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Caffeine dose effect on activation-induced BOLD and CBF responses

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Abstract

Caffeine is a popular psychostimulant, typically found in beverages. While low to intermediate doses of caffeine are associated with positive feelings and increased mental performance and alertness, high doses induce negative feelings such as insomnia, anxiety and nervousness. We investigate if this nonlinear dose response is present for caffeine's effects on functional activation. Twenty-seven healthy subjects were assigned randomly to four different groups: saline, 1mg/kg, 2.5mg/kg and 5mg/kg doses of caffeine. Simultaneous ASL/BOLD timeseries were collected both before and after an intravenous infusion of saline or caffeine and the task-induced CBF and BOLD percent changes were compared. The maximum increase in BOLD response was associated with the intermediate caffeine dose of 2.5mg/kg, which increased BOLD response by 32.2% and 32.5% in motor and visual areas respectively. The maximum increase in CBF response was associated with the highest caffeine dose of 5mg/kg. This difference could be related to different density of A_1 and A_{2A} adenosine receptors in the brain.

INTRODUCTION

Blood-oxygenation-level-dependent (BOLD) imaging is a noninvasive technique that is capable of investigating brain activities using magnetic resonance imaging. Despite its immense popularity, the relationship between BOLD and neural activity is not straightforward. BOLD is sensitive to levels of deoxyhemoglobin in the brain, which is dependent on many factors including cerebral blood flow (CBF), cerebral blood volume (CBV) and oxygen consumption. Since BOLD is typically reported as a percent change in signal relative to baseline, it is highly dependent on baseline BOLD signal, which can be affected by hypercapnia, hypocapnia and various vasoactive drugs such as methylxanthines (Li and Mathews, 2002).

In a recent study, Mulderink et al. reported a 37% and 26% increase in BOLD response in motor and visual areas respectively after ingestion of a 200mg caffeine pill (Mulderink et al., 2002). A similar result was reported in rats using another methylxanthine—theophylline (Morton et al., 2002). Mulderink et al. attributed this increase in BOLD response to caffeine's ability to decrease cerebral blood flow, which increases baseline deoxyhemoglobin levels, leading to a decrease in baseline BOLD signal. The authors concluded that caffeine could be

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used as a potential BOLD contrast booster. However, pharmokinetic studies have shown that caffeine has a nonlinear dose response curve. While low to intermediate doses of caffeine are associated with a positive affect, high doses of 300–800mg induce negative effects such as anxiety, nervousness and insomnia (Kaplan et al., 1997; Nehlig and Boyet, 2000). In order to use caffeine as an effective BOLD contrast booster, it is important to understand the dose response associated with caffeine's effects on BOLD contrast. For this study, we use a combination of BOLD and arterial spin labeling (ASL) to explore the effects of caffeine on functional activation.

As part of the methylxanthine family, caffeine has a wide range of biochemical effects in the brain. But the primary effect at normal consumption concentrations is binding to different adenosine receptors, which allows caffeine to modulate brain activity while simultaneously reducing CBF (Laurienti et al., 2003; Liu et al., 2004; Mathew and Wilson, 1985). Since the effects of caffeine are based on receptor binding, we hypothesize that the dose response curve of brain activity and CBF would approach a plateau at higher doses, due to saturation of receptors. To test this hypothesis, we randomly assign subjects to four groups, each receiving a different dose of caffeine: 0 mg/kg (saline), 1mg/kg, 2.5mg/kg and 5mg/kg. The activation-induced BOLD and CBF changes are compared between the groups to determine if the results correlate with caffeine dose. A recent PET study provided evidence of different distribution of adenosine receptors in various parts of the brain (Bauer et al., 2003), which could potentially lead to a variable caffeine dose response areas across the brain. To test this theory, data were collected from both motor and visual cortices simultaneously to determine if their dose response curves differ from each other.

MATERIALS AND METHODS

Images were acquired on a 3.0T Siemens MR scanner (Siemens TIM Trio, Erlangen, Germany) equipped with a twelve-channel receive-only head coil. 13 healthy subjects (8 males, 5 females, average age 29 ± 11 years) were randomly assigned to receive either 1mg/kg caffeine (n=6), 5mg/kg caffeine (n=7) or saline (n=6). Data from a fixed-dose study of 2.5mg/kg caffeine, collected from 14 healthy subjects (4 males, 10 females, average age 27 ± 10 years), were also included in the final analysis. The total amount of caffeine administered for each patient is listed in Table 1. Since 4 subjects repeated the study for either a different caffeine dose or saline, a total of 27 subjects were recruited for 33 exams. Subject recruitment was compliant with the guidelines of the university's Internal Review Board and informed consent was obtained from all subjects. Daily caffeine usage was estimated using a brief questionnaire. Subjects were requested to abstain from caffeine for 12–24 hours prior to the study. Two baseline blood samples were collected at the beginning of each study (one in the IV preparation room, the other when the subject was in position on the scanner table) to ensure that subjects complied with the caffeine abstinence requirement and to establish baseline plasma caffeine levels. Since it has been reported that plasma concentrations of methylxanthines closely resembles brain concentrations (Kaplan et al., 1993), blood samples were collected immediately after and at 10min intervals after infusion as a measure of brain concentrations of caffeine.

Data Acquisition

Each study was subdivided into two identical sessions, separated by a 10-minute infusion of either caffeine (mixed in 50ml of saline) or 50 ml of saline alone at a rate of 0.1 ml/s, followed by 15 ml of saline flush at a rate of 1ml/s. Simultaneous ASL and BOLD data were collected during the infusion period using a PICORE/Q2TIPS sequence (Luh et al., 1999) with gradient-echo echo planar imaging (EPI) readout: TI_1=700ms, TI_{1s} =1200ms, TI_2=1400ms, TR=2s, TE=23ms, flip angle=90°, two 5mm thick axial slices (2.5mm gap) placed above the corpus callosum. This scan will be referred to as the "trace" scan. The purpose of the trace scan was to monitor the temporal effects of caffeine on baseline CBF and BOLD signal as it enters the system.

During the pre- and post-caffeine sessions, two sets of data were collected

Block design BOLD—the same PICORE/Q2TIPS sequence used for tracing the caffeine infusion was used during a functional experiment. Six slices of 5mm thickness, 2.5 mm gap were oriented along a transverse to coronal oblique plane to capture both the motor and visual cortices. The functional paradigm consisted of grayscale checkerboard flashing at 4Hz for visual stimulus, and an auditory-cued finger tapping task at 2Hz for motor activation. The paradigm was a block design with 12s baseline, followed by four blocks of 30s ON/30s OFF. This experiment assessed activation induced changes for both BOLD and CBF.

Event-related BOLD—Gradient echo EPI with TE/TR/FA= 20ms/0.5s/45° was used to collect event-related data for a paradigm with 12s initial baseline, followed by 10 repetitions of a long trial event related design with 2s stimulus in the form of a flashing checkerboard and 28s rest. Eleven slices of 4mm thickness and no slice gap were positioned to cover both motor and visual cortices simultaneously. Subjects were instructed to open and close their fists rapidly during the flashing checkerboard. This long event-related experiment assessed temporal and magnitude changes in the BOLD response by directly mapping the hemodynamic response (HDR).

At the end of each study, high-resolution T_1-weighted images were also acquired for overlaying the functional data (MPRAGE sagittal orientation, 1mm isotropic resolution, TI=900ms, TR=2300ms, TE=2.91ms, 176 partitions).

Data Processing

All images were first motion corrected in BrainVoyager (Brain Innovations, Maastricht, The Netherlands). CBF and BOLD timecourses were calculated from the ASL runs using the surround averaging and subtraction method (Wong et al., 1997) in Matlab, and then imported back to BrainVoyager for analysis. The block design BOLD data were preprocessed with the following steps: 1) spatial smoothing with an 8mm FWHM Gaussian kernel, 2) linear trend removal, 3) temporal smoothing with a 6s (3 times TR) Gaussian kernel to remove high frequency fluctuations. Following motion correction, the CBF data were only spatially smoothed with an 8mm kernel, as it has been shown that temporal smoothing reduces statistical power (Wang et al., 2005). The event-related BOLD data were processed similarly to the block BOLD data except the temporal smoothing step, where a 1s (2 times TR) kernel was used instead, since the TR for this dataset was only 500ms.

For statistical analysis, all the preprocessed data were co-registered to the high resolution T_1-weighted images. Boxcar models of the block and event-related paradigms were convolved with a canonical form of the hemodynamic response function to generate a model for the expected response. This was then compared on a voxel-by-voxel basis using cross-correlation. Since areas of activation were known a priori, no correction for multiple comparisons was used. Regions-of-interest (ROIs) were chosen from voxels that exceed the correlation threshold of 0.23 (t-score 2.61, $p<0.01$ uncorrected) in left and right motor and visual areas from the CBF datasets. The BOLD datasets were thresholded at 0.3 (t-score 3.47, $p<0.0007$ uncorrected for block-design BOLD data N=124, and $p<2.6\times10^{-14}$ for event-related BOLD data N=622), and only supra-threshold voxels within the CBF ROIs were used to extract the BOLD timecourses. This voxel selection was based on the knowledge that CBF activations more accurately represent parenchyma, whereas BOLD activations are frequently biased by nearby draining veins (Luh et al., 2000). Activation-induced BOLD and CBF changes relative to baseline were calculated from the block design timecourses after averaging across the blocks. Baseline and activation were measured as the average of the final 15 s of the OFF and ON periods respectively to ensure signal has reached steady state. The effects of caffeine and caffeine dose were investigated using repeated measure analysis of variance (ANOVA). Post hoc analysis was performed using the difference between task-related activations before and after caffeine was assessed with univariate analysis of variance (ANOVA), with caffeine dose as the between-subject factor and baseline plasma caffeine concentration and caffeine usage as covariates (SPSS v.16.0, SPSS Inc., Chicago, IL). Caffeine usage was assigned to 3 levels: Low (<100mg/day), Medium (100–250mg/day) and High (>250mg/day). Average caffeine intake/day was calculated from the caffeine usage questionnaire by assuming caffeine contents for common sources of caffeine: coffee (100mg/8oz), tea (50mg/8oz), soda (35mg/12oz) and chocolate (9mg/bar) (Smith et al., 2007).

The resting-state trace data collected during the infusion were first motion-corrected, then CBF and BOLD image timeseries were calculated using the aforementioned surround subtraction and averaging method. For each of the two slices collected, gray matter masks were generated by thresholding the averaged CBF maps. These masks were applied to both CBF and BOLD images to generate a mean gray matter CBF and BOLD signal for each time point. In order to assess the change in signal fluctuations due to caffeine, the first and final sixty time points were averaged across subjects in each dose group, and an F-test was used to compare the variances between the groups at a significance level of 0.05.

RESULTS

Fig. 1 plots the plasma caffeine concentrations determined from the blood samples collected, averaged over all subjects in each group. The average baseline caffeine concentration for the four groups were: 0.56 ± 0.38 ug/ml, 0.60 ± 0.44 ug/ml, 0.34 ± 0.28 ug/ml and 0.57 ± 0.83 ug/ml. The caffeine concentration reached a maximum at the end of the infusion, and remained stable for the remaining duration of the experiment. The final measured concentrations of the three caffeine doses had a ratio of 1:2.0:4.5, which is close to the expected ratio of 1:2.5:5.

Figs. 2 and 3 show the results of the ASL trace sequence collected during infusion. Each data point was calculated as the mean of all the gray matter voxels in the two slices acquired for the trace scan. Both CBF and BOLD baseline remain stable throughout the saline infusion, but a significant decrease was observed for the caffeine infusions within one minute from the start of the infusion. The CBF signal dropped by 22%, 25% and 32% for caffeine doses of 1mg/kg, 2.5mg/kg, 5mg/kg, respectively. The BOLD baseline was unchanged for the lowest caffeine dose (1mg), but dropped by ~1.9% and 2.2% for the 2.5mg and 5mg doses. Notice that the onset of BOLD signal dropoff for both 2.5mg and 5mg doses appears to be delayed relative to the CBF signal dropoff. Also notice the two higher caffeine doses visibly reduced physiological fluctuations compared to the saline and 1mg cases. The standard deviations listed in Table 2 support this trend. One-tailed F-test for sample variances confirmed the reduction in variance for both BOLD and CBF datasets at the two higher doses of 2.5mg (CBF: $F=1.71$, $p=0.02$, BOLD: $F=3.95$, $p<0.01$) and 5mg (CBF: $F=2.78$, $p<0.01$, BOLD: $F=5.94$, $p<0.01$). The infusion of saline or 1mg/kg of caffeine did not produce a significant reduction in the signal fluctuation.

The group-averaged timecourses of the event-related BOLD scans before and after infusion are shown in Fig. 4. As expected, the pre- and post-infusion timecourses for the saline group appear identical and confirms the reproducibility of the experimental procedure. The 1mg/kg dose did not cause any change in the amplitude or rise time of the BOLD response, but visibly faster response time, particularly for the return to baseline, was observed for the 5mg/kg case. Repeated–measures ANOVA reveals a significant reduction in time after the peak amplitude to return to 50% (TA_{50}) for both motor (F(1,16)=10.51, p<0.01)and visual cortices (F(1,15) =10.23, p<0.01). Post-hoc Scheffe test shows a difference of the TA_{50} between the 5mg dose and saline (motor: p=0.03, visual: p=0.04). HDR data were not available for the 2.5mg/kg case since it was from a different experimental protocol.

Fig. 5 shows the plots of percent change in block design task-related activation for both BOLD and CBF in the motor and visual cortices after administration of caffeine or saline. A 20% change on this plot indicates a 20% increase after infusion in the BOLD response relative to the pre-infusion BOLD change. For example, if the BOLD response was 1% before caffeine, it will increase to 1.2% after caffeine. In both motor and visual cortices, the lowest dose (1mg/kg) produced the smallest increase in BOLD response: 12.8% (motor), 6.7% (visual). The largest increase was associated with the 2.5mg/kg dose: 32.2% (motor) and 32.5% (visual). The 5mg/kg dose increased BOLD response by 22% and 24.9% in motor and visual cortices respectively. For the CBF response, a linearly increasing trend was observed for increasing caffeine dose. Trend analysis reveals significant linear (motor: F (1,29) = 6.16, p = 0.019, visual: F (1,29) = 6.089, p = 0.02) and quadratic trends (motor: F (1,29) = 8.550, p = 0.007, visual: F (1,29) = 4.004, p = 0.055) in the BOLD data, and only linear trends (motor: F (1,29) = 15.255, p = 0.001, visual: F (1,29) = 20.553, p < 0.001) in the CBF data, confirming the different dose-response of the two measurements. Saline did not have a significant effect on either BOLD or CBF. Nor did the general trend of the dose responses differ between motor and visual areas.

For statistical analysis, a repeated-measures ANOVA on the pre- and post-caffeine BOLD and CBF activations with baseline caffeine and caffeine usage as covariates revealed both to be non-significant, therefore results from a repeated-measures ANOVA without covariates are reported (see Table 3). There was a significant main effect of caffeine in both cortices and measures. Post Hoc test was run on the percent difference between pre- and post-caffeine activations for further analysis. Post Hoc Tukey’s honestly significant difference (HSD) analysis indicated that the 2.5mg/kg dose significantly increased BOLD and CBF activations for all cases except visual CBF activation (p=0.06) compared to saline. Additionally, the 5mg/kg dose was significantly different from saline in CBF changes for both motor and visual tasks. These results are summarized in Table 4.

DISCUSSION

In this study, we examined the effects of three different doses of caffeine: 1mg/kg, 2.5mg/kg and 5mg/kg on task-induced CBF and BOLD activations. Given that the molecular weight of caffeine is 194.2g/mol, and assuming that the human body contains 60% water, the three doses of caffeine used in this study correspond to 0.009 mM, 0.021 mM and 0.043 mM respectively, which all fell within the range of normal caffeine consumption where the dominant effect is binding of caffeine to adenosine receptors (Fig 1 of Fredholm 1999 (Fredholm et al., 1999)). We also monitored how resting-state CBF and BOLD signals were affected using a simultaneous ASL and BOLD trace acquisition as the caffeine was administered through an IV infusion. The trace data showed that all three doses of caffeine decreased resting-state CBF in a dose-dependent manner, with the decreases ranging from 22% to 32%, which are in good agreement with prior studies (Cameron et al., 1990; Dager and Friedman, 2000; Field et al., 2003; Liu et al., 2004; Mathew and Wilson, 1985). Since binding of caffeine to A_1 adenosine receptors should increase neural activity (Koppelstaetter et al., 2008), oxygen consumption will most likely increase. Combined with the reduced CBF, this would lead to an increase in the deoxyhemoglobin level, and a decrease in resting-state BOLD signal is expected. This was indeed the case for the two higher caffeine doses, which decreased BOLD signal by 1.9%–2.2%. However, the lowest dose of caffeine had no effect on resting-state BOLD signal. A possible reason for this is that caffeine has higher affinity to A_{2A} receptors than A_1 receptors

(Varani et al., 2000), so its effect on neural activity does not become apparent until higher doses are used. It is also likely that the CBF reduction associated with the lowest dose is not sufficient to alter the BOLD signal. The trace results also show much smaller fluctuations in the two higher caffeine doses (see Figure 2, Figure 3 and Table 2). This may be a side effect of caffeine-induced vasoconstriction, which makes the blood vessels less prone to physiological fluctuations.

The trace data also demonstrates how rapidly the brain responds to an IV infusion of caffeine. On average, resting-state CBF and BOLD signals began to drop within a minute after the onset of infusion and reached steady–state during the 10 minute interval. This response is much faster than ingestion, the more commonly used form of caffeine administration (Bendlin et al., 2007; Dager et al., 1999; Laurienti et al., 2002, 2003), where 30–45 minutes are necessary for the caffeine to be absorbed through the gastrointestinal tract into the bloodstream (Dager and Friedman, 2000). As a result of the rapid response, caffeine administration via infusion can decrease total study time and minimize co-registration errors between pre- and post-caffeine session, since the subjects do not have to be removed from the scanner. It is also conducive to monitor resting-state CBF and BOLD response to caffeine in real-time. However, it is important to realize that infusion is not the primary method of caffeine intake in humans, so a direct comparison between the two methods is warranted.

A similar dose-response was observed in the event-related BOLD data used to map the HDR, where the 1mg/kg dose produced only a slight increase in BOLD amplitude, but no change in timing characteristics. The 5mg/kg not only increased the magnitude of the BOLD response, it also induced a measurable forward shift in the return to baseline after activation. This result is in excellent agreement with an earlier study that reported caffeine’s ability to alter the timing of the BOLD response (Liu et al., 2004). The same authors later proposed an arteriolar compliance model (Behzadi and Liu, 2005) which views total arteriolar compliance as the summation of muscular (active) and connective tissue (passive) compliances, and attributes caffeine’s ability to accelerate the BOLD response to the dominance of muscular compliance at small vessel radii.

In general, all three doses of caffeine increased %CBF and %BOLD responses to activation in both motor and visual cortices. While the increased %CBF response agrees with a recent caffeine study, the increased %BOLD response does not (Liau et al., 2008). The exact reason for this disagreement is unclear. A major difference between the two studies is Liau administered caffeine orally without controlling for subject body weight. In this study we used an infusion to administer a controlled dose to the subject without having to remove them from the magnet. This difference in experimental setup may account for the discrepancy in results. Closer examination of the current results reveal that the increases associated with the 1mg/kg dose were not significantly different from results obtained after saline infusion. This result is in good agreement with the trace and event-related BOLD results, which suggest that the lowest dose was insufficient to induce detectable group-averaged changes in either CBF or BOLD responses to functional tasks. The maximum BOLD increase was associated with the intermediate dose of 2.5mg/kg. The dose-response curve of the BOLD responses agrees with the biphasic dose-response reported in other studies (Daly and Fredholm, 1998). The CBF responses, on the other hand, increased linearly with increasing doses. The exact reason for this observation cannot be determined from our data. A plausible explanation is a higher abundance of A_{2A} receptors, found both on neurons in brain areas such as striatum, hippocampus and cortex, as well as on endothelial and smooth muscle cells in blood vessels (Chen et al., 1999; Ongini et al., 1997), may require a higher dose of caffeine to saturate the system and cause a detectable difference.

An important observation in the current study was the large inter-subject variations, evident in the extent of the error bars in Fig 5. This variation was not explained by either caffeine usage or baseline plasma concentration of caffeine. The non-significant effect of caffeine usage appears to be at odds with an earlier study by Laurienti et al., which reported that BOLD activations are significantly correlated with caffeine consumption (Laurienti et al., 2003). Since the aim of the current study was not to query the effects of caffeine usage, subject selection was not based on caffeine usage, therefore the current study does not have adequate sensitivity to detect the effect of caffeine usage. Another potential explanation could be the shorter echo time used in the current study, which was chosen to achieve concurrent CBF and BOLD contrast. The suboptimal BOLD contrast could hinder the detection of subtle differences due to caffeine usage. One should also note that these measurements were based on self report from subjects, and that beverage contents of caffeine are highly variable: a cup of coffee could contain anywhere between 40–180 mg of caffeine, depending on brewing method and duration (Fredholm et al., 1999), therefore the caffeine usage measurement in this study was only a rough estimate. The large inter-subject variations may also be a result of the non-quantitative nature of the current study. While variations in the resting-state BOLD signal pre- and post-caffeine were minimized by administering the caffeine through infusion, motion artifacts were still present due to the length of the study. These artifacts lead to changes in signal intensities, which affect the accuracy of comparison between runs. A more robust method would be to quantify the BOLD response based on changes in R_2^* as proposed by Perthen et al. in 2008 (Perthen et al., 2008).

CONCLUSION

We have demonstrated the rapid BOLD and CBF response to caffeine infusion using an ASL trace sequence. This study also showed that 2.5mg/kg is the minimum dose required to produce a significant increase in task-related BOLD and CBF activations. In most caffeine studies, a fixed 200mg over-the-counter pill is used for all subjects, regardless of body weight. But while 200mg for an 80kg adult is equivalent to a 2.5mg/kg dose, this dose increases to 4mg/kg for a 50kg adult. As this study has shown, there is a dose-dependent change in BOLD activation. Moreover, higher doses of caffeine may accentuate negative effects such as anxiety and insomnia; therefore a body-weight-adjusted dose should be used for caffeine studies.

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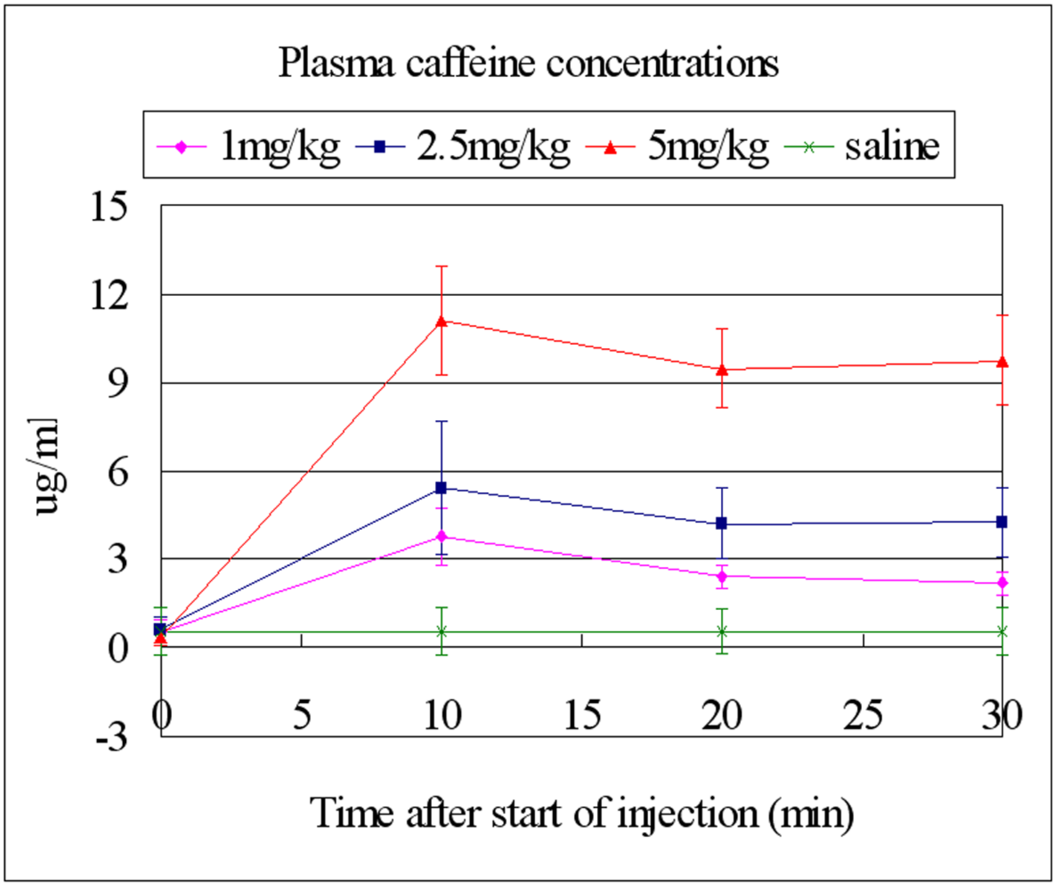

Figure 1.
Measured plasma caffeine concentrations from collected blood samples, averaged over all subjects in each group. Error bars represent standard errors for each group. The baseline timepoint was calculated as the average of the two baseline blood samples.

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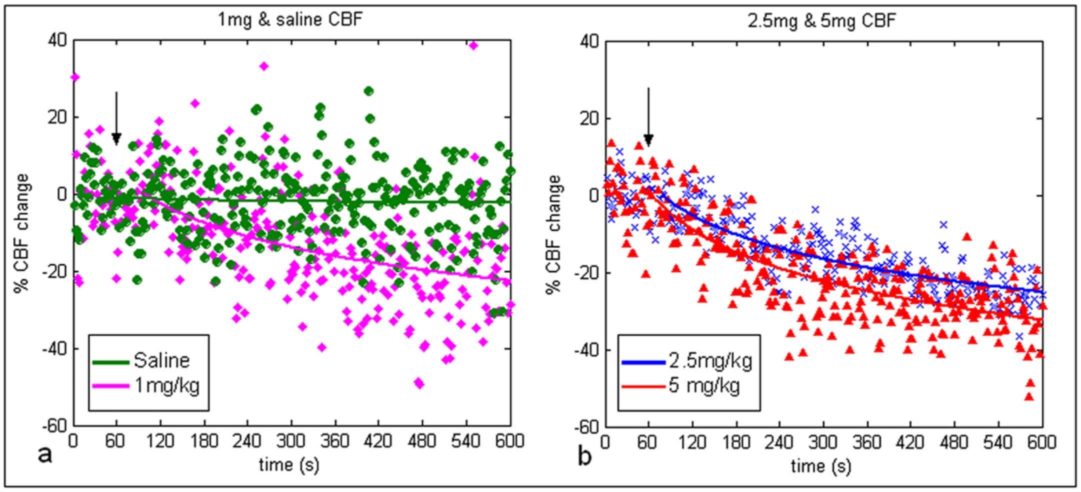

Figure 2.
CBF data collected during infusion, normalized as percent change from mean of first 60s. Each timepoint is the average signal of a whole brain gray matter region-of-interest. a) Saline (green) and 1mg (pink) data. b) 2.5mg (blue) and 5mg (red) data. Black arrow marks the onset of infusion. No change from baseline was observed for saline, but significant decrease was seen for the three caffeine doses after the initial 60s of baseline.

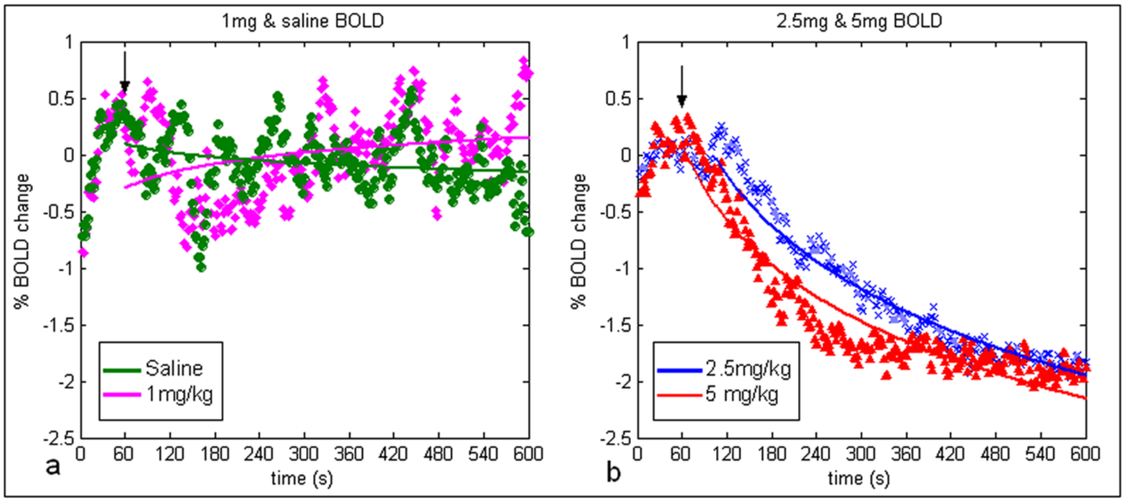

Figure 3.
BOLD data collected during infusion, normalized as percent change from mean of first 60s. Each timepoint is the average signal of a whole brain gray matter region-of-interest. a) Saline (green) and 1mg (pink) data. b) 2.5mg (blue) and 5mg (red) data. Black arrow marks the onset of infusion. No change from baseline was observed for the lowest caffeine dose and saline, but significant decrease was seen for the two higher doses.

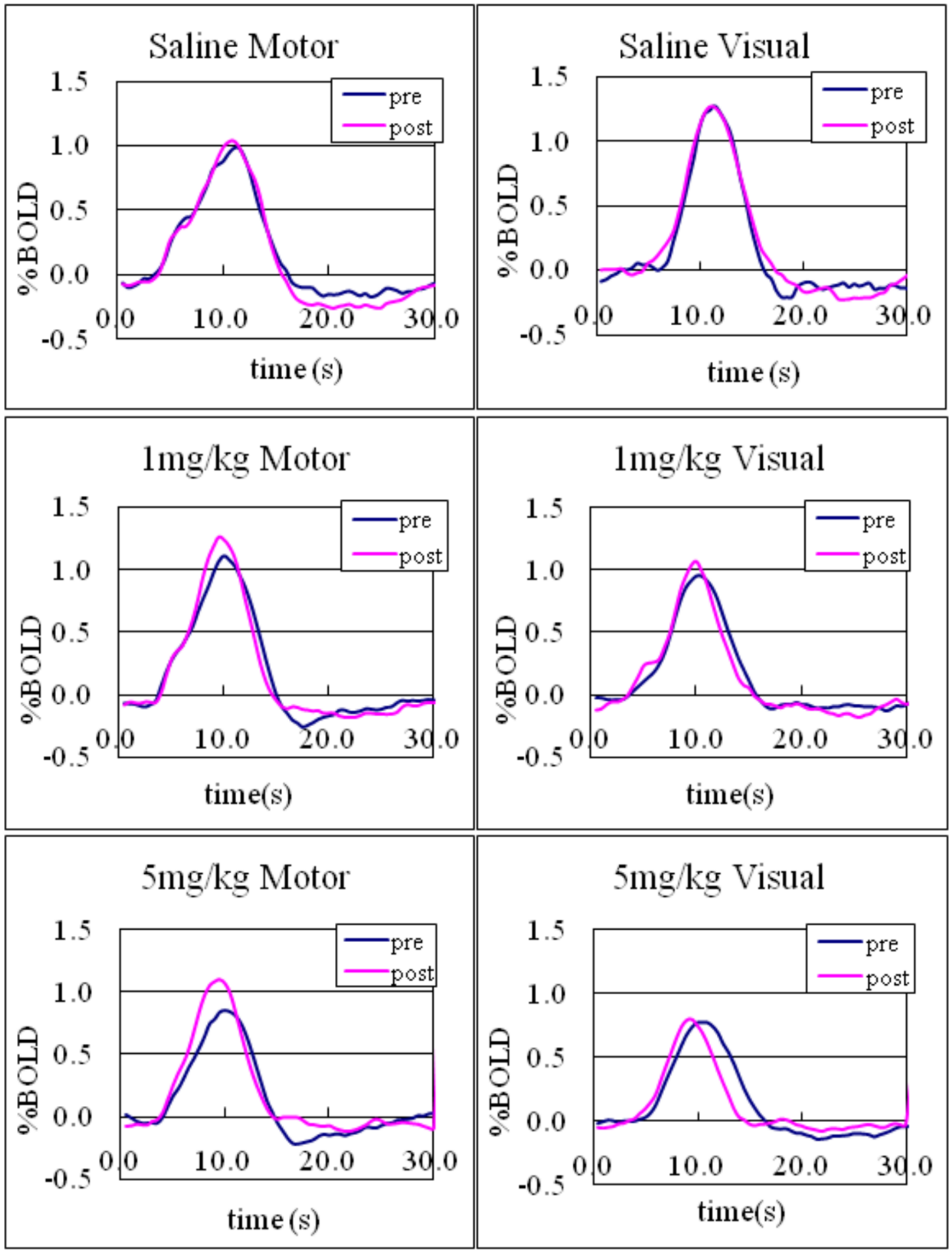

Figure 4.
Event-related BOLD timecourses for saline (top row), 1mg/kg (middle row) and 5mg/kg (bottom row) for both motor (left) and visual (right) cortices, averaged over all trials and all subjects.

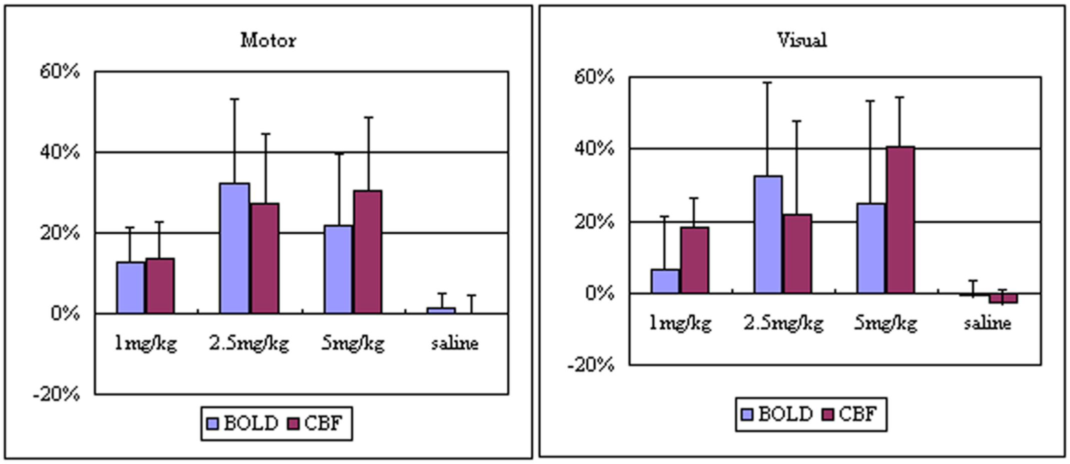

Figure 5.
Graph of percent change in task-related BOLD and CBF activation post-infusion relative to pre-infusion for the four groups.

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Table 1

Total amount of caffeine (in mg) and baseline plasma caffeine concentration (in ug/ml) for all subjects. Subjects were assigned to three caffeine usage groups: high (>250mg/day), medium (100–249mg/day), low (<100mg/day).

Subject No.	Dose (mg/kg)	Total Caffeine (mg)	Baseline Caffeine (ug/ml)	Estimated Caffeine Usage
1a	0	0	<0.5	High
2	0	0	<0.5	Low
3a	0	0	0	Low
4a	0	0	0	Low
5a	0	0	2.22	High
6	0	0	<0.5	Medium
7	1	69.8	0	High
8	1	63	0.63	Low
5b	1	56.3	0.86	High
9	1	67.5	<0.5	Low
10	1	91.4	0	Low
11	1	66.8	0	Low
12	2.5	159.5	<0.5	Low
3b	2.5	255.4	<0.5	Low
13	2.5	157.5	<0.5	High
14	2.5	191.3	0.56	High
15	2.5	198	0.70	High
16	2.5	144.1	0.52	Low
17	2.5	123.2	0.65	High
18	2.5	158.4	1.20	Medium
19	2.5	132	0.10	Low
20	2.5	193.6	0.36	Low
5c	2.5	137.5	1.83	High
21	2.5	196.9	0.61	Low
22	2.5	168.8	<0.5	Medium
23	2.5	213.8	<0.5	High
24	5	295	<0.5	Low
25	5	270	<0.5	Low
26	5	281.5	<0.5	Medium
27	5	330.8	0	Low
5d	5	279	0	High
4b	5	378	<0.5	Low
1b	5	450	<0.5	High

Table 2

Standard deviations (SD) of trace CBF and BOLD data for the different doses. SDs for the two higher doses were lower than saline and the 1mg dose.

	CBF	**BOLD**
Saline	10.31	0.26
1mg/kg	12.53	0.28
2.5mg/kg	4.61	0.09
5mg/kg	7.33	0.10

Table 3

Statistic results of repeated-measures ANOVA on the pre- and post-caffeine percent activations. There was a main effect of caffeine in BOLD and CBF activations in both cortices. All except visual BOLD had significant caffeine and dose interaction.

		Caffeine	**Caffeine Dose**
BOLD	Motor	$F(1,29)=41.008$, $p<0.001$	$F(3,29)=4.802$, $p=0.008$
	Visual	$F(1,29)=10.985$, $p=0.002$	$F(3,29)=2.657$, $p=0.067$
CBF	Motor	$F(1,29)=55.131$, $p<0.001$	$F(3,29)=6.169$, $p=0.002$
	Visual	$F(1,29)=30.328$, $p<0.001$	$F(3,29)=7.696$, $p=0.001$

Table 4

Summary of post-hoc Tukey's HSD results on the percent change in BOLD and CBF activation after infusion, p=p-value, CI=95% confidence interval for the difference between each pair of groups. Only comparisons with saline are shown here.

	BOLD			CBF		
	groups	**p**	**CI**	**groups**	**p**	**CI**
Motor	1 vs 0	0.43	[−11.1%,40.2%]	1 vs 0	0.25	[−6.9%, 38.7%]
	2.5 vs 0*	0.00*	[8.3%, 52.8%]	2.5 vs 0	0.00*	[6.2%, 45.8%]
	5 vs 0	0.16	[−5.3%, 46.0%]	5 vs 0*	0.00*	[6.5%, 52.1%]
Visual	1 vs 0	0.87	[−24.4%, 43.6%]	1 vs 0	0.10	[−3.6%, 55.4%]
	2.5 vs 0*	0.02*	[3.4%, 62.5%]	2.5 vs 0	0.06	[−0.7%, 50.5%]
	5 vs 0	0.20	[−8.8%, 51.4%]	5 vs 0*	0.00*	[14.0%, 73.0%]

* =statistical significance ($p<0.05$).