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The coupling of cerebral blood flow and oxygen metabolism with brain activation is similar for simple and complex stimuli in human primary visual cortex

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Abstract

Quantitative functional MRI (fMRI) experiments to measure blood flow and oxygen metabolism coupling in the brain typically rely on simple repetitive stimuli. Here we compared such stimuli with a more naturalistic stimulus. Previous work in primary visual cortex showed that direct attentional modulation evokes a blood flow (CBF) response with a relatively large oxygen metabolism ($CMRO_2$) response in comparison to an unattended stimulus, which evokes a much smaller metabolic response relative to the flow response. We hypothesized that a similar effect would be associated with a more engaging stimulus, and tested this by measuring the primary human visual cortex response to two contrast levels of a radial flickering checkerboard in comparison to the response to free viewing of brief movie clips. We did not find a significant difference in the blood flow-metabolism coupling (n=%ΔCBF/%Δ$CMRO_2$) between the movie stimulus and the flickering checkerboards employing two different analysis methods: a standard analysis using the Davis model and a new analysis using a heuristic model dependent only on measured quantities. This finding suggests that in the primary visual cortex a naturalistic stimulus (in comparison to a simple repetitive stimulus) is either not sufficient to provoke a change in flow-metabolism coupling by attentional modulation as hypothesized, that the experimental design disrupted the cognitive processes underlying the response to a more natural stimulus, or that the technique used is not sensitive enough to detect a small difference.

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Keywords

Calibrated BOLD; Functional MRI; Cerebral metabolic rate of oxygen ($CMRO_2$); Cerebral blood flow (CBF); Blood flow-oxygen metabolism coupling; visual cortex

1. Introduction

An interesting characteristic of neural activity is the divergent physiological responses of cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$). The brain's typical response to a stimulus involves a much greater CBF response than $CMRO_2$ response, and this is an essential component underlying the blood oxygen level dependent (BOLD) functional MRI (fMRI) signal (Fox and Raichle 1986, Lin, Fox et al. 2008). The ratio of the CBF and $CMRO_2$ responses is known as the coupling parameter, $n=\%\Delta CBF/\%\Delta CMRO_2$. Recent findings from our group and others suggest that not only do CBF and $CMRO_2$ change to different degrees, but their coupling in the brain is not constant depending instead both on the baseline state of the brain (Brown, Eyler Zorrilla et al. 2003, Perthen, Lansing et al. 2008, Griffeth, Perthen et al. 2011) and the stimulus (Lin, Fox et al. 2010, Moradi, Buracas et al. 2012, Liang, Ances et al. 2013, Moradi and Buxton 2013). These divergent responses suggest that, although they change in parallel, they are actually driven by separate mechanisms. For instance, neural activity may increase CBF by a feed forward mechanism through fast glutamate-mediated neural signaling associated with excitatory activity (Cauli, Tong et al. 2004, Hamel 2006, Cauli and Hamel 2010, Devor, Boas et al. 2012). Meanwhile $CMRO_2$ is likely a reflection of the overall evoked neural activity (associated with action potentials, postsynaptic effects of glutamate, and the cost of transporting ions) (Attwell and Iadecola 2002, Buxton 2010). If this is the case, changes in the type of neural activity or changes in the driving force behind the neural response could lead to changes in the coupling ratio. Here we examined the effect of a naturalistic stimulus in comparison to a simple repetitive stimulus on blood flow and oxygen metabolism coupling. We chose these stimuli to compare the typical fMRI experiment paradigm of the flickering checkerboard to a more naturalistic stimulus and to test whether this more engaging stimulus would alter the neurophysiological response and the coupling of blood flow and oxygen metabolism.

There is extensive literature examining the neurophysiologic response to flickering checkerboards at different frequencies, luminance and with different colors (Hoge, Atkinson et al. 1999, Vafaee and Gjedde 2000, Mohamed, Pinus et al. 2002, Lin, Fox et al. 2008), but the literature is sparser on how flow and metabolism in the visual cortex change in response to a more natural and complex stimulus. One study using a James Bond movie found maintained segregation and specialization of functional areas such that this complex movie stimulus and a simple block design stimulus both identified similar visual areas of the brain (Bartels and Zeki 2004). This was despite the brain having to respond simultaneously to many complex features of the movie. Other studies have found a significant level of voxel-by-voxel synchronization between individuals while watching a movie suggesting that these patterns of regional brain activation are preserved between subjects (Hasson, Nir et al. 2004, Jaaskelainen, Koskentalo et al. 2008). A third study concurrently measured the BOLD response and CBF using continuous arterial spin labeling (CASL) as subjects freely watched

a cartoon movie (Rao, Wang et al. 2007); this study found that CBF contrast provided higher statistical significance than BOLD contrast while confirming the maintained functional segregation of earlier studies. Notably this study relied on a single echo time (TE) to acquire BOLD and CBF data potentially biasing both data sets.

Here we compared the response in the human visual cortex to free viewing of a complex and engaging movie stimulus versus fixation on two contrast levels of a simple flickering checkerboard (10% and 40%) in order to test if the differences in these stimuli would create a difference in the coupling parameter. It was our hypothesis that the more complex movie stimulus would lead to recruitment of additional higher brain regions that would produce positive feedback on the visual cortex, increasing neural activity and $CMRO_2$. This would in turn lead to a reduction in the coupling parameter. We did not find this to be the case as there was no significant difference in the coupling of blood flow and oxygen metabolism between the movie and the flickering checkerboards.

2. Methods

2.1 Study design and acquisition

The study was performed on 16 healthy adults (9 female and 7 males, age 27.8±3.5) who had abstained from caffeine for at least 12 hours prior to study participation. The institutional review board at the University of California, San Diego approved the study, and written informed consent was obtained from all participants. Two scan sessions each consisting of three functional runs plus one functional localizer were performed for a total of 32 data sets (2 per subject). The functional runs were 6 min 20 sec long starting with a 45 s rest period followed by four cycles of a 20 s activation and a 55 s rest period followed by a final 35 s rest period. The activations switched between movie clips from “Earth: The Biography” (BBC Video) and either 10% contrast or 40% contrast black-white 8 Hz flickering radial checkerboards such that each stimulus type (movie, 10% and 40%) had a total of four 20 s blocks interspersed across the three runs to insure a consistent baseline state between the different stimuli. Contrast levels of 10% and 40% were chosen from preliminary data showing that these BOLD and CBF responses bracketed the response to the more complex movie stimulus. Rest periods consisted of a gray background with luminance normalized to that of the flickering checkerboards. Subjects were asked to fixate on a black cross in the middle of the screen during the baseline and flickering checkerboard tasks but not the movie stimulus. The functional localizer consisted of alternating 20 s periods of baseline with 20 s blocks of activation (either 100% contrast black-white 8 Hz flickering radial checkerboards or movie clips).

Using a spiral dual-echo ASL PICORE QUIPSS II (Wong, Buxton et al. 1998) pulse sequence, we simultaneously measured the CBF and BOLD responses to the stimuli during the three functional runs. For the single functional localizer a FAIR pulse sequence was used to increase sensitivity of the signal at the expense of quantitative accuracy, because FAIR does not control for the width of the tagged bolus. Sequence parameters included seven 6.8-mm slices with 0.2-mm gap aligned with the calcarine sulcus, TR 2.5 s, TI_1/TI_2 700/1500 ms, TE_1/TE_2 9.1/30 ms, 90° flip angle, FOV 240 mm, and matrix 64×64. A cerebral spinal fluid (CSF) reference scan and a minimum contrast scan were also acquired for use in

quantifying CBF as in Perthen et al. (Perthen, Lansing et al. 2008). A high-resolution anatomical image was acquired during each session using a magnetization prepared 3D fast spoiled gradient acquisition in the steady-state (FSPGR) pulse sequence with 172 sagittal slices, 1 mm slice thickness, TI 450 ms, TR 7.9 ms, TE 3.1 ms, 12° flip angle, FOV 25 cm, and matrix 256×256.

Cardiac pulse and respiratory effort data were monitored using a pulse oximeter (InVivo) and a respiratory effort transducer (BIOPAC), respectively. To synchronize the physiological data to the acquired images, scanner TTL pulse data were also recorded.

2.2 Image processing and general linear model analysis for ROI selection

The first four images of each ASL run were removed to allow the MRI signal to reach a steady state. These data along with the anatomical images were then registered to the first functional run using AFNI software (Cox 1996). Surround average (A) and difference (D) time courses were computed from the tag and control images as in Liu and Wong resulting in four time courses (two for each echo: A_{e1}, D_{e1}, A_{e2} and D_{e2}) (Liu and Wong 2005). The first echo difference data (D_{e1}) is closely related to CBF while the second echo average data (A_{e2}) is related to the BOLD signal. Noise regressors were identified from within the data itself using CompCor as described in (Behzadi, Restom et al. 2007). This process used a PCA analysis to identify five noise components from combined white matter and CSF data; additional nuisance regressors included constant and linear terms. Statistical analysis of the functional data was performed using a general linear model (GLM) approach to remove noise and identify an active region of interest (ROI) from the functional localizer data. Analysis occurred across the full data acquisition, and the stimulus-related regressor was obtained by convolving the block design stimulus pattern with a gamma density function (Boynton, Engel et al. 1996). To confirm our results were not dependent on the CompCor method, an alternate analysis was done in which the measured cardiac and respiratory data were used in the GLM as regressors rather than the five CompCor determined regressors.

An anatomical mask was drawn for each subject as triangular sections of the posterior third of the brain to include the visual cortex, and additional analysis was restricted to this area to avoid inclusion of other neurologic areas activated by the movie stimulus. Since our goal in this analysis was to identify an active ROI based on CBF data, only voxels passing a threshold of 40% of the mean baseline D_{e1} across the whole brain in the localizer run were included in the mask; this also increased the likelihood of including gray matter over white matter due to the higher CBF in gray matter, and of avoiding sulcal draining veins in the ROI. Analysis was further limited to voxels exhibiting a minimum signal to noise ratio of 200 in A_{e1} to avoid regions with low MR signal. A ROI was defined within this mask as voxels exhibiting activation in the first echo difference data of the functional localizer run. The desired ROI size was set to 100±10 voxels since the level of CBF change varies across subjects; this was achieved by adjusting the acceptable per-voxel p-value down from a max of p=0.01 until the desired ROI size was reached. Based on these studies and previous studies with a similar protocol, we have found that 100 voxels is reasonably representative of the active region size (Perthen, Lansing et al. 2008, Griffeth, Perthen et al. 2011). Voxels were also required to be in clusters of a size consistent with the whole cluster passing a

significance threshold of $\alpha \leq 0.05$ determined using AFNI AlphaSim (Cox 1996). Four scan sessions from three subjects were eliminated, as the number of active voxels did not reach this threshold. For summary statistics, the baseline was averaged over the 10 s prior to the start of the stimulus and the stimulus response was averaged over the last 10 s of the stimulus. To test whether our method of ROI determination biased our results, we also analyzed our data using a combined BOLD/CBF ROI.

2.3 Calculating BOLD and CBF responses

The source of the BOLD signal response is changes in blood oxygenation, which results in changes in the apparent rate of signal decay, R_2^*. The problem that usually confounds the interpretation of slow modulations in the BOLD signal is that it is sensitive to scanner drifts. To minimize this source of error, we directly calculated R_2^*. After averaging over the ROI, R_2^* was calculated from the surround average data (A_{e1} and A_{e2}) by modeling these signals as $A = A_0 \exp(-TE \cdot R_2^*)$ where TE_1 and TE_2 are known and A_0 is the theoretical signal at $TE = 0$. For display and analysis, we then calculated an equivalent BOLD response as a percent signal change using the definition $BOLD(t) = \exp(-TE_2 \cdot \Delta R_2^*(t)) - 1$ where ΔR_2^* is the change in R_2^* from the baseline or stimulus off period (Perthen, Lansing et al. 2008).

CBF time series were computed using the same signal model to determine the tag/control difference in the net magnetization (D_0) from the D_{e1} and D_{e2} time series. This net signal is proportional to the arterial spins delivered to the voxel (Liu and Wong 2005). Inhomogeneities in the coil sensitivity profiles were corrected using the smoothed minimum contrast images (Wang, Qiu et al. 2005) and then were converted to physiological units (mL/100mL/min) using the CSF as a reference signal (Chalela, Alsop et al. 2000).

Stimulus response averaging was performed over 5 time points from the 3rd time point (7.5s) after the stimulus was turned on to the 7th. Since we used the surround subtraction and average method, the last time point of the stimulus on period was not averaged into the stimulus response, because it is averaged with a time point for which the stimulus has been turned off for one TR. The undershoot was quantified using 4 consecutive time points from 10s to 17.5s after the stimulus was off.

To calculate $CMRO_2$ from normalized CBF and BOLD data, the Davis model (Davis, Kwong et al. 1998) was used:

$$BOLD(\%) = M(1 - f^{\alpha-\beta} r^{\beta}) \quad \text{(Eq. 1)}$$

This model describes the BOLD response as a function of the normalized (activation/baseline) values of CBF (f) and $CMRO_2$ (r). Values for the parameters $\alpha = 0.13$ and $\beta = 0.92$ were taken from a more detailed four compartment model of the BOLD response that includes effects left out of the original derivation including intravascular signal changes, volume exchange effects due to variation in blood volume, and unequal distribution of blood volume changes between vascular compartments (Griffeth and Buxton 2011, Griffeth, Blockley et al. 2013). The scaling parameter, M, was assumed to be 11.6% using hypercapnia data from similar subjects and adjusted for these values of α and β (Griffeth,

Perthen et al. 2011). To test whether bias in the Davis model parameters α, β or *M* would affect our conclusions, we also analyzed our data with the assumed value of *M*±30% with the same values of α and β, and with the following two sets of parameter values: α=0.2 and β=1.3 with *M*=8.6% (most common form currently used) or α=0.38, β=1.5 with *M*=8.5% (original Davis model) (Griffeth, Perthen et al. 2011). All BOLD, CBF, and $CMRO_2$ responses were expressed as a percent change from the pre-stimulus baseline and denoted %ΔBOLD, %ΔCBF, and %Δ$CMRO_2$.

To more directly examine the effects of different stimuli on the coupling of CBF and $CMRO_2$, we used a heuristic model we recently developed (Griffeth, Blockley et al. 2013). This model maintains the non-linear dependence of the BOLD signal on flow, reduces the number of parameters from three to two, and directly incorporates the coupling parameter, *n*. This simple model was inspired by work with the much more detailed model (Griffeth and Buxton 2011), which appeared to produce a very smooth BOLD surface across the CBF-$CMRO_2$ plane suggesting that the parameters α and β of the Davis model may be over-fitting the data. This new equation is:

$$BOLD(\%)=M(1-1/f)(1-\alpha_v-1/n) \quad \text{(Eq. 2)}$$

where *f* is the normalized CBF change and α_v is the exponent relating the CBF change to the venous CBV change. The power of this new model is that the coupling of CBF and $CMRO_2$ expressed as *n* can be directly compared without knowing the scaling parameter *M*. Instead, by creating a null hypothesis that *n* is the same for two stimulus types, the ratio of the two BOLD signals becomes:

$$BOLD_x/BOLD_{ref}=(1-1/f_x)/(1-1/f_{ref}) \quad \text{(Eq. 3)}$$

By performing a two-tailed paired t-test comparing the left and right sides of this equation, this hypothesis can be tested. If these ratios are significantly different, then the coupling parameter between the two stimulus types is different as long as α_v remains constant between the two stimulus types.

3. Results

We measured the BOLD and CBF responses to short movie clips and flickering checkerboards at both 10% and 40% contrast (Fig. 1). The two responses to the more complex movie stimulus were bracketed by the responses to the simpler flickering checkerboard. The BOLD stimulus responses and undershoots (mean ± standard deviation) for each stimulus type were as follows: 10% contrast (activation: 0.80±0.47%, $p<0.001$ and undershoot: −0.09±0.23, $p=0.048$), 40% contrast (activation: 1.3±0.49, $p<0.001$ and undershoot: −0.36±0.24, $p<0.001$) and movie (activation: 1.1±0.39, $p<0.001$ and undershoot: −0.35±0.28, $p<0.001$). The activation responses were all significantly different from one another: 10% contrast vs. 40% contrast ($p<0.001$), 10% contrast vs. movie stimulus ($p=0.001$) and 40% contrast vs. movie stimulus ($p<0.001$). The 10% contrast undershoot was significantly different from both the 40% contrast and movie stimulus (both $p<0.001$), but the 40% contrast undershoot was not significantly different than the movie stimulus undershoot ($p=0.84$).

The CBF responses for each stimulus type were: 10% contrast (activation: 18.6±16.1%, $p<0.001$ and undershoot: −1.4±8.0, $p=0.37$), 40% contrast (activation: 39.6±18.9, $p<0.001$ and undershoot: −7.8±8.3, $p<0.001$) and movie (activation: 29.0±16.2, $p<0.001$ and undershoot: −5.1±13.0, $p=0.048$). The CBF response to the 10% contrast was significantly different than the 40% contrast and movie stimulus responses ($p<0.001$ and $p=0.006$ respectively). Similarly the 40% contrast CBF response and movie response were also significantly different ($p=0.014$). For the CBF undershoots only the 10% contrast and 40% contrast differences reached significance ($p=0.001$) while the movie stimulus undershoot was not significantly different than either the 10% contrast or 40% contrast ($p=0.21$ and $p=0.39$ respectively). Similar results were found when the data were analyzed using a combined BOLD/CBF ROI and also with GLM regressors calculated from measured cardiac and respiratory data were rather than being calculated with CompCor.

Using the optimized Davis model, we also estimated the $CMRO_2$ responses to the three stimulus types (Fig. 2); lines corresponding to different values of n were plotted using the optimized Davis model and assumed value of M (11.6%). We found a significant increase in all the $CMRO_2$ responses ($p<0.005$) as well as significant differences from 10% contrast (6.7±11.0%) to 40% contrast (16.6±12.3%, $p<0.001$) and to the movie stimulus (11.8±10.2%, $p=0.031$). However there was not a significant difference between the movie $CMRO_2$ response and 40% contrast ($p=0.10$). The coupling of blood flow and oxygen metabolism for the three stimulus types is $n=2.79$ (10% contrast), $n=2.39$ (40% contrast), and $n=2.47$ (movie stimulus). Although differing by as much as 15%, no significant difference was found between these values of n (Fig 2).

Varying the assumed value of M by ±30% with the same values of α and β resulted in large changes in $CMRO_2$, but this did not affect the relationship of $CMRO_2$ between the states with the exception that for a much lower $M=8.1\%$ the difference between the increase in $\%\Delta CMRO_2$ from the movie stimulus compared to 10% contrast did not reach statistical significance ($p=0.09$). Using the more commonly employed form of the Davis model ($\alpha=0.2$ and $\beta=1.3$), the values of $\%\Delta CMRO_2$ are again similar: 10% contrast (6.7±10.9%), 40% contrast (16.4±12.1%), and movie clips (11.7±10.0%) with the 10% $CMRO_2$ response again reaching significance in comparison to both the 40% response ($p<0.001$) and the movie stimulus ($p=0.03$). The movie $CMRO_2$ response was not significantly different from 40% contrast ($p=0.11$). Similarly n was not found to differ significantly between the different stimuli when using the original Davis model, a combined BOLD/CBF localizer, or physiological noise regressors rather than CompCor regressors in the GLM.

We also tested whether there may be a difference in the flow-metabolism coupling using a new heuristic model (Griffeth, Blockley et al. 2013). We created a null hypothesis that there is no difference in the coupling parameter from the reference of 40% contrast to either the 10% contrast or movie stimulus responses. Looking within the same ROI, we tested this hypothesis by taking the ratio of the BOLD signals and the nonlinear combination of CBF signals (Eq [3]). This results in the scaling parameter, A, and coupling parameter term canceling. Again, we found no difference in these ratios; they fell close to the equality line (movie vs. 40%, $p=0.67$ and 10% vs. 40% $p=0.37$, Fig. 3). When the BOLD-CBF

intersection ROI was used, the results again showed a non-significant difference in *n* although the data points fell below the line of identity.

Unfortunately with regard to the undershoot data, there was a great deal of noise when application of the ratio method was attempted. This was due to the variation in the responses between the stimulus types leading to a great deal of scatter in the BOLD and blood flow ratios for different subjects. For this reason, it was not possible to draw any significant conclusions about $CMRO_2$ during the undershoot period.

4. Discussion

The relationship between evoked changes in cerebral blood flow and oxygen metabolism was initially of interest as the primary origin of the BOLD response, but recent findings suggest that variations in the balance of CBF and $CMRO_2$ is interesting in itself as a physiological phenomenon varying with the ROI, stimulus type, stimulus intensity, and baseline state of the brain (Hyder, Rothman et al. 2002, Brown, Eyler Zorrilla et al. 2003, Stefanovic, Warnking et al. 2006, Chiarelli, Bulte et al. 2007, Ances, Leontiev et al. 2008, Lin, Fox et al. 2008, Qiu, Ramani et al. 2008, Chen and Parrish 2009, Donahue, Blicher et al. 2009, Lin, Fox et al. 2010, Griffeth, Perthen et al. 2011). Most fMRI studies examining this relationship in the primary visual cortex use flickering radial checkerboards, because they are simple and produce robust responses (Hoge, Atkinson et al. 1999); whether these results are an accurate representation of how CBF and $CMRO_2$ respond to natural stimuli in the visual cortex remains to be answered, especially in light of studies showing differences in the neural response due to image component (specifically figure-ground) segregation (Lamme 1995, Zipser, Lamme et al. 1996), attention (Motter 1993, Ito and Gilbert 1999, McAdams and Reid 2005, Pooresmaeili, Poort et al. 2010, Ayzenshtat, Gilad et al. 2012), and the visual correlate of working memory (Super, Spekreijse et al. 2001). Our study design sought to remove the baseline state effects to isolate just the evoked responses by interspersing the stimulus types across the three functional runs and by studying young, healthy subjects who had refrained from caffeine consumption prior to the study.

In this study, we did not find a significant difference in the flow-metabolism coupling between the movie stimulus and the 10% or 40% contrast flickering checkerboards, based on two different analysis methods: a standard analysis using the Davis model and a new analysis using a heuristic model dependent only on measureable quantities. Figure 2 comparing the BOLD, CBF and $CMRO_2$ responses between these stimulus types shows that they appear to follow a single coupling parameter line as determined by the optimized Davis model (with M=11.6%). We confirmed this finding using the *ratio method*, which is based on the heuristic BOLD model for comparing flow-metabolism coupling between stimuli within the same ROI. These results do not support our hypothesis that a complex stimulus would result in top-down modulation of neural activity thereby altering the balance between CBF and $CMRO_2$ changes; however our results suggest the reliance on simple visual stimuli in fMRI experiments is reasonable and is also consistent with previous findings that stimulus type does not affect coupling in the human primary visual cortex (Hoge, Atkinson et al. 1999). Comparison of the 10% contrast to the 40% contrast also showed no significant difference in the coupling. Combined with previous findings showing increasing *n* with

increasing stimulus contrast from 1%, 4%, 9% and 100% this may reflect a ceiling effect on the CBF response as the stimulus intensity increases (Liang, Ances et al. 2013).

One draw back of our experimental design is that the 20s block stimuli could potentially disrupt the cognitive process that occurs in response to a natural situation, and could be a reason we did not measure a difference in the flow-metabolism coupling. It is also possible that the indirect attentional modulation due to the greater inherent interest of the movie clips was not enough to alter the coupling. In comparison, another experiment by our group directly and purposefully altered attention; in this study it was found that increased attention resulted in increased modulation of $CMRO_2$ and decreased the coupling ratio *n* (Moradi, Buracas et al. 2012). In the current experiment, subjects were told to freely watch the movie while during the flickering checkerboard they were told to fixate on a cross in the middle of the screen. It may be that this fixation command required a similar level of attention as the movie stimulus even if the movie clips were of greater interest. It is also possible that the technique used was not sensitive enough to detect a small 15% difference in *n* and that more subjects would be needed to test the hypothesis.

Another limitation of this study was that the scaling parameter, *M*, was not measured directly for each subject. This means that the absolute values of *n* for each subject were not determined. However, a feature of the current experimental design is that the coupling ratios for two stimuli can be compared even if *M* is not measured. The key to this is that the stimulus responses are all measured from the same baseline state for all responses measured in a subject. We tested this by varying the assumed value of *M*, but the most direct implementation of this idea is the ratio method. In short, with this approach the absolute values of *n* are not well determined, but with each subject serving as their own control, the presence of a different coupling ratio for different stimuli can be detected (Griffeth, Blockley et al. 2013). While this approach was appropriate for the current study goals, in general a calibration experiment is important for quantitatively assessing oxygen metabolism. An alternative to the standard hypercapnia experiment currently being developed for determining *M* without inhaled gases is measurement of R_2', which is a value closely related to *M* and the baseline brain state (Blockley, Griffeth et al. 2012). Future work combining measurements of *M* or R_2' with baseline blood flow and blood volume will potentially provide quantitative information about the dynamics of oxygen metabolism. This will include information on both the intrinsic evoked response studied here and also the baseline state of $CMRO_2$ that has been pursued in other studies (Griffeth, Perthen et al. 2011, Hyder, Herman et al. 2011).

Also of note in this study are the significant CBF undershoots for the 40% contrast and movie responses. It is interesting such large CBF undershoots have not been found before even within our group using similar stimuli (Griffeth, Perthen et al. 2011, Liang, Ances et al. 2013). This could be a reflection of improving techniques for measuring CBF or selection of ROI based on the CBF response. Unfortunately the noise in these measurements was not such that the *ratio method* could be applied in order to compare the CBF-$CMRO_2$ coupling in this response period. These results warrant further examination in future work.

5. Conclusions

Given that many studies are done with simple flickering checkerboards we sought to test whether there would be any difference in the evoked coupling of CBF and $CMRO_2$ changes with a complex and natural movie stimulus. Contrary to our hypothesis, no significant difference was found in the coupling of blood flow and metabolism in the visual cortex between free viewing of brief movie clips and fixation on a flickering checkerboard. It is possible that the top-down modulation due to factors such as attention, visual working memory and image component segregation was simply not sufficient to provoke such a modulation. Another possibility is that the study design itself with short block stimuli disrupted the cognitive processes underlying the response to the more natural stimulus. Nevertheless, these results support continued use of simple visual stimuli to examine primary visual cortex responses using block design experiments.

Abbreviations

(A_{e1}, A_{e2})	echo 1 and 2 average signal
(ASL)	arterial spin labeling
(BOLD)	blood oxygenation level dependent signal
(CASL)	continuous arterial spin label
(CBF)	cerebral blood flow
($CMRO_2$)	cerebral metabolic rate of oxygen
(CSF)	cerebral spinal fluid
(D_{e1}, D_{e2})	echo 1 and 2 difference signal
(FAIR)	flow alternated inversion recovery
(fMRI)	functional magnetic resonance imaging
(FOV)	field of view
(FSPGR)	fast spoiled gradient acquisition in the steady-state
(GLM)	general linear model
(MRI)	magnetic resonance imaging
(PCA)	primary component analysis
(PICORE)	proximal inversion with a control for off resonance effects
(QUIPSS II)	quantitative imaging of perfusion using a single subtraction version II
(ROI)	region of interest
(TE)	echo time
(TI)	inversion time
(TR)	repetition time

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Highlights

- Visual cortex responses to flickering checkerboards and movie clips were compared.
- Combined BOLD and cerebral blood flow measurements (CBF) were acquired.
- Evoked coupling of CBF and $CMRO_2$ changes were compared using two methods.
- The methods used were the well-established Davis model and a new ratio method.
- Similar responses detected in coupling of movie clips and flickering checkerboards.

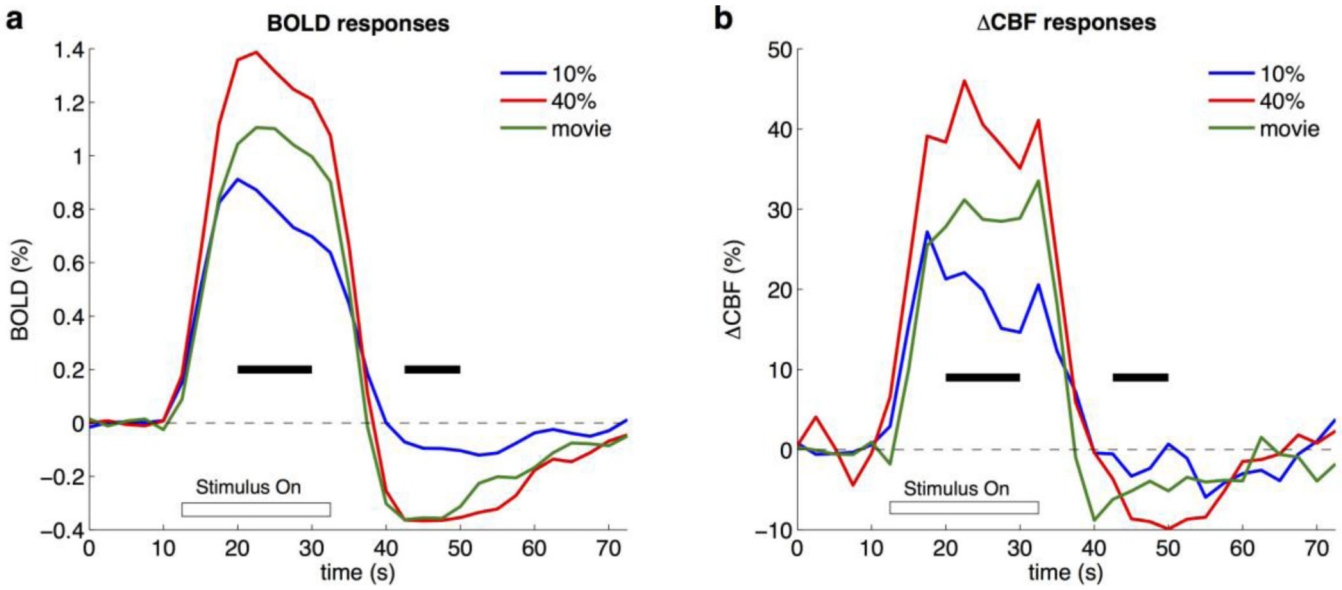

Figure 1. Average fractional changes in BOLD and CBF in response to for 10% contrast (blue), 40% contrast (red) and movie stimulus (green)

Evoked responses and undershoots are considered relative to the mean baseline preceding the stimulus. The BOLD (a) and CBF (b) stimulus responses and undershoots for each stimulus type are displayed.

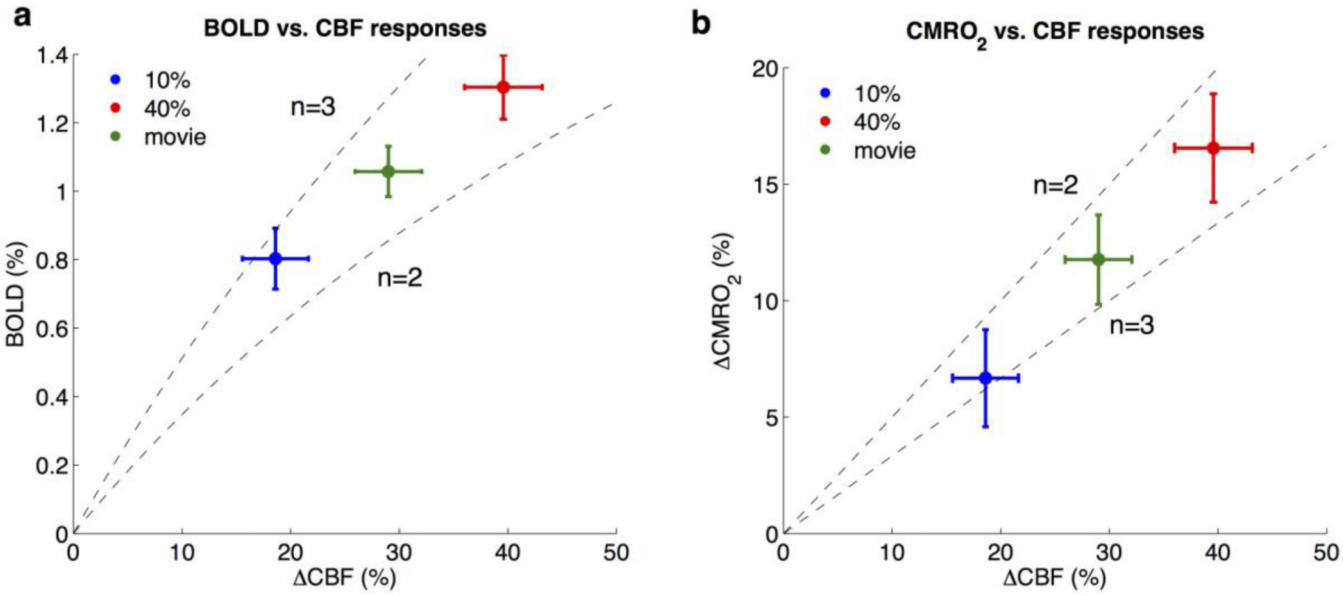

Figure 2. Measured BOLD and calculated $CMRO_2$ plotted against CBF for the three stimulus types

Crossbars on the data points represent standard error of the mean for CBF (horizontal) and either BOLD or $CMRO_2$(vertical). (a) Average BOLD and CBF data are plotted for the three stimulus types. (b) Again using the optimized Davis model, $CMRO_2$ responses were calculated. Lines for *n* are plotted using the optimized Davis model with M=11.6, α=0.13 and β=0.92. The data for the three stimulus types are not significantly different: 10% contrast (n=2.79, vs. 40% p=0.65), 40% contrast (n=2.39, vs. movie p=0.42) and movie stimulus (n=2.46, vs. 10% p=0.87).

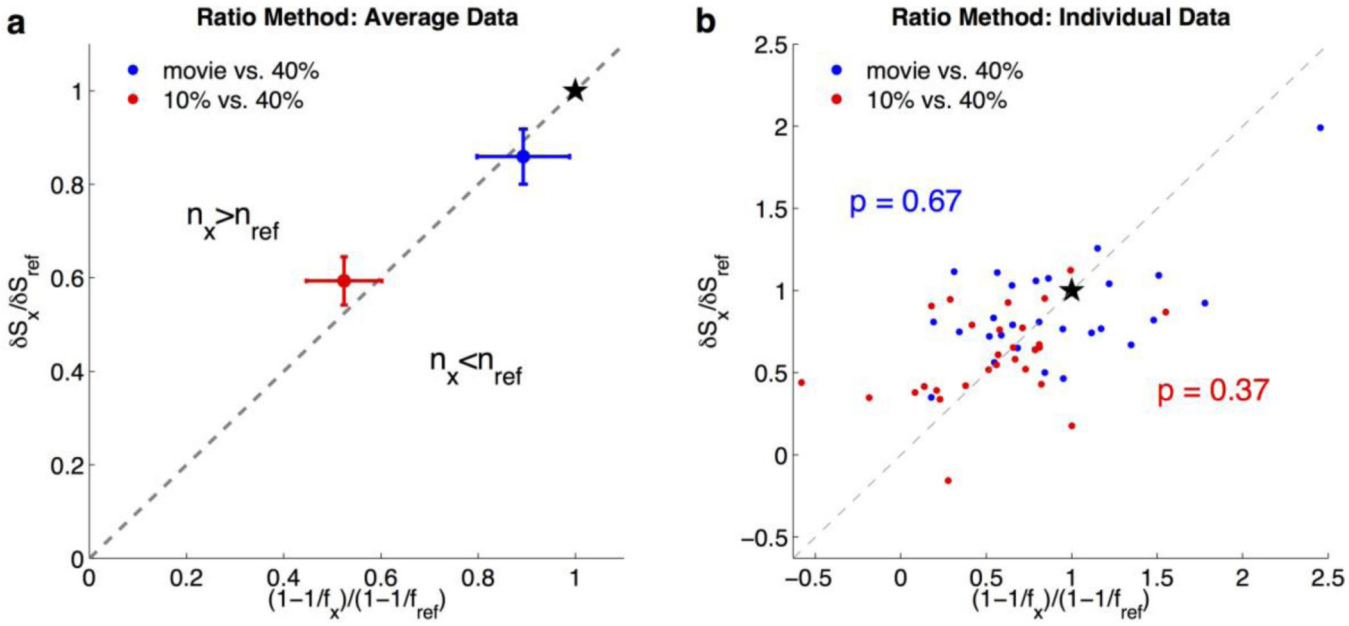

Figure 3. ***Ratio method*** **for comparison of CBF-CMRO$_2$ coupling**

In this figure the subscripted 'x' corresponds to the test state (either 10% contrast or movie stimulus) while 'ref' corresponds to the reference state (40% contrast). The star in each figure represents the case in which the BOLD and CBF responses to the test state and reference state are the same. The dashed black equality line represents the null hypothesis that *n* is the same between the two states; data points fall above the line when the test state has a higher *n* than the reference and below the line when the test state has a lower *n*. (a) Comparison of the average BOLD and non-linear CBF ratios. Error bars represent the standard error of the mean. (b) Comparison of the individual subject BOLD and non-linear CBF ratios. Using the ratio method no difference was found between the CBF-CMRO$_2$ coupling of the 40% contrast checkerboards in comparison to either the movie stimulus or 10% contrast checkerboards.