 seconds in length consisting of a baseline (20 seconds) followed by contrast stimulus (20 seconds), and a rest period (40 seconds). The signal in the 20 seconds prior to the contrast stimulus was averaged and subtracted from the entire segment and used to normalize each block to represent fractional change from baseline. For each contrast level, the 4 blocks from the 4 runs were then averaged to obtain single-block average CBF and BOLD responses specific to a particular contrast level. The mean amplitude of each functional response was determined as the mean response over the final 10 seconds of the task period (4 time points in total). No spatial or temporal smoothing beyond the surround subtraction processing were used.

Baseline CBF determination

The CBF time series from the baseline scan were corrected for inhomogeneities in the coil sensitivity profile using the smoothed minimum contrast images (Wang et al., 2005), and converted to physiological units (mL/100mL/min) using the CSF image as a reference signal to determine the fully relaxed magnetization of blood (Chalela et al., 2000). The mean resting CBF for each subject was calculated by averaging the CBF time series over all time points and over all voxels within the particular ROI.

Detection of differences in CBF/$CMRO_2$ coupling between stimuli

The theoretical framework for relating $CMRO_2$ changes to BOLD and CBF responses is based on the model for the BOLD effect introduced by Davis and coworkers (Davis et al., 1998). Taking f as the ratio of the CBF in the active state to its baseline value, and n as the CBF/$CMRO_2$ coupling ratio during activation (e.g., a 40% change in CBF accompanied by a 20% change in $CMRO_2$ would correspond to n=2), the model for the BOLD signal change can be written as:

$$\frac{\Delta S}{S_0} = M\left[1 - f^{\alpha-\beta}\left(1 + \frac{f-1}{n}\right)^{\beta}\right] \quad [1]$$

The parameter M is a scaling factor for the BOLD effect that defines the range for the BOLD response, and primarily reflects the amount of deoxyhemoglobin present in the local brain region in the baseline state of the experiment but also depends on details of the image acquisition. The parameter β was originally assumed to be 1.5 based on numerical

simulations (Davis et al., 1998). The venous CBV change is modeled in terms of the CBF change with the assumption that $v = f^{\alpha}$, with $\alpha = 0.38$ based on studies in animal models of how total CBV varies with CBF (Grubb et al., 1974; Mandeville et al., 1998).

Although widely used, the Davis model potentially suffers from several limitations. Intravascular signal changes and volume exchange effects were not included in the derivation, and recent experimental studies suggest that the venous volume changes with activation are weaker than the overall CBV changes, corresponding to a smaller value of α (Kim et al., 2007; Chen and Pike, 2009). Recent studies at 3T have also used a smaller value of β, so that $\alpha = 0.2$ and $\beta = 1.3$ is becoming a common assumption (Chen and Pike, 2009; Mark et al., 2011). In a recent study from our group, we analyzed the potential errors in the Davis model by developing a detailed multi-compartment model for the BOLD effect that incorporated recent experimental results and specifically included additional factors such as hematocrit and the baseline oxygen extraction fraction (Griffeth and Buxton, 2011). The primary finding was that the mathematical form of Eq [1] models the BOLD response quite well, but the accuracy of the model was improved by optimizing the values of the parameters α and β. For measurements with a 3 Tesla field strength and with fractional venous CBV changes assumed to be half of the total fractional CBV change, the optimized values were $\alpha = 0.14$ and $\beta = 0.9$. Note that with this optimization, the model parameters do not correspond to their original physical derivations, because they end up doing double duty to capture effects that were left out of the original derivation.

For the current study the primary goal was to measure responses to different stimuli in the same brain region and with the same baseline state. Thus for each stimulus the value of M is the same, so that for each subject we can work just with ratios of the BOLD responses to stimuli with different contrast levels and eliminate the need to know M for each subject. Specifically, for two stimuli the weaker response is normalized to the stronger response for both CBF and BOLD signals. From Eq [1] we can predict the relationship between these ratios if the CBF/$CMRO_2$ coupling parameter n is the same for both sets of responses. The model parameters that need to be specified are α, β, n and the CBF change for the stronger stimulus (f_{max}). We tested the sensitivity of the prediction to these parameter values by varying them over the following ranges: $\alpha = 0.1–0.4$, $\beta = 0.9–1.5$, $f_{max} = 1.4–1.6$, and $n = 2–4$. An overlay of 50 different curves calculated by randomly combining values for the model parameters from these ranges is shown in Figure 2 for comparison with the experimental data. The prediction of the model is that the BOLD ratio should be larger than the CBF ratio, and that prediction is relatively insensitive to the values of the model parameters. This prediction essentially follows from the idea that there is a ceiling on the BOLD response, so that the BOLD effect saturates as the CBF change continues to increase. In short, the mathematical form of the model makes a definite prediction that can be tested experimentally in a way that does not require a calibration experiment and does not require precise knowledge of the values of the model parameters.

The analysis above provides a way to test for the variability of n without having to do the calibration experiment that would make it possible to measure specific values of n. Nevertheless, it is important to estimate the range of variation of the $CMRO_2$ response that would be required to explain the current data. In a recent study we used a calibrated BOLD methodology with hypercapnia to measure n in a similar subject population with the same acquisition and analysis methods, and with a visual stimulus similar to that used for contrast level 4 of the current study (Perthen et al., 2008). In that study the mean value of n was about 2.3. To estimate $CMRO_2$ changes in the current study we assumed that $n=2.3$ for contrast 4 for each subject, and then calculated the $CMRO_2$ response and corresponding n values for the other contrast levels.

Statistical analysis

Differences in the CBF and BOLD response ratios for contrast levels 1–3, each normalized to the response to contrast level 4, were tested for overall significance with a one way repeated measures ANOVA. Post hoc comparisons with two-tailed paired t-tests were used to identify significant changes, where there was a significant omnibus F-statistic. Significance was accepted at the $p<0.05$ level. Our goal was to test whether the coupling parameter n was different for the weakest and strongest stimuli. The theory predicts that if n is the same, the BOLD ratio will be larger than the CBF ratio, and we tested this directly.

Results

Measured responses

The average resting baseline CBF within the active region of interest across all subjects was 54.5±14.2 mL/100 mL/ min (mean ± SD). Robust functional CBF and BOLD responses were observed for all subjects. Figure 1 shows the different visual stimulus luminance contrast levels and the corresponding mean CBF and BOLD responses across subjects. Both BOLD and CBF mean responses increased in magnitude with increasing luminance contrast. In addition to the primary positive response, there was evidence for a post-stimulus undershoot that was most prominent in the responses to the strongest contrast level. A detailed analysis of the post-stimulus undershoot will be presented in a separate paper, and in the remainder of this paper we focus on the primary positive CBF and BOLD responses.

The means of the peak responses are presented in Table 1 for both the primary ROI selection method based on CBF activation, and also the alternate method based on combined CBF and BOLD activation. The results are similar, and the rest of the analysis was applied to the data derived with the CBF activation ROI. Note that the BOLD responses to the four contrast levels were all significantly different from each other (paired t-test, $p<0.05$), and all of the CBF responses were significantly different from each other with the exception of contrasts 2 and 3. Although the responses to the lowest contrast level are relatively weak, they are both significantly different from zero ($p=0.0012$ for the BOLD response; $p=0.00033$ for the CBF response) because of the averaging across runs and over the ROI for each subject.

Comparison of BOLD and CBF response ratios

For each subject BOLD and CBF responses for contrast levels 1–3 were each normalized to the responses to contrast level 4, and the means and standard errors are plotted in Figure 2. For all contrast levels the means fall below the line predicted for equal n, consistent with a reduced value of n for the weaker stimuli. As a conservative test, the BOLD ratio for contrast level 1 (0.23 ± 0.13, mean ± standard deviation) is significantly lower than the corresponding CBF ratio (0.42 ± 0.18, $p=0.0054$). This is a conservative test because the prediction of the model is actually that the BOLD ratio is somewhat larger, rather than equal to, the CBF ratio if the coupling ratios are the same. Note that it is often useful to define the dynamic range as the inverse of this ratio (i.e. the response to the highest contrast level divided by the response to the lowest contrast level). The primary experimental result of this study is the finding that the dynamic range of the responses, defined in this way, is significantly *larger* for the BOLD response (~4.3 fold) than for the CBF response (~2.4 fold).

Tests for bias in ROI selection

Although the two ROI selection methods used gave similar results (i.e., considering only CBF activation and considering combined CBF and BOLD activation), we were still concerned that a bias may be present related to the ROI selection method. To test for the presence of a bias, the responses to contrast levels 2 and 3 were pooled and used to select the

active ROI based on CBF activation levels. The averages over this ROI were then calculated for contrast levels 1 and 4. In this way, the primary data being compared were not used for ROI selection. Compared to the original ROI selected using data for all contrast levels, the mean BOLD ratio (contrast 1/contrast 4) changed from 0.229 to 0.233 and the mean CBF ratio changed from 0.418 to 0.414. In short, these tests suggest that minimal bias in the calculated ratios was introduced by the ROI selection method.

Estimates of $CMRO_2$ changes

To estimate $CMRO_2$ changes in the current study we assumed that n=2.3 for contrast 4 for each subject, and then calculated the $CMRO_2$ response and corresponding n values for the other contrast levels (Table 2). The patterns of variation of n with stimulus contrast are similar for the two different choices of α and β, with n~1.7 for the weakest stimulus when n=2.3 is assumed for the strongest stimulus (significant difference with p=0.00016 for the classic values of α and β, and p=0.00034 for the optimized values). Taking the individual ratios of the $CMRO_2$ responses to contrast levels 1 and 4 gave a mean ratio of 0.58 ± 0.25, a significant difference from a value of 1 with p= 0.0011. Taking the inverse of this number, the dynamic range of $CMRO_2$ response from weakest to strongest contrast levels was ~1.7.

Figure 3 summarizes the experimental data and the CBF/$CMRO_2$ coupling relationship required to explain the measurements. Figure 3A shows the mean responses, averaged over the final 10 s of the stimulus block, plotted in the CBF/BOLD plane. The basic pattern of the observations, with the BOLD response increasing more rapidly than the CBF response, differs markedly from the pattern of BOLD saturation expected for a fixed value of n, illustrated by solid lines. Instead, the data are consistent with increasing n as stimulus contrast increases. Figure 3B shows the CBF/$CMRO_2$ relationship derived from the assumption of a fixed n=2.3 for contrast level 4, showing more directly the departure from a fixed CBF/$CMRO_2$ coupling ratio. This behavior is consistent with a curvilinear relationship between CBF and $CMRO_2$ responses, as suggested by the dashed line in Figure 3B. The curve drawn through the data is the best fit of a power law relationship between the fractional CBF and $CMRO_2$ responses. This power law relationship was combined with the BOLD signal model (Eq [1]) to generate the fitting curve relating the CBF and BOLD responses shown in Figure 3A.

Discussion

Although primarily driven by large CBF changes associated with neural activity, the BOLD response is strongly affected by the CBF/$CMRO_2$ coupling ratio n, defined as the ratio of the fractional changes. In this study our primary finding was that the BOLD response was more strongly modulated than the CBF response as the luminance contrast of a visual stimulus was varied. We assessed the dynamic range of the responses, taken as the ratio of the response to the highest contrast level divided by the response to the lowest contrast level. The dynamic range for the BOLD response was ~4.3, yet for the CBF response this ratio was only ~2.4. Interpreted in terms of a model for the BOLD effect, this result is consistent with the coupling ratio n increasing from ~1.7 to ~2.3 with increasing stimulus contrast, a dynamic range for the $CMRO_2$ response of only ~1.7. These results indicate that when comparing responses in the same brain region to different stimuli, the BOLD response magnitude should not be taken as a quantitative reflection of the magnitude of the underlying physiological changes.

For the quantitative interpretation of the BOLD response we used the Davis model (Davis et al., 1998) of how the BOLD response depends on the underlying changes in CBF and $CMRO_2$. We showed that the basic framework of the Davis model provides a way to test whether the CBF/$CMRO_2$ coupling ratio n differs for two stimuli activating the same brain

region. Specifically, for two levels of CBF change, the predicted ratio of the BOLD responses (weaker/stronger) from the Davis model for constant n is a function of the corresponding CBF ratios but is nearly independent of the exact values of the model parameters. The parameter M is automatically eliminated by working with BOLD response ratios, and the exact values of α and β have only a minor effect on the prediction (see Figure 1). Importantly, the prediction of the model is that for constant n the BOLD ratio is larger than the CBF ratio. This predicted pattern does not depend on the details of the model, but rather simply follows from the ceiling effect on the BOLD response: as CBF continues to increase the BOLD response approaches a saturation level. Our primary experimental finding was the opposite pattern: the BOLD response ratio (strongest/weakest) was larger than the corresponding CBF response ratio.

Comparison with previous studies

Our general finding of a graded BOLD response to increasing stimulus contrast is in agreement with earlier studies using BOLD measurements alone (Boynton et al., 1996; Goodyear and Menon, 1998; Mohamed et al., 2002). Two previous studies have measured both CBF and BOLD responses in human visual cortex to varying stimulus contrast, although these studies were not specifically analyzed to test whether n varies with contrast level. Hoge and colleagues (Hoge et al., 1999a), in a pioneering application of the calibrated BOLD methodology, varied the contrast of a drifting grating and found results consistent with a constant value of n. In that study, the ROI was taken as all of area V1 as determined by a retinotopic mapping study, in contrast to the current study where CBF activation across stimuli was used for ROI selection. Previous work from our group suggested that the methodology for choosing an ROI can significantly affect the estimates of n, possibly through the inclusion of signal changes dominated by draining veins (Leontiev et al., 2007). For this reason, we chose an ROI based on CBF activation in order to avoid draining veins.

More recently, Chiarelli and colleagues (Chiarelli et al., 2007a) reported a study in which contrast of a flickering checkerboard was changed by altering the “white” level to different shades of gray. In that study, the BOLD response appears to reflect the usual rolling off with increased CBF, in contrast to the current results. That experiment differs from the current one in that both contrast and average luminance were changed, rather than fixed average luminance with varying contrast. In the Chiarelli study the ROI was chosen as the overlap of BOLD and CBF responses. When we reanalyzed our current data using this ROI approach, we observed similar results compared to the method of choosing an ROI based on CBF activation (Table 1).

Studies varying the flicker frequency of a visual stimulus found a divergence of CBF and $CMRO_2$ responses similar to those found here for contrast variation. Using PET methods, Vafaee and Gjedde found that both CBF and $CMRO_2$ increased with stimulus frequency up to 4Hz, but for 8Hz the CBF response continued to increase while the $CMRO_2$ response decreased (Vafaee and Gjedde, 2000). In a recent calibrated BOLD study, Lin and clleagues found a similar divergence of the CBF and $CMRO_2$ responses, with n increasing as the stimulus frequency increased up to 8Hz and then remaining approximately constant at a high value (Lin et al., 2008). The basic experimental approach, measuring CBF and BOLD responses to the stimuli, was similar to our current study. From their average responses to 8Hz and 1Hz stimuli, the ratio of the CBF responses was ~1.9 while the ratio of the BOLD responses was ~2.5. In our current study there was a greater divergence of the ratios of the responses to the largest and smallest contrast levels (~4.3 for the BOLD response and ~2.4 for the CBF response), but the pattern is similar: as the stimulus intensity (flicker frequency or luminance contrast) increases, there is a growing divergence between the CBF and $CMRO_2$ responses, described by an increasing value of n.

A previous study from our group found a higher CBF/$CMRO_2$ coupling ratio in visual cortex compared with basal ganglia (Ances et al., 2008). In that study the evoked CBF response in basal ganglia was weaker, and the current study suggests that part of the difference in the observed CBF/$CMRO_2$ coupling could be an overall dependence of n on the magnitude of the response. However, the earlier study also found a larger CBF response to hypercapnia in visual cortex compared to basal ganglia, suggesting the possibility of an overall greater vascular responsiveness in visual cortex. Finally, it is also worthwhile to note that the pattern of CBF/$CMRO_2$ coupling we found in visual cortex with varying stimulus contrast may not hold for other brain regions. An early study in sensorimotor cortex by Kastrup and colleagues (Kastrup et al., 2002) found the opposite pattern: a faster rise in $CMRO_2$ compared to CBF. For these reasons, our finding of an anomalous relation between BOLD and CBF responses requires further investigation in other cortical and subcortical brain regions.

In a recent study we investigated the effects of attention on the BOLD and CBF responses to a weak visual stimulus, and concluded that attention was associated with an overall stronger response, but a reduction in n (Moradi et al., 2012). That is, the response ratio (unattended/attended) was larger for BOLD than CBF, the opposite pattern to our current finding with increasing stimulus contrast. Put another way, the earlier result is consistent with a basic pattern in which an unattended stimulus evoked a small CBF response but little $CMRO_2$ response, and attention then increased CBF but also increased $CMRO_2$ much more. The current study suggests that as the contrast of an attended stimulus increases, the CBF increases more than the $CMRO_2$ response.

Limitations of the current study

Whenever the same data is used to both select active regions and quantitatively compare responses in that region, we need to be concerned about introducing bias. To test for a bias, we selected active regions in three ways. The primary method was based on averaging the CBF responses to all contrast levels and using the CBF activation as the criterion for ROI selection. We also used a combined CBF and BOLD activation criterion and found similar values for the BOLD and CBF responses. As a final test, we used only the responses to contrast levels 2 and 3 to select the active ROI, and then examined the mean response ratios for levels 1 and 4 (i.e., the data compared were not used for ROI selection). The mean BOLD and CBF ratios were virtually identical to the original analysis, suggesting that bias effects related to ROI selection were minimal.

In addition, though, our ROI selection method, based on activation patterns rather than retinotopy, is likely to include multiple visual areas. We have essentially viewed the ROI as a homogeneous area, so that the observed change in CBF/$CMRO_2$ coupling is interpreted as differential modulation of CBF and $CMRO_2$ as stimulus contrast increases. Conceivably, this effect could also arise if heterogeneous visual areas within the ROI have fixed but different CBF/$CMRO_2$ coupling ratios and changing stimulus contrast alters the relative involvement of these areas. Future studies with detailed retinotopic mapping would help to answer this question as well as address the question of whether this contrast effect is consistent across visual areas.

Only a stimulus duration of 20s was examined in this study. The BOLD response is a complex dynamic process, and there is evidence for early and later steady-state phases that could have a different dependence on stimulus contrast. For example, Hoge and colleagues found an initial BOLD overshoot that was less sensitive to luminance contrast than the subsequent steady state level (Hoge et al., 1999b). However, if the early BOLD response is a sharp increase in CBF independent of stimulus contrast, we would expect the average CBF response measured in our study to be less strongly modulated by stimulus contrast than the

$CMRO_2$ response, opposite to our finding. In addition, venous blood volume changes could have a different relationship with CBF changes in the early and later phases of the BOLD response (Mandeville et al., 1999). Increased venous blood volume would be mistakenly interpreted as an increase of $CMRO_2$, as both serve to increase deoxyhemoglobin. This could artifactually enhance the modulation of $CMRO_2$, but again our finding was a greater modulation of CBF than $CMRO_2$. In addition, recent animal experiments found that venous blood volume increases were only detected after 30s of stimulation (Drew et al., 2011; Kim and Kim, 2011). Our choice of a 20s duration was primarily chosen as a representative block size, in order to estimate the degree of involvement of variable CBF/$CMRO_2$ coupling in typical experiments. Nevertheless, future studies examining the dynamics of CBF and $CMRO_2$ will be important for addressing these issues.

Conclusions

In conclusion, these results emphasize the need to better understand the coupling of CBF and $CMRO_2$ changes with activation in order to quantitatively interpret the BOLD response. In addition to regional variations across the brain (Chiarelli et al., 2007a; Ances et al., 2008), the current data suggests that the coupling ratio n also can vary with the strength of the stimulus. For this reason, the relative magnitudes of the BOLD responses for different stimuli cannot be taken as a quantitative reflection of the relative changes in CBF or $CMRO_2$. It is interesting to note, though, that in the current study this effect actually enhanced the sensitivity of the BOLD response to differences in the stimuli compared with the CBF response, in the sense that the dynamic range of the responses from the lowest to highest contrast was 80% larger for BOLD compared to CBF. The divergence of CBF and $CMRO_2$ responses implied by the current data could potentially provide insights into the mechanisms by which aspects of neural activity drive changes in flow and metabolism. Further studies manipulating the evoked response through adaptation, tuning and inhibition effects will be important for exploring this idea.

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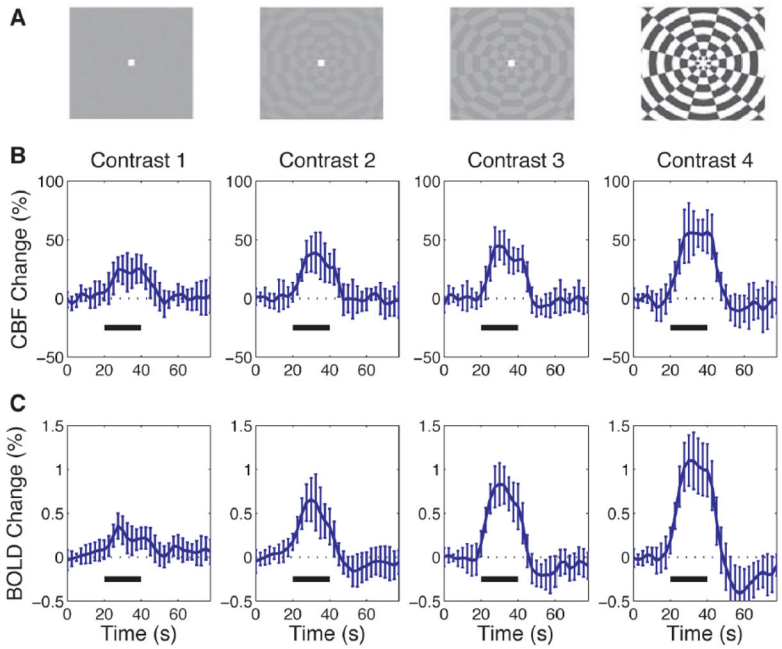

Figure 1. Measured CBF and BOLD responses to varying stimulus contrast

A) Stimuli were radial checkerboards with different luminance contrast flickering at 8 Hz, each presented in a 20s block followed by a 60s period of isoluminant gray with a fixation point. Four runs were measured on each subject, with each run containing one block of each of the four stimuli presented in random order. **B**) Average CBF responses for 9 subjects. **C**) Average BOLD responses for the same subjects. For all figures a solid black bar indicates the 20 second stimulus presentation and all error bars represent standard deviation across subjects. Both the CBF and BOLD responses increased as the stimulus luminance contrast increased. A post-stimulus undershoot was pronounced in the BOLD response to the highest contrast level, but much weaker in the CBF response.

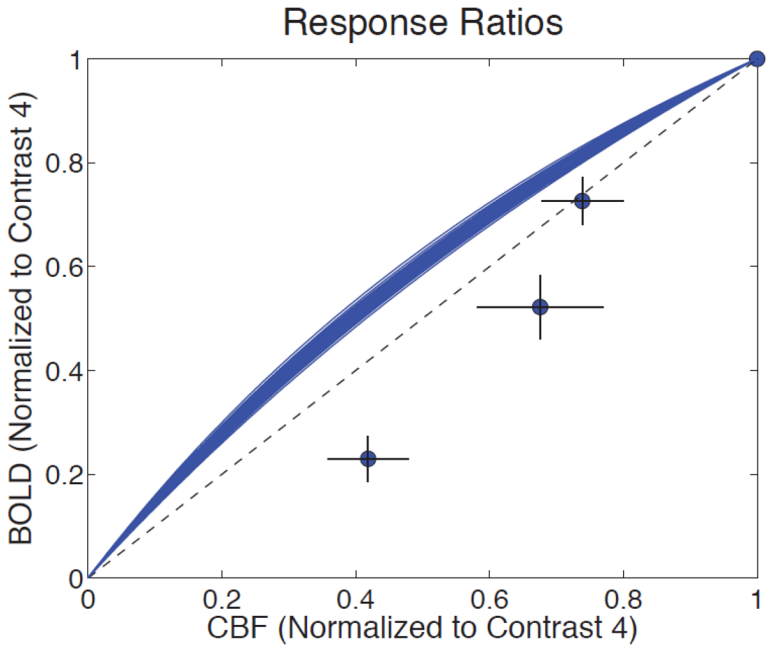

Figure 2. BOLD and CBF response ratios

For each subject the BOLD and CBF responses for contrast levels 1–3 were each normalized to the response to contrast level 4 and the mean values for the 9 subjects are plotted (error bars are the standard error of the mean). Both BOLD and CBF response ratios increase with increasing stimulus contrast. Theoretical predictions of the relationship between BOLD and CBF response ratios for a constant CBF/$CMRO_2$ coupling ratio are shown as a broad dark band, the compilation of 50 curves generated with Eq [1] for a range of model parameter values: α =0.1–0.4, β =0.9–1.5, maximum CBF response = 40–60%, CBF/$CMRO_2$ coupling ratio = 2–4. The data falling below the band indicates a reduced value of n for the weaker stimuli. For contrast level 1 (weakest response), the BOLD and CBF ratios are significantly different from each other (p=0.0054). Dashed line is the line of equality.

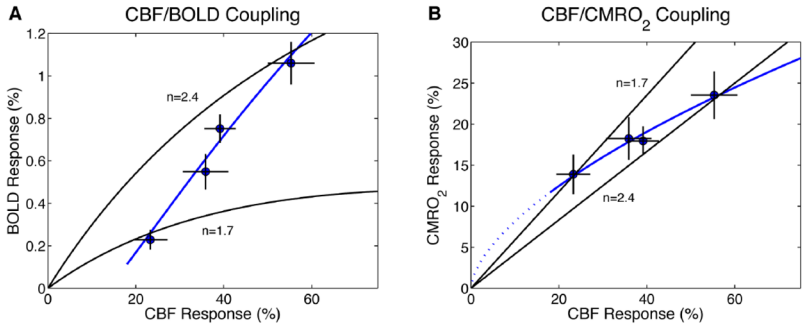

Figure 3. Coupling relationships based on the average data

The figure summarizes the relationships between CBF, $CMRO_2$ and BOLD responses to the 4 contrast levels. **A)** Measured BOLD response plotted as a function of the measured CBF response. The observed data are anomalous, in the sense that the curve does not show the characteristic saturation of the BOLD response expected for constant *n* as CBF increases. **B)** Calculated $CMRO_2$ response plotted as a function of the CBF response, based on Eq [1]. Also plotted (in blue) is the best-fit power-law through the data in (**B**) of the form $\%\Delta CMRO_2 = a(\%\Delta CBF)^k$ (with a=1.97 and k=0.62), with extrapolation of this power-law beyond the range of the data indicated by a dotted curve. Note that the extrapolation of this CBF/$CMRO_2$ coupling curve to small CBF changes would imply BOLD changes near zero or even negative values. Also shown in each plot are two curves corresponding to a fixed ratio *n* of the CBF and $CMRO_2$ changes. The calculated $CMRO_2$ values are based on Eq [1] with the assumption that $n=2.3$ for contrast level 4, based on an earlier calibrated BOLD study. These data are consistent with ~35% increase of *n* from the weakest to the strongest contrast stimulus. For both figures, error bars represent standard error of the mean for averaging over 9 subjects.

Table 1

Measured responses for different stimulus contrast levels for a region of interest (ROI) based on CBF activated voxels or combined CBF and BOLD activated voxels (mean ± standard deviation for 9 healthy subjects).

	ROI based on CBF activation		ROI based on combined CBF and BOLD activation	
Contrast	**BOLD response (%)**	**CBF response (%)**	**BOLD response (%)**	**CBF response (%)**
Level 1 (11 cd/m^2)	0.23 ± 0.14^{b,c,d}	23.3 ± 11.7^{b,c,d}	0.22 ± 0.12^{b,c,d}	23.6 ± 13.3^{b,c,d}
Level 2 (52 cd/m^2)	0.55 ± 0.25^{a,c,d}	35.9 ± 15.5^{a,d}	0.56 ± 0.24^{a,c,d}	36.0 ± 16.6^{a,d}
Level 3 (94 cd/m^2)	0.75 ± 0.20^{a,b,d}	39.1 ± 10.7^{a,d}	0.78 ± 0.22^{a,b,d}	40.0 ± 11.5^{a,d}
Level 4 (1036 cd/m^2)	1.06 ± 0.30^{a,b,c}	55.3 ± 15.9^{a,b,c}	1.11 ± 0.33^{a,b,c}	58.8 ± 15.6^{a,b,c}

^{a}significant difference with contrast 1 with $p < 0.05$

^{b}significant difference with contrast 2 with $p < 0.05$

^{c}significant difference with contrast 3 with $p < 0.05$

^{d}significant difference with contrast 4 with $p < 0.05$

Table 2

Estimated $CMRO_2$ responses and $CBF/CMRO_2$ coupling ratios (n) for different stimulus contrast levels (mean ± standard deviation for 9 healthy subjects), for two assumptions of the model parameters in Eq [1].

	α =0.4, β =1.5		**α =0.14, β =0.9**	
Contrast	**$CMRO_2$ response (%)**	***n* (dimensionless)**	**$CMRO_2$ response (%)**	***n* (dimensionless)**
Level 1 (11 cd/m^2)	13.8 ± 6.9[d]	1.75 ± 0.25[c,d]	15.2 ± 7.7[d]	1.63 ± 0.34[c,d]
Level 2 (52 cd/m^2)	18.3 ± 8.7	2.08 ± 0.53	19.3 ± 10.0	2.15 ± 0.99
Level 3 (94 cd/m^2)	18.1 ± 5.4[d]	2.21 ± 0.36[a]	18.3 ± 6.1	2.25 ± 0.64 [a]
Level 4 (1036 cd/m^2)	24.0 ± 6.9[a,c]	2.3 (assumed)	24.0 ± 6.9[a]	2.3 (assumed)

[a] significant difference with contrast 1 with $p < 0.05$

[b] significant difference with contrast 2 with $p < 0.05$

[c] significant difference with contrast 3 with $p < 0.05$

[d] significant difference with contrast 4 with $p < 0.05$