Published in final edited form as:

Neuroimage. 2009 May 15; 46(1): 56–63. doi:10.1016/j.neuroimage.2009.02.001.

Caffeine Reduces Resting-State BOLD Functional Connectivity in the Motor Cortex

Anna Leigh Rack-Gomer, Joy Liau, and Thomas T. Liu

Center for Functional Magnetic Resonance Imaging and Departments of Radiology and Bioengineering, University of California San Diego, La Jolla, CA

Abstract

In resting-state functional magnetic resonance imaging (fMRI), correlations between spontaneous low-frequency fluctuations in the blood oxygenation level dependent (BOLD) signal are used to assess functional connectivity between different brain regions. Changes in resting-state BOLD connectivity measures are typically interpreted as changes in coherent neural activity across spatially distinct brain regions. However, this interpretation can be complicated by the complex dependence of the BOLD signal on both neural and vascular factors. For example, prior studies have shown that vasoactive agents that alter baseline cerebral blood flow, such as caffeine and carbon dioxide, can significantly alter the amplitude and dynamics of the task-related BOLD response. In this study, we examined the effect of caffeine (200 mg dose) on resting-state BOLD connectivity in the motor cortex across a sample of healthy young subjects ($N = 9$). We found that caffeine significantly ($p < 0.05$) reduced measures of resting-state BOLD connectivity in the motor cortex. Baseline cerebral blood flow and spectral energy in the low-frequency BOLD fluctuations were also significantly decreased by caffeine. These results suggest that caffeine usage should be carefully considered in the design and interpretation of resting-state BOLD fMRI studies.

Introduction

In resting-state functional magnetic resonance imaging (fMRI), low-frequency (typically less than 0.08 Hz) fluctuations in the blood oxygenation level dependent (BOLD) signal are generally thought to reflect underlying fluctuations in neural activity. The correlation between BOLD fluctuations in different brain regions is then used as a measure of functional connectivity between these regions. A change in BOLD correlation is often interpreted as a change in the functional connectivity of the respective brain regions.

Biswal and colleagues (Biswal et al. 1995) were the first to demonstrate the presence of synchronous BOLD fluctuations within the motor cortex at rest. Since then, resting-state connectivity has been evaluated in many other functional networks including the default mode network, which comprises regions of the brain that routinely decrease activity in the presence of a task (Lowe et al. 1998; Hampson et al. 2002; Greicius et al. 2003). The strength of functional connections in the default mode network has been shown to correlate with performance on working memory tasks (Hampson et al. 2006), providing evidence that spontaneous activity can yield important information regarding cognitive ability and function.

Correspondence to: Thomas T. Liu, Ph.D., UCSD Center for Functional MRI, 9500 Gilman Drive, MC 0677, La Jolla, CA 92093-0677, Phone: 858-822-0542; Fax: 858-822-0605; E-mail: E-mail: ttlui@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Also, a growing number of studies have demonstrated that resting-state connectivity is altered in disorders such as Alzheimer's disease, schizophrenia, multiple sclerosis, and epilepsy (Lowe et al. 2002; Greicius et al. 2004; Garrity et al. 2007; Lui et al. 2008). In these studies, decreases in BOLD connectivity are often interpreted as an impairment of the neural connections between related brain regions. However, this interpretation can be complicated by the dependence of the BOLD signal on both neural and vascular factors.

Functional MRI provides an indirect measure of neural activity, where the BOLD response is a complex function of changes in oxygen metabolism, cerebral blood flow (CBF), and blood volume that follow changes in neural activity (Buxton et al. 2004). As a result, the BOLD signal may be affected by alterations in the neurovascular coupling that links neural activity to hemodynamic changes. Studies with task-related BOLD fMRI have shown that changes in neurovascular coupling, due to factors such as disease, age, medication, and diet, can significantly alter the BOLD response (D'Esposito et al. 2003). In particular, factors that alter baseline CBF can modulate the temporal dynamics of the BOLD response through alterations in the strength of neurovascular coupling (Behzadi and Liu 2005). Hypercapnia, which increases baseline CBF through vasodilation, has been shown to reduce the magnitude and speed of the visual BOLD response (Kemna and Posse 2001; Cohen et al. 2002). These changes indicate that the BOLD response may be less sensitive to neural stimulation when baseline CBF is increased. Additional studies by our research group and others have shown that caffeine and hypocapnia, which both reduce baseline CBF, increase the speed of the visual BOLD response (Cohen et al. 2002; Liu et al. 2004; Behzadi and Liu 2006; Liao et al. 2008b), suggesting that neurovascular coupling is strengthened when CBF decreases. The amplitude of the task-related BOLD response has also been shown to have an inverse dependence on baseline CBF (Cohen et al. 2002; Mulderink et al. 2002; Liao et al. 2008a), both across subjects and pharmacological conditions. Overall, these studies suggest that the sensitivity of the BOLD response to stimulated neural activity may be inversely related to baseline CBF.

Changes in neurovascular coupling may also influence resting-state BOLD connectivity measures. A number of studies have examined the effect of altered baseline CBF on low-frequency fluctuations in cerebral hemodynamics (Dirnagl et al. 1993; Hudetz et al. 1995; Obrig et al. 2000). For example, Dirnagl et al. (1993) found that nitrous oxide synthase blockade, which constricts blood vessels, produced an increase in low-frequency oscillations in CBF as measured with laser-Doppler flowmetry in rats anaesthetized with α -chloralose. In another study of anaesthetized (pentobarbital + 0.5% halothane) rats, Hudetz et al. (1995) showed that nitrous oxide synthase blockade increased flow oscillations, while hypercapnia reduced the oscillations. Obrig et al. (2000) used near infrared spectroscopy to show that hypercapnia also attenuates spontaneous fluctuations in cerebral oxygenation in awake humans. In general, the prior studies suggest that vasodilation reduces the amplitude of spontaneous fluctuations, while vasoconstriction tends to increase fluctuations.

Similar to task-related studies, the results of these resting-state studies suggest that the strength of neurovascular coupling between spontaneous neural activity and hemodynamic fluctuations is inversely related to baseline CBF. Furthermore, a resting-state fMRI experiment found that hypercapnia (5% CO₂) decreased BOLD connectivity measured in the motor cortex in addition to reducing low-frequency BOLD fluctuations (Biswal et al. 1997). A number of animal studies have found that mild levels of hypercapnia do not affect neural activity (Jones et al. 2005; Sicard and Duong 2005; Zappe et al. 2008). Jones et al. (2005) used optical imaging spectroscopy, laser Doppler flowmetry, and multi-channel electrophysiology (EEG) to measure the hemodynamic and neural responses to whisker stimulation in rats anesthetized with urethane. They found that 5% CO₂ did not significantly alter the cerebral metabolic rate of oxygen (CMRO₂) responses or the EEG responses. In another study with spontaneously breathing isoflurane anesthetized rats, Sicard et al. (2005) showed that both baseline

CMRO₂ levels and CMRO₂ increases with forepaw stimulation were not significantly changed by 5% CO₂. Zappe et al. (2008) found a trend toward hypercapnia reduced spontaneous neural activity as measured with intracortical recordings in the visual cortex of remifentanyl anesthetized macaque monkeys, but it was not significant at 3% CO₂ (equivalent to ~5% CO₂ in humans). These findings suggest that a decrease in BOLD connectivity caused by hypercapnia most likely reflects a reduction in the strength of neurovascular coupling, as opposed to an alteration in neural activity. Therefore, factors that change baseline CBF may be significant confounds in resting-state fMRI studies, as a decrease in BOLD connectivity due to a reduction in the strength of neurovascular coupling might be incorrectly interpreted as a decrease in neural connectivity. An improved understanding of the effects that vascular changes produce on BOLD connectivity is especially important for clinical populations where disease and medication may alter both neural connectivity and neurovascular coupling.

In this study, we examined the effect of a 200 mg caffeine dose on resting-state BOLD measures in the motor cortex. Caffeine is a commonly used neural and metabolic stimulant that readily binds to adenosine receptors, thereby competitively inhibiting adenosine activation and decreasing baseline CBF (Cameron et al. 1990; Ngai et al. 2001). As prior work with task-related BOLD fMRI suggests that caffeine may increase the sensitivity of the BOLD signal to stimulated neural activity, we hypothesized that caffeine would also increase the sensitivity of BOLD fluctuations to spontaneous neural activity and therefore produce an increase in resting-state BOLD connectivity.

Methods

Experimental protocol

Eleven healthy volunteers participated in this study after providing informed consent. After exclusion of data from 2 subjects due to excessive motion (greater than 0.4mm root mean squared displacement), the sample consisted of 9 subjects (5 males and 4 females, ages 23 to 41 years). Participants were instructed to refrain from ingesting caffeine for at least 12 hours prior to being scanned. The estimated daily caffeine usage for each subject based on self-reports of coffee, tea, and soda consumption is presented in Table 1. The assumed caffeine contents for an 8-oz cup of coffee, an 8-oz cup of tea, and a 12-oz soda were 100 mg, 40 mg, and 20 mg respectively (Fredholm et al. 1999).

Each subject participated in two imaging sessions: a caffeine session and a control session, in that order. The two imaging sessions were separated by at least 6 weeks. The caffeine session consisted of a pre-dose and a post-dose imaging section, each lasting around 45 minutes each. Upon completion of the pre-dose section, participants ingested a 200 mg caffeine pill and then rested for approximately 30 minutes outside of the magnet before starting the post-dose section. This procedure is similar to our previous protocols using caffeine (Liu et al. 2004; Behzadi and Liu 2006). The first resting-state scan of the post-dose section began approximately 45 minutes after the caffeine pill was ingested to achieve approximately 99% absorption of caffeine from the gastrointestinal tract (Fredholm et al. 1999). Control sessions used the same protocol, but without the administration of caffeine between sections, similar to the protocol used in (Perthen et al. 2008). Subjects were not given a placebo during the control session. However, for convenience, we will still refer to the two scan sections as the “pre-dose” and “post-dose” sections, even though a dose was not administered.

Each scan section consisted of (1) a high-resolution anatomical scan, (2) a bilateral finger tapping block design, (3) CBF baseline and quantification scans, and (4) two five-minute resting-state BOLD scans. Bilateral finger tapping was self-paced and the block design run consisted of 20s rest followed by 5 cycles of 30s tapping and 30s resting. Subjects were instructed to tap while a flashing checkerboard was displayed and then to rest during the display

of a control image, consisting of a white square situated in the middle of a gray background. During resting-state scans, the control image was displayed for the entirety of the scan and subjects were asked to maintain attention on the white square.

Image acquisition

Imaging data were collected on a GE Signa 3 Tesla whole body system with an eight channel receive coil. Laser alignment was used to landmark subjects and minimize differences in head position between pre-dose and post-dose sections.

Functional data were collected over six oblique 6-mm thick slices prescribed through the primary motor cortex. The finger tapping scan and a CBF baseline scan were acquired with a PICORE QUIPSS II (Wong et al. 1998) arterial spin labeling (ASL) sequence (TR = 2s, TI₁/TI₂ = 600/1500 ms, 10 cm tag thickness, 1 cm tag-slice gap) with dual echo spiral readout (TE₁/TE₂ = 9.2/30 ms, FOV = 24 cm, 64 × 64 matrix, and flip angle = 90°). The two resting-state BOLD scans were acquired using BOLD-weighted imaging with spiral readout (TE = 30ms, TR = 500 ms, FOV = 24 cm, 64 × 64 matrix, and flip angle = 45°).

Additional CBF quantification scans were acquired using the same in-plane parameters as the functional scans, but the number of slices was increased to ensure coverage of the lateral ventricles. These scans included a cerebrospinal fluid (CSF) reference scan consisting of a single-echo, single repetition sequence (TE = 9.1ms), and a minimum contrast scan (TR = 2s, TE = 11 ms). The high-resolution anatomical scan was acquired with a magnetization prepared 3D fast spoiled gradient (FSPGR) sequence (TI=450 ms, TR=7.9 ms, TE=3.1 ms, 12° flip angle, FOV 25 cm, matrix 256 × 256 × 256).

Cardiac pulse and respiratory effort data were monitored using a pulse oximeter (InVivo) and a respiratory effort transducer (BIOPAC), respectively. The pulse oximeter was placed on the subject's index finger, and the respiratory effort belt was placed around the subject's abdomen. The pulse oximeter was not worn during the bilateral finger tapping scan. Physiological data were sampled at 40 samples per second using a multi-channel data acquisition board (National Instruments).

Data analysis

Images from each scan section were co-registered using AFNI software (Cox 1996). In addition, the anatomical volume from each post-dose section was aligned to the anatomical volume of its respective pre-dose section, and the rotation and shift matrix used for this alignment was then applied to the post-dose functional images. The outer two slices of the functional data were discarded to minimize partial volume effects associated with the rotation of the post-dose data, and the first 10s of each functional run were not included. In addition, voxels from the edge of the brain were not included in the analysis in order to minimize the effects of motion.

The second echo data from the finger tapping scans were used to generate BOLD activation maps of the motor cortex. This was accomplished using a general linear model (GLM) approach for the analysis of ASL data (Mumford et al. 2006; Restom et al. 2006). The stimulus-related regressor was produced by the convolution of the square wave stimulus pattern with a gamma density function (Boynton et al. 1996). Constant and linear trends were included in the GLM as nuisance regressors. In addition, the data were pre-whitened using an autoregressive model of order 1 (Burock and Dale 2000; Woolrich et al. 2001). The statistical maps were based on the square root of the F-statistic, which is equal to the t-statistic in the case of one nuisance term (Liu et al. 2001). Active voxels were defined using a method based on activation mapping as a percentage of local excitation (AMPLE) (Voyvodic 2006). In summary, the $\sqrt{F}$ maps were

separated into left and right hemispheric regions. The highest value in each region was identified and then every voxel was converted to a percentage of the peak statistical value for the region ($\text{AMPLE value} = (\sqrt{F} \text{ value} / \text{peak } \sqrt{F} \text{ value}) \times 100$). Active voxels were required to exceed an AMPLE value of 45% and a $\sqrt{F}$ value of 2 ($p < 0.05$). The final activation maps were defined from the intersection of voxels active in both pre-dose and post-dose scan sections. Regions of interest (ROIs) were then defined for the left and right motor cortices from these activation maps. Thus, the same ROIs were used in the comparison of pre-dose and post-dose functional connectivity within an imaging session.

Baseline CBF images were calculated from the average difference between the tag and control images in the CBF baseline scan. The mean ASL images were corrected for coil sensitivity and B1 field inhomogeneities with the minimum contrast scan (Wang et al. 2005), and converted to physiological units, $\text{ml}/(100\text{g} \cdot \text{min})$, using the CSF reference scan (Chalela et al. 2000). Average baseline CBF values were extracted from each subject's motor cortex, defined as the union of the right and left motor ROIs.

The time series data from the BOLD resting-state runs were used to generate measures of resting-state functional connectivity. First, nuisance parameters were removed from the raw data through linear regression. These regressors included constant and linear trends, physiological noise terms from the measured cardiac and respiratory signals (Glover et al. 2000), and six motion parameters obtained during image co-registration. Data were then temporally low-pass filtered using a finite impulse response function (Diniz et al. 2002) with a cutoff frequency of 0.08 Hz. This cutoff frequency was chosen for consistency with previous functional connectivity studies (Biswal et al. 1997; Cordes et al. 2001; Fox et al. 2005). The average signal from either the left ROI or the right ROI was used to define a reference time course. This reference time course was then correlated with all other voxel time series within the brain to generate a functional connectivity map. The correlation coefficients (r values) in these maps were converted to z scores in a method similar to that used by Fox et al. (2005). For a qualitative presentation of functional connectivity changes, average z score maps for the pre-dose and post-dose sections were calculated for each subject by averaging across the two resting-state runs and thresholding at $z = 2.58$ ($p < 0.005$). Voxels were required to have at least 2 nearest neighbors for inclusion in the resting-state connectivity maps.

Two metrics were used to quantitatively assess the strength of resting-state functional connectivity in the motor cortex. For the first metric, the mean z score was extracted from either the right or left ROI (defined with the AMPLE method as described above), where the average signal from the opposite ROI was used as a reference time course. The second metric, referred to as the percent overlap, was first used by Biswal et al. (1995, 1997), and is described in more detail in these references. To summarize, the quantity $\bar{n}_{LR}/n_L$ is the percent of voxels in the left ROI that are significantly correlated with all voxel time courses from the right ROI (mean $r > 0.42$, $p = 0.001$), where the subscripts R and L refer to right and left, respectively. In this method, $\bar{n}_{LR}/n_L$ provides a measurement of connectivity between hemispheres, with $\bar{n}_{LR}/n_L$ necessarily equal to $\bar{n}_{RL}/n_R$. Two additional measures of connectivity within hemispheres were calculated as well. These quantities, $\bar{n}_{LL}/n_L$ and $\bar{n}_{RR}/n_R$, represent the percent of voxels in the left and right ROIs, respectively, that are significantly correlated with all other voxel time series from within the respective ROIs. Two-tailed paired t -tests were performed between the pre-dose and post-dose results to assess changes in these metrics of functional connectivity. In addition, a repeated measures two-way analysis of variance (ANOVA) was performed for each metric (Keppel and Wickens 2004). Also, the correlation coefficient threshold used in the percent overlap method was varied between 0.24 and 0.42 ($0.05 \geq p \geq 0.001$) in increments of 0.02 to assess the robustness of this measurement.

For frequency domain analysis, power spectra were calculated from the pre-processed (but unfiltered) resting-state data using a minimum 4-term Blackman-Harris window. Average power spectra were extracted from the motor cortex (union of right and left ROIs) in the pre-dose and post-dose sections for each subject. A quantitative measurement of energy in the low-frequency BOLD fluctuations was calculated as the sum of the average power below 0.08 Hz. Paired *t* tests were performed to assess changes in energy between the pre-dose and post-dose sections.

Additional processing steps were taken to determine whether changes in resting-state functional connectivity might be due to changes in low-frequency respiration variations. This was accomplished by adding an extra regressor to the original nuisance matrix. The additional term was either the global signal (Fox et al. 2005) or the respiration volume per time (RVT) signal (Birn et al. 2006). In order to minimize bias that can be introduced when motor cortex fluctuations are included in the regression (Murphy et al. 2008), the global signal was extracted from the anterior portion of the brain in the four slices that were analyzed. Because both the global and RVT signals were obtained in arbitrary units, we first normalized the global and RVT signals to their mean values to derive percent changes of these signals. We then computed the energy below 0.08 Hz and the standard deviation of the percent change signals.

To assess changes in the finger tapping BOLD response to caffeine, average % Δ BOLD block responses were extracted from the combined motor cortex ROI. Each subject's % Δ BOLD block response was interpolated to a time resolution of 0.25s and the following timing parameters were computed: 1) time to reach 50% of the peak response (T_{50}), 2) time at which the response falls to 50% of the peak response (TA_{50}), and 3) the full-width half-maximum ($FWHM = TA_{50} - T_{50}$). In addition, the peak BOLD response amplitudes were calculated for each subject.

Results

Figure 1 provides a qualitative summary of the results. Resting-state functional connectivity maps obtained by using the average signal from the left ROI as a reference are shown for a representative slice from each subject. The top two rows display each subject's average pre-dose and post-dose maps for the caffeine session. The extent of significantly ($p < 0.005$) correlated voxels in the post-dose connectivity maps has been visibly diminished in most subjects, as compared to the pre-dose maps. In contrast, for most subjects the control session maps in the bottom two rows do not demonstrate an obvious difference in connectivity between pre-dose and post-dose conditions.

Metrics of connectivity strength are graphed in Figure 2. The scatter plots in the top row show the mean *z* scores for each subject (post-dose vs. pre-dose) from the (a) caffeine session and (b) control session, with the solid black lines representing equality between the pre-dose and post-dose sections. The caffeine session plot shows a significant ($t(8) = 3.3$, $p = 0.01$) caffeine-induced decrease in mean *z* score across subjects. In contrast, the mean *z* scores in the control session are clustered about the centerline, without a significant ($t(8) = -0.51$, $p = 0.62$) change between pre-dose and post-dose conditions. These results are consistent with a repeated measures two-way ANOVA, which showed that the interaction between period (pre-dose vs. post-dose) and session (caffeine vs. control) is significant ($F(1,8) = 9.8$, $p = 0.01$). In addition, the baseline (pre-dose) mean *z* scores in panels (a) and (b) are not significantly different ($t(8) = 0.36$, $p = 0.73$). The mean *z* scores presented here were determined using the average time course from the left motor ROI as a reference. If the average time course from the right ROI is used as a reference instead, the caffeine-induced decline in mean *z* score is still significant ($t(8) = 2.8$, $p = 0.02$).

The bar charts in the bottom row of Figure 2 present the group average pre-dose and post-dose percent overlap values for the (c) caffeine session and (d) control session. Caffeine significantly ($t(8) > 2.3$, $p < 0.05$) reduced the three metrics, $\bar{n}_{LL}/n_L$, $\bar{n}_{LR}/n_L$, and $\bar{n}_{RR}/n_R$. In contrast, no significant ($|t(8)| < 0.6$, $p > 0.56$) change exists between the pre-dose and post-dose metrics in the control session. These results are consistent with a repeated measures two-way ANOVA, which showed that the interaction between period (pre-dose vs. post-dose) and session (caffeine vs. control) is significant ($F(1,8) > 5.1$, $p \leq 0.05$). The pre-dose group average percent overlap values are not significantly different ($|t(8)| < 0.51$, $p > 0.62$) between the caffeine and control sessions (blue bars in panels (c) and (d)). The percent overlap results shown in Figure 2 correspond to a correlation coefficient threshold of 0.42 ($p = 0.001$). This threshold was adjusted between 0.24 and 0.42 ($0.05 \geq p \geq 0.001$) to assess the robustness of the metric. The caffeine-induced decreases in $\bar{n}_{LR}/n_L$ and $\bar{n}_{RR}/n_R$ were significant ($t(8) > 2.3$, $p < 0.05$) at every threshold. The quantity $\bar{n}_{LL}/n_L$ exhibited a post-dose decrease across subjects at each threshold, but the paired t-test p values varied from 0.2 to 0.04. The reduction in $\bar{n}_{LL}/n_L$ was significant ($t(8) > 2.3$, $p < 0.05$) for thresholds of $r \geq 0.42$.

Inspection of the physiological data collected during imaging showed that caffeine did not significantly alter the group average heart rate ($t(8) = 1.5$, $p = 0.18$) or breathing rate ($t(8) = -0.92$, $p = 0.38$). We also found no significant changes in the standard deviation of the RVT signal ($t(8) = 0.7$, $p = 0.5$) or global signal ($t(8) = -1.5$, $p = 0.2$). In addition, there were no significant changes in the group average energy (< 0.08 Hz) for the RVT signal ($t(8) = 0.28$, $p = 0.8$) or for the global signal ($t(8) = -1.9$, $p = 0.09$). Thus, differences in low-frequency respiration variations do not appear to be responsible for the caffeine-induced decreases in functional connectivity observed. Consistent with these results, we found that the metrics of functional connectivity (mean z score, $\bar{n}_{LL}/n_L$, $\bar{n}_{LR}/n_L$, and $\bar{n}_{RR}/n_R$) were not significantly ($|t(17)| < 1.1$, $p > 0.3$) altered when RVT signal regression was included in the analysis. In addition, all caffeine-related decreases were still significant ($t(8) > 2.5$, $p < 0.04$) after inclusion of either the global signal or the RVT signal in the analysis.

Baseline CBF values are shown in Table 1. Caffeine produced a significant ($t(8) = 7.4$, $p = 7.4 \times 10^{-5}$) decrease in baseline CBF across subjects, with an average decline of 30%. No significant change in baseline CBF was observed in the control session data ($t(8) = -0.34$, $p = 0.74$). Consistent with previous findings by our group (Liu et al. 2004; Behzadi and Liu 2006; Liao et al. 2008b), we found that the caffeine-induced decreases in CBF were accompanied by an acceleration in the temporal dynamics of the BOLD block response. Caffeine produced a 25% decrease in T_{50} ($t(8) = 2.6$, $p = 0.03$) and a 5% decrease in TA_{50} ($t(8) = 3.6$, $p = 0.01$). We did not find significant changes in the FWHM ($t(8) = 1.5$, $p = 0.17$) or peak BOLD amplitude ($t(8) = -1.1$, $p = 0.31$). As addressed in the Introduction, we had expected that the acceleration of the BOLD temporal dynamics with the caffeine-induced reduction in CBF would tend to increase resting-state functional connectivity, but instead found a decrease in connectivity. Furthermore, the caffeine-related decreases in mean z-scores and percent overlap metrics were not significantly correlated with either pre-dose baseline CBF ($|r| < 0.28$, $p > 0.46$) or the changes in baseline CBF observed ($|r| < 0.24$, $p > 0.53$).

Spectral analyses of the resting BOLD fluctuations are shown in Figure 3. Scatter plots of the spectral energy below 0.08 Hz (post-dose vs. pre-dose session) are provided for the (a) caffeine session and (b) control session. Caffeine ingestion led to a significant ($t(8) = 3.4$, $p = 0.01$) reduction in the energy of low-frequency fluctuations. On the other hand, the control session results shown do not demonstrate a significant ($t(8) = 0.41$, $p = 0.69$) change in energy between the pre-dose and post-dose conditions. In addition, there was not a significant difference between the pre-dose energy values in the caffeine and control conditions ($t(8) = -1.1$, $p = 0.29$). Representative power spectra from Subject 2 are shown for the (c) caffeine session and (d) control session. The graphs present a qualitative comparison of the spectral power (in units

of $|\% \Delta \text{BOLD}|^2/\text{Hz}$) in the motor cortex between the pre-dose and post-dose conditions. The spectral energy below 0.08 Hz is visibly diminished by caffeine, whereas the power spectra from the control imaging session are similar for both the pre-dose and post-dose sections.

Discussion

We have shown that caffeine reduces resting-state BOLD connectivity in the motor cortex. This reduction was apparent in the connectivity maps from a representative subject and in the quantitative metrics of connectivity obtained for all subjects. In addition, we found that baseline CBF and the magnitudes of the spontaneous BOLD fluctuations were decreased by caffeine.

Physiological confounds, such as changes in respiration and arterial CO_2 levels, can alter BOLD signal fluctuations. Regression-based removal of global and respiration volume per time (RVT) signals has been shown to reduce the influence of these physiological confounds (Wise et al. 2004; Birn et al. 2006). When we included the regression of the RVT or global signal in our data analysis we found that the post-dose connectivity metrics were still significantly ($p < 0.05$) decreased. In fact, RVT signal regression did not significantly alter the functional connectivity metrics ($p > 0.3$). Furthermore, we found that the variance or energy in the global and RVT signals were not significantly altered by caffeine ($p > 0.3$). These findings suggest that the caffeine-induced reduction in BOLD connectivity is not primarily due to respiration changes.

In agreement with the literature, we found that the caffeine-reduced reduction in CBF was associated with an acceleration of the BOLD response to a finger tapping task (Cohen et al. 2002; Liu et al. 2004; Behzadi and Liu 2006; Liau et al. 2008b). In addition, vasoconstriction due to either hypocapnia or nitric oxide synthase blockade has been found to increase low-frequency fluctuations in CBF (Dirnagl et al. 1993; Hudetz et al. 1995). We have previously presented a biomechanical model that explains how vasoconstriction can increase the dynamic compliance of the arterioles and thus increase the responsiveness of the vasculature to neural stimulus and fluctuations (Behzadi and Liu 2005). However, in this study we found that both the spectral amplitude of low-frequency BOLD fluctuations and the coherence between resting BOLD fluctuations were diminished by caffeine, suggesting that an increase in biomechanical responsiveness was not a dominant factor.

Vasoconstriction due to caffeine is thought to primarily reflect the antagonism of adenosine A_2 receptors (Fredholm et al. 1999). As binding of adenosine to A_2 receptors is associated with vasodilation, caffeine-related antagonism may reduce the ability of adenosine to contribute to functional increases in cerebral blood flow. In a recent study, we found that a 200 mg oral dose of caffeine led to a significant decrease in the absolute functional CBF change (as measured in physiological units) in response to a visual stimulus but resulted in a significant increase in the percent CBF change (Liau et al. 2008b). These results indicated that the drop in the absolute functional CBF change was primarily related to a drop in baseline CBF as opposed to reflecting an impairment of neurovascular coupling. Also consistent with our prior work and the results of this study, Liau et al. (2008b) did not find a significant change in the BOLD response. Taken together, these results indicate that caffeine's effect on adenosine-related vasodilation does not significantly reduce the task-related functional BOLD response. If task-related and resting-state BOLD activity share a common neurovascular coupling pathway, then the task-related BOLD results suggest that an impairment of adenosine-related vasodilation was probably not the dominant factor in the reduced functional connectivity observed in this study. Further studies elucidating the similarities and differences in neurovascular coupling for task-related and resting-state BOLD signals would be useful.

In addition to its vasoconstrictive effects, caffeine directly influences neural activity. Caffeine stimulates the central nervous system by antagonizing adenosine A₁ receptors throughout the brain. This blocks the inhibitory actions of adenosine, which include hyperpolarization of membrane potentials and the inhibition of neurotransmitter release (Dunwiddie and Masino 2001). Although caffeine acts as a neurostimulant, previous work has shown that a 200 mg dose of caffeine reduces the power of resting electroencephalography (EEG) activity in the alpha, beta, and theta bands (Dimpfel et al. 1993; Siepmann and Kirch 2002). In addition, the coherence of anterior cortex neural fluctuations in the alpha and theta bands is decreased by caffeine when compared to periods of caffeine abstinence (Reeves et al. 2002). Simultaneous EEG/fMRI recordings have shown that resting-state BOLD fluctuations are significantly correlated with EEG power fluctuations in the alpha band (Goldman et al. 2002; Laufs et al. 2003a; Moosmann et al. 2003; de Munck et al. 2007; Ritter et al. 2008), the beta band (Laufs et al. 2003b), and the theta band (Scheeringa et al. 2008). These prior findings suggest that the reduction in resting-state BOLD fluctuations and connectivity found in this study may primarily reflect changes in neural power fluctuations. Although the physiological effects of caffeine are often beneficial, such as enhanced mood, attention, wakefulness, and motor speed (Lieberman et al. 1987; Kaplan et al. 1997; Fredholm et al. 1999), a 200 mg dose has been shown to impair several types of memory tasks, including motor learning of a finger tapping task (Mednick et al. 2008). In light of our findings, this observed decrease in motor learning might reflect a caffeine-induced decrease in resting-state neural connectivity. Further experiments with simultaneous EEG/fMRI would be useful to determine if caffeine-induced changes in neural power fluctuations are directly related to the observed reduction of BOLD connectivity.

In addition to caffeine, a number of pharmacological agents have been found to alter resting-state BOLD connectivity. Both hypercapnia (Biswal et al. 1997) and cocaine (Li et al. 2000) have been shown to reduce the magnitude and coherence of resting-state BOLD fluctuations, while anesthesia (Kiviniemi et al. 2005; Peltier et al. 2005; Vincent et al. 2007; Liu et al. 2008) appears to have varying effects depending on the specific agent and brain region. Cognitive disorders such as Alzheimer's disease, schizophrenia, multiple sclerosis, and epilepsy have also been shown to modulate BOLD connectivity (Lowe et al. 2002; Greicius et al. 2004; Garrity et al. 2007; Lui et al. 2008). While changes in resting-state BOLD connectivity are typically interpreted as changes in coherent neural activity across spatially distinct brain regions, changes to the neurovascular system may also alter connectivity. For example, as mentioned in the Introduction, hypercapnia appears to decrease BOLD connectivity by weakening the neurovascular coupling between spontaneous neural activity and resting-state BOLD fluctuations. Since many pharmacological agents and diseases are likely to affect both the neural and vascular systems, a greater understanding of the neural and vascular mechanisms that give rise to resting-state BOLD connectivity will be critical for the correct interpretation of changes in connectivity.

Similar to a prior study examining the effect of caffeine on baseline oxygen metabolism (Perthen et al. 2008), we used a control session to examine potential changes in baseline CBF, resting-state functional connectivity, and low-frequency BOLD fluctuations that might have been caused by differences in the subject's state between the pre-dose and post-dose scan sections. We did not find significant differences between the pre-dose and post-dose results obtained during the control session, indicating that the caffeine-induced decrease in BOLD connectivity was not due to factors, such as subject fatigue, associated with participating in two scan sections. As our protocol did not involve the administration of a placebo dose, it is possible that psychological effects associated with taking a dose could have affected the functional connectivity measures. Future studies would be useful to assess the effect of a placebo dose on resting-state BOLD connectivity.

There was a range of caffeine usage in the current sample of subjects. Prior work has demonstrated variability in the task-related BOLD response due to differences in dietary caffeine consumption. Inter-subject differences in caffeine consumption may also influence the effect of caffeine on resting-state BOLD connectivity. In addition, subjects in this study were asked to abstain from caffeine for at least 12 hours prior to being scanned. Caffeine withdrawal has been shown to alter EEG power in the alpha and theta bands (Jones et al. 2000; Reeves et al. 2002). It is possible that caffeine's effect on resting-state BOLD connectivity will differ based on the subject's state of withdrawal during the pre-dose scan section. Further investigation of the effects of dietary consumption and withdrawal on caffeine-induced changes in BOLD connectivity will be helpful.

The work presented here shows that caffeine reduces resting-state BOLD connectivity in the motor cortex, most likely by reducing the amplitude and coherence of neural power fluctuations. As the distribution of adenosine receptors varies across the brain (Dunwiddie and Masino 2001), it is possible that the effect of caffeine on functional connectivity will vary with the local receptor concentration. While future work is necessary to determine whether caffeine alters connectivity in other functional networks, the findings of this study indicate that caffeine usage should be carefully considered in the design and interpretation of studies involving resting-state BOLD connectivity.

Acknowledgments

This work was supported in part by a grant from the National Institutes of Health (ROINS051661). The authors thank Joanna Perthen and Kara Yopak for their assistance with this study.

References

- Behzadi Y, Liu TT. An arteriolar compliance model of the cerebral blood flow response to neural stimulus. *Neuroimage* 2005;25(4):1100–11. [PubMed: 15850728]
- Behzadi Y, Liu TT. Caffeine reduces the initial dip in the visual BOLD response at 3 T. *Neuroimage* 2006;32(1):9–15. [PubMed: 16635577]
- Birn RM, Diamond JB, Smith MA, Bandettini PA. Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *Neuroimage* 2006;31(4):1536–48. [PubMed: 16632379]
- Biswal B, Hudetz AG, Yetkin FZ, Haughton VM, Hyde JS. Hypercapnia reversibly suppresses low-frequency fluctuations in the human motor cortex during rest using echo-planar MRI. *J Cereb Blood Flow Metab* 1997;17(3):301–8. [PubMed: 9119903]
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo planar MRI. *Magn Reson Med* 1995;34:537–541. [PubMed: 8524021]
- Boynton GM, Engel SA, Glover GH, Heeger DJ. Linear systems analysis of functional magnetic resonance imaging in human V1. *J Neuroscience* 1996;16:4207–4221.
- Burock MA, Dale AM. Estimation and detection of event-related fMRI signals with temporally correlated noise: a statistically efficient and unbiased approach. *Human Brain Mapping* 2000;11:249–260. [PubMed: 11144754]
- Buxton RB, Uludag K, Dubowitz DJ, Liu TT. Modeling the hemodynamic response to brain activation. *Neuroimage* 2004;23(Suppl 1):S220–33. [PubMed: 15501093]
- Cameron OG, Modell JG, Hariharan M. Caffeine and human cerebral blood flow: a positron emission tomography study. *Life Sci* 1990;47(13):1141–6. [PubMed: 2122148]
- Chalela JA, Alsop DC, Gonzalez-Atavales JB, Maldjian JA, Kasner SE, Detre JA. Magnetic resonance perfusion imaging in acute ischemic stroke using continuous arterial spin labeling. *Stroke* 2000;31(3):680–7. [PubMed: 10700504]
- Cohen ER, Ugurbil K, Kim SG. Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. *J Cereb Blood Flow Metab* 2002;22(9):1042–53. [PubMed: 12218410]

- Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME. Frequencies contributing to functional connectivity in the cerebral cortex in “resting-state” data. *AJNR Am J Neuroradiol* 2001;22(7):1326–33. [PubMed: 11498421]
- Cox RW. AFNI-software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 1996;29:162–173. [PubMed: 8812068]
- D’Esposito M, Deouell LY, Gazzaley A. Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. *Nat Rev Neurosci* 2003;4(11):863–72. [PubMed: 14595398]
- de Munck JC, Goncalves SI, Huijboom L, Kuijter JP, Pouwels PJ, Heethaar RM, Lopes da Silva FH. The hemodynamic response of the alpha rhythm: an EEG/fMRI study. *Neuroimage* 2007;35(3):1142–51. [PubMed: 17336548]
- Dimpfel W, Schober F, Spuler M. The influence of caffeine on human EEG under resting conditions and during mental loads. *Clin Investig* 1993;71(3):197–207.
- Diniz, PSR.; da Silva, EAB.; Netto, SL. *Digital Signal Processing: System Analysis and Design*. Cambridge University Press; Cambridge: 2002.
- Dirnagl U, Lindauer UA, Villringer. Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. *Neuroscience Letters* 1993;149:43–46. [PubMed: 8469377]
- Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci* 2001;24:31–55. [PubMed: 11283304]
- Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A* 2005;102(27):9673–8. [PubMed: 15976020]
- Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999;51(1):83–133. [PubMed: 10049999]
- Garrity AG, Pearlson GD, McKiernan K, Lloyd D, Kiehl KA, Calhoun VD. Aberrant “default mode” functional connectivity in schizophrenia. *Am J Psychiatry* 2007;164(3):450–7. [PubMed: 17329470]
- Glover GH, Li TQ, Ress D. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med* 2000;44(1):162–7. [PubMed: 10893535]
- Goldman RI, Stern JM, Engel J Jr, Cohen MS. Simultaneous EEG and fMRI of the alpha rhythm. *Neuroreport* 2002;13(18):2487–92. [PubMed: 12499854]
- Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A* 2003;100(1):253–8. [PubMed: 12506194]
- Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer’s disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci U S A* 2004;101(13):4637–42. [PubMed: 15070770]
- Hampson M, Driesen NR, Skudlarski P, Gore JC, Constable RT. Brain connectivity related to working memory performance. *J Neurosci* 2006;26(51):13338–43. [PubMed: 17182784]
- Hampson M, Peterson BS, Skudlarski P, Gatenby JC, Gore JC. Detection of functional connectivity using temporal correlations in MR images. *Hum Brain Mapp* 2002;15(4):247–62. [PubMed: 11835612]
- Hudetz AG, Smith JJ, Lee JG, Bosnjak ZJ, Kampine JP. Modification of cerebral laser-Doppler flow oscillations by halothane, PCO₂, and nitric oxide synthase blockade. *Am J Physiol* 1995;269(1 Pt 2):H114–20. [PubMed: 7543253]
- Jones HE, Herning RI, Cadet JL, Griffiths RR. Caffeine withdrawal increases cerebral blood flow velocity and alters quantitative electroencephalography (EEG) activity. *Psychopharmacology (Berl)* 2000;147(4):371–7. [PubMed: 10672630]
- Jones M, Berwick J, Hewson-Stoate N, Gias C, Mayhew J. The effect of hypercapnia on the neural and hemodynamic responses to somatosensory stimulation. *Neuroimage* 2005;27(3):609–23. [PubMed: 15978844]
- Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard JE, Cotreau MM, Harmatz JS, Shader RI. Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. *J Clin Pharmacol* 1997;37(8):693–703. [PubMed: 9378841]
- Kemna LJ, Posse S. Effect of respiratory CO₂ changes on the temporal dynamics of the hemodynamic response in functional MR imaging. *Neuroimage* 2001;14(3):642–9. [PubMed: 11506537]

- Keppel, G.; Wickens, TD. *Design and Analysis: A Researcher's Handbook*. Pearson Prentice Hall; Upper Saddle River: 2004.
- Kiviniemi VJ, Haanpaa H, Kantola JH, Jauhiainen J, Vainionpaa V, Alahuhta S, Tervonen O. Midazolam sedation increases fluctuation and synchrony of the resting brain BOLD signal. *Magn Reson Imaging* 2005;23(4):531–7. [PubMed: 15919598]
- Laufs H, Kleinschmidt A, Beyerle A, Eger E, Salek-Haddadi A, Preibisch C, Krakow K. EEG-correlated fMRI of human alpha activity. *Neuroimage* 2003a;19(4):1463–76. [PubMed: 12948703]
- Laufs H, Krakow K, Sterzer P, Eger E, Beyerle A, Salek-Haddadi A, Kleinschmidt A. Electroencephalographic signatures of attentional and cognitive default modes in spontaneous brain activity fluctuations at rest. *Proc Natl Acad Sci U S A* 2003b;100(19):11053–8. [PubMed: 12958209]
- Li SJ, Biswal B, Li Z, Risinger R, Rainey C, Cho JK, Salmeron BJ, Stein EA. Cocaine administration decreases functional connectivity in human primary visual and motor cortex as detected by functional MRI. *Magn Reson Med* 2000;43(1):45–51. [PubMed: 10642730]
- Liau, J.; Perthen, J.; Liu, TT. The Relation between BOLD amplitude and Baseline Cerebral Blood Flow Depends on the Analysis Scale. *Proceedings of the 16th ISMRM; Toronto*. 2008a. p. 854
- Liau J, Perthen JE, Liu TT. Caffeine reduces the activation extent and contrast-to-noise ratio of the functional cerebral blood flow response but not the BOLD response. *Neuroimage* 2008b;42(1):296–305. [PubMed: 18514545]
- Lieberman HR, Wurtman RJ, Emde GG, Roberts C, Coviella IL. The effects of low doses of caffeine on human performance and mood. *Psychopharmacology (Berl)* 1987;92(3):308–12. [PubMed: 3114783]
- Liu TT, Behzadi Y, Restom K, Uludag K, Lu K, Buracas GT, Dubowitz DJ, Buxton RB. Caffeine alters the temporal dynamics of the visual BOLD response. *Neuroimage* 2004;23(4):1402–13. [PubMed: 15589104]
- Liu TT, Frank LR, Wong EC, Buxton RB. Detection power, estimation efficiency, and predictability in event-related fMRI. *Neuroimage* 2001;13(4):759–73. [PubMed: 11305903]
- Liu, X.; Zhu, XH.; Zhang, Y.; Chen, W. Tightly Coupled Spontaneous EEG and CBF Signals in the Anesthetized Rat Brain. *Proceedings of the 16th ISMRM; Toronto*. 2008. p. 755
- Lowe MJ, Mock BJ, Sorenson JA. Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage* 1998;7(2):119–32. [PubMed: 9558644]
- Lowe MJ, Phillips MD, Lurito JT, Mattson D, Dzemidzic M, Mathews VP. Multiple sclerosis: low-frequency temporal blood oxygen level-dependent fluctuations indicate reduced functional connectivity initial results. *Radiology* 2002;224(1):184–92. [PubMed: 12091681]
- Lui S, Ouyang L, Chen Q, Huang X, Tang H, Chen H, Zhou D, Kemp GJ, Gong Q. Differential interictal activity of the precuneus/posterior cingulate cortex revealed by resting state functional MRI at 3T in generalized vs. Partial seizure. *J Magn Reson Imaging* 2008;27(6):1214–20. [PubMed: 18504738]
- Mednick SC, Cai DJ, Kanady J, Drummond SP. Comparing the benefits of caffeine, naps and placebo on verbal, motor and perceptual memory. *Behav Brain Res* 2008;193(1):79–86. [PubMed: 18554731]
- Moosmann M, Ritter P, Krastel I, Brink A, Thees S, Blankenburg F, Taskin B, Obrig H, Villringer A. Correlates of alpha rhythm in functional magnetic resonance imaging and near infrared spectroscopy. *Neuroimage* 2003;20(1):145–58. [PubMed: 14527577]
- Mulderink TA, Gitelman DR, Mesulam MM, Parrish TB. On the use of caffeine as a contrast booster for BOLD fMRI studies. *Neuroimage* 2002;15:37–44. [PubMed: 11771972]
- Mumford JA, Hernandez-Garcia L, Lee GR, Nichols TE. Estimation efficiency and statistical power in arterial spin labeling fMRI. *Neuroimage* 2006;33(1):103–14. [PubMed: 16860577]
- Murphy, K.; Birn, RM.; Bandettini, PA. The Impact of Global Signal Regression on Anti-Correlated Networks in Resting State Connectivity Analyses. *Proceedings of the 14th Human Brain Mapping; Melbourne, Australia*. 2008. p. 183
- Ngai AC, Coyne EF, Meno JR, West GA, Winn HR. Receptor subtypes mediating adenosine-induced dilation of cerebral arterioles. *Am J Physiol Heart Circ Physiol* 2001;280(5):H2329–35. [PubMed: 11299238]
- Obrig H, Neufang M, Wenzel R, Kohl M, Steinbrink J, Einhaupl K, Villringer A. Spontaneous low frequency oscillations of cerebral hemodynamics and metabolism in human adults. *Neuroimage* 2000;12(6):623–39. [PubMed: 11112395]

- Peltier SJ, Kerssens C, Hamann SB, Sebel PS, Byas-Smith M, Hu X. Functional connectivity changes with concentration of sevoflurane anesthesia. *Neuroreport* 2005;16(3):285–8. [PubMed: 15706237]
- Perthen JE, Lansing AE, Liao J, Liu TT, Buxton RB. Caffeine-induced uncoupling of cerebral blood flow and oxygen metabolism: A calibrated BOLD fMRI study. *Neuroimage* 2008;40(1):237–47. [PubMed: 18191583]
- Reeves RR, Struve FA, Patrick G. Topographic quantitative EEG response to acute caffeine withdrawal: a comprehensive analysis of multiple quantitative variables. *Clin Electroencephalogr* 2002;33(4):178–88. [PubMed: 12449850]
- Restom K, Behzadi Y, Liu TT. Physiological noise reduction for arterial spin labeling functional MRI. *Neuroimage* 2006;31(3):1104–15. [PubMed: 16533609]
- Ritter P, Moosmann M, Villringer A. Rolandic alpha and beta EEG rhythms' strengths are inversely related to fMRI-BOLD signal in primary somatosensory and motor cortex. *Hum Brain Mapp.* 200810.1002/hbm.20585
- Scheeringa R, Bastiaansen MC, Petersson KM, Oostenveld R, Norris DG, Hagoort P. Frontal theta EEG activity correlates negatively with the default mode network in resting state. *Int J Psychophysiol* 2008;67(3):242–51. [PubMed: 17707538]
- Sicard KM, Duong TQ. Effects of hypoxia, hyperoxia, and hypercapnia on baseline and stimulus-evoked BOLD, CBF, and CMRO₂ in spontaneously breathing animals. *Neuroimage* 2005;25(3):850–8. [PubMed: 15808985]
- Siepmann M, Kirch W. Effects of caffeine on topographic quantitative EEG. *Neuropsychobiology* 2002;45(3):161–6. [PubMed: 11979068]
- Vincent JL, et al. Intrinsic functional architecture in the anaesthetized monkey brain. *Nature* 2007;447(7140):83–6. [PubMed: 17476267]
- Voyvodic JT. Activation mapping as a percentage of local excitation: fMRI stability within scans, between scans and across field strengths. *Magn Reson Imaging* 2006;24(9):1249–61. [PubMed: 17071346]
- Wang J, Qiu M, Constable RT. In vivo method for correcting transmit/receive nonuniformities with phased array coils. *Magn Reson Med* 2005;53(3):666–74. [PubMed: 15723397]
- Wise RG, Ide K, Poulin MJ, Tracey I. Resting fluctuations in arterial carbon dioxide induce significant low frequency variations in BOLD signal. *Neuroimage* 2004;21(4):1652–64. [PubMed: 15050588]
- Wong EC, Buxton RB, Frank LR. Quantitative imaging of perfusion using a single subtraction (QUIPSS and QUIPSS II). *Magn Reson Med* 1998;39(5):702–708. [PubMed: 9581600]
- Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage* 2001;14(6):1370–86. [PubMed: 11707093]
- Zappe AC, Uludag K, Oeltermann A, Ugurbil K, Logothetis NK. The Influence of Moderate Hypercapnia on Neural Activity in the Anesthetized Nonhuman Primate. *Cereb Cortex.* 200810.1093/cercor/bhn023

Figure 1.

Resting-state functional connectivity maps ($p < 0.005$) over a representative slice for all 9 subjects, in which the average signal from the left ROI was used as a reference. The top two rows display each subject's average pre-dose (blue) and post-dose (red) maps for the caffeine session, while the bottom two rows display the average pre-dose (blue) and post-dose (red) maps for the control session.

Figure 2.

Scatter plots of the mean z score (post-dose vs. pre-dose) for each subject are shown from the (a) caffeine session and (b) control session, in which the average signal from the left ROI was used as a reference. The solid black lines represent equality between the pre-dose and post-dose sections. Group average percent overlap values are shown from the (c) caffeine session and (d) control session. The length of each error bar represents the standard error across subjects. Probability (p) values were obtained with paired t tests.

Figure 3.

Average energy below 0.08 Hz in the motor cortex is plotted (post-dose vs. pre-dose) for each subject from the (a) caffeine session and (b) control session. Probability (p) values were obtained with paired t tests. Representative power spectra from Subject 2, with units of $|\% \Delta \text{BOLD}|^2/\text{Hz}$, from the pre-dose and post-dose sections are shown for the (c) caffeine session and (d) control session. The length of each error bar represents the standard error across the motor cortex voxels.

Table 1

Pre-dose and post-dose mean baseline CBF values

Subject	Estimated daily caffeine usage (mg)	Caffeine Session CBF ml/(100 g min)		Control Session CBF ml/(100 g min)	
		Pre	Post	Pre	Post
1	<30	53.4	35.7	53.1	50.3
2	<30	78.7	56.1	72.3	71.7
3	340	65.2	57.1	76.2	73.5
4	<30	49.7	29.4	51.9	48.1
5	50	74.8	45.9	71.6	70.8
6	<30	64.2	40.4	58.1	63.9
7	50	51.2	31.8	48.6	47.5
8	<30	38.6	23.0	46.3	55.7
9	225	63.5	57.4	69.7	70.7
Mean	90.6	60.0	41.9	60.9	61.4
SD	113	12.7	12.9	11.6	10.96
p-value	-	7.4 e-5		0.74	

Mean values were computed across voxels in each subject's motor cortex ROI (union of left and right motor cortices). Significance values were computed with paired *t* tests (two-sided).