ELSEVIER

NeuroImage

www.elsevier.com/locate/ynimg
NeuroImage 23 (2004) 148–155

# Coupling of cerebral blood flow and oxygen consumption during physiological activation and deactivation measured with fMRI

Kâmil Uludağ,* David J. Dubowitz, Elizabeth J. Yoder, Khaled Restom, Thomas T. Liu, and Richard B. Buxton

*0677 Center for Functional MRI, Department of Radiology, University of California, San Diego, La Jolla, CA 92093-0677, USA*

Received 7 January 2004; revised 7 May 2004; accepted 11 May 2004

**The physiological basis of the blood oxygenation level dependent (BOLD) signal and its dependence on baseline cerebral blood flow (CBF) were investigated by comparing responses to a visual stimulus after physiological changes of the baseline. Eight human subjects were imaged with 3 and 4 T MRI scanners, and both BOLD signal and CBF were simultaneously measured. Subjects viewed a flickering radial checkerboard in a block design experiment, alternating between eyes open or closed during the off periods. Compared to a baseline state with eyes open in a darkened room, substantial deactivation (average change: 2.9 ± 0.3% BOLD, 22 ± 2.1% CBF) in the occipital cortex was observed when the eyes were closed. The absolute response during stimulation (average change: 4.4 ± 0.4% BOLD, 36.3 ± 3.1% CBF) was independent of the preceding resting condition. We estimated the fractional change in CBF to be approximately 2.2 ± 0.15 times greater than the fractional change in metabolic rate of oxygen ($CMRO_2$). The changes in CBF and $CMRO_2$ were consistently linearly coupled during activation and deactivation with CBF changes being between approximately 60% and 150% compared to baseline with eyes open. Relative to an assumed baseline oxygen extraction fraction (OEF) of 40%, the estimated OEF decreased to 33 ± 1.4% during activation and increased to 46 ± 1.2% during rest with eyes closed. In conclusion, we found that simply closing the eyes creates a large physiological deactivation in the visual cortex, and provides a robust paradigm for studying baseline effects in fMRI. In addition, we propose a feed-forward model for neurovascular coupling which accounts for the changes in OEF seen following baseline changes, including both the current physiological perturbations as well as previously reported pharmacologically induced changes.**


*Keywords:* Cerebral blood flow; Oxygen metabolism; Neurovascular coupling; fMRI; Deactivation; Visual stimulation

Functional magnetic resonance imaging (fMRI) to map brain activation relies on the detection of hemodynamic changes that accompany neural activity. The primary observation is that with increased neuronal activity, cerebral blood flow (CBF) increases substantially, while the cerebral metabolic rate of oxygen ($CMRO_2$) increases only moderately, so the oxygen extraction fraction (OEF) is reduced (Fox and Raichle, 1986). For example, in visual stimulation experiments, a CBF increase of up to 50% is observed, whereas the $CMRO_2$ increase is only approximately 25% (Hoge et al., 1999a). Most functional MRI techniques are sensitive either to the decreased OEF, through blood oxygenation level-dependent (BOLD) signal increases (Kwong et al., 1992; Ogawa et al., 1990), or to the increased CBF, through arterial spin labeling methods (Calamante et al., 1999; Wong et al., 1997). More rarely, decreases in BOLD signal and CBF are observed. Increased BOLD or CBF signals are usually interpreted as activation and decreased signals as deactivation. As commonly used, the terms activation and deactivation are relative terms (Gusnard and Raichle, 2001), so the choice of an appropriate task to define the neural activity of the baseline condition plays a critical role in an fMRI experiment (Stark and Squire, 2001).

The relationship between CBF and $CMRO_2$ during activation and deactivation is still under investigation (Buxton and Frank, 1997; Hyder et al., 1998). A convenient way to empirically characterize this relationship is with the ratio $n$ of the fractional change in CBF to the fractional change in $CMRO_2$ with activation. If $n > 1$, then the oxygen extraction fraction decreases with activation. Hoge et al. (1999a) have shown with fMRI that during graded visual stimulation the data was well-described with $n = 2$, and similar results have been reported by other groups ($n = 3$, Davis et al., 1998; $n = 2$, Marrett and Gjedde, 1997; $n = 2–5$, Kastrup et al., 2002; $n = 3$, Kim et al., 1999; $n = 3$, Seitz and Roland, 1992). However, some earlier investigations using PET techniques have reported smaller changes in $CMRO_2$ ($n = 6$, Fox and Raichle, 1986; $n = 10$, Fox et al., 1988) or even no change during stimulation ($n = \infty$, Fujita et al., 1999). It is not clear whether the variability of these experimental results is due to variability of the underlying physiology or to errors of the measurement techniques. The previous fMRI studies of coupling between CBF and $CMRO_2$ have investigated activation from the

* Corresponding author. 0677 Center for Functional MRI, Department of Radiology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0677.

*E-mail address:* kuludag@ucsd.edu (K. Uludağ).

**Available online on ScienceDirect (www.sciencedirect.com.)**

1053-8119/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2004.05.013

baseline condition. Here, we extend this work to explore the coupling in the deactivation regime (Hyder et al., 2002a).

In addition, the physiological baseline, defined by CBF and $CMRO_2$, can have a strong effect on the hemodynamic response to activation and the resulting BOLD signal changes. Recent studies found that increased baseline CBF following $CO_2$ inhalation (Corfield et al., 2001; Davis et al., 1998; Hoge et al., 1999a; Kastrup et al., 2002; Kim et al., 1999) or acetazolamide administration (Brown et al., 2003) significantly reduced the BOLD signal response to the same stimulation. However, in those studies in which CBF was measured, the increment of CBF change remained constant despite the change in baseline CBF. In apparent contradiction to this picture of additive CBF changes, Hyder et al. (2002b) have reported that when the resting state is varied in rats with different levels of anesthesia, the final CBF in the activated state is constant regardless of the baseline CBF.

Following the observation of Raichle et al. (2001) that the eyes closed state might have a lower baseline value of CBF in the visual cortex compared with the eyes open state, we used a visual stimulation paradigm in human subjects using both eyes open and eyes closed as resting conditions to (a) investigate simultaneously the coupling between CBF and $CMRO_2$ during activation (visual stimulation vs. resting with eyes open) and during deactivation (resting with eyes closed vs. resting with eyes open), and (b) determine whether the previous baseline condition affects the subsequent absolute stimulus response.

One advantage of this approach is that the brain physiology is studied under normal conditions, avoiding the potential confounding non-physiological effects associated with exogenous vasoactive agents and anesthesia. Using a quantitative arterial spin labeling fMRI technique, we measured both BOLD signal and CBF simultaneously, and used these data to estimate ranges for the $CMRO_2$ and OEF during different states. Finally, we propose a model of feed-forward neurovascular coupling that is consistent with present and prior experimental observations. A preliminary version of this work was presented in abstract form (Uludag et al., 2003).

## Methods

### *Subjects and study design*

We measured BOLD signal and CBF in a total of 12 scanning sessions on eight healthy subjects at 3 or 4 T. Five independent measures at each magnetic field strength were made. Subjects were recruited from the neuroscience community (age range 24–49). Our Institutional Review Board approved the imaging procedures and subjects gave informed consent.

All experiments used a full-field flickering black and white radial checkerboard stimulation alternating with periods of darkness with a small white fixation cross displayed, and during the eyes open condition subjects fixated on the cross. The scanning room was darkened so only a small amount of ambient light was present. In two preliminary experiments, subjects viewed the stimulus for four blocks (20 s on, 40 s off), with 40 s rest at the beginning (total 280 s). In the first of these preliminary experiments, the subjects had their eyes open during all rest periods, and in the second experiment their eyes were closed, with an auditory cue to open (close) their eyes at the beginning (ending) of the stimulus. The data from these preliminary experiments were used solely to select activated brain regions and construct a region-of-interest for averaging in the subsequent experiment. In the main experiment, the number of stimulation blocks was increased to eight (each 20 s on, 40 s off), with an additional 80 s of rest at the beginning and 40 s at the end, for a total of 600 s. The resting blocks alternated between eyes open or eyes closed (see Fig. 1). A verbal cue was used to prompt the subject to open or close their eyes at the beginning/ending of the stimulus.

### *Data acquisition*

The subjects were imaged with 3 and 4 T whole body imaging systems (Varian NMR systems, Palo Alto, CA) using a custom-built 26 cm volume coil on the 3-T system and a 34-cm volume coil on the 4-T system (Nova Medical, Inc. Wakefield, MA). An axial localizer scan was used to ensure correct positioning of the subject's head in the scanner followed by a sagittal localizer scan (9 slices, 4 mm thick) to identify the calcarine sulcus. For the functional studies, four oblique slices parallel to the calcarine sulcus were acquired with a voxel size of 3.75 × 3.75 × 4 mm. For the arterial spin labeling (ASL) acquisitions, we measured interleaved control and tagged images using a PICORE tagging scheme with the QUIPSS II modification (Wong et al., 1998) that enables simultaneous collection of BOLD signal and quantitative CBF data (10 cm tag width, 1 cm tag slice gap). The BOLD signal is constructed as the running average of the control and tagged images and the CBF as the running difference (Wong et al., 1998). The use of a pre-saturation pulse on the image plane in this sequence minimizes cross-contamination of the BOLD signal and CBF (Wong et al., 1997), that is, the average of the fully relaxed signal in the control image and the inversion recovery in the tag image approximates a saturation recovery. Thus, the inflowing blood and the blood in the image plane follow

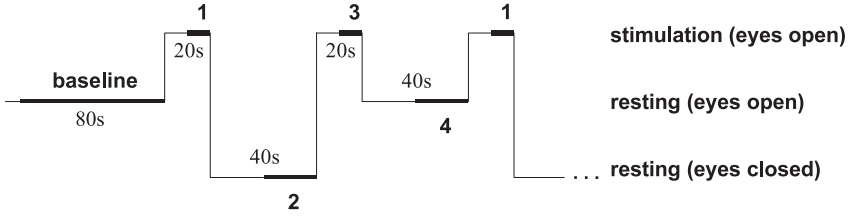


Fig. 1. Design of the main experiment. Eighty seconds of baseline condition (resting with open eyes) was included at the beginning of the experiment. The stimulation cycle consisted of 20 s full-field checkerboard stimulation followed by 40 s resting, alternating eyes closed and open. The cycle was repeated four times. For the calculation of the mean values of the four different states (labeled in bold), only the second half of each condition was included to avoid transient features and obtain steady-state values.

approximately the same recovery curve and hence the average signal of control and tag image is only sensitive to susceptibility changes and not to the changes in the inflowing blood. Because of the non-zero TE, a 'cross talk' from the BOLD signal to CBF still exists. However, compared to the magnitude of the changes during functional activation this 'cross talk' is small (Wong et al., 1998), that is, if the BOLD change is 3% and the CBF change 30%, then a 3% change in the CBF is due to cross talk. The scanning parameters were: TR = 2 s, TE = 26 ms, TI1/TI2 = 700 ms/1400 ms.

*Data analysis*

To avoid a selection bias in the result of the main experiment, the voxels used for analysis were selected using only the data from the preliminary experiments. The BOLD time series for each voxel was correlated with a reference function constructed assuming a gamma-variate hemodynamic response ($\tau = 2.4$ s, order = 3, delay = 2 s, see Friston et al., 2000), and the correlation coefficient threshold was varied until about 50 voxels in the visual cortex were activated for each subject. The individual thresholds for each subject are given in Table 1. The BOLD signal and CBF time courses of these selected voxels were then averaged in the main experiment. Due to higher noise in the CBF measurement, its time course was first smoothed using a running average of five data points. Baseline CBF and BOLD signal were determined as the average of the first 10–80 s and the last 20 s of the run and the average curves were expressed as fractional changes from this baseline (see Fig. 1). We averaged the responses for four steady-state conditions, defined as:

state 1: activation following rest with eyes open
state 2: rest with eyes closed
state 3: activation following rest with eyes closed
state 4: rest with eyes open.

In these averages we avoided transient periods after a change of state. For the activation states (1 and 3), the last 10 s after stimulus onset were averaged and for the resting states (2 and 4), the last 20 s before the stimulus were averaged (see Fig. 1). We have tested whether the four different states deviate significantly from the baseline both for CBF and BOLD signal. For all statistical tests, we used a two-tailed $t$ test. Differences were considered significant for $P < 0.05$. In three cases for which the baseline value was biased due to movement or high oscillations, one of the intervals was chosen manually for the calculation of the baseline BOLD signal and CBF. The errors are given as standard error of the mean.

*Estimation of $CMRO_2$ change from changes in CBF and BOLD signal*

The BOLD signal change ΔS can be modeled as a function of the relative changes in CBF and $CMRO_2$ as follows (see Davis et al., 1998 or Buxton, 2002 for a derivation):

$$\frac{\Delta S}{S_0} = A \times \left[1 - \left(\frac{\mathrm{CBF}}{\mathrm{CBF}_0}\right)^{\alpha-\beta} \times \left(\frac{\mathrm{CMRO}_2}{\mathrm{CMRO}_{2,0}}\right)^{\beta}\right] \quad (1)$$

The subscript 0 denotes the baseline values. In steady-state, $\alpha$ is the exponent that relates the cerebral blood volume (CBV) to CBF with a power law expression, and was previously determined from steady-state studies in monkeys to be 0.38 (Grubb et al., 1974). The term $\beta$ is a numerical parameter that expresses the super-linear dependence of the relaxation rate on deoxyhemoglobin concentration in blood and is calculated from simulations to be 1.5 for the field strength of 1.5 T (Davis et al., 1998). The parameter $A$ is a local scaling factor, which corresponds to the maximum BOLD change for complete removal of deoxyhemoglobin in the voxel.

Since there are two unknowns to be solved in Eq. (1) ($A$ and $\Delta\mathrm{CMRO}_2/\mathrm{CMRO}_{2,0}$), an additional experiment is usually required to calculate the scaling factor $A$ and thereby calibrate the BOLD signal. Hypercapnia is commonly used (Davis et al., 1998; Hoge et al., 1999a; Kastrup et al., 2002; Kim et al., 1999), with the

Table 1
Mean BOLD signal and CBF steady state values of the different states (top: 3 T, bottom: 4 T)

| Subject | ΔCBF1/3 (%) | ΔCBF2 (%) | ΔCBF4 (%) | ΔBOLD1/3 (%) | ΔBOLD2 (%) | ΔBOLD4 (%) | Threshold |
|---|---|---|---|---|---|---|---|
| *3 T* | | | | | | | |
| 1a | 35.77 | −23.32 | −20.15 | 3.33 | −2.46 | −1.42 | 0.81 |
| 2a | 13.25 | −12.21 | −4.99 | 2.94 | −1.98 | −1.03 | 0.50 |
| 3 | 29.76 | −21.73 | −0.40 | 2.67 | −1.32 | −1.21 | 0.66 |
| 4 | 29.64 | −15.70 | −1.95 | 6.04 | −3.53 | −1.86 | 0.90 |
| 5a | 52.98 | −23.45 | −18.71 | 4.60 | −3.53 | −1.75 | 0.88 |
| 5b | 45.49 | −36.30 | −15.26 | 6.11 | −4.01 | −1.21 | 0.83 |
| Average | 34.48 ± 5.66 | −22.12 ± 3.38 | −10.24 ± 3.59 | 4.28 ± 0.62 | −2.81 ± 0.42 | −1.41 ± 0.13 | |
| *4 T* | | | | | | | |
| 1b | 25.22 | −15.93 | −7.22 | 6.17 | −3.01 | −1.74 | 0.78 |
| 2b | 41.32 | −26.35 | −14.19 | 5.03 | −4.90 | −1.28 | 0.72 |
| 6 | 41.96 | −26.28 | −22.53 | 5.70 | −3.33 | −1.46 | 0.77 |
| 7 | 32.64 | −10.58 | 23.78* | 4.09 | −2.73 | −1.42 | 0.49 |
| 8a | 38.32 | −31.30 | −2.23 | 2.85 | −1.75 | −0.49 | 0.72 |
| 8b | 48.80 | −20.91 | −6.42 | 2.90 | −2.31 | −1.07 | 0.72 |
| Average | 38.04 ± 3.34 | −21.89 ± 3.12 | −10.52 ± 3.25 | 4.45 ± 0.57 | −3.01 ± 0.44 | −1.24 ± 0.17 | |

Individual correlation coefficients to obtain the approximately 50 highest correlated voxels from the first experiment are shown at the right. Repeated measurements on subjects are labeled with a and b. There was no significant difference in BOLD signal or CBF values between for the 3 and 4 T data. Subject 7 was excluded from the averaging.

assumption that inhaled $CO_2$ increases CBF but does not affect $CMRO_2$. To estimate the range of $\Delta CMRO_2/CMRO_{2,0}$ compatible with the data without a hypercapnia experiment, we assume that $A$ is 0.25 both for 3 T and 4 T, extrapolating from the value of A determined at 1.5 T ($A = 0.22$ in Hoge et al., 1999a, $A = 0.15$ in Hoge et al., 1999b) presuming an approximately linear dependence on the field strength ($A \sim B_0^{\beta}$) and a linear dependence on the echo time TE ($A \sim TE$).

Because of the uncertainty of the value of $A$, we calculated $CMRO_2$ using values of $A$ between 0.15 and 0.45. In addition, to explore the dependence of the calculated $CMRO_2$ on our assumed values of $\alpha$ and $\beta$, we varied both parameters within the expected physiological range. The Grubb exponent $\alpha$ was varied from 0.15 to 0.45, which, for a CBF change of 50%, would describe a CBV change from approximately 6% to 20%. From theoretical considerations and experimental evidence (Buxton, 2002), it is expected that for higher field strengths, the BOLD signal is mainly extravascular corresponding to a $\beta$ value close to 1. Therefore, we assumed values between 1 and 1.6.

We can examine the relationship between the changes in CBF and $CMRO_2$ by defining the ratio $n$ as a measure of their coupling:

$$n = \frac{\Delta CBF}{CBF_0} \Big/ \frac{\Delta CMRO_2}{CMRO_{2,0}} \tag{2}$$

A constant $n$ in a certain range describes a tight coupling between oxygen metabolism and blood flow. A value of $n = 1$ would correspond to equal fractional changes in CBF and $CMRO_2$.

The calculated $n$ allows us to determine the oxygen extraction fraction (OEF), defined as:

$$OEF = OEF_o \times \frac{CMRO_2}{CMRO_{2,0}} \Big/ \frac{CBF}{CBF_0} \tag{3}$$

Assuming that resting $OEF_0 = 40\%$ (Raichle et al., 2001), the OEF for both activation and deactivation is calculated from the value of $n$ that is consistent with the data.

## Results

The time courses of the averaged BOLD signal and CBF over all data sets are shown in Fig. 2a (blue: BOLD; red: CBF). The voxels were chosen from the first preliminary experiment. (Choosing the voxels from the second preliminary experiment, with eyes closed, resulted in very similar time courses and is therefore not shown.) The $y$-axis scale in Figs. 2a and b indicates the percent change of BOLD signal on the left-axis, and the percent change of CBF on the right-axis. The time courses show typical stimulus responses with increases in signal following a visual stimulus, and a post-stimulus undershoot. However, during rest with eyes closed, the signal decreases to a much lower value compared to rest with eyes open. All four states, as defined in Methods, showed significant deviation from the baseline. All data were free from movement artifacts caused by the stimulus or from closing and opening the eyes. There was no significant difference between the peak activation levels following both resting conditions (states 1 and 3), that is, with activation both the BOLD signal and CBF reached the same final level independent of the preceding resting condition (see Table 1 for the magnitude of the changes). Therefore, in the following analysis, states 1 and 3 are treated as one. State 2 (resting, eyes closed) showed a significantly reduced value from state 4 (resting, eyes open). This pattern of increase and decrease of the BOLD signal and CBF can be seen on results from individual subjects and even on single voxels (data not shown). In Fig. 2b, the data from the four blocks are averaged over a single cycle (20 s stimulation with alternating open and closed eyes as the rest condition). The beginnings of the stimuli occur at 20 and 80 s. The shape of the time courses of the BOLD signal and CBF follows a similar pattern and both have a post-stimulus undershoot.

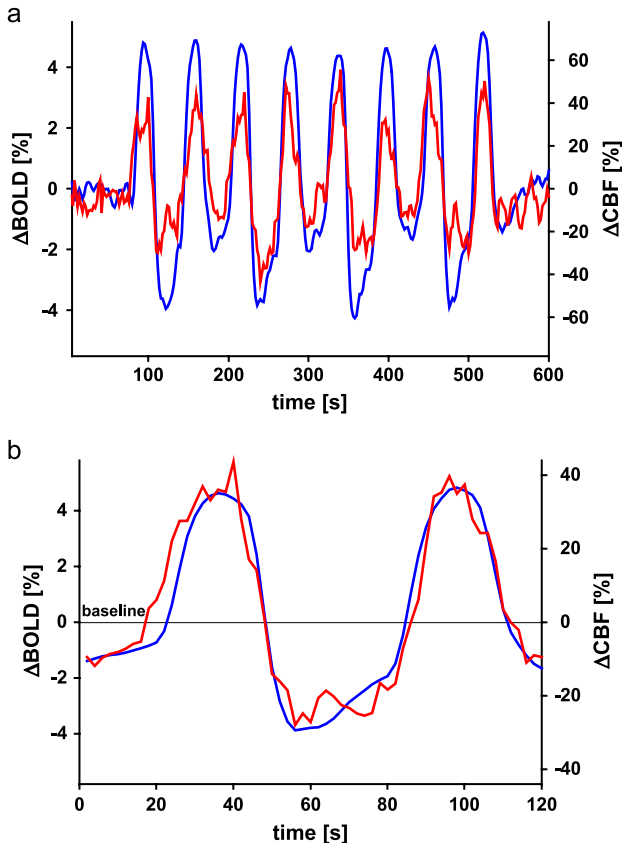


Fig. 2. Grand average of all data sets (blue: BOLD, red: CBF). The $y$-axis scale on the left indicates the range of BOLD signal percent change and on the right indicates the percent change in CBF. During stimulation BOLD signal and CBF increased to the same level independent of the prior condition. In addition, the levels during rest with eyes closed were lower than during rest with eyes open.

In Table 1, the mean values for the different states and the individual statistical thresholds are given. The data for the studies at 3 T are presented in the upper part of the table, and data for 4 T in the lower part. There is no significant difference in the averaged mean values for BOLD signals and CBF percent changes between 3 and 4 T data. The BOLD signal was expected to be larger at 4 T, but our inability to detect a difference is likely due to the small number of subjects and inter-subject variability. In the following, no distinctions are made between 3 and 4 T data and averages are calculated for the pooled data. We observed a substantial decrease in signal relative to baseline ($2.9 \pm 0.3\%$ BOLD, $22 \pm 2.1\%$ CBF) when the eyes were closed, compared with an increase in signal

(4.4 ± 0.4% BOLD, 36.3 ± 3.1% CBF) during stimulation. There was evidence for a post-stimulus undershoot of the BOLD signal, with lower signal in the 'eyes open' rest periods following a stimulus block than in the initial rest period (1.3 ± 0.1% BOLD, 10.4 ± 2.3.0% CBF).

*Calculation of OEF*

The plot of CBF vs. $CMRO_2$, both normalized to their baseline values and calculated from Eq. (1) with assumed values $A = 0.25$, $\alpha = 0.38$, $\beta = 1.5$, is shown in Fig. 3. The distribution can be approximated by a linear function with a slope of $n \sim 2.2 \pm 0.15$. Note that this linear relationship is only valid over a limited range because extrapolation to CBF = 0 gives a non-zero value of approximately 54% for $CMRO_2$, which is physically impossible. This simply indicates that the $CBF/CMRO_2$ relationship, although approximately linear over a broad physiological range, is intrinsically a nonlinear function.

The values of $A$, $n$, and OEF are summarized in Table 2. Assuming a baseline OEF of 40% (Raichle et al., 2001), the average OEF decreased to 33 ± 1.4% during activation (states 1 and 3) and increased to 46 ± 1.2% during rest with eyes closed (state 2). During the rest period with eyes open, the BOLD signal and CBF did not fully return to baseline, and the calculated average OEF during this period is 43 ± 1.2%.

In Fig. 4, the sensitivity of the calculated value of $n$ to the assumed values of $A$, $\alpha$, and $\beta$ is shown. Each plot shows the calculated value of $n$ as the assumed value of one of the parameters is changed, and the values of the other two parameters held constant are indicated. Varying $A$ from 0.15 to 0.45 results in values of $n$ in the interval of approximately 1.6 to 2.4, showing that the estimate of $n$, and thus the estimate of $CMRO_2$, is not strongly sensitive to the assumed value of $A$. Varying $\alpha$ or $\beta$ gives values of $n$ from approximately 1.6 to 2.6.

Note also that the effect of assuming a smaller value of $A$ is to make the $CMRO_2$ estimate smaller in the activated state and larger in the deactivated state (or vice versa). In other words, the effect of varying the assumed value of $A$ is to tip the plotted curve in Fig. 3, essentially by rescaling the $CMRO_2$ axis. The key result is that both activation and deactivation data vary along the same curve, even if there remains uncertainty about the precise value of the slope. (The maximum of $n$ occurs because this rescaling is different for activation and deactivation.)

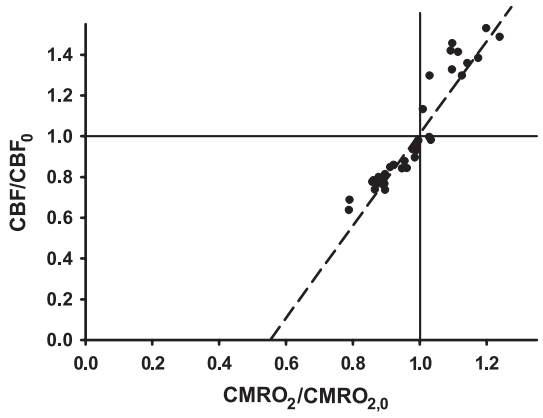


Fig. 3. Average single cycle of the grand average. Initial baseline value is indicated. The shape of the time courses of BOLD signal and CBF are almost identical, both showing a post-stimulus undershoot.

Table 2
Summary of the values for A ('scaling constant'), $n$ ('coupling factor') and OEF ('oxygen extraction fraction') for assumed $\alpha = 0.38$ and $\beta = 1.5$

| | | |
|---|---|---|
| $A$ | | 0.25 |
| $n = \frac{\Delta CBF/CBF_0}{\Delta CMRO_2/CMRO_{2,0}}$ | | 2.2 ± 0.15 |
| OEF | baseline (assumed) | 40% |
| | 1/3 (activation) | 33 ± 1.4% |
| | 2 (deactivation) | 46 ± 1.2% |
| | 4 (undershoot) | 43 ± 1.2% |

See text for details.

## Discussion

In functional neuroimaging studies, the goal is to measure patterns of neural activity in the brain. However, fMRI is not directly sensitive to the neural activity, but rather to the blood flow and oxygenation changes which are linked to the energetic costs of the neural activity. In this study, we have used fMRI to investigate how cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$) are coupled during activation and deactivation. Compared to a baseline state defined as lying quietly in a darkened room with eyes open, the deactivation (measured by the fractional change in CBF) in visual cortex associated with simply closing the eyes was about two thirds as strong as the activation with eyes open viewing a flashing checkerboard. Our results indicate that in the visual cortex, CBF and $CMRO_2$ co-vary in a consistent fashion during both activation and deactivation from this baseline state with eyes open. Hoge et al. (1999a) found a close coupling of CBF and $CMRO_2$ in a range of activation states, and our results partially extend these earlier results into the deactivation regime. For both activation and deactivation, the relationship between the fractional changes in CBF and $CMRO_2$ is reasonably linear, with a slope greater than one. Model estimates based on a wide range of

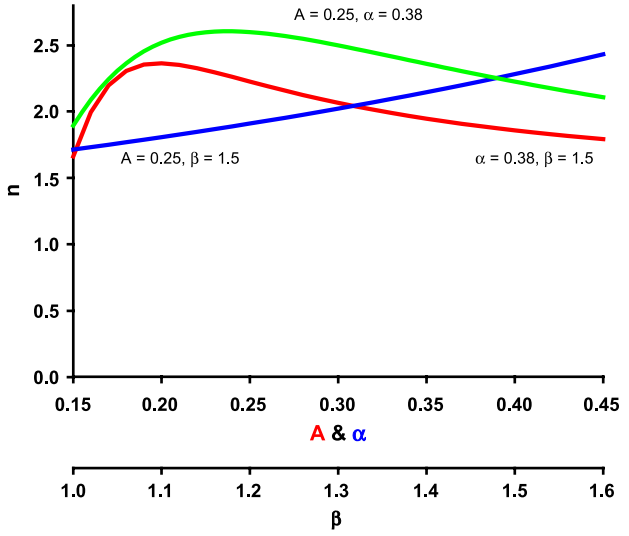


Fig. 4. Normalized CBF measured as a function of the normalized maximum $CMRO_2$ calculated for assumed parameter values of $\beta = 1.5$ and $A = 0.25$. The slope $n$, which is a measure of the coupling of CBF and $CMRO_2$, is approximately 2.2.

parameter values indicate that the slope $n$ is in the range 1.6–2.6. It is important to note, however, that because $n$ is not equal to one, this local linear behavior is only an approximation to an intrinsically nonlinear relationship.

We also found that both the BOLD signal and CBF reached the same level in the activated state, regardless of the preceding resting state. This result in human subjects complements the results of Hyder et al. (2002b) in animal studies. After lowering the metabolic resting activity pharmacologically with different levels of anesthesia in rats, they found that a fixed stimulus raised the CBF to a new level that was independent of the level of the resting activity. In addition, the authors observed proportionality between spiking frequency ('neuronal activity') and $CMRO_2$. Assuming a tight coupling of CBF and $CMRO_2$, the implication is that CBF level reflects the neuronal activity. Note that in the study of Hyder et al., the source of the deactivation (the anesthetic) was not withdrawn during the periods of activation, whereas in our study, the source of deactivation (closing the eyes) was not present anymore during activation. Hence, our current results support the basic picture of a close coupling between neuronal activity and CBF with a more physiological manipulation of the baseline state.

In addition, in this work, we have developed a new approach to partially calibrate the BOLD signal. With a hypercapnia experiment, it is possible to calibrate the BOLD effect and determine the fractional change in $CMRO_2$ with activation, provided the assumption that hypercapnia does not change $CMRO_2$ is true (Horvath et al., 1994; Yang and Krasney, 1995). However, we have shown that even without such a calibration experiment, it is possible to estimate the possible range of $CMRO_2$ change consistent with the data by assuming a range for the unknown scaling factor. In fact, we found that varying this assumed value has little effect on the change of estimated $CMRO_2$.

An interesting side result was that both the BOLD signal and CBF were reduced in the resting condition with eyes open following a stimulus compared to the initial baseline with eyes open. A post-stimulus undershoot of the BOLD signal is often present in fMRI data, and it is not uncommon for this effect to appear as a lowering of the baseline after a stimulation period (Buxton et al., 1998). However, a post-stimulus undershoot of CBF is not as common (Hoge et al., 1999c), and this has led to models in which CBV returns to baseline more slowly than CBF, creating an undershoot of the BOLD signal without an undershoot of the CBF signal (Mandeville et al., 1999). However, the undershoot in the BOLD signal can also be explained as a reduction of the neuronal activity after stimulation (Logothetis, 2003), which would lead to a matching undershoot in CBF as observed in this study. The sources of the post-stimulus undershoot are not well established, and these data suggest that there may be several. That is, a mismatch in the post-stimulus recovery of CBF and CBV, or a CBF undershoot following reduction of neuronal activity, or a combination of both.

The paradigm employed here, comparing eyes open and eyes closed, provides a simple physiological manipulation of the resting state. In addition to its ease of implementation, this approach was motivated by the observation of Raichle et al. (2001) that CBF might be lowered when the eyes are closed compared to the eyes open state. Based on a meta-analysis of PET studies (Mazoyer et al., 2001), they proposed that the OEF could be used as a measure of an absolute metabolic baseline for each cerebral area. Specifically, an OEF of 40% defines a set-point for each brain region, and a reduction of OEF corresponds to an activation and an increase of OEF corresponds to a deactivation. Because the $CMRO_2$ and CBF vary substantially over the entire brain, the uniformity of OEF suggests that evolutionary and individual development ensures that energy consumption, reflected in $CMRO_2$, and energy supply, reflected in CBF, are matched to the local energy needs. Our fMRI results are consistent with this hypothesis. Specifically, in the visual cortex, the hypothesis based on the PET data is that the state with eyes closed is a deactivation from the baseline state with eyes open. In this study, we have shown with arterial spin labeling fMRI that closing the eyes does indeed lower the resting value of the BOLD signal and CBF, consistent with an increase in OEF from 40% to 46 $\pm$ 1.2%.

Models of oxygen transport to tissue support the central role of the OEF in defining the metabolic state (Buxton and Frank, 1997; Buxton et al., 1998). In these models, the OEF decrease with activation serves to raise the capillary $O_2$ content and increase the $O_2$ gradient driving $O_2$ from capillaries to mitochondria in neurons and glia. Our analysis indicates that the fractional $CMRO_2$ change in the current experiments was approximately 45% of the fractional CBF increase, so that OEF decreased from 40% to 33 $\pm$ 1.4% during activation.

In our study, the final activated state was characterized by the same BOLD signal and CBF, even when the preceding resting state was altered substantially by closing the eyes. However, recent studies manipulating the baseline CBF with vasoactive agents (Brown et al., 2003; Davis et al., 1998; Hoge et al., 1999b; Kastrup et al., 2002; Kim et al., 1999) found that the effect of the stimulus was additive to the effects of the administered agent, with the *increment* of CBF change with activation remaining constant, rather than the final value. However, we propose that a mechanistic model of *feed-forward neurovascular coupling* can reconcile both observations. Specifically, we hypothesize that neural activity produces and/or releases vasoactive agents that increase or decrease CBF, so that the change in CBF directly reflects the change in neural activity. In this way, the change in CBF for a given stimulus does not depend on the baseline CBF, but it does depend on the baseline neural activity. In the $CO_2$ experiments, baseline neural activity presumably remained constant, despite the alteration of baseline CBF, so the increment of CBF change with activation remained the same. In our experiment, neural activity was presumably reduced with eyes closed, and the corresponding reduction in CBF was a result of neurovascular coupling. The larger increment of CBF change in our experiment then reflects the larger change in neural activity between the resting and activated states. We are currently evaluating the same paradigm using simultaneous EEG-fMRI to estimate the associated neuronal changes (Goldman et al., 2002). The feed-forward model of neurovascular coupling can be used to investigate the mechanisms of action of other exogenous agents that alter CBF, such as caffeine or alcohol (or a mix of both) (Field et al., 2003; Mulderink et al., 2002; Schmalbruch et al., 2002; Seifritz et al., 2000). Are these drugs altering baseline CBF through direct effects on the vasculature, or is the CBF change simply following neuronal changes?

Note that this feed-forward model describes the *mechanism* for neurovascular coupling, but does not address its *function*. A critical question is: if the function served by the CBF increase with activation is to deliver $O_2$ to support an increased $CMRO_2$, and breathing $CO_2$ has already raised CBF, why does CBF need to increase any further with activation? Part of the problem in answering this question and integrating all of these results in the

framework of a biological explanation is that the function served by the high CBF sensitivity to $CO_2$ is unknown. These questions, and the general question of the functions served by a large CBF increase with brain activation, are the focus of ongoing research.

In conclusion, this study shows that combined measurements of BOLD signal and CBF provide a versatile and easy method for studying human brain activity under physiological conditions. Analyzing these data with a few conservative assumptions can set reasonably tight limits on OEF values in different states. We found that simply closing the eyes creates a large physiological deactivation in the visual cortex, and provides a simple paradigm for studying baseline effects in fMRI. Our data, and earlier data exploring the effects of altering baseline CBF with vasoactive agents, are consistent with a model of feed-forward neurovascular coupling. In this model, the increment of CBF change between a resting and activated state directly reflects the change in neural activity, independent of the resting level CBF. However, the BOLD effect depends on both the CBF change *and* the resting CBF. This suggests that the BOLD effect may be more variable in practice, and that measurement of the CBF change with ASL techniques may be a more reliable reflection of the change in neural activity.

## Acknowledgments

We thank Gregory G. Brown and Martin Sereno for helpful discussions. This work was supported by National Institutes of Health Grants NS-36722 and NS-042069.

## References

Brown, G.G., Zorilla, L.T.E., Georgy, B., Kindermann, S.S., Wong, E.C., Buxton, R.B., 2003. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. J. Cereb. Blood Flow Metab. 23, 829–837.

Buxton, R.B., 2002. Introduction to Functional Magnetic Resonance Imaging: principles and techniques. Cambridge Univ. Press, New York.

Buxton, R.B., Frank, L.R., 1997. A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. J. Cereb. Blood Flow Metab. 17, 64–72.

Buxton, R.B., Wong, E.C., Frank, L.R., 1998. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. Magn. Reson. Med. 39, 855–864.

Calamante, F., Thomas, D.L., Pell, G.S., Wiersma, J., Turner, R., 1999. Measuring cerebral blood flow using magnetic resonance imaging techniques. J. Cereb. Blood Flow Metab. 19, 701–735.

Corfield, D.R., Murphy, K., Josephs, O., Adams, L., Turner, R., 2001. Does hypercapnia-induced cerebral vasodilation modulate the hemodynamic response to neural activation? NeuroImage 13, 1207–1211.

Davis, T.L., Kwong, K.K., Weisskoff, R.M., Rosen, B.R., 1998. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. Proc. Nat. Acad. Sci. U. S. A. 95, 1834–1839.

Field, A.S., Laurienti, P.J., Yen, Y.F., Burdette, J.H., Moody, D.M., 2003. Dietary caffeine consumption and withdrawal: confounding variables in quantitative cerebral perfusion studies? Radiology 227, 129–135.

Fox, P.T., Raichle, M.E., 1986. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation of human subjects. Proc. Nat. Acad. Sci. U. S. A. 83, 1140–1144.

Fox, P.T., Raichle, M.E., Mintun, M.A., Dence, C., 1988. Nonoxidative glucose consumption during focal physiologic neural activity. Science 241, 462–464.

Friston, K.J., Mechelli, A., Turner, R., Price, C.J., 2000. Nonlinear responses in fMRI: the Balloon model, Volterra kernels, and other hemodynamics. NeuroImage 12, 466–477.

Fujita, H., Kuwabara, H., Reutens, D.C., Gjedde, A., 1999. Oxygen consumption of cerebral cortex fails to increase during continued vibrotactile stimulation. J. Cereb. Blood Flow Metab. 19, 266–271.

Goldman, R.I., Stern, J.M., Engel Jr., J., Cohen, M.S., 2002. Simultaneous EEG and fMRI of the alpha rhythm. Neuroreport 13, 2487–2492.

Grubb Jr., R.L., Raichle, M.E., Eichling, J.O., Ter-Pogossian, M.M., 1974. The effects of changes in PaCO2 on cerebral blood volume, blood flow, and vascular mean transit time. Stroke 5, 630–639.

Gusnard, D.A., Raichle, M.E., 2001. Searching for a baseline: functional imaging and the resting human brain. Nat. Rev., Neurosci. 2, 685–694.

Hoge, R.D., Atkinson, J., Gill, B., Crelier, G.R., Marrett, S., Pike, G.B., 1999a. Linear coupling between cerebral blood flow and oxygen consumption in activated human cortex. Proc. Nat. Acad. Sci. U. S. A. 96, 9403–9408.

Hoge, R.D., Atkinson, J., Gill, B., Crelier, G.R., Marrett, S., Pike, G.B., 1999b. Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model. Magn. Reson. Med. 42, 849–863.

Hoge, R.D., Atkinson, J., Gill, B., Crelier, G.R., Marrett, S., Pike, G.B., 1999c. Stimulus-dependent BOLD and perfusion dynamics in human V1. NeuroImage 9, 573–585.

Horvath, I., Sandor, N.T., Ruttner, Z., McLaughlin, A.C., 1994. Role of nitric oxide in regulating cerebrocortical oxygen consumption and blood flow during hypercapnia. J. Cereb. Blood Flow Metab. 14, 503–509.

Hyder, F., Rothman, D.L., Shulman, R.G., 2002a. Total neuroenergetics support localized brain activity: implications for the interpretation of fMRI. Proc. Nat. Acad. Sci. U. S. A. 99, 10771–10776.

Hyder, F., Rothman, D.L., Shulman, R.G., 2002b. A model for the regulation of oxygen cerebral delivery. J. Appl. Physiol. 85, 554–564.

Hyder, F., Shulman, R.G., Rothman, D.L., 1998. A model for the regulation of cerebral oxygen delivery. J. Appl. Physiol. 85, 554–564.

Kastrup, A., Kruger, G., Neumann-Haefelin, T., Glover, G.H., Moseley, M.E., 2002. Changes of cerebral blood flow, oxygenation, and oxidative metabolism during graded motor activation. NeuroImage 15, 74–82.

Kim, S.G., Rostrup, E., Larsson, H.B., Ogawa, S., Paulson, O.B., 1999. Determination of relative CMRO2 from CBF and BOLD changes: significant increase of oxygen consumption rate during visual stimulation. Magn. Reson. Med. 41, 1152–1161.

Kwong, K.K., Belliveau, J.W., Chesler, D.A., Goldberg, I.E., Weisskoff, R.M., Poncelet, B.P., Kennedy, D.N., Hoppel, B.E., Cohen, M.S., Turner, R., Cheng, H.-M., Brady, T.J., Rosen, B.R., 1992. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc. Natl. Acad. Sci. U. S. A. 89, 5675–5679.

Logothetis, N.K., 2003. The underpinnings of the BOLD functional magnetic resonance imaging signal. J. Neurosci. 23, 3963–3971.

Mandeville, J.B., Marota, J.J., Ayata, C., Zaharchuk, G., Moskowitz, M.A., Rosen, B.R., Weisskoff, R.M., 1999. Evidence of a cerebrovascular postarteriole windkessel with delayed compliance. J. Cereb. Blood Flow Metab. 19, 679–689.

Marrett, S., Gjedde, A., 1997. Changes of blood flow and oxygen consumption in visual cortex of living humans. Adv. Exp. Med. Biol. 413, 205–208.

Mazoyer, B., Zago, L., Mellet, E., Bricogne, S., Etard, O., Houde, O., Crivello, F., Joliot, M., Petit, L., Tzourio-Mazoyer, N., 2001. Cortical networks for working memory and executive functions sustain the conscious resting state in man. Brain Res. Bull. 54, 287–298.

Mulderink, T.A., Gitelman, D.R., Mesulam, M.M., Parrish, T.B., 2002. On the use of caffeine as a contrast booster for BOLD fMRI studies. NeuroImage 15, 37–44.

Ogawa, S., Lee, T.M., Kay, A.R., Tank, D.W., 1990. Brain magnetic reso-

nance imaging with contrast dependent on blood oxygenation. Proc. Nat. Acad. Sci. U. S. A. 87, 9868–9872.
Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc. Nat. Acad. Sci. U. S. A. 98, 676–682.
Schmalbruch, I.K., Linde, R., Paulson, O.B., Madsen, P.L., 2002. Activation-induced resetting of cerebral metabolism and flow is abolished by beta-adrenergic blockade with propranolol. Stroke 33, 251–255.
Seifritz, E., Bilecen, D., Hanggi, D., Haselhorst, R., Radu, E.W., Wetzel, S., Seelig, J., Scheffler, K., 2000. Effect of ethanol on BOLD response to acoustic stimulation: implications for neuropharmacological fMRI. Psychiatry Res. 99, 1–13.
Seitz, R.J., Roland, P.E., 1992. Vibratory stimulation increases and decreases the regional cerebral blood flow and oxidative metabolism: a positron emission tomography (PET) study. Acta Neurol. Scand. 86, 60–67.
Stark, C.E., Squire, L.R., 2001. When zero is not zero: the problem of ambiguous baseline conditions in fMRI. Proc. Nat. Acad. Sci. U. S. A. 98, 12760–12766.
Uludağ, K., Yoder, E.J., Dubowitz, D.J., Restom, K., Buxton, R.B., 2003. Physiological perturbations of the baseline state in fMRI: evidence for consistent coupling of CBF and CMRO2 during activation and deactivation. Program-Soc., vol. 236.1. Society for Neuroscience, Washington, D.C.
Wong, E.C., Buxton, R.B., Frank, L.R., 1997. Implementation of quantitative perfusion imaging techniques for functional brain mapping using pulsed arterial spin labeling. NMR Biomed. 10, 237–249.
Wong, E.C., Buxton, R.B., Frank, L.R., 1998. Quantitative imaging of perfusion using a single subtraction (QUIPSS and QUIPSS II). Magn. Reson. Med. 39, 702–708.
Yang, S.P., Krasney, J.A., 1995. Cerebral blood flow and metabolic responses to sustained hypercapnia in awake sheep. J. Cereb. Blood Flow Metab. 15, 115.