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# Caffeine's effects on cerebrovascular reactivity and coupling between cerebral blood flow and oxygen metabolism☆

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ABSTRACT

The blood-oxygenation-level-dependent (BOLD) signal is dependent on multiple physiological factors such as cerebral blood flow (CBF), local oxygen metabolism ($CMRO_2$) and cerebral blood volume (CBV). Since caffeine affects both CBF and neural activity, its effects on BOLD remain controversial. The calibrated BOLD approach is an excellent tool to study caffeine because it combines CBF and BOLD measures to estimate changes in $CMRO_2$. The present study used the calibrated BOLD approach with 5% $CO_2$ to determine if a 2.5 mg/kg intravenous injection of caffeine changes the coupling between CBF and $CMRO_2$ during motor and visual tasks. The results show that caffeine decreases $n$, the CBF:$CMRO_2$ coupling ratio, from 2.58 to 2.33 in motor ($p=0.006$) and from 2.45 to 2.23 in visual ($p=0.002$) areas respectively. The current study also demonstrated that caffeine does not alter cerebrovascular reactivity to $CO_2$. These results highlight the importance of the calibrated BOLD approach in improving interpretation of the BOLD signal in the presence of substances like caffeine.



## Introduction

Caffeine is a widely used psychostimulant that is present in many foods and drinks, primarily in coffee and tea. According to a recent consumption report, 54% of adults in the United States drink coffee every day, with an average daily consumption of 3.1 cups/person (>500mg) (Field et al., 2003). Caffeine belongs to the methylxanthine family, which are cerebral vasoconstrictors and systemic vasodilators (Mulderink et al., 2002). It is widely accepted that caffeine's effect on the central nervous system is because it is an antagonist for adenosine receptors, especially types $A_1$ and $A_{2A}$ (Tarter et al., 1998). Since adenosine inhibits the release of excitatory neurotransmitters and affects neuronal firing rate through activation of type $A_1$ receptors, binding of an antagonist such as caffeine leads to increased neural stimulation through disinhibitory mechanism (Koppelstaetter et al., 2008). This is likely the reason why subjects report better performance and higher alertness after ingestion of caffeine. On the other hand, the $A_{2A}$ receptors are responsible for the vasoconstrictive effects of caffeine. Given caffeine's nonspecific binding to both types of receptors, it is capable of altering the coupling between blood flow and neural activity depending on the ratio between the two types of receptors in different areas of the brain (Laurienti et al., 2003).

Blood-oxygenation-level-dependent (BOLD) imaging is a popular method used to measure brain activity through changes in blood oxygenation. However, it is only an indirect measure because BOLD is based on the interaction between many factors, including oxygen consumption and cerebral blood flow (CBF) (Matthews and Jezzard, 2004). In a recent study, Mulderink et al. reported that caffeine increases the BOLD response by 37% and 26% in motor and visual areas respectively, and concluded that caffeine can be used as a BOLD contrast booster (Mulderink et al., 2002). The authors attributed this result to caffeine's ability to decrease CBF, which decreases BOLD baseline, allowing for a larger capacity of BOLD response to activation. However, due to the complexity of BOLD and inter-subject variability in the metabolism of caffeine, caffeine's effect on BOLD remains controversial (Bendlin et al., 2007; Koppelstaetter et al., 2008; Laurienti et al., 2003). Given the worldwide popularity of caffeine, it is imperative to understand how it affects BOLD.

In order to separate the metabolic and vascular components of functional activity, Davis et al. introduced the calibrated BOLD approach (Davis et al., 1998). This approach uses functional ASL to establish a mathematical relationship between CBF, BOLD and cerebral metabolic rate of oxygen ($CMRO_2$):

$$\frac{\Delta BOLD}{BOLD_0} = M\left(1-\left(\frac{CMRO_2}{CMRO_{2,0}}\right)^{\beta}\left(\frac{CBF}{CBF_0}\right)^{\alpha-\beta}\right) \quad (1)$$

where $M$ is the maximum BOLD contrast observable should all deoxyhemoglobin (dHb) be replaced with fully oxygenated blood, and is related to echo time (TE), a proportional constant $A$ which is field-

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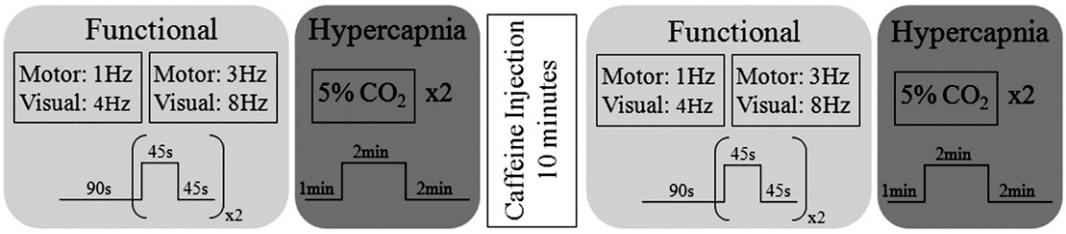


**Fig. 1.** Experimental design. Each study was divided into two identical sessions by a 10 min injection of 2.5 mg/kg caffeine diluted in a 50 ml saline solution. Each of the sessions consisted of two functional scans and two hypercapnia scans. During the functional scans, subjects performed auditory-cued bilateral finger tapping and viewed a grayscale flashing checkerboard simultaneously. Two levels of stimulation frequency were used for the functional scans to ensure a good fit for the coupling ratio, *n*. 5% $CO_2$ was used for all resting–state hypercapnia scans. Functional paradigms for the functional and hypercapnia scans are also shown.

strength and sample-specific, as well as baseline (denoted by subscript 0) cerebral blood volume (CBV) and dHb:

$$M = \mathrm{TE} \cdot A \cdot \mathrm{CBV}_0 \cdot [\mathrm{dHb}]_0^{\beta} \quad (2)$$

The constant α, also known as Grubb's constant, describes the relationship between CBF and CBV and is assumed to be 0.38 (Grubb et al., 1974). $\beta$ is a constant that relates BOLD to field strength and oxygenation, and is typically set to 1.5 (Boxerman et al., 1995; Davis et al., 1998; Hoge et al., 1999a; Leontiev and Buxton, 2007). The basic idea of the calibrated BOLD approach is to use a vasoactive agent such as $CO_2$ that has minimal effect on $CMRO_2$ to "calibrate" the BOLD contrast against known CBF levels by estimating *M*. The estimated *M* can then be applied to BOLD and CBF changes from functional data to calculate relative changes in $CMRO_2$:

$$\frac{\mathrm{CMRO_2}}{\mathrm{CMRO_{2,0}}} = \left(1 - \frac{(\Delta \mathrm{BOLD}/\mathrm{BOLD_0})}{M}\right)^{1/\beta} \left(\frac{\mathrm{CBF}}{\mathrm{CBF_0}}\right)^{\alpha-\beta} \quad (3)$$

The calibrated BOLD approach is an excellent tool for studying the effects of substances such as caffeine on BOLD as it can provide a measurement of the CBF:$CMRO_2$ coupling ratio–*n*, which is expected to change given the neurostimulative and vasoactive effects of caffeine. In this study, we use the calibrated BOLD approach to examine how caffeine alters CBF:$CMRO_2$ coupling during motor-visual task.

## Materials and methods

### *Experimental setup*

Fourteen healthy subjects (4 males, 10 females, average age 27 ± 10 years) were recruited in accordance with the university's Institutional Review Board and written informed consent was obtained from every subject. All scans were completed before noon to minimize diurnal and dietary fluctuations. Subjects were asked to abstain from caffeine for 12–24 h prior to each study. A brief questionnaire was used to determine each subject's daily caffeine usage.

Prior to each study, two baseline blood samples were collected to ensure subjects complied with the caffeine abstinence requirement. Each study consisted of two identical sessions (see Fig. 1), separated by a caffeine injection consisting of 2.5 mg/kg body weight of caffeine diluted in 50 ml of saline injected at a rate of 0.1 ml/s intravenously (8.3 min duration), followed by 15 ml of saline flush at a rate of 1 ml/s. Each session consisted of two functional scans, each at a different stimulation frequency. The functional paradigm began with a 90 s baseline, followed by two blocks of 45 s on and 45 s off. For the motor task, subjects performed bilateral finger tapping to an auditory cue, paced at either 1 Hz or 3 Hz. For visual stimulation, graded stimulation was achieved by adjusting the flashing frequency of a grayscale checkerboard to 4 Hz and 8 Hz. The choice of these levels of stimulation was based on previous studies (Hoge et al., 1999b; Sadato et al., 1997), which have shown good separation between the respective BOLD and CBF responses at these frequencies, ensuring a better fit for calculating the coupling ratio. The visual stimulus was projected onto a screen placed at the end of the magnet bore, which subjects viewed through a mirror affixed on top of the head coil. During the rest blocks a black screen with a small white cross in the center was displayed. All functional stimuli were programmed and presented using Cogent (Wellcome Department of Cognitive Neurology, London, England) and Matlab (The MathWorks,Inc., Natick, MA).

Two hypercapnia scans were also collected during each of the sessions before and after caffeine injection, and averaged for data analysis. A gas mixture containing 5:21:74% $CO_2/O_2/N_2$ was used to induce hypercapnia. A disposable snorkel-like mouthpiece and nose clip (see Fig. 2a) were used to deliver the gas from a 100 L non-diffusible bag to the subjects. The setup was optimized such that the gas can be delivered to the subjects without discomfort and excessive motion. During the breathing of $CO_2$ gas, the bag was being filled at a rate of 10 L/min to minimize breathing resistance. For subject comfort, the

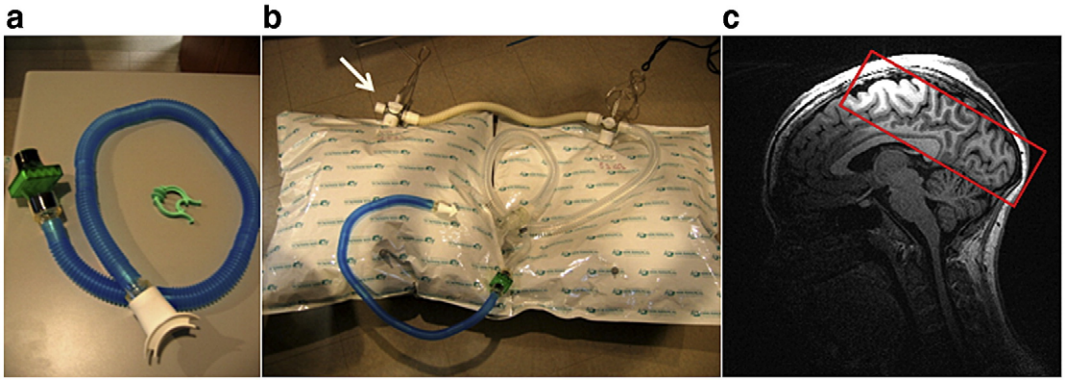


**Fig. 2.** (a) Disposable mouthpiece and nose clip used for delivery of $CO_2$. (b) A 100 L nondiffusable bag used for storage of $CO_2$ gas mixture. White arrow marks the valve used to switch between gas mixture in bag and room air. (c) Red rectangle marks the position of the slices acquired in the functional and hypercapnia scans.

**Table 1**
Physiological parameters measured pre- and post-caffeine, shown as mean ± SD

| | Precaffeine | Postcaffeine |
|---|---|---|
| Heart rate (beats per minute) | 68 ± 11 | 65 ± 7 |
| Systolic arterial pressure (mmHg) | 121.9 ± 10.9 | 122.6 ± 12.0 |
| Diastolic arterial pressure (mmHg) | 64.3 ± 10.4 | 65.5 ± 6.0 |
| $SpO_2$[1] (%) | 98.6 ± 1.3 | 98.5 ± 0.6 |
| Hemoglobin | 12.9 ± 1.4 | 12.6 ± 1.2 |
| Baseline $EtCO_2$ [2](mmHg) | 32.9 ± 4.6 | 34.4 ± 4.9 |
| Hypercapnia $EtCO_2$ (mmHg) | 49.8 ± 2.6 | 49.6 ± 3.1 |
| $\Delta EtCO_2$ (mmHg) | 14.1 ± 3.4 | 12.8 ± 2.1 |

[1] Saturation percentage of oxygen
[2] End-tidal $CO_2$

mouthpiece and nose clip were only in place during the hypercapnia scans. Each hypercapnic scan was 5 min with the following alternation between room air and $CO_2$-enriched gas mixture: 1 min air/2 min $CO_2$/2 min air. The alternation between room air and $CO_2$-enriched gas was achieved through a manual valve located at the top of the non-diffusible bag (see Fig. 2b). End tidal $CO_2$ ($etCO_2$) were monitored during the hypercapnia scans at the end of the snorkel tubing on the exhaust side of a one-way valve, and vital signs such as heart rate, oxygen saturation ($spO_2$), systolic and diastolic blood pressure were monitored throughout the study (InVivo Patient Monitor).

*Data acquisition*

All data were acquired on a 3 T Siemens whole-body scanner (Siemens TIM Trio, Erlangen, Germany) with the posterior half of a twelve-channel receive-only head coil (six channels). An additional four-channel carotid coil was placed above the motor cortex area to increase signal-to-noise ratio (SNR) in this region. A vacuum pillow was used to minimize head movement. Headphones were used to minimize scanner noise, as well as deliver auditory cues for motor task.

A sagittal functional localizer scan was run at the beginning of the study to locate the functionally active areas of the motor and visual cortices. This information was then used to position six slices of 5 mm thickness, 2.5 mm apart and in-plane resolution of 3.45 × 3.45 $mm^2$ along a transverse to coronal oblique plane that captured the motor and visual cortices in the same acquisition (see Fig. 2c) for all subsequent scans. During the functional and hypercapnia scans, data were acquired using a PICORE/Q2TIPS sequence (Luh et al., 1999; Wong et al., 1997) with gradient-echo echoplanar imaging (EPI) readout, $TI_1$ = 700 ms, TI1 s = 1200 ms, $TI_2$ = 1400 ms, tag size 20 cm, TR = 3 s, TE = 23 ms, flip angle = 90°. High-resolution $T_1$-weighted images were also acquired using a 3D anatomic scan (MPRAGE sagittal orientation, 1 mm isotropic resolution, TI = 900 ms, TR = 2300 ms, TE = 2.91 ms, 176 partitions). During the post-caffeine session, blood samples were collected immediately after the caffeine injection and every 10 min to ensure that the plasma caffeine concentration remains stable.

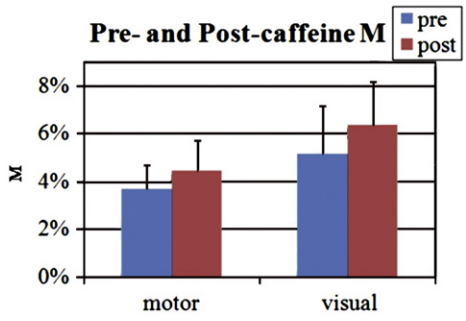


**Fig. 4.** Average *M* values calculated by fitting Equation [1] to the hypercapnia data from all subjects. Error bars denote standard deviation. In both motor and visual cortices, caffeine increased *M*, signifying a change in resting state physiology.

*Data analysis*

All data were first motion corrected in BrainVoyager (Brain Innovations, Maastricht, The Netherlands). The surround subtraction and averaging method (Wong et al., 1997) was used to generate raw motion-corrected CBF and BOLD timeseries in Matlab (The

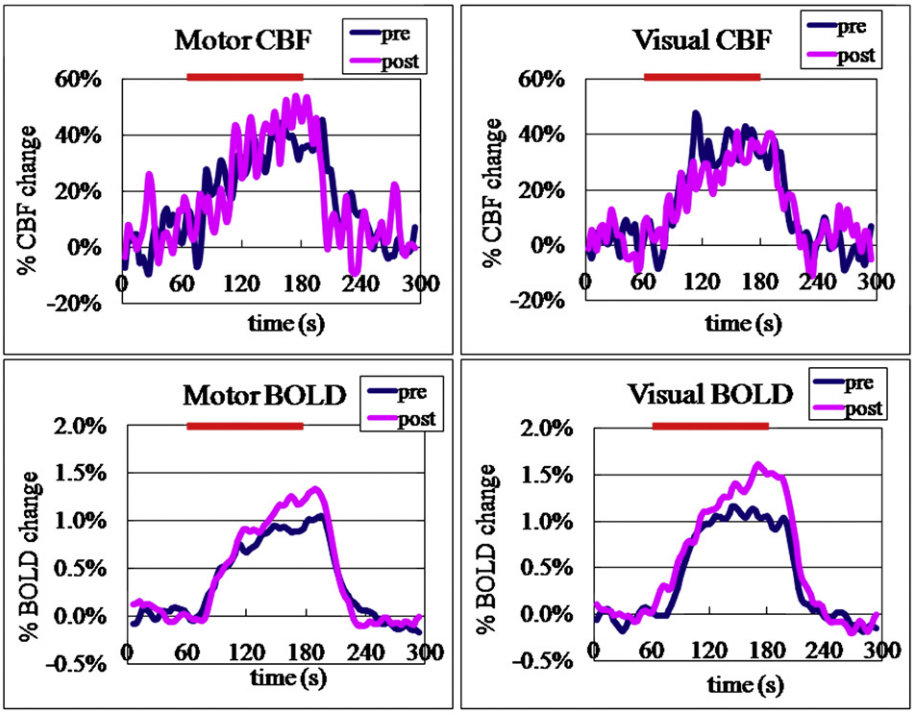


**Fig. 3.** CBF and BOLD timecourses collected during the hypercapnia scans, averaged over the two scans in each session for all 14 subjects. The thick horizontal line at the top of the graph indicates $CO_2$ administration. Notice the pre- and post-caffeine CBF timecourses appear identical, whereas the BOLD amplitude increased after caffeine.

**Table 2**
Literature estimates of *M*, compared to pre-caffeine estimates of *M* (mean±SD) in current study

| | Motor *M* (%) | Visual *M* (%) |
|---|---|---|
| (Stefanovic et al., 2006) | 6.1±1.1 | 7.6±1.3 |
| (Chiarelli et al., 2007) | 4.3±3.5 | 6.6±3.4 |
| (Davis et al., 1998) | – | 7.9±2.2 |
| Current study (pre-caffeine) | 3.7±1.0 | 5.1±2.0 |

MathWorks,Inc., Natick, MA). The separated CBF and BOLD timeseries were then imported back into BrainVoyager for processing. BOLD data were processed as follows: 1) spatial smoothing with a 8 mm FWHM Gaussian kernel, 2) linear trend removal and 3) temporal smoothing over 6 s (2 TRs). The CBF data were spatially smoothed with a 8 mm Gaussian kernel. No temporal smoothing was applied to the CBF data as it reduces statistical power (Wang et al., 2005). All image series were co-registered to the high-resolution anatomic images.

Activation maps were generated by cross-correlation to a reference function generated by convolving the boxcar function of the paradigm with a canonical hemodynamic response function. ROIs were selected in the left and right motor and visual areas based on the CBF correlation maps threshold at $r>0.23$ (t-score 2.22), and applied to both the BOLD and hypercapnia data to extract timecourses. Percent changes relative to baseline were calculated from these timecourses after averaging over the functional blocks. Data from left and right hemispheres were also averaged. To avoid the inclusion of post-stimulus undershoot, baseline was calculated from the initial rest period and the final 15 s of the "off" period between blocks. Activation was calculated from the final 30 s of each functional block to ensure the signal has reached steady state. Similarly for the hypercapnia data, baseline was defined from the initial 1 min rest period and the final 1 min at the end of each run, and activation was calculated from the final 1 min of each hypercapnic block. For statistical analysis, the pre-caffeine results were compared to post-caffeine results using two-tailed paired t-test. The results were considered statistically significant when $p<0.05$.

## Results

The physiological parameters averaged over all subjects are listed in Table 1. The differences between pre- and post-caffeine sessions were not statistically significant. Breathing the $CO_2$ enriched gas increased $etCO_2$ by 14.1 mmHg and 12.8 mmHg respectively before and after caffeine injection. This difference was not significant.

Fig. 3 shows the BOLD and CBF timecourses for the hypercapnia scans. The CBF timecourses pre- and post-caffeine appear identical in amplitude and timing characteristics, suggesting that caffeine does not affect cerebrovascular reactivity to $CO_2$. However, an increase in amplitude was measured in the post-caffeine BOLD timecourses for both motor and visual cortices. For further analysis, the average BOLD responses to hypercapnia pre- and post-caffeine for each subject were calculated by averaging timepoints of the timecourses between 150 s–210 s and compared using two-tailed paired t-test. The increase in BOLD response post-caffeine was statistically significant for both motor ($p=0.04$) and visual ($p=0.01$) areas.

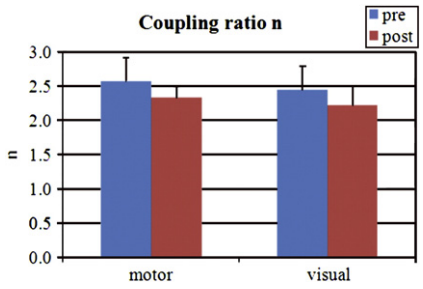


**Fig. 6.** Average CBF:CMRO2 coupling ratios for motor and visual cortices pre- and post-caffeine administration. Notice caffeine decreases the coupling ratio in both cortices.

The average *M* values for pre- and post-caffeine motor and visual areas are plotted in Fig. 4. Error bars represent standard deviation. A statistically significant increase in *M* was observed in both motor and visual areas after caffeine (paired t-test, visual: $p=0.02$, motor: $p=0.01$). Since *M* was estimated by fitting Equation [1] to hypercapnia data collected while subjects were not performing any functional tasks, the change in *M* post-caffeine suggests caffeine has the ability to alter resting state physiology, likely through a combination of caffeine's effects on BOLD and CBF. This result highlights the importance of accounting for the effect of caffeine in functional MRI studies, as this type of inter-subject variation could be a source of noise in group analyses. For comparison, *M* values estimated from other studies in recent literature are listed in Table 2.

$\Delta CMRO_2$ values calculated from the functional scans using Equation [3] are plotted against ΔCBF in Fig. 5. This plot shows that caffeine increases $\Delta CMRO_2$ more than ΔCBF, which decreases the coupling ratio n from 2.58 to 2.33 in the motor cortex ($p=0.006$, two-tailed paired t-test), and a similar change of 2.45 to 2.23 ($p=0.002$) was observed for the visual cortex (see Fig. 6).

## Discussion

There has been much debate on the relationship between baseline perfusion and BOLD due to the complexity of the BOLD signal. The calibrated BOLD approach is an excellent tool for examining how BOLD is coupled to changes in perfusion and neural activity. In the pre-caffeine portion of the study, we used the calibrated BOLD approach to calculate the CBF:$CMRO_2$ coupling ratio in both motor and visual

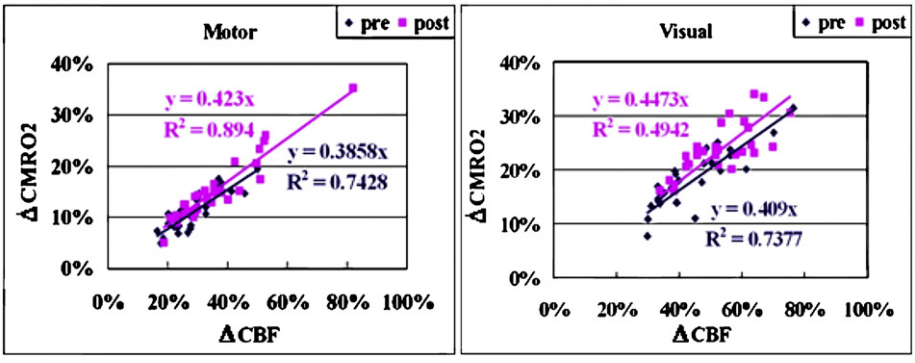


**Fig. 5.** %$\Delta CMRO_2$ vs. %ΔCBF plots for motor and visual tasks measured pre- (diamond, solid line) and post-caffeine (square, dashed line). In both cortices, caffeine increases the slope of the fitted line, signifying a larger increase in $\Delta CMRO_2$ per unit increase in ΔCBF during activations.

cortices. The M values obtained from the current study (motor: 3.7 ± 1.0, visual: 5.1 ± 2.0) are close to the values reported by previous studies but slightly lower (see Table 2), likely due to the shorter TE used in the current study. The current results agree with the observation of other studies that the visual cortex has a greater *M* value than the motor cortex, which may be attributed to the higher concentration of venules in the visual cortex (Davis et al., 1998). The coupling ratios for motor and visual were 2.58 and 2.45, which are within the range of values reported in previous studies (Davis et al., 1998; Hoge et al., 1999a; Kastrup et al., 2002; Uludag et al., 2004).

An important finding in this study is that caffeine increases *M* in both motor and visual cortices. This increase is primarily due to the increase in BOLD response to hypercapnia while CBF response remained unchanged (see Fig. 3). The increase in BOLD response is related to caffeine's ability to reduce resting state CBF. In the current study, caffeine reduced resting state CBF by 26%±8%, similar to that reported by earlier studies using ASL and PET (Cameron et al., 1990; Field et al., 2003; Liu et al., 2004). This reduction in resting state CBF causes a corresponding decrease in $CBV_0$, as well as an increase in $[dHb]_0$. As CBV is a small quantity, the change in $CBV_0$ would be a minor contribution to the change in *M*. Given that $\beta$ used for this study is 1.5, the $[dHb]_0$ term has a larger contribution, and *M* is expected to increase. It is important to note that this increase in BOLD response may not be entirely due to vascular effects, but also include contribution from caffeine's neurostimulative effects through binding with $A_1$ receptors. Support for this is the larger increase in BOLD response to hypercapnia in the visual cortex. A recent PET study used a novel $^{18}$F-labeled $A_1$ receptor antagonist to image $A_1$ receptor distribution in vivo and found higher accumulation of the radio-labeled antagonist in the occipital lobe compared to the sensorimotor cortex (Bauer et al., 2003). Given the higher distribution of $A_1$ receptors, it is possible that caffeine's effect on BOLD may be greater in the visual cortex.

A few previous studies have used BOLD fMRI as a method to investigate cerebrovascular reactivity (CVR) to hypercapnia (Kastrup et al., 2001; van der Zande et al., 2005; Vesely et al., 2001). Although BOLD fMRI is an attractive alternative to transcranial Dopper (TCD) ultrasound, the traditional method for measuring CVR, it is important to remember that the BOLD signal is based on a combination of many physiological processes. When a substance such as caffeine, which is capable of altering both neural activity and CBF, is administered, the BOLD signal is no longer an accurate measure of CVR. ASL, on the other hand, provides a direct measure of CBF, and is therefore a more realistic measure of vascular reactivity. The timecourses in Fig. 3 show that caffeine does not alter vascular response to $CO_2$ in both motor and visual cortices. This supports the results of a recent study that used TCD to investigate blood velocity changes in the middle cerebral artery (MCA) and found no change in vasomotor reactivity to $CO_2$ after caffeine (Blaha et al., 2007). Since the MCA is a major blood vessel to the brain, the TCD study focused on the global effect of caffeine, whereas the current study extends the results by investigating local effects of caffeine.

Results from the functional scans demonstrated that caffeine decreases the coupling ratio n in both motor and visual activations. A possible explanation for this change in coupling could be that caffeine increases the total number of neurons firing during activation, which is supported by the observation that caffeine increases glucose utilization in rats (Nehlig et al., 1986, 1984). Given that caffeine decreases baseline CBF, oxygen extraction fraction (OEF) is expected to increase in order to support the increased metabolic demand, since OEF is inversely proportional to n. However, OEF cannot increase by a large amount without disrupting the cerebrovascular physiology balance, so it is possible that anaerobic mechanisms will also be involved to sustain the increased metabolic demand. A second explanation for the altered coupling could be that caffeine increases oxygen consumption without altering the number of neurons firing. But this is rather counterintuitive as the combination of an increased oxygen demand and decreased CBF (hence oxygen supply) would mimic a hypoxic situation. This may explain why the brain attempts to adjust for this imbalance by upregulating $A_1$ receptors in habitual caffeine users, leading to a higher baseline CBF level (Field et al., 2003). Since the calibrated BOLD model does not offer insight into the actual mechanism underlying the uncoupling of CBF and $CMRO_2$, additional studies using non-flow based methods such as electroencephalogram (EEG) and FDG-PET are needed to better understand the mechanism of caffeine.

A major difference between the present study and other studies on caffeine is the method of caffeine administration. Other studies have used either over-the-counter caffeine pills (Field et al., 2003; Koppelstaetter et al., 2008; Laurienti et al., 2002, 2003; Liu et al., 2004; Mulderink et al., 2002) or beverages (Bendlin et al., 2007; Dager et al., 1999), which are both oral administrations, while the current study administered caffeine through an injection. One reason for this choice is to eliminate the need to remove and reposition the subject, which is necessary for the oral methods as swallowing is a hazard in supine position. Changes in subject position alter field homogeneity, shimming, and partial volume effects, resulting in vastly different BOLD baselines, thus making comparison between before and after caffeine sessions difficult. An injection, on the other hand, can occur while the subject is inside the scanner. Additionally, over-the-counter pills deliver a fixed amount of caffeine regardless of body weight, which affects metabolic rates of caffeine. This can be accounted for by injecting a fixed caffeine dose based on body weight as has been done in the current study. Alternatively, it is also possible to have a pharmacist develop individually-dosed pills, but when caffeine is ingested through the gastrointestinal tract, it takes about 45 min for 99% absorption in humans (Fredholm et al., 1999). Given the length of the current study, an additional 45 min is not practical.

A limitation of this study is that the measurements are not absolute. Although subjects remain inside the scanner throughout the study, the length of the study and the need for subjects to position the $CO_2$ apparatus makes it difficult for subjects to remain perfectly still. Although slight changes in position can be rectified by motion correction, BOLD baselines could be significantly different, leading to errors in the comparison between scans. This is one reason why so many subjects were necessary for the results to be statistically significant.

Another weakness of the current study is the low BOLD contrast observed, which is related to the simultaneous ASL/BOLD sequence. Since both ASL and BOLD signal were acquired in a single echo, compromise between the two signals was needed. For this study, the minimum TE of 23 ms was chosen to optimize the ASL signal at the expense of BOLD sensitivity, as the ASL signal suffers from very low signal to noise ratio. A good solution to this problem without running separate ASL and BOLD scans is to use a dual-echo spiral sequence which collects the ASL data in the first echo and the BOLD data in the second echo (Perthen et al., 2008).

## Conclusion

We have demonstrated that the calibrated BOLD approach is a useful method for studying the effects of substances such as caffeine on fMRI. Our findings demonstrate that caffeine does not alter CVR during hypercapnia, but it decreases the CBF:$CMRO_2$ coupling ratio in both motor and visual cortices during task-related activations, potentially through a combination of increased OEF and anaerobic metabolism.

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