NeuroImage **15,** 37–44 (2002)
doi:10.1006/nimg.2001.0973, available online at http://www.idealibrary.com on IDEAL®

On the Use of Caffeine as a Contrast Booster for BOLD fMRI Studies

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Received March 13, 2001

This study explored the possible use of caffeine as an agent to improve the BOLD (blood oxygen level-dependent) signal response in fMRI. Previous research has demonstrated that caffeine has the ability to reset the level of coupling between blood flow and neuronal activity. In the present study, it has been shown that caffeine causes a decrease in cerebral perfusion by as much as 13.2% without a change in performance. Caffeine is a cerebral vasoconstrictor that causes an increase in the concentration of deoxyhemoglobin and thus a decrease in the BOLD baseline resting signal by 4.4%. During activation, the vasculature responds from below-normal baseline levels with a normal increase in blood flow and volume, resulting in an overall increase in the BOLD contrast. This increase can be as large as 22–37% during the performance of a visually cued motor task. The benefit of such a large increase in the BOLD contrast could be used to improve the image resolution, the acquisition scheme, or the task design of fMRI experiments. Caffeine has the potential to be used as a contrast booster for fMRI experiments.

INTRODUCTION

In functional MRI the fact remains that a finite amount of signal change is required for these experiments to work within the bounds of the statistical assumptions (Parrish *et al.*, 2000). If it were possible to improve the contrast of the blood oxygen level-dependent (BOLD) signal, it would result in an improvement in current fMRI experiments and could enable subtler fMRI designs, which do not generate sufficient signal under standard scanning conditions.

Caffeine and theophylline belong to the methylxanthine family of drugs. These drugs act as vasoconstrictors in the brain and vasodilators in the peripheral vascular system. However, caffeine is one of the most widely used psychostimulants in the world. It is believed that the effects of caffeine on the central nervous system and related physiologic parameters are a result of adenosine receptor antagonism by caffeine (Nehlig *et al.*, 1992; Fredholm, 1995; Fredholm *et al.*, 1999). Although there are other theories about the mechanisms of caffeine including neuronal hyperexcitability, the adenosine receptor blocking theory is the most plausible based on the caffeine levels found in the bloodstream subsequent to typical consumption (Nehlig *et al.*, 1992; Fredholm *et al.*, 1999). Peak blood levels occur approximately 30–40 min postconsumption. Cerebral glucose metabolism is directly related to the caffeine level, which might be attributed to the wakefulness felt after its ingestion (Nehlig *et al.*, 1992; Fredholm *et al.*, 1999). Caffeine, at low-level doses (200 mg or about two to three cups of coffee), is a cerebral vasoconstrictor that can decrease the resting-state cerebral blood flow (CBF) in humans by as much as 20–30% (Mathew and Wilson, 1985; Cameron *et al.*, 1990). Therefore, we investigated the use of caffeine as an agent to decrease the resting CBF and increase the deoxyhemoglobin concentration in the baseline condition, allowing an overall increase in the BOLD response during the active condition.

METHODS

Subjects

Eighteen (14 caffeine, 8 controls) young, healthy, right-handed Northwestern University students were enrolled in the study after signing the consent form. Enrollment of subjects was approved by the Northwestern institutional review board. The subjects were told that they were entering a study on caffeine. They were asked to refrain from ingesting any food or drink (coffee, tea, soft drinks, chocolate, etc.) that contained caffeine 12 h prior to their imaging time. Each subject filled out a questionnaire documenting his or her level of caffeine use.

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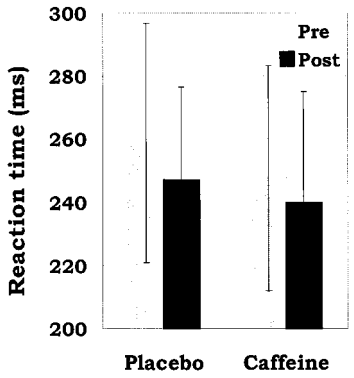

FIG. 1. Reaction times (milliseconds) from the motor task. Note how similar all the measurements are, signifying that all the experiments had similar performance.

Setup and Equipment

All MRI was done on a 1.5-T Siemens Vision equipped with echo-planar gradients. The head coil was outfitted with a mirror system to enable visualization of the stimuli. MR-compatible headphones (Avotec, Jensen Beach, FL) were used to communicate with the subject. The subject's head was placed in a vacuum restraint pillow (Vac-Fix, Bionix), which was then clamped to the head coil to make it more rigid. This setup has been shown to reduce the level of movement during a typical functional run to less than 1 mm (Parrish *et al.*, 1998). All behavioral responses were acquired with a fiberoptic button connected to a Macintosh PowerPC computer running Superlab software (Cedrus Corp., Phoenix, AZ). All stimuli were displayed via an LCD projector onto a rear projection screen.

Experimental Protocol

The design of this study used two groups, caffeine and control. Each group was studied in the pre- and posttreatment phases using fMRI and a resting state perfusion imaging method. All studies were completed over a $2\frac{1}{2}$-hour period. All subjects completed each phase of the experiment and four subjects participated in both groups on different days.

The pre- and posttreatment studies were identical for both groups. The orientation of the slices was prescribed on sagittal scout images. All slices (functional and anatomic) were oriented in an oblique axial plane such that they passed through both the primary motor and visual cortices. The 3D anatomic data was transformed into Talairach space. The same transformation was applied to the functional data. The normalization of the data allowed for group analysis and controlled for head position errors due to the two different MRI studies. The functional experiments were performed first to ensure that the subject was alert. The anatomic images were acquired next, followed by the resting state perfusion measurement. The subject was then removed from the magnet and administered either caffeine or placebo. The caffeine preparation was an over-the-counter caffeine tablet containing 200 mg of caffeine. The subject was instructed to remain in the waiting area at the MRI center. Approximately 30–40 min following the administration of drug or placebo, the subject was placed back in the magnet and the imaging protocol was repeated.

Functional Experiment

The functional experiment used an event-related design with a long intertrial interval. This design allowed the direct investigation of the hemodynamic response with adequate time for the MR signal to return to

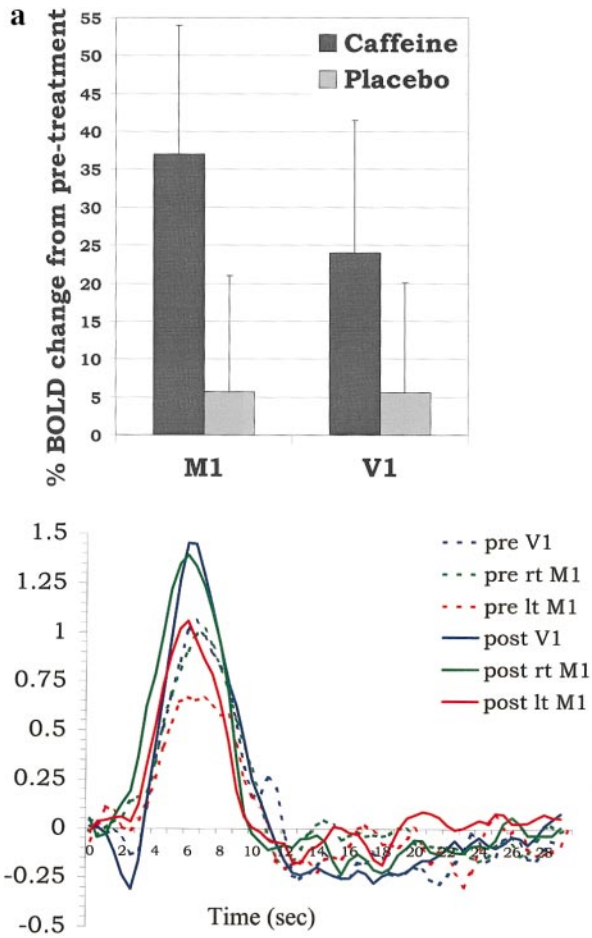

FIG. 2. The percentage BOLD signal change relative to the pretreatment level was measured. (a) Percentage change is plotted versus location. After the placebo, the signal change was not different from the pretreatment condition. After caffeine, there was a marked increase in the BOLD response in the motor (M1, 37%) and visual (V1, 22%) cortices. (b) The time course from a single subject is shown. The pretreatment time courses are denoted with the dashed lines and the posttreatment courses (caffeine) are shown by solid lines. In this subject all three regions had a large increase in the peak BOLD response but the magnitude of the poststimulus undershoot is the same.

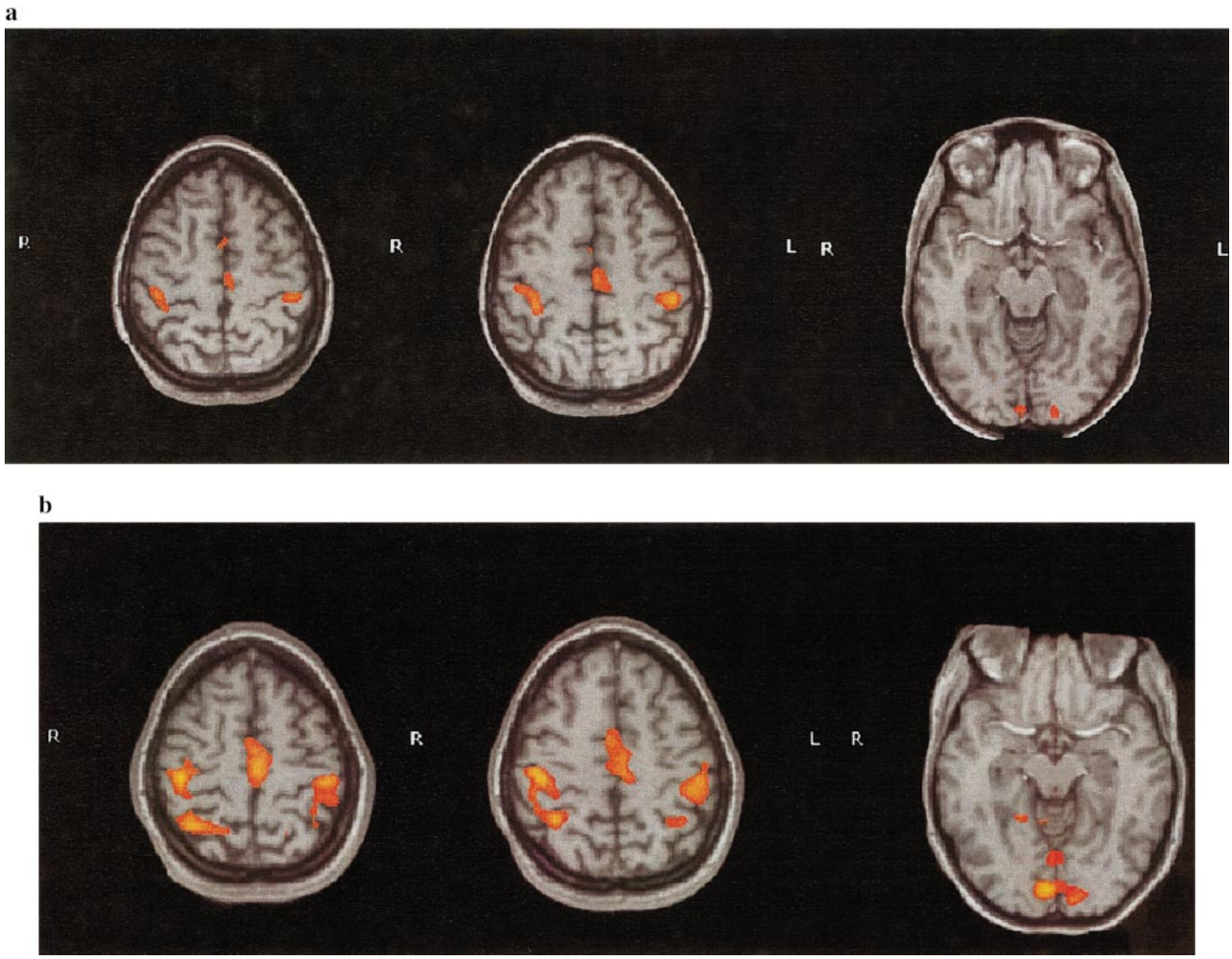

FIG. 3. All of the functional maps were generated with a cross-correlation threshold of $r > 0.3$. (a) Selected slices taken from the pretreatment functional map are shown. (b) The same slice levels are shown as in (a), but this experiment (identical paradigm) was conducted after the administration of caffeine. The extent of activation has increased in both the motor and visual cortices.

baseline before the next trial. The single-shot echo-planar sequence, which acquired six 6-mm slices with a 3-mm gap, had the following parameters, TR = 500 ms, TE = 40 ms, flip angle = 58°, matrix = 64 × 64, FOV = 240 mm (in-plane resolution of 3.75 × 3.75 mm). The functional experiment used 10 trials of the visually cued motor task. This required acquisition of 600 volumes in addition to 20 initial volumes to allow the signal to reach steady-state. The paradigm used a 2-s duration stimulus of an 8-Hz flashing checkerboard. During the flashing checkerboard, the subject was instructed to perform a bilateral, complex finger movement, which consisted of opposing the thumb with the index finger followed by the ring finger, the middle finger, and lastly the little finger. The movement was repeated throughout the flashing checkerboard period (2 s).

Motor reaction times were measured to assess behavioral performance. Subsequent to the visually cued motor task, a reaction time task was performed. The format of the experiment was similar to that described above except that a red checkerboard (target, duration of 125 ms) was inserted into the flashing black and white checkerboard. The subject was instructed to press the button when the red checkerboard was displayed. The target appeared two to three times during each trial and the reaction time was measured from the onset of the target's arrival via a fiberoptic button. Imaging data were collected during the reaction time task. The differences in reaction time between groups and treatment were assessed using a repeated-measures ANOVA with the statistical software package SuperAnova (Abacus Concepts, Berkeley CA).

Functional data analysis was performed using Brain Voyager (Version 4.4, Brain Innovation, The Netherlands) subsequent to 3D motion correction, spatial smoothing using a 5-mm full width half-maximum (FWHM) Gaussian kernel (<2 voxels), and temporal

smoothing using a 1-s FWHM Gaussian kernel. The spatial smoothing was used to increase the signal-to-noise since so few trials were collected. The temporal smoothing was employed to reduce rapid oscillations that were superimposed on the hemodynamic response function (HRF). The HRF was generated by localizing the ROIs within the motor and visual cortices on the anatomic data. Within these regions, the time course data from five individual voxels were averaged together to form the average HRF function. The number of voxels used to generate the average HRF was intentionally small to minimize any effects from larger draining veins. The three areas investigated were left and right primary motor cortex (M1) and primary visual cortex (V1). The average BOLD signal amplitude response was determined from these curves. In addition to time course data, standard functional maps were generated by cross-correlation with a reference function generated by convolving the event time course with a canonical HRF. The functional maps were generated with a correlation threshold of $r \geq 0.3$. The justification for this threshold level was that the regions of interest (M1 and V1) were predetermined a priori, reducing the number of multiple comparisons.

To investigate the group comparison, all functional and anatomic data were transformed into Talairach space. A multisubject comparison was made using cognitive subtraction and a fixed effects general linear model as implemented by Brain Innovation. A threshold of $Z > 4.0$ was used to account for the multiple comparisons made.

Additional analysis was completed to measure the signal-to-noise ratio (SNR) in the non activated BOLD data pre- and posttreatment. A large region of interest (ROI) was chosen in a slice that contained the motor cortex. The mean signal of the ROI was divided by the standard deviation of the values within the ROI to obtain the SNR value. The posttreatment SNR change relative to the pretreatment SNR was calculated in all subjects.

Perfusion Experiment

A pulsed arterial spin-labeling sequence called Quantitative Imaging of Perfusion with a Single Subtraction, Version II (QUIPSSII), was used to measure the resting state perfusion level (Wong *et al.*, 1998, 1999). The method of pulsed arterial spin labeling tags the arterial blood with an inversion pulse and allows the tagged blood to arrive and mix with the slice of interest, and then an image is acquired. This image is interleaved with a control image in which no inversion tag is applied. By subtracting the perfusion-weighted image from the control image, it is possible to obtain a measurement proportional to local perfusion (Wong *et al.*, 1998, 1999; Detre and Alsop, 1999). The signal-to-noise was improved by averaging this signal over several minutes, which removed the influence of pulsation, respiration, and any low-frequency drift in the MR signal. The perfusion data were acquired over a 3-min period during both experimental phases, while the subject rested quietly. The analysis of the data required the investigator to define a ROI located in visual and motor cortices and white matter. The anatomic data were used to localize the ROI for the perfusion analysis. The percentage signal change relative to baseline was calculated for all subjects and regions.

Anatomic Data

The 3D anatomic images were acquired in the same plane as the functional slices and were used to overlay the functional maps. The parameters of the 3D FLASH sequence were TR = 15 ms, TE = 5.6 ms, flip angle = 20°, FOV = 240 mm, matrix = 256 × 256.

RESULTS

The result of the repeated-measures ANCOVA of the reaction time measurements showed (a) no significant difference in reaction time pre and post placebo administration, (b) no significant difference in reaction time pre and post caffeine administration, (c) no significant difference between the placebo or caffeine groups, and (d) no interaction between treatment type and group (see Fig. 1). This demonstrates a similar level of performance within and across the groups as well as across treatment. The analysis of the functional data acquired during the reaction time task showed little activation due to the limited number of button pushes during the 10 trials.

Following caffeine, the average percentage signal change of the BOLD response relative to baseline showed significant changes in both motor and visual areas (see Fig. 2a). The motor region exhibited the largest percentage signal change of 37% relative to the pretreatment state ($P < 0.03$). The visual cortex demonstrated a 26% increase in the amplitude of the BOLD response subsequent to caffeine relative to the pretreatment condition ($P < 0.05$). During the placebo condition, both M1 and V1 exhibited a BOLD response that was larger than that in the pretreatment period by 6 and 4%, respectively. These results are from data averaged over each group, which resulted in large standard deviations. The large deviations could reflect the differences in dose of caffeine (200 mg of caffeine was given independent of body mass) as well individual differences in the metabolism of caffeine. Nevertheless, the differences in the motor and visual cortices were significant. An example time course for a single subject is shown in Fig. 2b.

Functional maps were created pre- and posttreatment for all the individual subjects; see Fig. 3 for example maps. Ten of the fourteen caffeine subjects

had similar changes in their functional maps. Whereas three controls had an increase in the extent, three had decreases and two had no change. However, group analysis of the data using a direct subtraction, $(Caf_{post} - Caf_{pre}) - (Placebo_{post} - Placebo_{pre})$, demonstrated the increased activity in the motor and visual cortices in Fig. 4.

The pre- and posttreatment SNR levels are shown in Fig. 5. There was little change in the SNR level of the baseline BOLD data in the placebo group. However, there was a significant decrease ($P < 0.001$) in the SNR following the administration of caffeine. The average SNR decrease across all caffeine subjects was 4.4% relative to the pretreatment state.

The result of the perfusion measurements relative to baseline averaged across all subjects is shown in Fig. 6. The resting state perfusion measurement in the motor cortex showed a significant change ($P < 0.005$) from baseline following the caffeine treatment: −13.2% compared with the placebo change of −0.8%. A significant decrease ($P < 0.05$) was also detected in the visual cortex subsequent to caffeine ingestion. The resting state perfusion in V1 decreased relative to pretreatment with caffeine by −11% compared with the presence of the placebo, −2.8%. A similar trend was seen in white matter but was not significant.

DISCUSSION

Recently, Morton *et al.* (2000) used theophylline, a vasoconstrictor, in rats to study the effect on the BOLD response. They found that the BOLD response was significantly increased (60–65%) over the pretreatment BOLD response after administration of the vasoconstrictor (Morton *et al.*, 2000). They also demonstrated that the amplitude of the resting state BOLD signal was decreased compared with the pretheophylline condition. These signal changes are indicative of cerebral vasoconstriction.

Exploring the effects of a vasodilator, other researchers have shown that a vasodilator (acetazolamide) results in an increase in the BOLD sensitive signal in rats (Graham *et al.*, 1994) and in humans (Bruhn *et al.*, 1994; Kleinschmidt *et al.*, 1995; Hedera *et al.*, 1996) in the resting state condition. The increase in blood flow without a proportional increase in oxygen consumption leads to an increase in the oxygen concentration and thus an increase in the resting state BOLD signal (Vorstrup *et al.*, 1984; Davis *et al.*, 1998; Hoge *et al.*, 1999; Kastrup *et al.*, 1999). However, this leads to decreased *BOLD contrast* because there is a smaller difference between the rest and active states (Bruhn *et al.*, 1994). The vasodilator increases the baseline CBF and cerebral blood volume, and dilutes the deoxyhemoglobin pool. The dilution of the deoxyhemoglobin and increased resting CBF lead to a reduction in the BOLD contrast (Bruhn *et al.*, 1994; Li *et al.*, 2000).

In our study, we found that caffeine administration results in a profound decrease in the resting state blood flow level and increase in BOLD contrast. Vasoconstriction is believed to be the mechanism (Mathew and Wilson, 1985; Cameron *et al.*, 1990; Nehlig *et al.*, 1992; Fredholm *et al.*, 1999). During vasoconstriction CBF is reduced and the concentration of deoxyhemoglobin is increased, thus making the BOLD signal more sensitive to changes related to activation. Caffeine has also been shown to increase glucose metabolism, resulting in hyperexcitability of neurons (Nehlig *et al.*, 1992; Fredholm *et al.*, 1999). Caffeine is one of a few compounds that is capable of resetting the level of coupling between CBF and energy metabolism (Nehlig *et al.*, 1992; Fredholm *et al.*, 1999). Furthermore, recent MRI studies have shown that the vascular response to a stimulus is the same magnitude independent of the prestimulus vascular condition (Li *et al.*, 2000; Corfield *et al.*, 2001). By exploiting the cerebral vasoconstrictive property of caffeine and its ability to reset the level of coupling between CBF and neuronal activity, it is possible to increase the BOLD signal response (Figs. 2–4).

The amplitude of the baseline BOLD signal decreased with the administration of caffeine (Fig. 5), as a result of vasoconstriction and reduced CBF. The average perfusion level decreased by 11–13% without a change in performance as measured by reaction time, suggesting that a new level of coupling between CBF and neuronal activity had been set. At this new level, a small change in the deoxyhemoglobin concentration may result in a higher sensitivity in the BOLD signal or, alternatively, a similar-magnitude vascular response may result in a larger percentage change due solely to the lower baseline CBF level. Using breath holding to alter the CO_2 levels, several groups have monitored changes in CBF due to breath holding alone, stimulation alone, and a combined task (Li *et al.*, 2000; Corfield *et al.*, 2001). They found that the baseline level of CBF had no effect on the CBF change associated with the visual stimulus. In the case of caffeine, the baseline CBF is decreased compared with pretreatment level, which results in a larger percentage change in CBF following neuronal stimulation. This change in CBF may be amplified into a larger overall BOLD signal response. The BOLD signal increase could be applied to many issues in fMRI such as resolution, acquisition schemes, robustness of activation, or more complex paradigms, which would normally invoke a smaller neuronal and BOLD response.

Not all subjects responded uniformly to the caffeine treatment. The dose of caffeine (200 mg) was administered without consideration for weight. Neither the metabolism of the caffeine pill nor subject's food intake prior to the study was monitored. All of these factors could be attributed to the variability of the response. Furthermore, the dose–response curve for caffeine is not linear, and at higher doses of caffeine, it is possible

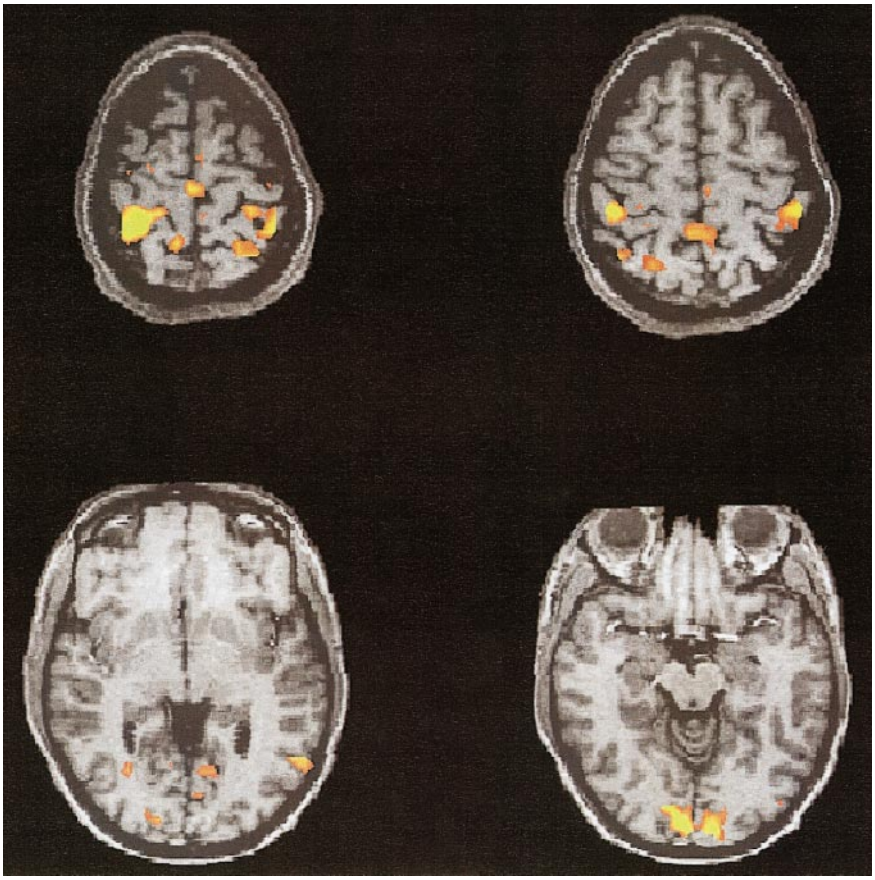

FIG. 4. The result of the group analysis demonstrates activation in the motor and visual cortex after the direct subtraction ($Caf_{post} - Caf_{pre}$) − ($Placebo_{post} - Placebo_{pre}$). The group analysis map was generated using a threshold of $Z > 4.0$.

to reduce the physiologic response (Kaplan *et al.*, 1990, 1992, 1997). A contradictory study has been reported by Bruhn *et al.* (2000) that showed a decrease in the BOLD response subsequent to administration of therapeutic levels of theophylline (Bruhn *et al.*, 2000). The dose level of methylxanthines may play a critical role in the enhancement of the BOLD response. The choice of therapeutic levels of theophylline may be too large. Caffeine and theophylline are both methylxanthines;

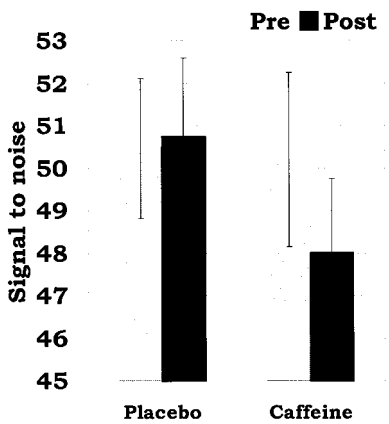

FIG. 5. The signal to noise measurement does not change after administration of the placebo. However, after caffeine there is a significant decrease in signal to noise of 4.4%. This is an indication of the vasoconstriction property of caffeine. Signal to noise is measured as the mean signal divided by the standard deviation of the data within a specific region of interest.

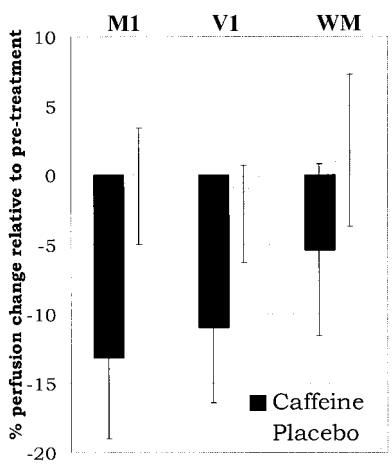

FIG. 6. Perfusion changes in motor cortex (M1), visual cortex (V1), and white matter (WM) following caffeine administration. Note that the change in M1 and V1 is significant.

however, their potencies as adenosine antagonists differ (theophylline greater than caffeine) (Daly, 1982).

Another issue is the uniformity of enhancement throughout the brain. Since caffeine is an adenosine antagonist, the vasoreactivity changes induced by caffeine may be a function of the density of the adenosine receptors. In the current study, a visually cued motor task was used to investigate several brain regions. The nonuniformity of the results may reflect the regional impact of caffeine or the dependency on the capacity for the local vasculature to adjust to different flow conditions. Nevertheless, the effect, while nonuniform, was in the same positive direction for the BOLD response. However, this needs to be studied further before it can be applied to all fMRI experiments.

In the present study, there was no attempt to measure the baseline level of caffeine in the blood or saliva of the subjects. The subjects were asked to refrain from caffeine consumption 12 h prior to the study. However, there was no enforcement or measure of compliance. It is possible to detect plasma levels of caffeine through a saliva sample (Lee *et al.*, 1996). In the future, performance, physiologic measures, and BOLD response changes need to be compared with actual levels of caffeine in the blood to determine appropriate dose of caffeine.

Many researchers have investigated the effect of caffeine on sensory and cognitive performance. The results are mixed, with some showing increases in performance (Durlach, 1998), some showing decreases (Bovim *et al.*, 1995; Smit and Rogers, 2000), and some showing no change (Battig *et al.*, 1984; Nehlig *et al.*, 1992; Edwards *et al.*, 1996; Stein *et al.*, 1996; Rogers and Dernoncourt, 1998; Fredholm *et al.*, 1999). The contradictory results are perhaps due to the complexity of the different tests and the dose-dependent effects of caffeine. The impact of hyperexcitable neurons, reduced CBF, and increased glucose consumption on performance needs to be better understood before caffeine is employed as a contrast booster for fMRI.

CONCLUSION

This study has shown the benefits of using caffeine to improve BOLD signal contrast. We found a 22–37% BOLD signal increase over the baseline signal after the administration of a low dose of caffeine. Future work will investigate the physiologic mechanisms for the BOLD enhancement. The widespread use of caffeine makes it an ideal candidate for use with fMRI. The impact of caffeine on performance of functional tasks during fMRI needs to be studied further, but at low doses, caffeine does not affect simple task performance. Further, the impact of prior caffeine use (naive to heavy) needs to be studied to demonstrate that the BOLD signal boost is applicable to the general population.

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