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Chapter 6

The effect of caffeine on working memory load-related brain activation in middle-aged males

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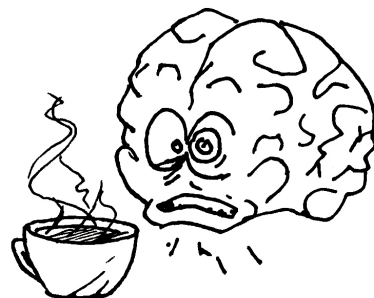
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ABSTRACT

Caffeine is commonly consumed in an effort to enhance cognitive performance. However, little is known about the usefulness of caffeine with regard to memory enhancement, with previous studies showing inconsistent effects on memory performance. We aimed to determine the effect of caffeine on working memory (WM) load-related activation during encoding, maintenance and retrieval phases of a WM maintenance task using functional magnetic resonance imaging (fMRI). 20 healthy, male, habitual caffeine consumers aged 40 to 61 years were administered 100 mg of caffeine in a double-blind placebo-controlled crossover design. Participants were scanned in a non-withdrawn state following a workday during which caffeinated products were consumed according to individual normal use (range = 145 – 595 mg). Acute caffeine administration was associated with increased load-related activation compared to placebo in the left and right dorsolateral prefrontal cortex during WM encoding, but decreased load-related activation in the left thalamus during WM maintenance. These findings are indicative of an effect of caffeine on the fronto-parietal network involved in the top-down cognitive control of WM processes during encoding and an effect on the prefrontal cortico-thalamic loop involved in the interaction between arousal and the top-down control of attention during maintenance. Therefore, the effects of caffeine on WM may be attributed to both a direct effect of caffeine on WM processes, as well as an indirect effect on WM via arousal modulation. Behavioural and fMRI results were more consistent with a detrimental effect of caffeine on WM at higher levels of WM load, than caffeine-related WM enhancement.

* This chapter is based on data from fMRI study 2.

INTRODUCTION

Caffeine (a methylxanthine) is one of the most widely consumed psychoactive substances in the world, with coffee generally accounting for the majority of dietary caffeine intake (Nehlig, 1999). As a result of its stimulant properties, caffeine is often consumed in an attempt to enhance performance and combat the detrimental effects of fatigue. However, although caffeine has consistently been shown to diminish tiredness, increase energy and improve mood, findings regarding its ability to enhance cognitive performance are more complex (Nehlig, 2010; Snel et al., 2004).

Relatively consistent effects have been found with regard to the enhancement of psychomotor function (e.g. speeded reaction time) and sustained attention following caffeine consumption. Effects on learning and memory performance, however, appear to be more variable. Variable effects may be attributed to the diversity of caffeine research protocols in terms of caffeine dosage, cognitive task type and the characteristics (e.g., age, gender and habitual caffeine consumption levels) and state (e.g., fatigue level, arousal level and caffeine abstinence) of the participant. Fortunately, insight into the effects of psychoactive agents, such as caffeine, on cognitive processes (regardless of whether significant changes in performance are evident at the behavioural level) can be provided by functional magnetic resonance imaging (fMRI). In the present study, we used fMRI to investigate the effect of caffeine on working memory (WM).

To date, just one study has investigated the effect of caffeine on learning or memory using fMRI. Koppelstaetter et al. (2008) demonstrated increased WM load-related activation (N-back task, block design) following caffeine (compared to placebo) administration in prefrontal cortex areas (PFC) associated with executive and attentional functions (in the absence of a significant effect on WM performance). This finding was suggested to reflect a direct effect of caffeine on WM processes. Behavioural findings, on the other hand, are generally considered to indicate an indirect effect of caffeine on WM via changes in arousal state.

We aimed to extend on findings by Koppelstaetter et al. (2008) by investigating the effect of caffeine on WM load-related activation during encoding, maintenance and retrieval phases of a WM maintenance task (modified Sternberg task) using an event-related fMRI design. In contrast to Koppelstaetter et al. (2008), we tested participants following a day of habitual caffeine consumption, rather than requiring an extended period of caffeine abstinence prior to testing. The advantage of this approach is that the effects of caffeine are investigated in the context of each participant's usual levels of caffeine in the body, instead of in the context of a caffeine withdrawal state. This design addresses the criticism that the beneficial effects of caffeine in abstained habitual consumers may in fact reflect the removal of the negative effects of caffeine withdrawal (Field et al., 2003; Heatherley, 2011; James, 1994). Caffeine withdrawal is associated with headaches, fatigue, dysphoric mood changes and flu-like somatic symptoms (Ozsungur et al., 2009), as well as changes in cerebral blood flow (CBF), the effect of which will be elucidated further below in relation to fMRI. In line with Koppelstaetter et al. (2008), we examined the effects of a 100 mg dose of caffeine (equivalent to 1 – 1.5 cups of coffee) in male caffeine consumers (250 – 800 mg per day).

Our study focused on middle-aged males aged 40 to 61 years. Cognitive performance, including WM, has already started to decline by middle age (e.g. Bopp and Verhaeghen, 2005; Myerson et al., 2003; Park et al., 2002), as have energetic resources (Salthouse, 1988). Nevertheless, middle-aged adults commonly still work fulltime in positions that demand a high level of cognitive performance. There is some indication that caffeine can exert a more pronounced enhancement of cognition in older individuals (Nehlig, 2010). Therefore, the possible memory enhancing effects of caffeine are of particular interest in this age group; middle-aged adults may stand to benefit more from caffeine in terms of work-related performance enhancement than young adults (or retired old adults in whom daily cognitive demands are probably reduced). Since the memory enhancing effects of caffeine may be more evident when participants are in a suboptimal state (Nehlig, 2010), we tested participants immediately after a full day of work, when work-related fatigue levels are high.

When investigating the effect of caffeine on the blood oxygen level-dependent (BOLD) response utilised by fMRI as an indirect measure of neural activity, it is important to recognise that changes in the BOLD response can arise from both neural and cerebrovascular mechanisms (for a review see Koppelstaetter et al., 2010; Laurienti et al., 2003). Caffeine, a non-selective adenosine receptor antagonist (Pelligrino et al., 2010), elicits: 1) neurostimulant effects, primarily via A_1 receptors and the dopamine system (Ferre, 2008; Pelligrino et al., 2010); 2) cerebrovascular effects, primarily via A_{2A} and A_{2B} receptors located on blood vessels (Pelligrino et al., 2010); and 3) arousal enhancing effects, via A_{2A} receptors and the histaminergic arousal system (Ferre, 2008); with tolerance thought to arise as the brain regulates its population of adenosine receptors to reach a new state of equilibrium in response to levels of caffeine chronically present in the body (Jacobson et al., 1996; Ralevic and Burnstock, 1998; Sousa et al., 2011). Studies have shown that administration of 200 - 250 mg of caffeine results in reduced CBF (Addicott et al., 2009; Field et al., 2003; Laurienti et al., 2003; Liau et al., 2008; Mulderink et al., 2002; Perthen et al., 2008), possibly accompanied by a reduction in the baseline BOLD signal (during simple motor and visual tasks) (Chen and Parrish, 2009b; Perthen et al., 2008) and changes in the magnitude (Griffeth et al., 2011; Laurienti et al., 2002) and temporal dynamics of the BOLD response (Liau et al., 2008; Liu et al., 2004; Rack-Gomer et al., 2009). Furthermore, increased CBF has been found during caffeine withdrawal (Addicott et al., 2009; Field et al., 2003). Therefore, findings from studies examining the effect of caffeine on brain activation in the context of caffeine withdrawal (compared to the withdrawal state as a control condition) may represent the resolution of withdrawal-related activation changes as well as changes relating to the effects of caffeine on CBF and cognition (Field et al., 2003). However, there is evidence that lower caffeine doses do not affect the amplitude or timing of the BOLD response (Chen and Parrish, 2009a) and have a smaller effect (Chen and Parrish, 2009a), or no significant effect (Kennedy and Haskell, 2011), on CBF. Furthermore, caffeine-related CBF changes are smaller in non-abstained higher consuming habitual users (Addicott et al., 2009). Therefore, our decision to administer a lower caffeine dosage to non-abstained moderate to high habitual caffeine consumers was intended to minimise the potential cerebrovascular effects of caffeine administration and avoid caffeine withdrawal effects.

In summary, we investigated the effect of caffeine on WM load-related activation in middle-aged, male habitual caffeine consumers. Participants were tested following a workday during which they consumed caffeine according to their own habitual regime. Based on findings by Koppelstaetter et al. (2008), we hypothesised that (although this dose may not significantly affect WM performance at the behavioural level) caffeine would increase WM load-related activation in the fronto-parietal network associated with the top-down control of attention and executive functions.

METHODS

Participants

Twenty-one right-handed Dutch male participants (aged 40 – 61) were recruited via advertisements in local newspapers. Inclusion criteria specified a high level of education, fulltime employment in a white-collar profession (to ensure greater sample homogeneity with regard to cognitive demands throughout the workday and the resulting cognitive fatigue state) and consumption of 250 to 800 mg of caffeine daily. Daily caffeine consumption estimates at screening were based on participants' self-reports, with the estimate in mg calculated according to a recent overview of caffeine-containing products in the Netherlands (Wendte et al., 2003). The most commonly consumed products were estimated as follows: one cup (125 ml) of filter coffee = 85 mg of caffeine, instant coffee (125 ml) = 60 mg, espresso (50 ml) = 65 mg, and black tea (125 ml) = 30 mg. Exclusion criteria were significant past or present physical or psychiatric illness, medication use, illicit substance or nicotine use, or MRI contraindications. Females were not included due to gender differences in caffeine consumption and metabolism (Carrillo and Benitez, 1996; Magkos and Kavouras, 2005). The study was approved by the medical ethical committee at Maastricht University academic hospital. Volunteers gave informed consent prior to their (paid) participation.

Design

The study employed a randomised double-blind placebo-controlled crossover design. Participants were tested once after drinking caffeinated coffee (caffeine condition) and once after decaffeinated coffee (placebo condition) (treatment order was counterbalanced). Test sessions took place after a full workday (starting between 1600 and 1800 h), including the participant's habitual regime of caffeine consumption (with the second session scheduled on the same day of the week, at the same time, as the first session, with one week between sessions). Participants and experimenters were blind to the caffeine content of the coffee preparation. Moreover, participants were not informed prior to testing that they would receive caffeinated and decaffeinated coffee; they were simply asked to drink a cup of coffee at each session. This addressed the role of expectations held by caffeine consumers regarding the effects of coffee on cognitive performance and mood (Dawkins et al., 2011; Kirsch and Weixel, 1988), as expectancy effects were kept constant across the placebo and caffeine conditions. Participants were informed about the caffeine and placebo conditions during debriefing.

Procedure

To start with, participants completed a diary recording food and beverage type, quantity and time of consumption during one workday (the same workday as the test sessions were subsequently held on). The diary provided a more precise estimate of the amount of caffeine consumed by each participant during the workday. Furthermore, the diary ensured consistency with regard to the concentration of caffeine in the body at the start of the two test sessions; each participant's pattern of caffeine consumption reported in the diary was used as a schedule for their caffeine consumption on both test days, with the exception that caffeine sources normally consumed during the 2 h prior to the start of the test sessions were removed. This abstinence period of at least 2 h (exact times depended on individual habits) ensured that caffeine levels were relatively low at the beginning of the test sessions (but that participants were not in caffeine withdrawal) and that subsequent coffee administration did not result in excessive levels of caffeine in the body. Participants confirmed compliance to their caffeine schedule by recording actual consumption times throughout each test day.

During a training session, participants practiced the fMRI tasks in a dummy scanner to minimise practice effects during the test sessions and to become familiarised with the scanning environment. During the test sessions, participants filled out pre-coffee subjective rating scales and provided a baseline (time 0) saliva sample before drinking the coffee preparation and entering the MRI scanner. The scanning session began with a structural scan (10 min after coffee consumption), followed by saliva sample collection (time 1), the visual control task (30 min after coffee), and the WM task. Therefore, at the time of the first functional scan, the majority of the caffeine dose was expected to be absorbed and distributed in the brain (Liguori et al., 1997). After exiting the scanner, participants completed the post-coffee subjective rating scales and provided another saliva sample (time 2).

Caffeine manipulation

In the caffeine condition, 100 mg of caffeine (anhydrous) was mixed into a preparation of decaffeinated instant coffee. This dose was chosen to reflect caffeine dosages commonly consumed by habitual coffee drinkers and to minimise physiological side effects such as heart rate, blood pressure, CBF and BOLD response changes (Chen and Parrish, 2009a; Kennedy and Haskell, 2011; Koppelstaetter et al., 2008). The placebo treatment was a cup of decaffeinated instant coffee. Although decaffeinated coffee is not caffeine free (it contains < 3 mg caffeine), it is considered an effective placebo as behavioural effects are not apparent at this dose and effects on the BOLD response do not differ between decaffeinated coffee and water (Bendlin et al., 2007).

Subjective rating scales

The Karolinska sleepiness scale (KSS) was administered as a measure of subjective feelings of sleepiness/alertness (1 = very alert, 9 = very sleepy) (Akerstedt and Gillberg, 1990), with demonstrated sensitivity to caffeine effects (Mednick et al., 2008). The Dutch short visual analogue scale (VAS) version of the Profile of Mood States (POMS) (Wald and Mellenbergh, 1990) was administered; the fatigue (6 items: 0 = low fatigue, 100

= high fatigue) and vigour (5 items: 0 = low vigour, 100 = high vigour) subscales are recommended measures of the mood of energy and fatigue in investigations that are short in duration (O'Connor, 2006) and the vigour scale has shown sensitivity to caffeine effects (Childs and de Wit, 2008; Herz, 1999). Finally, several additional VAS items (0 = 'not at all', 100 = 'extremely') were administered (post-coffee only) targeting caffeine-related experiences (Liguori et al., 1997) including: "Have you felt any caffeine effects?" (also used as an evaluation of blinding effectiveness), "Have you felt any good effects?", and "Have you felt any bad effects?"

Salivary caffeine concentrations

Saliva samples were collected pre-coffee (time 0) and 25 min (time 1) and 90 min (time 2) post-coffee using Salivettes (Sarstedt, the Netherlands). Saliva sampling is considered a good method for frequent measurement of caffeine pharmacokinetics (Newton et al., 1981; Suzuki et al., 1989), with a linear relationship reported between caffeine concentrations in saliva and plasma, and salivary caffeine levels reported to be around 80% of plasma levels (Perera et al., 2011; Zylber-Katz et al., 1984). Collection, storage and analysis procedures are supplied as Supplementary Material (pp124).

fMRI visual control task

The visual control task (Goebel et al., 1998) was administered to assess whether caffeine exerted a global effect on the BOLD response (Iannetti and Wise, 2007). Details of the task, fMRI acquisition and analysis are provided as Supplementary Material (pp124).

fMRI working memory task

Participants performed a parametric version of the letter Sternberg task (3, 4, 5 or 6 letter strings). The task consisted of 144 trials (36 for each letter string length) organised in a fast event-related design and presented in a fixed pseudorandom order (stimulus letters, but not trial order, varied per task version). Experimental trials consisted of encoding, maintenance and retrieval phases. Participants were instructed to memorise a letter string (lower case consonants) presented on the screen (for 4 s) during the encoding phase. The maintenance phase followed, consisting of a fixation cross in the centre of the screen (for a jittered interval of 3 to 6 s). Finally, in the retrieval phase, participants responded to a probe letter by pressing a button to indicate whether the probe letter was part of the previous letter string (right button press) or not (left button press). Trials were separated by a 3 s pause (during which time three asterisks were shown in the centre of the screen). In addition to jittering, partial trials were also included to reduce the problem of multicollinearity arising from the delayed nature of the BOLD response inherent to tasks, such as the Sternberg task, with a fixed stimulus event order (Dale, 1999; Miezin et al., 2000; Ollinger et al., 2001a; Ollinger et al., 2001b; Serences, 2004). The partial trial technique allows the unique estimation of the different trial phases and has been shown to be more effective than jittering or slow event-related designs for the detection of cue-related activity in the PFC (Goghari and MacDonald, 2008). 50% of all trials consisted of the encoding and maintenance phases only and a further 17% consisted of the encoding phase only. Participants were therefore not required to respond to these incomplete trials,

but were required to encode and maintain, or encode only, respectively. The task was administered in three blocks of approximately 11 min each and 48 trials per block.

MRI data acquisition

Images were acquired in a 3 Tesla head scanner (Siemens MAGNETOM Allegra, Siemens Medical systems, Erlangen, Germany) with an 8 channel head coil. During each of the three WM task blocks, approximately 330 Echo-planar imaging (EPI) scans were made (TR = 2000 ms, TE = 30 ms, number of slices = 32, image matrix = 64 x 64, voxel size = 3.5 x 3.5 x 3.5 mm) of the cortex. A T1 weighted anatomical (ADNI) scan was also acquired for anatomical reference and coregistration of the two test sessions (image matrix = 256 x 256, number of slices = 192, voxel size = 1 x 1 x 1 mm).

MRI data analysis

SPM8 (Statistical Parametric Mapping: Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London) was used to preprocess the fMRI scans. Preprocessing steps included: slice time correction, realign and unwarp, coregistration (session 2 scans were coregistered to session 1 scans), spatial normalisation (MNI space using individual spatial normalisation parameters obtained during structural image segmentation), and smoothing (FWHM 6 mm).

Working memory task data were analysed in the context of the general linear model, using boxcar regressors convolved with the canonical hemodynamic response to model activation during each event type. Encoding events were modeled as 4 s blocks, maintenance events as blocks with duration dependent on jittering time and retrieval events as blocks with duration dependent on response time. Additionally, we extracted the average fMRI time series from white matter and cerebrospinal fluid (CSF) using each participants segmented anatomical scan in the REST toolbox (Song et al., 2011) and added these as regressors to the model as a measure to correct for possible changes in physiological noise resulting from caffeine administration.

Individual activation contrasts were created by modeling activation to the WM load levels (3, 4, 5, 6 letters) during encoding, maintenance and retrieval using a parametric load contrast (-2 -1 1 2). Contrasts were entered into three second-level Full Factorial models to assess the effects of caffeine condition on load-related activation in each of the task phases. The effect of caffeine condition on whole-brain activation was examined at $p(\text{uncorrected}) < .001$ with a cluster threshold > 5 voxels (masked inclusively with task-related activation to restrict the search for activation differences to those areas showing an effect of WM load).

Region of interest (ROI) analysis was used to examine the effect of caffeine (at $p < .05$, corrected for multiple comparisons) within select fronto-parietal areas consistently modulated by WM load (as well as within any additional areas showing an effect of caffeine on load-related activation at the whole-brain level) using the MarsBaR toolbox for SPM (Brett et al., 2002). ROIs were constructed by creating a sphere (10 mm radius) around peak load-related activation coordinates obtained from a previous study using the same task in a similar population of 30 males aged 25 – 60, and then masking this

sphere with a grey matter mask. ROIs included the bilateral dorsolateral prefrontal cortex (DLPFC) (MNI coordinates $x = (-)39$, $y = 42$, $z = 30$; Brodmann area [BA] 46), precentral gyrus (coordinates $x = (-)51$, $y = 3$, $z = 42$; BA 6), superior parietal (coordinates $x = (-)24$, $y = -66$, $z = 54$; BA 7) and inferior parietal cortex (coordinates $x = (-)51$, $y = -45$, $z = 48$; BA 40). Additionally, we used the MarsBaR toolbox to extract the fitted event time course from the ROIs showing a significant effect of caffeine to determine whether caffeine affected the temporal dynamics (time to peak) of the BOLD response underlying activation differences.

Salivary caffeine, subjective ratings and working memory performance data analysis

Analyses were carried out using PASWStatistics (version 18.0). Repeated measures analysis of variance (ANOVA) were used to examine the effect of caffeine condition and time on caffeine concentration in saliva and subjective ratings on the KSS and the POMS subscales, and the effect of caffeine condition and WM load on performance accuracy and reaction time (RT) on the WM task.

RESULTS

One participant was excluded for failing to follow their workday caffeine consumption schedule leaving 20 participants for the analysis (mean age = 49.2, SD = 5.6, range = 40 – 61). Data was missing from one participant on the visual control task. Missing salivary caffeine values, due to an inadequate amount of saliva for analysis, were replaced with the value from the other test session (for baseline values) or with the individual's average from the previous and following time points (for time 1 and time 2 values, also using a time 3 measurement, 120 min after coffee consumption, not otherwise included in the analyses).

Caffeine consumption and salivary concentrations

Estimates of caffeine consumption during the workday prior to the test session indicated an average consumption of 335 mg (SD = 104 mg, range = 145 – 595). The mean amount of caffeine administered per kg of body weight was 1.22 mg/kg (SD = 0.17, range = 0.89 – 1.47 mg/kg).

The concentration of caffeine in saliva is shown in Figure 1. A main effect of caffeine condition ($F(1, 19) = 8.55$, $p = .009$) and time point was found ($F(2, 38) = 7.87$, $p = .005$), as well as a trend for an interaction between caffeine condition and time point ($F(2, 38) = 2.92$, $p = .069$). Follow-up tests indicated that caffeine concentrations did not differ between conditions at baseline (time 0), but were significantly higher in the caffeine than the placebo condition at time 1 ($t(19) = 2.93$, $p = .009$) and this difference neared significance at time 2 ($t(19) = 2.02$, $p = .058$).

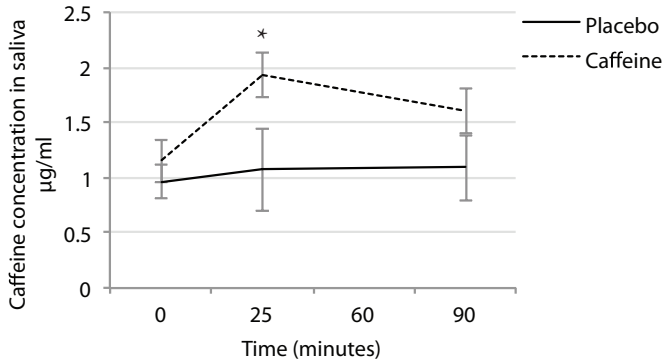


Figure 1 | Caffeine concentration in saliva at baseline (time 0) and at each post-coffee measurement (indicated in minutes after coffee consumption). Error bars show standard error and * indicates a significant difference between placebo and caffeine conditions.

Subjective ratings

Ratings on each of the scales are shown in Table 1. Caffeine condition had no significant effect on ratings on the KSS or the POMS fatigue or vigour scales. A main effect of time point was found on each of the scales indicating increased sleepiness ($F(1,19) = 32.64, p < .001$) and fatigue ($F(1,19) = 34.28, p < .001$), and decreased vigour ($F(1,19) = 30.80, p < .001$) after the scanning session compared to the pre-coffee baseline, regardless of caffeine condition. Scores also did not differ between caffeine and placebo conditions on the additional caffeine VAS items targeting caffeine-related experiences, indicating that participants had no awareness of caffeine condition.

Table 1 | Mean (standard deviation) scores on the subjective rating scales

	Placebo		Caffeine	
	Pre-coffee	Post-coffee	Pre-coffee	Post-coffee
Karolinska sleepiness scale ^(ns)	3.2 (1.4)	4.6 (1.8)	3.0 (1.2)	4.8 (2.1)
POMS Fatigue ^(ns)	25.0 (17.5)	37.2 (22.0)	25.0 (17.4)	39.7 (23.7)
POMS Vigour ^(ns)	79.9 (14.5)	66.2 (21.3)	78.0 (19.0)	65.0 (22.3)
Q1: caffeine effects experienced ^(ns)		30.0 (20.6)		34.8 (24.2)
Q2: positive caffeine effects experienced ^(ns)		32.2 (18.7)		31.4 (19.8)
Q3: negative caffeine effects experienced ^(ns)		24.7 (19.7)		27.3 (18.5)

Visual control task

Visual task-related activation is provided in Supplementary Material (pp124). No activation differences were found between placebo and caffeine conditions at $p(\text{uncorrected}) < .001$, with a cluster threshold > 5 voxels, small volume corrected to restrict the search for activation differences to the visual cortex.

Working memory task behavioural results

Accuracy and RT on the task are shown in Figure 2. A main effect of WM load was found on performance accuracy ($F(3, 57) = 5.09, p = .006$), reflecting a decrease in accuracy with increasing load. A trend for an interaction between the effect of caffeine condition

and WM load on accuracy was also found ($F(3, 57) = 2.68, p = .058$), with follow-up tests showing significantly lower accuracy on six letter trials in the caffeine than in the placebo condition ($t(20) = 2.15, p = .044$). A main effect of WM load was found on RT ($F(3, 57) = 20.40, p < .001$), reflecting increased RT with increasing WM load ($F(1, 19) = 12.70, p = .002$). However, caffeine condition did not significantly affect RT.

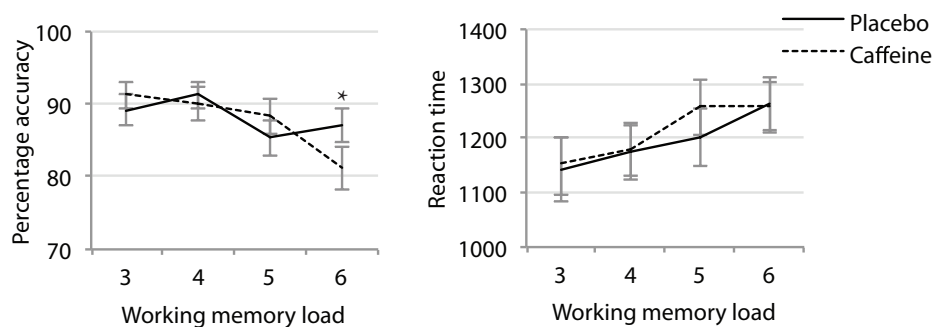


Figure 2 | Percentage accuracy and reaction time to each level of working memory load (3, 4, 5, or 6 letters) on the fMRI working memory task in the placebo and caffeine conditions. Error bars show standard error and * indicates a significant difference between placebo and caffeine conditions.

Working memory task fMRI results

Whole brain analysis: Load-related activation during encoding, maintenance and recognition in the placebo and caffeine condition is shown in Table 2. Load-related encoding activation was greater in the caffeine than the placebo condition in the right DLPFC (peak coordinates $x = 36, y = 36, z = 39$; t -value = 4.79; cluster size = 23; BA 9/46). Load-related maintenance activation was greater in the placebo than the caffeine condition in the left thalamus (peak coordinates $x = -15, y = -9, z = 6$, t -value = 4.24, cluster size = 8). No load-related activation differences were found during retrieval.

Region of interest analysis: Activation in ROIs showing load-related activation differences in the placebo and caffeine condition are shown in Figure 3. ROI analysis confirmed greater load-related encoding activation in the caffeine than the placebo condition in the right DLPFC ($t(19) = 2.73, p = .038$) and additionally showed a trend for the same effect in the left DLPFC ($t(19) = 2.59, p = .053$). In the right DLPFC, load-related activation was evident in the placebo condition ($t(19) = 2.32, p = .013$), and further increased in the caffeine condition ($t(19) = 4.88, p < .001$). In the left DLPFC, activation in the placebo condition was not modulated by WM load ($t(19) = 0.61, p = .727$), but showed a load-related increase in the caffeine condition ($t(19) = 1.79, p = .041$).

Greater load-related maintenance activation in the placebo than in the caffeine condition in the left thalamus ($t(19) = 3.09, p = .020$) reflected a load-related increase in the placebo condition ($t(19) = 2.38, p = .011$), but somewhat of a load-related decrease in activation in the caffeine condition ($t(19) = 1.58, p = .061$). ROI analysis did not reveal any additional activation differences between the placebo and caffeine conditions. Furthermore, we found no indication of differences in the temporal dynamics of the BOLD response in the DLPFC and thalamus between the placebo and caffeine conditions.

Table 2 | Brain areas showing working memory load-related activation during the encoding,

Region		BA	MNI coordinates			t-value	Cluster size
			PLACEBO				
			x	y	z		
<i>Encoding</i>							
Dorsolateral PFC	R						
Dorsomedial PFC	L/R	6/32	-3	3	57	9.59	282**
Insula	R	48	39	15	6	3.92	16
Premotor cortex	L	6	-51	-6	48	10.66	790**
	<i>L</i>	6	-54	6	21	8.02	
	R	6	57	-3	45	6.12	288**
	<i>R</i>	4	48	-9	39	5.82	
Temporo-parietal	L	41	-48	-39	24	5.12	33
Hippocampal formation	L	-	-24	-24	-6	5.19	43**
	R	-	24	-27	-3	5.76	24
Occipital	L	18	-24	-90	21	9.89	
	R	18	30	-84	15	12.05	2489**
Pallidum	L	-	-21	0	6	7.03	50**
Putamen	R	-	30	18	3	4.15	35*
Thalamus	L	-	-12	-18	0	4.41	10
<i>Maintenance</i>							
Dorsolateral PFC	L	46	-27	48	21	4.40	43**
	L	44	-51	21	33	3.83	47**
	R	46	36	42	30	4.19	121**
Dorsomedial PFC	L	6	-3	3	57	4.88	24
Premotor cortex	L	6	-45	-3	54	5.66	159**
	<i>L</i>	44	-51	21	33	5.04	
Temporal pole	L	48	-54	12	-3	7.09	161**
Insula	L	48	-30	18	9	4.00	22
Lateral temporal	L	21	-63	-24	-3	3.69	10
	R	21	48	-33	-3	3.72	12
Temporo-parietal	L	42	-60	-42	21	5.69	78**
Inferior parietal	L	40	-45	-48	54	4.82	107**
	<i>L</i>	7	-36	-60	48	4.34	
	R	40	48	-48	45	4.39	42**
Thalamus	L	-	-15	-9	6	4.01	22
<i>Retrieval</i>							
Dorsomedial PFC	L	6	-9	9	51	5.08	54**
	<i>L</i>	6	9	12	54	5.04	
Ventrolateral PFC	L	48	-48	21	21	4.82	51**
	<i>L</i>	45	-48	30	18	3.68	
	L	44	-36	3	30	4.00	61**
	<i>L</i>	6	-48	3	24	4.31	
	L	45	-45	36	3	4.12	13
Insula	L	48	-45	15	3	3.89	10

* sig at cluster-level at $p(\text{FWE}) < 0.1$, ** sig at cluster-level at $p(\text{FWE}) < 0.05$. Italics show additional peak

maintenance and retrieval phases in the placebo and caffeine conditions.

Region		BA	CAFFEINE			t-value	Cluster size
			MNI coordinates				
			x	y	z		
<i>Encoding</i>							
Dorsolateral PFC	R	46/9	36	36	36	5.20	131**
Dorsomedial PFC	L/R	6/32	-3	3	57	9.44	707**
Insula	R	48	39	15	3	4.15	20
Premotor cortex	L	6	-51	-3	48	11.13	781**
	L	6	-54	6	21	7.63	
	R	6	57	-3	45	7.00	707**
	R	6	54	-3	36	6.59	
Temporo-parietal	L	41	-48	-39	24	5.24	38*
Hippocampal formation	L	-	-24	-30	0	4.34	13
	R						
Occipital	L	18	-24	-90	21	11.11	
	R	18	30	-84	5	11.84	2599**
Pallidum	L	-	-21	0	6	6.96	83**
Putamen	R	-	21	9	0	3.52	11
Thalamus	L	-	-12	-18	6	3.86	13
<i>Maintenance</i>							
Dorsolateral PFC	L	46	-27	51	21	4.62	42**
	L	44	-54	15	36	4.54	19
	R	46	36	30	33	3.71	11
		46	30	45	33	4.05	13
Dorsomedial PFC	L	6	-3	6	57	5.02	18
Premotor cortex	L	6	-42	-3	57	4.98	51**
	L	43	-63	-3	24	4.35	20
Temporal pole	L	48	-57	12	0	4.79	18
Insula	L						
Lateral temporal	L						
	R						
Temporo-parietal	L	41	-51	-39	18	3.91	32
Inferior parietal	L	40	-48	-51	48	3.84	23
	L	40	-39	-45	45	3.25	
	R						
Thalamus	L						
<i>Retrieval</i>							
Dorsomedial PFC	L						
	L						
Ventrolateral PFC	L						
	L						
	L						
	L						
Insula	L	47	-30	27	0	5.19	13

activation coordinates within a cluster

Post-hoc correlations: To gain insight into the relationship between the effect of caffeine on load-related activation and WM performance, we correlated activation within the left DLPFC, right DLPFC and left thalamus ROIs with performance accuracy and RT (examined at $p < .05$, corrected for multiple comparisons). A significant positive correlation was found between load-related activation in the right DLPFC and performance accuracy in the caffeine condition ($t(19) = 2.95, p = .013$). We also examined the relationship between load-related activation in the three ROIs and the factors age, daily caffeine consumption level and caffeine dose in mg/kg. However, no significant correlations were found.

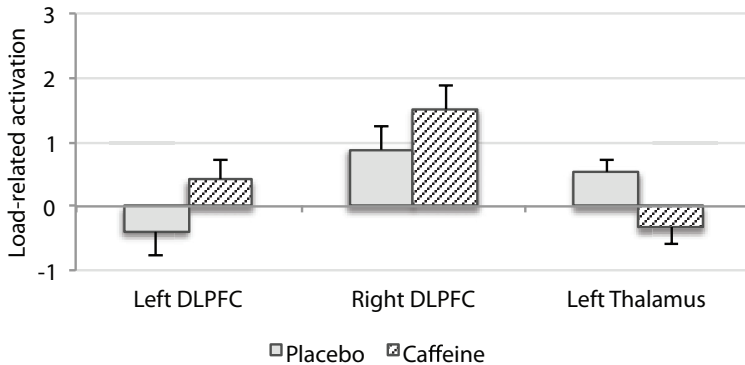


Figure 3 | Working memory load-related activation (percentage signal change, with error bars showing standard error) in the dorsolateral prefrontal cortex (DLPFC) during encoding and the left thalamus during maintenance in the placebo and caffeine condition.

DISCUSSION

We examined the effects of 100 mg of caffeine (compared to placebo) on WM load-related activation in non-withdrawn male habitual caffeine users at the end of the workday (when work-related fatigue levels were expected to be relatively high). The measurement of caffeine concentration in saliva confirmed that concentrations were significantly higher in the caffeine compared to the placebo condition. Functional MRI showed an effect of caffeine on WM load-related activation in the bilateral DLPFC during encoding and in the left thalamus during maintenance, whereas no effects were found during retrieval.

The increase in load-related activation in the DLPFC in the caffeine compared to the placebo condition is suggestive of a direct effect of caffeine on WM processes. This finding is somewhat consistent with the study by Koppelstaetter et al. (2008) in which caffeine also increased WM load-related activation in the PFC. Although, activation changes in that study were evident in the bilateral medial frontopolar cortex (BA 10), extending into the ACC. This difference in the areas modulated by caffeine may be attributed to one or all of several important differences between the two studies including the type of WM task, fMRI design, and participant age and abstinence state. Nevertheless, both studies point towards a direct effect of caffeine on the fronto-parietal WM network, with our study specifically indicating an effect on this network during WM encoding.

Studies have consistently demonstrated that the DLPFC is a key area in the brain's response to varying levels of WM load and have suggested a primary role for this area in the top-down control of attention and WM (Toro et al., 2008). Thus, it may be expected that enhanced DLPFC activation would be indicative of the facilitation of top-down cognitive

control, thereby leading to enhanced WM encoding and a higher level of performance. Indeed, we found a positive correlation between load-related activation in the right DLPFC and overall performance accuracy in the caffeine condition. However, performance actually tended to worsen at the higher WM load levels in the caffeine compared to the placebo condition; somewhat longer RTs were evident in response to five letters followed by a decrease in accuracy at six letters. These behavioural findings are consistent with the suggestion, based on previous behavioural studies, that although caffeine appears to facilitate performance at low levels of WM demand, it is associated with impaired performance at higher levels of demand (Anderson and Revelle, 1983; Loke, 1993; Nehlig, 2010). Therefore, we suggest that although greater load-related recruitment of the right DLPFC during encoding in the caffeine condition was associated with better performance, the evident detrimental effect of caffeine on performance at higher levels of WM load overall indicates that the effect of caffeine on other processes (such as arousal, discussed below) may have been more instrumental in determining WM retrieval performance.

Load-related activation in the maintenance phase of the task was greater in the placebo than in the caffeine condition in the left thalamus. An increase in activation in the left thalamus in response to greater WM load has been demonstrated in previous WM studies (Altamura et al., 2007; Choo et al., 2005; Nyberg et al., 2009). Furthermore, a specific role for the thalamus during WM maintenance has been proposed by Ashby et al. (2005), in which activation during the delay interval is sustained by an excitatory prefrontal cortico-thalamic loop (in conjunction to the fronto-parietal excitatory loop). As such, the thalamus may be differentially activated in an effort to support the maintenance of information in the absence of input from external stimuli.

An earlier fMRI study also demonstrated decreased thalamic activation following caffeine administration (compared to a sleep deprivation condition) (Portas et al., 1998) and attributed this finding to the proposed role of the thalamus in arousal regulation (Schiff, 2008). Specifically, higher levels of thalamic activation are found in relation to low levels of arousal (Chee et al., 2006; Foucher et al., 2004). Hence, we suggest that lower arousal levels in the placebo condition necessitated greater thalamic activation to support WM maintenance, whereas higher arousal levels in the caffeine condition necessitated less support from the thalamus. In relation to our behavioural findings, it has been suggested that the caffeine-related increase in arousal state, in combination with increased arousal resulting from high task demands, may lead to over-arousal and a subsequent decline in performance (Nehlig, 2010). Indeed, Foucher et al. (2004) showed that the left thalamus is modulated by both endogenous arousal levels as well as arousal changes in response to task stimuli. Therefore, although thalamic activation was not directly correlated with WM performance, it may have reflected an indirect effect of caffeine on performance via changes in arousal state. Specifically, increased arousal levels in the caffeine condition may have contributed to poorer WM performance at high WM load levels in this condition. Overall, findings are suggestive of a modulatory effect of caffeine on activation in the prefrontal cortico-thalamic loop involved in the interaction between arousal and the top-down control of attention during maintenance.

A major strength of the present study is that participants were not tested in a state of withdrawal. Therefore, differences found between the placebo and caffeine conditions

cannot be attributed to resolution of the withdrawal state. Furthermore, additional use of a caffeine dosage equivalent to 1-1.5 cups of coffee means that results are more indicative of effects of caffeine as it is consumed in daily life. Although, the administration of this low dose to caffeine-tolerant habitual consumers probably also accounts for the absence of a significant effect of caffeine on subjective fatigue, vigour and sleepiness ratings and the subjective experience of caffeine-related effects. Similarly, the overall lack of a statistically significant effect of caffeine on behavioural measures in the present study (in particular RT) is not unusual in studies using a low dose in non-abstained habitual consumers (Christopher et al., 2005; Heatherley et al., 2005), whereas significant effects are more consistently found in studies testing participants in a withdrawal state using higher doses. Nevertheless, there are other advantages to using this low dose including minimal physiological side effects and effective blinding.

Our decision to administer caffeine in the form of a cup of coffee is consistent with our aim to examine the effect of caffeine as it is usually consumed in daily life. However, we note that the effects of a cup of coffee may be attributable to factors other than caffeine alone. Specifically, although there is no reason to expect differential caffeine expectancy effects in the placebo and caffeine conditions, as participants were simply asked to drink a cup of coffee in both conditions, we cannot rule out the possibility that expectancy effects may have contributed to results on our outcome measures in both conditions. Furthermore, we cannot rule out possible effects of other active compounds in coffee on mood or cognition (Cropley et al., 2012) (which may have obscured caffeine effects), or a possible synergistic relationship between the effects of these active compounds and caffeine.

Interpretations of our fMRI results are subject to one primary caveat. Namely, that caffeine can influence the BOLD response via both neural and vascular pathways. However, minimal vascular effects were expected due to the administration of a low caffeine dose to habitual users. Results from the visual control task support the expected absence of a global effect of caffeine on the magnitude of BOLD response, as the BOLD response to visual stimulation did not differ significantly between the caffeine and placebo conditions. Furthermore, no differences were evident in the temporal dynamics of the BOLD response in areas showing a significant effect of caffeine. Moreover, as explained by Koppelstaetter et al. (2008), the regional specificity of the effects of caffeine on the BOLD response during the WM task argues against an explanation solely based on vascular effects, as A₁ receptors responsible for the neuroexcitatory effects of caffeine are expressed throughout the cortex. Similarly, the specificity of caffeine effects to the particular WM task phases in the present study is again inconsistent with a global effect of caffeine on parameters of the BOLD response. Finally, the use of a parametric contrast avoids the direct comparison of the BOLD response itself between caffeine and placebo conditions. Nevertheless, we note that the present study would have been improved by the addition of a measure providing direct insight into vascular effects (such as arterial spin labelling). Furthermore, although we endeavoured to correct for any possible effects of increases in physiological noise resulting from caffeine administration by including the average CSF and WM time series as regressors in the fMRI model, ideally, future studies should include physiological measures such as heart and respiration rate in order to properly monitor and correct for

possible physiological changes.

In conclusion, the present study provides evidence for an effect of 1-1.5 cups of coffee on load-related activation during WM encoding and maintenance in 40 to 61 year-old male habitual caffeine consumers. During WM encoding, caffeine modulated activation in the right DLPFC, indicating an effect on the top-down cognitive control of WM. During WM maintenance, caffeine modulated activation in the left thalamus, indicating an effect on arousal. Therefore, the effects of caffeine on WM may be attributed to both a direct effect of caffeine on WM processes, as well as an indirect effect via changes in arousal. This finding is consistent with both the neurostimulant and arousal enhancing effects of caffeine primarily via the dopamine (Ferre, 2008; Pelligrino et al., 2010) and histaminergic arousal (Ferre, 2008) systems respectively. However, behavioural and fMRI results were more consistent with a detrimental effect of caffeine on WM at higher levels of WM load, than caffeine-related WM enhancement.

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SUPPLEMENTARY MATERIAL

Salivary caffeine concentrations

The Salivette tube contains a swab that participants placed in their mouth for 2 min. Samples were frozen at -80°C until analysed for caffeine content. Caffeine concentration was determined by capillary zone electrophoresis (CZE) using theophylline as an internal standard. Saliva was extracted from Salivettes by centrifugation for 10 min at 3000 g. 100 μL of saliva was mixed with 100 μL of potassium phosphate buffer (100mM, pH 7.4) with 10 $\mu\text{g}/\text{mL}$ theophylline. Hereafter, 500 μL dichloromethane with propanol-2 (4:1) was added and mixed for 30 s on a Vortex mixer. Organic phase was separated from the aqueous phase by centrifugation at 1000 g for 5 min. 300 μL of the organic phase was evaporated and solved in 30 μL CZE running buffer (50mM glycine/50 mM SDS, pH 10.5). Samples were run on CZE using caffeine in water as a standard.

Visual control task

In the visual control task, alternating blocks (12 s) of stationary dots, moving dots or no dots were presented (Goebel et al., 1998). Participants were instructed to look at a fixation-cross presented in the centre of the screen throughout all blocks. During the task, approximately 110 EPI scans were made (TR = 2000 ms, TE = 30 ms, number of slices = 28, image matrix = 128 x 128, voxel size = 2 x 2x 2 mm) over the visual cortex. Preprocessing steps included: slice time correction, realign and unwarp, coregistration (session 2 scans were coregistered to session 1 scans), spatial normalisation (MNI space using individual spatial normalisation parameters obtained during structural image segmentation), and smoothing (FWHM 6 mm).

Data were analysed in the context of the general linear model, using boxcar regressors convolved with the canonical hemodynamic response to model experimental trial blocks. Additionally, we extracted the average fMRI time series from white matter and cerebrospinal fluid (CSF) using each participants segmented anatomical scan in the REST toolbox (Song et al., 2011) and added these as regressors to the model as a measure to correct for possible changes in physiological noise resulting from caffeine administration. Individual activation contrasts comparing activation during moving dot blocks to stationary dot blocks were entered into a second level paired-samples t-test to compare visual cortex activation in the placebo and caffeine conditions. Effects were tested at $p(\text{uncorrected}) < .001$, with a voxel threshold of 5 voxels, small volume corrected to restrict the search for activation differences to the visual cortex.

Visual task-related activation in the placebo condition in response to stimulation with moving compared to stationary dots was found bilaterally throughout the primary and secondary visual areas (peak coordinates: e.g., $[x = -22, y = -90, z = 28]$, $[x = -6, y = -84, z = 0]$, $[x = 8, y = -82, z = -2]$, $[x = 26, y = -86, z = 16]$; t-values = 26.74, 25.28, 21.52, 21.07; cluster size = 4373, BA 17/18).