The Effects of Dietary Caffeine Use and Abstinence on Blood Oxygen Level-Dependent Activation and Cerebral Blood Flow

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Background: Caffeine is a known vasoconstrictor that reduces resting cerebral blood flow (CBF) throughout the brain. This effect may be problematic in functional magnetic resonance imaging (fMRI) research, as the blood oxygen level-dependent (BOLD) signal is a complex interaction of CBF and other factors that are dependent on changes in neural activity. It is unknown whether changes in the BOLD signal during an fMRI experiment could be affected by subjects' recent use or abstinence from dietary caffeine.

Methods: Here, we report two similar studies ($n=45$ and 17) that measure the effects of caffeine on BOLD activation, BOLD time course parameters, and CBF. Using a factorial design, low, moderate, and high caffeine consumers received either caffeine (250 mg) or placebo during normal caffeine use (satiated state) or after 30 hours of abstinence (abstinent state). The fMRI of a reaction time task and resting-state CBF were collected.

Results: In general, acute caffeine administration reduced the time to peak and full width at half maximum of the BOLD time course, and CBF across both studies. Caffeine also produced a small reduction in BOLD activation. The majority of these reductions across measures were moderated by neither the level of caffeine use, nor the abstinent or satiated state.

Conclusions: These results suggest that dietary caffeine use does not produce a significant effect on task-related BOLD activation.

Introduction

CAFFEINE IS A WIDELY used stimulant known for its positive effects on mood and alertness (for review see Refs.^{1–4}). Dietary caffeine use among research subjects presents a unique problem in functional magnetic resonance imaging (fMRI). fMRI indirectly measures neural activity with the blood oxygenation level-dependent (BOLD) signal, which represents the extent and magnitude of neural activity needed to perform a behavioral task. However, the BOLD signal is a complex interaction of cerebral blood flow (CBF) and other variables that are dependent on changes in neural activity. Any drug that has vascular effects could interfere with the BOLD response. Caffeine is unique, as it produces both neurostimulant and vasoconstrictive effects. Caffeine is a nonselective competitive antagonist of adenosine, which is one of the molecules that couples neural activity to the hemodynamic response, thus giving rise to the BOLD signal.^{5,6} By antagonizing adenosine receptors, caffeine indirectly increases neural activity by facilitating firing rates⁷ and decreases CBF via vasoconstriction.⁶

Caffeine, at normal dietary levels, is a competitive antagonist of adenosine receptors in vascular smooth muscle and en-

dothelial cells.⁸ A 250 mg dose of caffeine has been shown to reduce resting CBF between 22% and 30%.^{9–11} With this in mind, many investigators request that their subjects maintain overnight abstinence of caffeine use before undergoing an MRI experiment. However, tolerance is believed to develop for the effects of daily caffeine use on CBF,¹² and caffeine abstinence among daily consumers has been associated with increased resting CBF levels^{10,13} and increased CBF velocities.¹⁴ Therefore, abstinence in habitual caffeine users could result in abnormally high CBF levels that could also contribute to undesirable variability in the BOLD signal. Studies have shown that an acute dose of caffeine can affect the extent and magnitude of BOLD activation; however, the direction of this effect has been inconsistent.^{15–18} Another approach to investigating BOLD activation is to extract the BOLD signal time course. When plotted across time, the BOLD signal is a waveform reflecting the rise and fall of the hemodynamic response to neural activation. Studies have shown similar effects of caffeine on time-course parameters, despite reporting different effects on BOLD activation.^{16,19} Potentially, time-course analyses will provide more consistent evidence for the effects of caffeine on the BOLD signal than the voxel-wise contrast analyses.

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It is unknown whether the caffeinated state or the level of daily caffeine use can affect BOLD activation or whether the state or level of use mediates the effects of an acute dose of caffeine on the BOLD signal. Here, we report the effects of caffeine on BOLD activation, BOLD time-course parameters, and CBF from two separate, but similar, studies. Both studies used the same design, caffeine (250 mg) or placebo was administered in a satiated state (after normal dietary caffeine use) and in an abstained state (after 30 hours of caffeine abstinence). Data from the first study were collected on a 1.5T magnet using low, moderate, and high caffeine consumers. Data from the second study were collected on a 3T magnet using moderate caffeine consumers. Establishing the effects of caffeine and the caffeinated state on BOLD activation is an important step for future fMRI-based caffeine experiments as well as for all fMRI studies that include daily caffeine consumers in their subject samples.

Methods

The overall designs of Studies 1 and 2 were nearly identical. The following protocol description applies to both studies, except where indicated. CBF measurements from Study 1¹² and behavioral results from Study 2²⁰ have been previously published, along with detailed participant characteristics, salivary caffeine concentrations, and perfusion imaging methods. The data presented here are the fMRI BOLD activation results of these studies, which have not been previously reported. Pertinent information is reviewed next.

Subjects

Healthy adults (ages 18–50 years) were recruited from the Winston-Salem, North Carolina area with flyers, newspaper advertisements, and by word of mouth. Potential volunteers were assessed with a physical medical screening before inclusion in the study, and individuals who met the following criteria were invited to participate: (1) no history of neurologic and vascular trauma or disease, (2) no current symptoms of depression or anxiety, (3) no current abuse of alcohol or illicit drugs, and (4) no MR contraindications. Participants were identified as low (<200 mg/day), moderate (200–600 mg/day), and high (>600 mg/day) caffeine users based on a 7-day caffeine consumption diary²¹ completed the week after the screening session. This diary is a modified version of the Caffeine Consumption Questionnaire,²² and estimates are based on caffeine content information of common beverages (Center for Science in the Public Interest www.cspinet.org). Participants provided written informed consent, and the protocols for these studies were approved by the Wake Forest Institutional Review Board.

Forty-five participants were included (20 men) in Study 1. There were 18 low consumers (mean $\pm$ SD: 45 $\pm$ 38 mg/day), 9 moderate consumers (405 $\pm$ 103 mg/day), and 18 high consumers (950 $\pm$ 237 mg/day); the average age was 32 $\pm$ 9 years. Seventeen moderate (200–600 mg/day) consumers were included (eight men) in Study 2; the average age was 30 $\pm$ 8 years. The average caffeine intake was 375 $\pm$ 101 mg/day. There was no overlap in participant populations between the two studies.

Procedure

Using a within-subject repeated-measures design, participants underwent fMRI on four separate occasions, separated

by at least 1 week. On two occasions, the subjects were instructed to maintain normal caffeine use until 90 minutes before the scan (satiated state); on the other two occasions, the subjects were instructed to abstain from caffeine for 30 hours before the scan (abstained state). In each state, participants were administered caffeine (250 mg anhydrous) on 1 day and a matching placebo capsule on the other day in a double-blind manner. The four State by Drug conditions are abbreviated as follows: abstained placebo (AP), abstained caffeine (AC), satiated placebo (SP), and satiated caffeine (SC). State and Drug conditions were counter-balanced and pseudo-randomized to eliminate time as a variable.

To verify compliance, saliva samples were obtained on arrival to the study visit and 45 minutes after capsule ingestion.

MRI scans began 1 hour after capsule administration, and participants were scanned at approximately the same time of day across the four sessions. Visual stimulation during the scan was presented via MR-compatible visual display goggles (Resonance Technology, Inc.), and responses were recorded via a button box placed under the participant's right hand. Computer tasks were programmed with E-Prime (Psychology Software Tools). Wakefulness was monitored from the control room with an infrared camera positioned inside the visual display goggles.

fMRI task. In Study 1, the fMRI task was presented in a single scan lasting 5 minutes 31 seconds. The stimulus was a black and white checkerboard. Trials consisted of single flashes (250 milliseconds) and double flashes (100 milliseconds on, 50 milliseconds off, and 100 milliseconds on) randomly presented. Participants responded by pressing a button with their index finger for a single flash, and by pressing a second button with their middle finger for a double flash. There were 98 trials (50% single flashes). The inter-stimulus interval varied and averaged 2.9 $\pm$ 2.1 seconds.

In Study 2, the fMRI task was presented in two scans, each lasting 5 minutes 51 seconds. These scans were identical except for the sequence of inter-stimulus intervals. The stimulus was a black and white checkerboard; a single red square appeared randomly in one of the four quadrants during each presentation (250 milliseconds). Participants pressed a left or right button with their index or middle finger to indicate whether the red square was on the left or the right side of the checkerboard. There were 156 trials (50% left). The inter-stimulus interval varied and averaged 3.7 $\pm$ 2.7 seconds.

Image acquisition

Study 1. Images were collected with a 1.5T GE scanner (Twin-speed; GE Healthcare) with a 4-channel neurovascular headcoil (MEDRAD, Inc.). An initial T₁-weighted structural scan was obtained using an Inversion Prepared 3D Spoiled Gradient Echo; IR-3DSPGR²³: TE/TR/TI = 2.60/9.00/600 milliseconds; bandwidth = 16.93 kHz; flip angle = 20°; field of view (FOV) = 240 $\times$ 240 mm; acquisition matrix = 256 $\times$ 256; 124 slices; 1.5 mm thickness; zero slice gap. BOLD-weighted images were acquired with a gradient echo EPI sequence with TE/TR = 40/2100 milliseconds, flip angle = 90°; FOV = 240 $\times$ 240 mm; acquisition matrix = 64 $\times$ 48, slice thickness = 5 mm, zero slice gap. Twenty-eight slices were acquired sequentially and paralleled the AC-PC line. Original voxel resolution was 3.75 $\times$ 3.75 $\times$ 5.00 mm³. The first nine volumes of each scan were used to achieve a steady state of contrast and were discarded before data analysis.

Functional images were processed using SPM99 (Wellcome Trust Center for Neuroimaging). BOLD-weighted images were motion corrected, spatially normalized to an EPI template (Montreal Neurological Institute), re-sampled to $4 \times 4 \times 5$ mm, and smoothed with an 8 mm full width at half-maximum isotropic gaussian filter. Images were globally normalized and high-pass filtered.

Study 2. Images were collected with a 3T GE Signa Excite scanner with twin-speed gradients (GE Healthcare) and an eight-channel neurovascular headcoil. An initial T_1 -weighted structural scan was acquired using a 3D Spoiled Gradient Echo (TI/TE=450 milliseconds/3.4–13.0 milliseconds, bandwidth=15.63 kHz, flip angle=25°, FOV=240×240 mm, acquisition matrix=256×256, 124 slices, 1.5 mm thickness, zero slice gap). BOLD-weighted images were acquired with an EPI sequence (TE/TR=28/1500 milliseconds, flip angle=68°, FOV=240×240 mm, acquisition matrix=64×48, slice thickness=5 mm, zero slice gap). Twenty-eight slices were acquired sequentially and paralleled the AC-PC line. Original voxel resolution was $3.75 \times 3.75 \times 5$ mm³. The first 13 volumes of each scan were used to achieve a steady state of contrast and were discarded before data analysis.

Images were processed and analyzed with SPM5 (The Wellcome Trust Center for Neuroimaging). BOLD-weighted and perfusion images were motion corrected, spatially normalized to an EPI template (Montreal Neurological Institute), re-sampled to $4 \times 4 \times 5$ mm, and smoothed with an 8 mm full width at half-maximum (FWHM) isotropic gaussian filter. The BOLD-weighted data were modeled with the hemodynamic response function, including the time and dispersion derivatives.

Resting-state CBF was measured following the functional scans using a previously described method.¹² Participants were instructed to keep their eyes open and gaze at a fixation cross. One hundred and twenty images were collected (60 pairs) for analysis during a 6 minutes 30 second scan. Fifteen slices were acquired parallel to the AC-PC line (TR=3000 milliseconds, TE=23.9 milliseconds, TI=2000 milliseconds, FOV=240×240 mm, acquisition matrix 640×640, slice thickness=8.0 mm, zero slice gap).

CBF processing steps have been previously described.¹² The processing performed here was identical, except the T_1 of blood was assumed to be 1490 milliseconds at 3T.²⁴ Perfusion scans were cleaned of paired images that were poorly

aligned (e.g., >2.5 standard deviations from the mean) due to motion artifact.²⁵

Data analysis

Whole-brain BOLD contrast. In Study 1, the main effects of state were analyzed with a 1-sample *t*-test of the first level contrasts comparing the abstained conditions to the satiated conditions. The main effects of the drug were tested with a one-sample *t*-test of the first-level contrasts by comparing the placebo conditions with the caffeine conditions. *t*-tests comparing low, moderate, and high caffeine consumers were conducted based on the contrast difference between the drugs in each state (i.e., AP-AC, SP-SC), as a factorial model analysis was not a design option in SPM99. In Study 2, the two simple reaction time runs were concatenated in a fixed-effects analysis, then a second level analysis was performed using a 2 (State)×2 (Drug) full factorial analysis of variance (ANOVA). For both studies, cluster-size correction for multiple comparisons ($p < 0.05$) was determined via Monte-Carlo simulations²⁶ using an uncorrected $p < 0.001$ with an extent threshold (K_E) of 17 contiguous voxels. Figures were created using MRICro.²⁷

BOLD signal time course. For both Study 1 and 2, unique activation masks were created for each participant using a conjugate analysis of the four scan sessions (AP, AC, SP, and SC) of the simple reaction time tasks. Areas of activation were limited to *a priori*-defined regions of interest (ROIs) that were created using WFU pickatlas²⁸ and consisted of bilateral visual cortices and left motor cortex. This process created a mask of voxels that were significantly active across the four scans (family-wise error correction [FWE] $p < 0.05$) while ensuring that the same number of voxels was analyzed from each scan. Time courses of the event-related hemodynamic response were extracted from these voxels using a Tikhonov regularization method²⁹ and interpolated to TR/6. Time courses were averaged across all trials per scan session. Examples of BOLD time courses from a single subject, extracted from the primary visual cortex, are shown in Figure 1. Time to peak (TTP, seconds), peak amplitude (AMP, arbitrary units), and FWHM (seconds) were measured. Several subjects were dropped from the time course analyses, because their time courses did not resemble waveforms (i.e., there was no rise from baseline); thus, parameters (TTP, AMP, and FWHM) could not be calculated. From Study 1, one moderate

FIG. 1. Blood oxygen level-dependent time courses from a single subject, extracted from the primary visual cortex in (a) the abstained state and (b) the satiated state.

and one high caffeine consumer were dropped from the visual cortex analysis, and four low, one moderate, and three high consumers were dropped from the motor cortex analysis. From Study 2, one subject was dropped from both analyses. In Study 1, time courses were interpolated to 0.35 seconds. Parameters were analyzed separately with $2 (\text{State}) \times 2 (\text{Drug}) \times 3 (\text{Caffeine use})$ ANOVAs. In Study 2, time courses were interpolated to 0.25 seconds. Parameters were analyzed with $2 (\text{State}) \times 2 (\text{Drug})$ ANOVAs.

Cerebral blood flow. In Study 1, the average whole-brain gray matter CBF values were analyzed using a $2 (\text{State}) \times 2 (\text{Drug}) \times 3 (\text{Caffeine use})$ ANOVA (see Ref.¹²). In Study 2, the average whole-brain gray matter CBF values were analyzed using a $2 (\text{State}) \times 2 (\text{Drug})$ repeated-measures ANOVA. Due to artifacts, CBF data were excluded from two conditions between two subjects. Missing data were replaced with the group mean for the ANOVA. Time course and CBF data were analyzed using SPSS 16.0; results were considered significant if $p < 0.05$.

Results

Whole-brain BOLD contrast

In Study 1, there was a main effect of drug during the simple reaction time (RT) task. In the placebo > caffeine contrast, there were three clusters: one located in the posterior cingulate gyrus and two in white matter tracts (Fig. 2a and Table 2). There was no main effect of state. There were no differences in state, drug, or state $\times$ drug effects between caffeine use levels (low, moderate, and high). In Study 2, there was a main effect of drug during the simple RT task. This was due to a greater BOLD contrast in the placebo condition compared with the caffeine condition in the left thalamus, extending into the caudate nucleus (Fig. 2b). There was no main effect of state or a state $\times$ drug interaction effect.

BOLD signal time course

In Study 1, caffeine reduced the TTP in the visual cortex (Drug effect: $F[1,40] = 19.0$, $p < 0.001$), and this reduction was greater in the abstained state than in the satiated state (State $\times$ Drug effect: $F[1,40] = 5.0$, $p < 0.05$). Caffeine also reduced the

FWHM (Drug effect: $F[1,40] = 13.5$, $p < 0.005$), although there were no interactions with either state or caffeine use level. AMP was not affected by caffeine or the state; however, there was an interaction effect between the drug and caffeine use level (Drug $\times$ Caffeine use: $F[2,40] = 3.5$, $p < 0.05$). This was due to a drug effect among the moderate consumers, where caffeine increased the AMP (Drug effect: $F[1,7] = 23.6$, $p < 0.005$), although this effect was not present among the low or high consumers. In the motor cortex, caffeine increased AMP (Drug effect: $F[1,34] = 10.8$, $p < 0.005$) and reduced FWHM (Drug effect: $F[1,34] = 14.7$, $p < 0.005$). Neither of these effects had an interaction with state or caffeine use level. TTP was not affected by the state or drug conditions (Table 1).

In Study 2, caffeine reduced the TTP (Drug effect: $F[1,15] = 13.6$, $p < 0.005$) and the FWHM (Drug effect: $F[1,15] = 4.8$, $p < 0.05$) in the visual cortex, but produced no effect on AMP. Nor was there an effect of caffeinated state or an interaction effect on these parameters. In the motor cortex, caffeine also reduced TTP (Drug effect: $F[1,15] = 8.7$, $p < 0.05$) and FWHM (Drug effect: $F[1,15] = 7.3$, $p < 0.05$), but had no effect on AMP. Again, there was no effect of caffeinated state or an interaction effect on these parameters.

Cerebral blood flow

In Study 1, caffeine decreased the average global CBF by 27%, and this decrease was larger in the abstained state (−33%) than in the satiated state (−20%). In the placebo condition, the moderate and high consumers had greater CBF in the abstained state than in the satiated state. There was no difference between these two states among low consumers. For complete results, see Addicott *et al.*¹²

In Study 2, caffeine decreased the average global CBF by 28% across states, but caffeine decreased CBF more in the abstained state (−34.2%) than in the satiated state (−20%) (State $\times$ Drug effect: $F[1,16] = 18.0$, $p < 0.005$; paired *t*-tests: AP > AC $t[16] = 10.9$, $p < 0.001$; SP > SC $t[16] = 4.7$, $p < 0.001$). There was also greater CBF in the AP condition than in the SP condition ($t[16] = 6.1$, $p < 0.001$), and in the SP condition than in the AC condition ($t[16] = 4.8$, $p < 0.001$), see Table 1 for summaries of average gray matter CBF from Studies 1 and 2.

Discussion

Summary

Overall, our results show a consistent effect of caffeine across both studies. The bolus dose (250 mg) of caffeine reduced the BOLD time course parameters TTP, FWHM, and CBF, as well as slightly diminished BOLD contrast activation. There was no main effect of state on any of the measures; the abstained and satiated states were not significantly different, nor were there any effects or interactions involving caffeine use level. The acute effects of caffeine were similar in both states and across caffeine use groups. We hypothesized in Study 1 that the level of daily caffeine use would mediate the effects of caffeine on BOLD activation. Instead, the results suggest that caffeine reduced the BOLD response to the same extent in low and high users. We hypothesized in Study 2 that caffeine would have a greater effect on measures in the abstained state than in the satiated state. This was true for CBF, but not for the BOLD contrast or time-course parameters.

FIG. 2. Brain regions showing a reduction in activation after caffeine (placebo > caffeine) during the reaction time task in (a) Study 1 and (b) Study 2. Uncorrected $p < 0.001$, $K_E = 17$ voxels (cluster-corrected for multiple comparisons, $p < 0.05$). Color images available online at www.liebertonline.com/jcr

TABLE 1. BLOOD OXYGEN LEVEL-DEPENDENT TIME-COURSE PARAMETERS, AND CEREBRAL BLOOD FLOW VALUES [MEAN (SD)] FROM STUDY 1 AND STUDY 2 ACROSS THE FOUR CONDITIONS

	n		AP	AC	SP	SC	Main effects p<0.05
Time course parameters (seconds & % change from baseline) from simple RT task							
Study 1	43	Visual cortex					
		TTP	4.3 (1.0)	3.8 (0.7)	4.1 (0.9)	3.9 (0.8)	Drug, State×Drug
		AMP	5.0 (1.5)	4.5 (1.6)	4.4 (1.6)	4.7 (1.4)	Drug×Caffeine use
		FWHM	4.7 (1.2)	4.1 (0.8)	4.6 (1.6)	4.2 (0.8)	Drug
Study 1	37	Motor cortex					
		TTP	4.3 (1.4)	3.9 (1.1)	4.2 (1.3)	4.2 (1.2)	ns
		AMP	3.6 (1.7)	4.1 (2.0)	3.7 (2.1)	4.3 (1.7)	Drug
		FWHM	4.3 (1.5)	3.6 (0.8)	4.2 (1.5)	3.7 (0.7)	Drug
Study 2	16	Visual cortex					
		TTP	3.4 (0.4)	3.0 (0.4)	3.3 (0.5)	3.1 (0.4)	Drug
		AMP	8.7 (2.6)	7.9 (1.6)	9.2 (2.8)	8.6 (2.6)	ns
		FWHM	3.8 (0.5)	3.4 (0.5)	3.7 (0.7)	3.5 (0.5)	Drug
Study 2	16	Motor cortex					
		TTP	2.8 (0.4)	2.6 (0.4)	2.8 (0.4)	2.7 (0.4)	Drug
		AMP	5.5 (1.7)	6.2 (1.2)	6.4 (1.8)	6.4 (2.4)	ns
		FWHM	3.3 (0.6)	3.0 (0.4)	3.2 (0.7)	3.0 (0.5)	Drug
Cerebral blood flow [mL/(100 g·min)]							
Study 1	45	Average GM	85 (20)	57 (13)	72 (16)	58 (14)	State×Drug×Caffeine use
Study 2	17	Average GM	63 (10)	41 (8)	49 (12)	39 (10)	State×Drug

SD, standard deviation; AP, abstained placebo; AC, abstained caffeine; SP, satiated placebo; SC, satiated caffeine; FWHM, full-width at half-maximum; TTP, time to peak; AMP, peak amplitude; ns, nonsignificant; GM, gray matter; RT, reaction time.

Providing subjects with instructions regarding caffeine use (in addition to other psychoactive drugs and medications) before a scan is prudent in order to avoid confounds in both cognitive performance and BOLD activation. However, asking daily caffeine users to abstain from caffeine may also increase resting CBF and trigger symptoms of caffeine withdrawal, such as fatigue, difficulty in concentrating, and headache,³⁰ which could also produce adverse effects on performance. Based on the results from the studies reported here, it appears that there is no appreciable effect of dietary caffeine use or abstinence from use on BOLD activation or time-course parameters. Although subjects may be instructed to limit their fluid intake before being confined to a scanner for an hour to avoid potential bladder discomfort, we recommend that subjects are scanned in their normal caffeine-satiated state. For daily caffeine consumers, the satiated state is more comfortable and is likely to be more representative of “normal” cognitive function and resting CBF levels.

Whole-brain BOLD contrast

No main effects or interactions with caffeinated state or caffeine use level were found. Caffeine reduced BOLD activation overall, but only in a small number of regions and at a low

(cluster-corrected) threshold. These activation differences are not evident with FWE or false discovery rate (FDR) correction, and should be interpreted with caution. Although it is possible that these regions support the performance of the simple RT task, it is difficult to attribute these contrast differences to changes in behavioral task performance (i.e., reduced reaction time) resulting from caffeine administration. The lack of a significant effect of caffeine on the BOLD activation in visual and motor cortices is in agreement with the absence of a significant drug effect on the AMP of the time courses extracted from the motor and visual ROIs. We conclude that caffeine does not have a significant effect on task-related BOLD activation or amplitude.

BOLD signal time course

Our results showed that acute caffeine administration reduced TTP and FWHM in both the visual and motor cortices, with one exception (Study 1, motor cortex TTP). This reduction agrees with previous studies.^{16,19,31} However, AMP was mostly unaffected by caffeine. There was no reliable effect of caffeine state or caffeine use level on time-course parameters. The Drug $\times$ Caffeine use effect in the visual cortex of Study 1 is most likely due to the smaller sample size of

TABLE 2. BRAIN REGIONS AND THRESHOLD VALUES FOR WHOLE-BRAIN BLOOD OXYGEN LEVEL-DEPENDENT CONTRAST ANALYSES DURING REACTION TIME PERFORMANCE

	N	Brain region	R/L	Contrast	x,y,z	K _E	Threshold (T)
Study 1	45	White matter	R	Pla > Caf	36,-52,25	23	4.87
		White matter	L	Pla > Caf	-24,20,20	26	4.26
		Posterior cingulate gyrus	R	Pla > Caf	8,-36,30	36	3.94
Study 2	17	Thalamus/caudate nucleus	L	Pla > Caf	-12,0,10	28	4.21

R/L, right/left; Pla, placebo; Caf, caffeine; x,y,z, MNI co-ordinates (mm); K_E, cluster extent.

the moderate group ($n=9$), resulting in more variance. Caffeine also increased the AMP in the motor cortex of Study 1, but since this was not reproduced in Study 2, the results are equivocal.

Cerebral blood flow

The pattern of CBF results reported in Study 2 (AP > SP > AC = SC) reproduces the results from Study 1.¹² In both studies, caffeine reduced CBF more in an abstained state than in a satiated state, and CBF was greater in the AP condition than in the SP condition. Earlier, we concluded that moderate caffeine users had developed tolerance to their daily caffeine use, and the increased CBF during abstinence could be the result of adenosine binding to an upregulated number of receptors. Adenosine receptor upregulation (e.g., increase in receptor number) is the mechanism believed to underlie the development of tolerance,^{32–35} although sensitization (e.g., conversion to high-affinity state) has also been reported.³³ However, a potential neural or astrocytic role in the chronic effects of caffeine on CBF cannot be eliminated.³⁶

Salivary caffeine concentrations, obtained 45 minutes after drug administration in Study 2, were similar after the 250 mg dose in the abstained state (AC: $2.9 \pm 2.0 \mu\text{g/mL}$) and in the satiated state after placebo (SP: $2.5 \pm 2.7 \mu\text{g/mL}$).²⁰ However, CBF was significantly lower in the AC condition [$41 \pm 8 \text{ mL}/(100 \text{ g} \cdot \text{min})$] than in the SP condition [$49 \pm 12 \text{ mL}/(100 \text{ g} \cdot \text{min})$]. This suggests that the bolus dose is not the physiological equivalent of normal caffeine use. Alternatively, the effects of the acute bolus dose are possibly being measured during the absorption phase of caffeine, while the effects of regular consumption are measured during the elimination phase.

Potential mechanisms of action

A number of previous studies have investigated the acute effects of caffeine on CBF and the BOLD signal. Although the vasoconstrictive effects of caffeine have been consistent, the effects on the BOLD response have varied. Initially, caffeine was shown to enhance the BOLD response and time course AMP; this was thought to be caused by a vasoconstriction-induced increase in the difference between resting and activity-evoked deoxyhemoglobin levels.¹⁵ While these results have been replicated,¹⁶ other investigations have reported a decrease³⁷ or no change in the BOLD magnitude or AMP after caffeine administration.^{19,38} Despite these differences, caffeine reliably reduces TTP;^{16,19} Behzadi and Liu proposed that caffeine reduces the TTP of the BOLD response because arterioles dilate more quickly in response to neural activity at a vasoconstricted baseline.³¹ The outcomes of the current studies are consistent with this hypothesis.

The modulation of the effects of acute caffeine by consumption habits and caffeinated state was previously investigated by our laboratory. Low (mean 41 mg/day) and high (mean 648 mg/day) caffeine consumers were presented with visual stimulation during an fMRI scan; resting-state CBF was also measured. High consumers had greater CBF in an AP condition and greater BOLD signal change after caffeine administration.^{10,18} Laurienti *et al.* proposed that neural adenosine receptors may upregulate to a greater degree than vascular receptors, which would allow for a greater neurostimulatory response and a heightened BOLD signal among high consumers.¹⁸ However, no relationship was found be-

tween the decrease in CBF and the difference in the BOLD response between the caffeine and placebo conditions,³⁹ which suggests that the vasoconstrictive action of caffeine is not directly responsible for changes in BOLD amplitude. This corresponds with the results of the current studies, although we did not reproduce the differences between high and low consumers. Additional studies are needed in order to understand the unique contributions of cerebrovascular and neural adenosine receptors to the action of caffeine on the hemodynamic response.

Although the methods and outcomes of these studies all vary widely, taken together, caffeine does not unequivocally enhance the BOLD response to primary sensory stimuli. The most consistent effect is the reduction of the TTP. This is unexpected, as the neurostimulant effects of caffeine could hypothetically enhance the BOLD response. A potential explanation is that by antagonizing adenosine receptors on cerebrovascular smooth muscle, caffeine interferes with the hemodynamic response to neural activation.³⁶ Therefore, putative amplitude gains produced by the neurostimulant effects of caffeine may be lost due to its vasoconstrictive effects. The difficulty in obtaining consistent outcomes with acute caffeine administration illustrates the complexities and challenges of indirectly measuring neural activity with the BOLD signal. As noted by Liau *et al.*, the CBF response is a potentially more direct, and less variable, measure of neural activity than the BOLD response.³⁸ Future studies may be able to better distinguish and interpret the neural from the vascular effects of caffeine using a combined measure of the BOLD/CBF response.

Limitations

The limitations of our study include the re-positioning of subjects in the scanner for each session. Given our design, it was not possible to collect these data during 1 day. The differences in slice prescriptions could have reduced the overlap in the voxels chosen for the time-course extraction and diminished our effect. Second, despite an identical pattern of change in CBF, the absolute values of CBF are lower in Study 2 than in Study 1, possibly due to scanner differences or an underestimation of the gray matter due to T_1 -image inhomogeneities from the 3T scanner. Third, instead of a 250-mg dose, we could have adjusted the dose by body weight or by daily caffeine use. Lastly, lost data from the CBF and time-course measures could have affected statistical analyses.

Conclusions

Caffeine is a widely used psychostimulant that has both neural and cerebrovascular effects. This combination of effects presents a unique challenge to fMRI research, given the reliance of the BOLD signal on changes in CBF; therefore, the dietary caffeine use of research subjects is of interest to many researchers. These studies found no main effects of state or caffeine use level, suggesting that subjects may be scanned in their normal caffeinated state.

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Author Disclosure Statement

The authors Addicott, Peiffer, and Laurienti have no conflicts of interest to disclose.

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