Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

L-Theanine and caffeine improve target-specific attention to visual stimuli by decreasing mind wandering: a human functional magnetic resonance imaging study

Chanaka N. Kahathuduwa[a,b,c], *Chathurika S. Dhanasekara*[a], *Shao-Hua Chin*[a], *Tyler Davis*[c,d], *Vajira S. Weerasinghe*[b], *Tharaka L. Dassanayake*[b,e], *Martin Binks*[a,*]

[a] *Behavioral Medicine and Translational Research Lab, Department of Nutritional Sciences, Texas Tech University, Lubbock, TX, USA*
[b] *Department of Physiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*
[c] *Department of Human Development and Family Studies, Texas Tech University, Lubbock, TX, USA*
[d] *Department of Psychological Sciences, Texas Tech University, Lubbock, TX, USA*
[e] *School of Psychology, The University of Newcastle, New South Wales, Australia*

ARTICLE INFO

Article history:
Received 2 June 2017
Revised 31 October 2017
Accepted 12 November 2017

Keywords:
Caffeine
Echo planar magnetic resonance imaging
Clinical trial
Tea
Attention

ABSTRACT

Oral intake of L-theanine and caffeine supplements is known to be associated with faster stimulus discrimination, possibly via improving attention to stimuli. We hypothesized that L-theanine and caffeine may be bringing about this beneficial effect by increasing attention-related neural resource allocation to target stimuli and decreasing deviation of neural resources to distractors. We used functional magnetic resonance imaging (fMRI) to test this hypothesis. Solutions of 200 mg of L-theanine, 160 mg of caffeine, their combination, or the vehicle (distilled water; placebo) were administered in a randomized 4-way crossover design to 9 healthy adult men. Sixty minutes after administration, a 20-minute fMRI scan was performed while the subjects performed a visual color stimulus discrimination task. L-Theanine and L-theanine–caffeine combination resulted in faster responses to targets compared with placebo (Δ = 27.8 milliseconds, P = .018 and Δ = 26.7 milliseconds, P = .037, respectively). L-Theanine was associated with decreased fMRI responses to distractor stimuli in brain regions that regulate visual attention, suggesting that L-theanine may be decreasing neural resource allocation to process distractors, thus allowing to attend to targets more efficiently. L-Theanine–caffeine combination was associated with decreased fMRI responses to target stimuli as compared with distractors in several brain regions that typically show increased activation during mind wandering. Factorial analysis suggested that L-theanine and caffeine seem to have a synergistic action in decreasing mind wandering. Therefore, our hypothesis is that L-theanine and caffeine may be decreasing deviation of attention to distractors (including mind wandering); thus, enhancing attention to target stimuli was confirmed.

Abbreviations: BOLD, blood-oxygen-level-dependent; fMRI, functional magnetic resonance imaging; FSL, FMRIB Software Library.

* *Corresponding author at:* Department of Nutritional Sciences, College of Human Sciences, Texas, Tech University, 1301 Akron St, Box 1270, Lubbock, Texas 79409-1270, USA. Tel.: +1 919 485 9215.
E-mail address: m.binks@ttu.edu (M. Binks).

https://doi.org/10.1016/j.nutres.2017.11.002
0271-5317/

1. Introduction

L-Theanine and caffeine are 2 natural compounds that are purported to improve multiple cognitive functions including alertness and attention [1]. L-Theanine is a non–protein-forming L-amino acid that has structural similarities to glutamic acid. Caffeine is a natural plant alkaloid. Caffeine is primarily consumed as an ingredient in coffee, and both L-theanine and caffeine exist naturally in tea. Both of these compounds are freely available for purchase over the counter in tablet and powder forms as nutritional supplements in the United States. Moreover, both L-theanine and caffeine are used as additives in a variety of food and beverages, including some beverages that claim to improve alertness [2-5]. Thus, both L-theanine and caffeine are present in the food chain in varied forms.

Pharmacokinetics of both L-theanine and caffeine have been extensively studied. In animal studies, L-theanine has been shown to be readily absorbed from the small intestine and peaks in the plasma within 30 minutes of oral administration [6]. Forty minutes after oral administration of 200 mg of L-theanine, a significant increase in α electroencephalographic activation was observed, suggesting that L-theanine level in the brain reaches a therapeutic concentration within 40 minutes of administration [7]. L-Theanine is completely cleared from the plasma and the brain within 24 hours of administration [8]. Similarly, caffeine is almost entirely absorbed from the intestines within 45 minutes of oral administration [9] and reaches a peak level in the plasma within 30-75 minutes [10]. Overall elimination half-life of 200 mg of caffeine is 3.46 hours [11]; thus, caffeine can also be thought to be completely cleared from plasma within 24 hours of oral administration. However, metabolism of caffeine could be influenced by smoking and cyclical changes in estrogen and progesterone levels in females [12,13]. Taken together, both L-theanine and caffeine enter the brain and affect brain functions within 45 minutes of oral administration, and both substances are almost completely cleared from the body within 24 hours of oral administration.

Despite the fact that L-theanine and caffeine are often believed and at times advertised to improve alertness and attention, little work has been conducted to examine the effects of these compounds and their combination (as they occur naturally in tea; *Camellia sinensis*) on attention in human subjects. Our previous findings indicate that L-theanine, caffeine, and their combination improve visual color stimulus discrimination reaction time and increase the P300 event-related potential amplitude in an auditory oddball paradigm (which is an electrophysiological marker of allocation of attention-related neural resources for target stimulus identification and stimulus discrimination [14]) compared with a placebo [15]. Our subsequent analyses also revealed that L-theanine and caffeine have additive effects in increasing neural resource allocation for stimulus discrimination (as indicated by auditory P300 amplitude) and also in increasing visual color stimulus discrimination [15,16].These findings are compatible with behavioral and electrophysiological findings of many others, suggesting that L-theanine, caffeine, and their combination improve cognitive processing, visual stimulus discrimination, and possibly visual attention [1,15-20].

While informative, these data provide an incomplete picture because there is no direct evidence demonstrating neural activation in specific brain regions that would support conclusions regarding mechanisms of action of L-theanine and caffeine in mediating the observed cognitive effects. First, during a visual choice reaction time task, the brain regions that are known to process color stimuli (eg, the primary visual cortex, the lateral occipital cortex, V4) are likely to be involved in differentiating the color stimuli at the visual cortical level [21,22]. Thus, it is logical to speculate that the primary visual cortex as well as the lateral occipital cortex could be potential targets of L-theanine and caffeine if the compounds act on the visual cortical level. Second, a theoretical network of brain regions known as the default-mode network is known to show decreased activity when attention is focused on processing task-related stimuli while showing increased activity when an individual's attention drifts from processing task-related stimuli to task-independent thoughts (known as mind wandering) [23-25]. Faster reaction times associated with L-theanine, caffeine, and their combination could theoretically be due to decreased mind wandering as reflected by decreased task-related activity in brain regions forming the default mode network (eg, ventromedial prefrontal cortex, posterior cingulate cortex, and precuneus). Third, alteration in neuronal activity in a brain region known as the anterior cingulate cortex is thought to generate the auditory P300 event-related potential during the shifting of attention from a standard stimulus to a target stimulus [14]. Furthermore, there is converging evidence to suggest that the anterior cingulate cortex is involved in detecting and allocating attentional resources to resolve conflicts in response selection in reaction time tasks while being minimally influenced by the characteristics of stimuli per se [26,27]. Therefore, if the mechanisms of action of L-theanine and caffeine involve response selection and allocation of attentional resources for response selection, the anterior cingulate cortex is likely to be a potential target of L-theanine and caffeine. Taken together, improved (ie, faster) reaction times observed in a visual stimulus discrimination task following administration of L-theanine, caffeine, and their combination could be due to either (1) increased activity of brain regions that process visual stimuli (eg, primary visual cortex and visual association areas) in response to target stimuli as compared with distractor stimuli; (2) decreased activity in the brain regions involved in mind wandering (ie, the default mode network); or (3) increased activity of the anterior cingulate cortex.

Advances in functional magnetic resonance imaging (fMRI) have provided a means to test hypotheses regarding neurophysiological processes underlying improvements in the performance of neuropsychological tasks (eg, a visual color stimulus discrimination task) by visualizing the alterations in blood flow to specific regions in the brain while the subjects engage in such tasks [28]. This tool is ideal for taking the next logical step in examining the mechanism through which L-theanine, caffeine, and their combination improve attention, especially in relation to stimulus discrimination, by allowing for examination of the brain regions that show activations/deactivations during stimulus discrimination in subjects who have ingested these compounds.

Improving human attention in relation to stimulus discrimination has implications for day-to-day functioning (eg, driving), skilled professions (eg, pilots, military), and education as well as certain clinical conditions (eg, attention-deficit/hyperactivity disorder). Given that there is evidence to suggest that L-theanine, caffeine, and their combination are likely to improve the speed of stimulus discrimination, outlining the potential neurophysiological mechanisms leading to such improvements could result in use of these substances and possibly food or beverages containing these substances as performance enhancers as well as therapeutic agents.

Therefore, in a pilot 4-way crossover fMRI study, we aimed to examine the neurophysiological responses of the human brain while the subjects performed a visual color stimulus discrimination reaction time task 60 minutes after ingestion of a 150-mL solution of distilled water containing (1) 200 mg of L-theanine, (2) 160 mg of caffeine, or (3) their combination compared with (4) a distilled water–only placebo. We specifically hypothesized that L-theanine and caffeine may be improving the speed of stimulus discrimination by the brain via increased allocation of attention-related neural resources to process target stimuli and decreased deviation of neural resources in the brain to distractor stimuli (ie, external distractors) as well as mind wandering (ie, internal distractors) as measured by blood-oxygen-level-dependent (BOLD) fMRI responses while the subjects engage in a visual color stimulus discrimination reaction time task. We further hypothesized that L-theanine and caffeine may have additive effects in allocating attention-related neural resources to process target stimuli and in decreasing deviation of neural resources to internal and external distractors.

2. Methods and materials

2.1. Ethics

All materials and methods were approved by the Human Research Protection Program of the Texas Tech University. All procedures were conducted in accord with the Helsinki Declaration revised in 2000 [29]. Informed written consent was obtained from all subjects who met the eligibility criteria. The protocol was Clinical Trials Registry Number NCT02770105 (https://clinicaltrials.gov/ct2/show/NCT02770105).

2.2. Subjects

Nine adult men (age 18-60 years) were recruited from Texas Tech University and or surrounding Lubbock Texas community. Potential subjects were screened for eligibility in an initial telephone prescreening interview conducted using a standard script. Individuals who had absolute contraindications to undergo magnetic resonance imaging; gross visual, auditory, or motor impairments; severe physical, psychiatric, or neurological impairments; a current or past history of exposure to medications, toxins, or substances known to affect neurological function; history of alcohol or substance abuse; and diseases that could be aggravated by L-theanine or caffeine consumption (eg, tea or coffee) were excluded from the study. Individuals who were on medications that included caffeine, adenosine receptor blockers, or phosphodiesterase inhibitors and individuals who were on medications that are known to interact with caffeine were also excluded. Finally, individuals who were not willing to participate in the interventional study within the given time period were excluded.

2.3. Design

A repeated-measures, placebo-controlled, 4-way crossover design was implemented that included 5 weekly in-person visits (Fig. 1). During visit 1, the subjects were rescreened for eligibility, and informed consent was obtained. The subjects were subsequently trained to perform a recognition visual reaction time task on a laptop computer and were allowed to practice the task for 10 minutes.

On arrival for each of visits 2 through 5, the subjects consumed 200 mg of L-theanine, 160 mg of caffeine, a combination of 200 mg of L-theanine and 160 mg of caffeine, or distilled water (placebo) during each consecutive visit. The doses of the substances were determined based on the results of our prior study and studies of several others which suggested that administration of 200 mg of L-theanine (equivalent to the dose found in many commercially available supplements or 8 cups of tea), 160 mg of caffeine (equivalent to the dose found in 8 cups of tea), and their combination seems to improve visual stimulus discrimination reaction times and auditory P300 event-related potential amplitudes. Furthermore, evidence derived from a pilot clinical experiment [16] and a subsequent dose-response study (unpublished data) also suggested that attention-enhancing effects of L-theanine appear to peak at 200 mg. Tea was not administered in a separate arm because our prior studies indicated that a cup of Ceylon black tea is unlikely to elicit significant neurophysiological or behavioral responses in a stimulus discrimination task compared with a placebo [15]. The order of substance administration by visit was randomized using a random number generator in R statistical software (version 3.2.4). At each visit, the subjects arrived and were given the randomly assigned solution to consume. They were then allowed to practice the recognition visual reaction time task for 10 minutes and then remained seated and relaxed until 60 minutes elapsed from the time of administration of the solution. Subsequently, they underwent an approximately 20-minute scanning session.

2.4. Treatment

L-Theanine and caffeine were purchased in the purified powder form (Powder City Inc, York, PA, USA) and were stored in opaque airtight containers in room temperature. The doses of L-theanine and caffeine were measured using a microscale with a precision of 1 mg. L-Theanine, caffeine, and their combination were administered after dissolving in 200 mL of distilled water to obtain clear solutions. Two hundred milliliters of distilled water was administered as the placebo. Each solution was prepared within 4 hours of the time of administration. The subjects consumed each 200-mL solution in a disposable plastic cup within 1 minute.

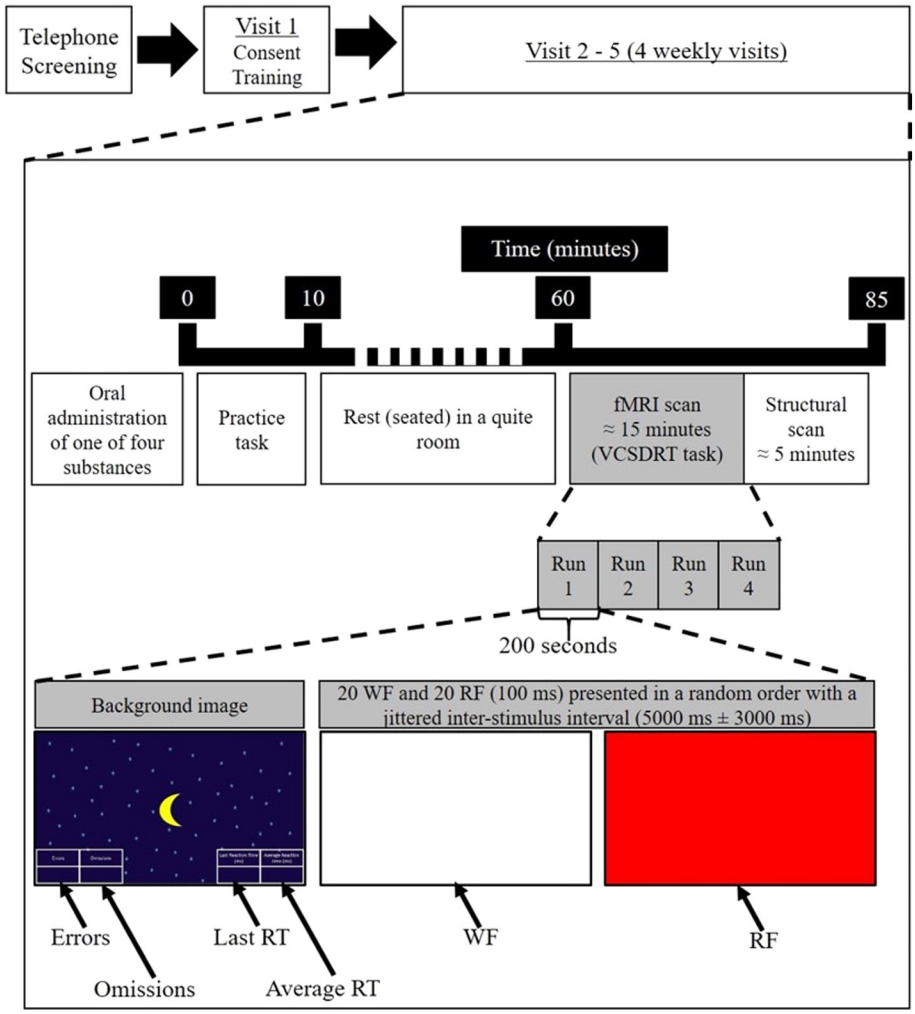

Fig. 1 – Data collection protocol. VCSDRT, visual color stimulus discrimination reaction time; WF, white flash (ie, target stimulus); RF, red flash (ie, distractor stimulus); RT, reaction time.

2.5. *Imaging paradigm*

All scanning sessions were conducted at the Texas Tech Neuroimaging Institute using a 3.0-T Siemens Skyra scanner equipped with a 20-channel head coil. First, 4 fMRI runs were performed followed by a T1-weighted structural magnetic resonance imaging scan. During each functional run, the recognition visual reaction time task was presented on an LCD screen and was reflected using a mirror attached to the head coil of the scanner. The subjects responded by pressing a button in a handheld fiber-optic device with the index finger of the right hand.

The recognition visual reaction time task adopted from our previous work [15] was programmed and presented using PsychoPy 2.0 (University of Nottingham, UK). A blue-colored background was presented. Within each functional run, 20 white-colored 100-millisecond flashes (ie, target stimuli) and 20 red-colored 100-millisecond flashes (ie, distractor stimuli) were presented on the background in a random order with a jittered interstimulus interval (5000 ± 3000 milliseconds). The subjects were instructed to press the fiber-optic response button as quickly as possible only in response to the white-colored flashes while ignoring the red-colored flashes. The importance of accuracy and speed was emphasized. The reaction time (ie, the time between the presentation of the stimulus and the response) of each target stimulus trial and the averaged reaction time to target stimuli within each functional run were displayed on the screen. Responses to distractor stimuli were counted as errors. Target stimuli for which the subjects did not respond within 1000 milliseconds were counted as omissions. Omissions and errors committed within each run were also displayed on the screen. For fMRI scans, the stimuli were displayed on an LCD screen and were reflected using a mirror attached to the head coil of the scanner, while the subjects responded by pressing a button in a handheld fiber-optic device with the index finger of the right hand irrespective of the handedness.

fMRI data were acquired using an echo planar imaging sequence with the following parameter settings: repetition time = 2140 milliseconds; echo time = 25; flip angle = 70; field of view = 192 mm × 192 mm; acquisition matrix = 64 × 64; slice thickness = 2.5 mm; and 42 ascending axial slices. Slices were tilted approximately 30° from the anterior commissure–posterior commissure line to minimize orbitofrontal cortical

signal dropout [30]. A T1-weighted MPRAGE scan was also collected using the following parameters: repetition time = 1900 milliseconds; echo time = 2.49; flip angle = 9; field of view = 240 mm × 240 mm; acquisition matrix = 256 × 256; slice thickness = 0.9 mm; and 192 slices in the sagittal plane. Each scanning session was completed within approximately 20-25 minutes.

2.6. *Statistical analyses*

Because of the lack of previous comparable fMRI studies to derive effect sizes, sample size was determined based on the effect size of the relative increase in auditory P300 event-related potential amplitude (which is an electrophysiological marker of allocation of attention-related neural resources for target stimulus identification and stimulus discrimination) with L-theanine compared with placebo in a previous randomized, placebo-controlled, crossover trial conducted among healthy adult men [15] using R statistical software (version 3.2.4). Based on the outcomes of the power calculation, a minimum of 9 subjects was required to confirm the hypothesis of the current study.

All reaction time and error data were imported to R statistical software (version 3.2.4). A mixed-effects model was constructed using the nlme package in R to predict the reaction time using the administered substance, accounting for the variability within the levels of subjects, scans, and functional runs. Placebo was used as the reference category; thus, the regression coefficients of other categories were interpreted as differences in reaction times between the respective substance and the placebo. A similar mixed-effects model was constructed to predict errors. A separate factorial mixed-effects analysis was conducted to examine for the additive and/ or synergistic effects of L-theanine and caffeine, including L-theanine, caffeine, and their interaction as regressors. $P < .05$ was considered significant.

All structural and functional raw data images were converted to NIfTI format using the dcm2nii converter [31]. The Freesurfer (autorecon1) [32,33] was used for structural image preprocessing, and FMRIB Software Library (FSL; Version 6.00, Oxford, UK) was used for functional image preprocessing and analysis. Preprocessing of functional images included the following: motion correction by aligning each functional volume to the center volume within each functional run with 6-DOF sinc interpolation using the MCFLIRT tool in FSL [34]; skull-stripping using the BET tool in FSL [35]; registration to high-resolution structural space by the BBR algorithm and subsequently to the standard space by 12-DOF using the FLIRT tool in FSL [36]; spatial smoothing using a Gaussian kernel of FWHM 8.0 mm; grand-mean intensity normalization of the entire 4D data set by a single multiplicative factor; and high-pass temporal filtering (Gaussian-weighted least squares straight line fitting, with σ = 50.0 s) and FILM prewhitening [37].

Standard 3-level analysis in the FEAT tool in FSL was used to analyze the fMRI data. In level 1 models, single-run functional time series were modeled with task-based regressors for stimuli (ie, target stimuli, distractor stimuli, correctly identified target stimuli, omissions, errors, and correctly ignored distractor stimuli). Task-based regressors were convolved using a canonical double-γ hemodynamic response function. Confound variables to control for motion effects included the 6 motion parameters, their temporal derivatives, and regressors to scrub (ie, censor) volumes that exceeded a frame-wise displacement of 0.9 mm [38]. An autocorrelation correction was included to account for serial dependencies between samples not accounted for by the task and confound variables. The contrast between correctly identified target stimuli and correctly ignored distractor stimuli was modeled. In addition, the contrast between correctly identified target stimuli and the baseline and correctly ignored distractor stimuli and the baseline was examined. In the level 2 analyses, the level 1 experimental variables of all functional runs of each subject were averaged using a fixed-effects model [39-41]. In the third-level analysis, the level 2 averages were regressed by 9 variables that represented each subject and 3 variables that represented L-theanine, caffeine, and their combination using a mixed-effects model with subject as a random effect for population inference (FLAME 1). Specifically, the L-theanine vs placebo, caffeine vs placebo, and L-theanine–caffeine vs placebo contrasts were examined. A separate level 3 factorial analysis was performed to examine for the additive and/or synergistic effects of L-theanine and caffeine by regressing the level 2 outputs of the scans by variables representing each of the 9 subjects, L-theanine, caffeine, and their interaction. The final statistical maps were corrected for multiple comparisons at $P < .05$ at a Z threshold of 1.96 via the Gaussian random field theory–based approach in FSL. The Harvard-Oxford cortical and subcortical structural atlases and the atlasquery tool in FSL were used to identify the brain regions showing statistical significance.

3. Results

All enrolled subjects completed the study (see Fig. 2 for a CONSORT diagram). Baseline characteristics of the 9 subjects are shown in Table 1. Analysis of reaction time data using a mixed-effects model revealed that L-theanine and theanine-caffeine combination significantly improved visual color stimulus discrimination reaction times compared with the placebo by 27.8 milliseconds (SE = 11.6, t = –2.40, P = .018) and 26.7 milliseconds

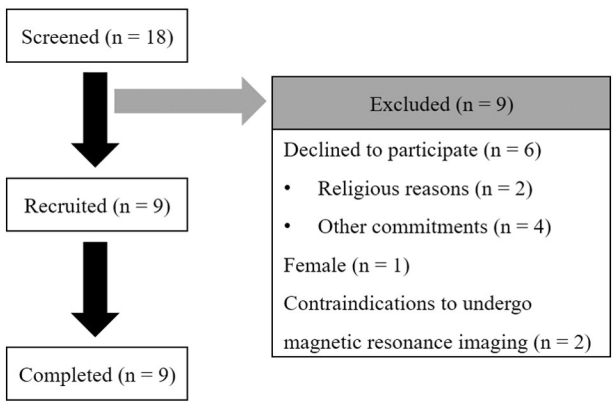

Fig. 2 – Eligibility and randomization of study participants and completion of data collection: CONSORT diagram.

Table 1 – Baseline characteristics of the subjects participating in the 4-way crossover trial

Parameter	Values [a]
Age (y)	28.11 ± 9.36
Weight (kg)	86.47 ± 35.68
Height (cm)	176.39 ± 5.84
Body mass index (kg/m^2)	27.52 ± 10.28

Adult male subjects (N = 9) were recruited to undergo a 4-way, repeated-measures, crossover fMRI study in which task-related fMRI responses while performing a visual color stimulus discrimination task were examined following administration of each of 200 mg of L-theanine, 160 mg of caffeine, their combination, and a placebo (water) in a randomized order.
[a] Values are means ± SDs.

(SE = 12.1, t = –2.21, P = .037), respectively. Improvement observed with caffeine (22.5 milliseconds) compared with the placebo approached significance (SE = 12.1, t = –1.86, P = .075).

Analysis of errors using a mixed-effects model revealed that, with placebo, 0.78 error was committed on average during a scanning session, and this was significantly different from zero (SE = 0.02, t = 3.33, P = .001). Mean of errors caused when L-theanine was administered was also 0.78 (SE = 0.19, t = 0.00, P = 1.000). Administration of caffeine and L-theanine–caffeine combination was associated with reductions of means of errors by 0.17 and 0.19 vs placebo, respectively; however, these improvements in accuracies were not significant (SE = 0.19, t = –0.89, P = .380 and SE = 0.19, t = –1.01, P =.307, respectively).

Factorial analyses of reaction times revealed a significant main effect for L-theanine (SE = 11.60, t = –2.40, P = .018) and a trend for caffeine (SE = 12.10, t = –1.86, P = .075). However, the interaction of L-theanine and caffeine was not significant (SE = 16.9, t = 1.39, P = .176).

Administration of L-theanine as compared with the placebo was associated with a significant decrease in fMRI BOLD responses in several brain regions including the bilateral frontal medial cortex, bilateral lateral occipital cortex, and the right occipital pole when distractor stimuli were presented (Table 2; Supplemental Table S1, Fig. 3; Supplemental Figure S1). Given that the ventromedial prefrontal cortex is part of a broader network (known as the default mode network), which shows increased activity during mind wandering (ie, occurrence of stimulus-independent thoughts) and shows decreased activity when mind wandering decreases and attentional resources are allocated to a specific task-related stimulus [23-25], our finding indicates that L-theanine may be increasing vigilance to detect distractor stimuli. Decreased responsiveness of the regions that process visual stimuli (eg, occipital pole and lateral occipital cortex [21,22]) to distractor stimuli may be another mechanism through which L-theanine, when administered alone or in combination with caffeine, may be associated with faster reaction times to target stimuli in the visual

Table 2 – Brain regions that showed significant task-related fMRI responses following intake of L-theanine, caffeine, and their combination compared with a placebo

Substance	Stimulus	Response compared with placebo	Brain regions	Functional significance
L-Theanine	Target	Not significant		
	Distractor	Decreased (P = .001)	Bilateral frontal medial cortex	Decreased mind wandering while processing distractor stimuli
		Decreased (P < .001)	Bilateral lateral occipital cortex	Decreased visual attention to distractor stimuli
		Decreased (P < .001)	Right occipital pole	Decreased visual attention to distractor stimuli
	Target vs distractor	Increased (P = .049)	Right inferior frontal gyrus	Recruitment of neural resources for target detection
Caffeine	Target	Not significant		
	Distractor	Not significant		
	Target vs distractor	Not significant		
L-Theanine–caffeine combination	Target	Decreased (P < .001)	Right precuneus cortex	Decreased mind wandering while processing target stimuli
			Right occipital cortex	Decreased recruitment of resources to process target stimuli
	Distractor	Decreased (P < .001)	Left lateral occipital cortex	Decreased visual attention to distractor stimuli
			Left occipital pole	Decreased visual attention to distractor stimuli
	Target vs distractor	Decreased (P < .001)	Left posterior cingulate cortex	Decreased mind wandering while processing target vs distractor stimuli
			Left anterior cingulate cortex	Decreased mind wandering while processing target vs distractor stimuli

Adult male subjects (N = 9) consumed 200 mg of L-theanine, 160 mg of caffeine, their combination, and a placebo (water) in a randomized order in a 4-way, repeated-measures, crossover trial. fMRI was performed while the subjects engaged in a visual color stimulus discrimination task. Analyses were conducted using FSL to examine fMRI responses observed in each stimulus condition (ie, target vs distractor) in each treatment condition as compared with the placebo. Third-level random-effects models were constructed using the FLAME-1 algorithm in FSL. Only the clusters that survived the Z threshold of 1.96 and P threshold of .05 after family-wise error correction are reported.

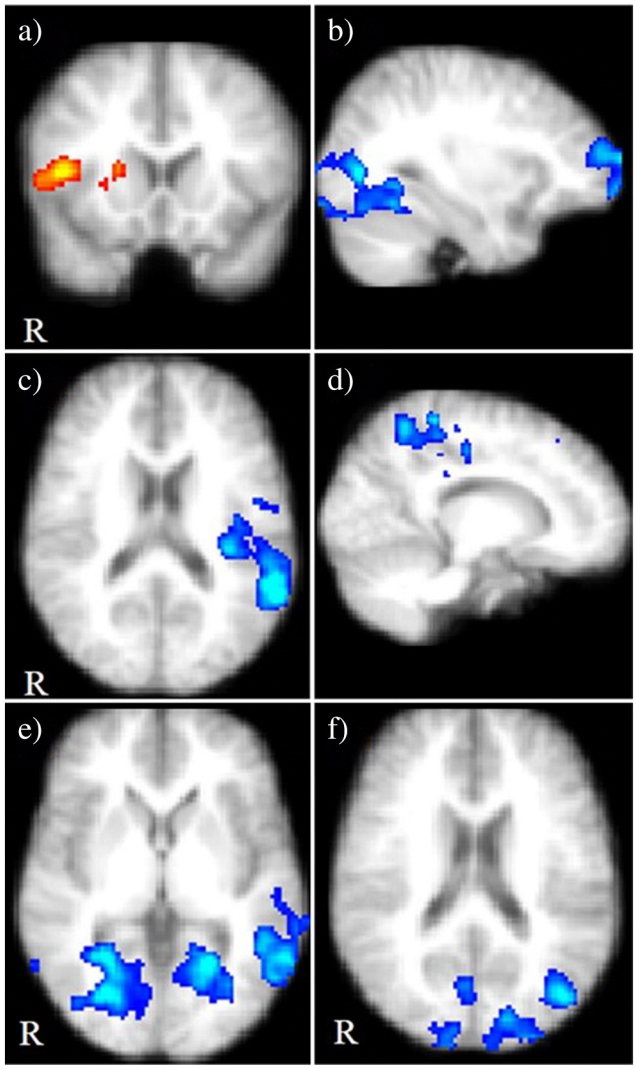

Fig. 3 – Brain regions showing significant task-related fMRI responses following intake of L-theanine, caffeine, and their combination compared with a placebo. Compared with the placebo, (A) brain regions that showed greater fMRI responses to target stimuli as compared with distractor stimuli with L-theanine (coronal section); (B) brain regions that showed lesser fMRI responses to distractors stimuli with L-theanine (sagittal section on the right side); (C) brain regions that showed lesser fMRI responses to target stimuli as compared with distractor stimuli with the L-theanine–caffeine combination (horizontal section); (D) brain regions that showed lesser fMRI responses to target stimuli as compared with distractor stimuli with the L-theanine–caffeine combination (midsagittal section); (E) brain regions that showed lesser fMRI responses to target stimuli with the L-theanine–caffeine combination (horizontal section); and (F) brain regions that showed lesser fMRI responses to distractor stimuli with L-theanine–caffeine combination (horizontal section).Adult male subjects (N = 9) consumed 200 mg of L-theanine, 160 mg of caffeine, their combination, and a placebo (water) in a randomized order in a 4-way, repeated-measures, crossover trial. fMRI was performed while the subjects engaged in a visual color stimulus discrimination task. Analyses were conducted using FSL to examine fMRI responses observed in each stimulus condition (ie, target vs distractor) in each treatment condition as compared with the placebo. Third-level random-effects models were constructed using the FLAME-1 algorithm in FSL. Only the clusters that survived the Z threshold of 1.96 and P threshold of .05 after family-wise error correction are shown.

color stimulus discrimination reaction time task. Administration of L-theanine (vs placebo) was also associated with increased fMRI BOLD responses when target stimuli were presented as compared with distractor stimuli in the right inferior frontal gyrus, which is also a brain region that is known to be involved in target detection [42]. Therefore, L-theanine appears to improve the speed of visual color stimulus discrimination possibly via decreasing mind wandering, increasing recruitment of neural resources for target detection, and decreasing neural resource allocation for processing distractor stimuli at visual cortical level.

Administration of caffeine was not associated with a significant change in fMRI BOLD responses as compared with the placebo when each of the target and distractor stimuli was presented. However, as compared with the placebo, oral intake of L-theanine–caffeine combination was associated with decreased fMRI BOLD responses of the right precuneus and several regions in the right occipital cortex

Table 3 – Brain regions that showed significant task-related fMRI responses in a factorial analysis of L-theanine and caffeine factors and their interaction

Factor	Stimulus	Response vs placebo	Brain regions	Functional significance
L-Theanine	Target	Decreased ($P < .001$)	Right precuneus cortex	Decreased mind wandering while processing target stimuli
		Decreased ($P < .001$)	Right occipital cortex	Decreased recruitment of resources to process target stimuli
	Distractor	Decreased ($P < .001$)	Left frontal medial cortex	Decreased recruitment of resources to process target stimuli
		Decreased ($P < .001$)	Bilateral lateral occipital cortex	Decreased visual attention to distractor stimuli
	Target vs distractor	Not significant		
Caffeine	Target	Decreased ($P < .001$)	Right precuneus cortex	Decreased mind wandering while processing target stimuli
		Decreased ($P < .001$)	Bilateral intracalcarine cortex	Decreased recruitment of resources to process target stimuli
		Decreased ($P < .001$)	Right occipital fusiform cortex	Decreased recruitment of resources to process target stimuli
	Distractor	Not significant		
	Target vs distractor	Decreased ($P < .001$)	Bilateral paracingulate cortex	Decreased mind wandering while processing target vs distractor stimuli
L-theanine × caffeine interaction	Target	Decreased ($P < .001$)	Right precuneus cortex	Decreased mind wandering while processing target vs distractor stimuli
		Decreased ($P < .001$)	Right lateral occipital cortex	Decreased recruitment of resources to process target stimuli
	Distractor	Not significant		
	Target vs distractor	Decreased ($P < .001$)	Left posterior cingulate cortex	Decreased mind wandering while processing target vs distractor stimuli

Adult male subjects (N = 9) consumed 200 mg of L-theanine, 160 mg of caffeine, their combination, and a placebo (water) in a randomized order in a 4-way, repeated-measures, crossover trial. fMRI was performed while the subjects engaged in a visual color stimulus discrimination task. Factorial analyses were conducted using FSL to examine the fMRI responses observed in each stimulus condition (ie, target vs distractor) with L-theanine and caffeine factors and their interaction. Third-level random-effects models were constructed using the FLAME-1 algorithm in FSL. Only the clusters that survived the Z threshold of 1.96 and P threshold of .05 after family-wise error correction are reported.

when target stimuli were presented (Table 2), and of the left lateral occipital cortex and occipital pole when distractor stimuli were presented. When target vs distractor stimulus conditions were compared, with administration of the L-theanine caffeine combination, left posterior cingulate cortex showed decreased fMRI BOLD responses. Precuneus and posterior cingulate cortex represents the posterior node of the default mode network that shows increased activity when an individual is engaged in mind wandering [23-25]. Lateral occipital cortex and occipital pole are involved in early processing of visual stimuli [21,22]. Therefore, our findings suggest that L-theanine–caffeine combination appears to result in faster visual color stimulus discrimination reaction times, possibly via decreasing mind wandering when target stimuli are presented and decreasing early processing of distractor stimuli.

However, there were several unexpected findings in the L-theanine–caffeine vs placebo comparison. First, decreased, rather than increased, fMRI BOLD responses were observed in the anterior cingulate cortex (particularly on the left hemisphere) in response to target stimuli despite improved reaction times with L-theanine–caffeine combination compared with the placebo. This finding suggests the possibility of an action of L-theanine–caffeine combination that is independent of the attention regulatory mechanisms in the anterior cingulate cortex given that the anterior cingulate cortex has been shown to regulate attention and arousal to visual stimuli [43] and attention control over response selection [44,45]. Second, decreased fMRI BOLD responses in the visual information processing occipital regions (eg, lateral occipital cortex) were noted with L-theanine–caffeine combination in response to both target and distractor stimuli, also suggesting the possibility that when a combination of L-theanine and caffeine is administered, an individual may not have to rely on increasing attentional resources at the stimulus processing level or response selection level, as mind wandering is minimized and attentional resources are focused on task-related stimuli (as evidenced by decreased reactivity of the posterior cingulate cortex and precuneus) to a greater extent compared with when either L-theanine or caffeine is administered alone.

In the factorial analysis of BOLD responses to distractor stimuli, the L-theanine was found to be associated with decreased mind wandering when processing target stimuli (as evidenced by decreased fMRI BOLD responses in right precuneus [23-25]) and also decreased early visual attention to both target and distractor stimuli (Table 3; Supplemental Table S2). Similarly, caffeine was associated with decreased mind wandering when processing target stimuli (ie, decreased fMRI BOLD responses in right precuneus [23-25]) and decreased early visual attention to target stimuli only. A significant interaction of L-theanine and caffeine factors was found in the right precuneus cortex and right lateral occipital cortex for target stimuli suggesting that L-theanine and caffeine seem to have synergistic effects in decreasing mind wandering in the presence of target stimuli and decreasing

early processing of target stimuli, respectively. The target vs distractor stimulus contrast also showed a significant interaction in the left posterior cingulate cortex, further substantiating the fact that L-theanine and caffeine seem to have a synergistic effect in decreasing mind wandering especially when discriminating between target vs distractor stimuli.

4. Discussion

Although previous studies have examined the effects of L-theanine, caffeine, and their combination on various electrophysiological and behavioral measures (including reaction time), evidence regarding the specific brain regions that appear to be affected by L-theanine, caffeine, and their combination in improving performance in behavioral tasks is scarce. In this first-of-its-kind study, we examined the effects of oral administration of 200 mg L-theanine, 160 mg of caffeine, and their combination compared with placebo on reaction times and related BOLD responses of the human brain using fMRI in a 4-way crossover trial.

We found significantly faster reaction times to the target stimuli with L-theanine and L-theanine–caffeine combination compared with the placebo. We also found a trend of improvement of reaction time for the target stimuli with a moderate effect size in the caffeine condition. The small sample size in our study may have contributed to our not observing all anticipated effects. Together, these findings do however corroborate our previous findings and findings of others indicating that L-theanine, caffeine, and their combination seem to be associated with faster stimulus discrimination reaction times [1,15-20].

Factorial analysis of reaction times revealed a significant main effect for L-theanine and a trend for caffeine. However, the L-theanine × caffeine interaction was not significant. In a previous study, we have shown that, upon oral administration, 200 mg L-theanine and 160 mg caffeine appear to have additive but not synergistic effects in improving visual color stimulus discrimination reaction times and also the P300 auditory event-related potential amplitudes [15]. Thus, the findings of the factorial analysis of the present study substantiate our previous findings suggesting that, in relation to improving (ie, giving rise to faster) reaction times in a stimulus discrimination task, L-theanine and caffeine seem to have additive effects.

Our fMRI findings suggested that L-theanine appears to improve the speed of visual color stimulus discrimination possibly via decreasing mind wandering, increasing recruitment of neural resources for target detection, and decreasing neural resource allocation for processing distractor stimuli at visual cortical level, thus potentially improving the efficiency of neural resources attending to target stimuli and discriminating target stimuli from distractor stimuli. In 2 previous studies, Gomez-Ramirez et al [46,47] observed decreased background electroencephalographic α oscillations in the brain and increased attention-related α oscillations in subjects who engaged in an intersensory and a visuospatial attention task following administration of 250 mg of L-theanine as compared with a placebo. Our findings are consistent with the results of these 2 studies which suggest that L-theanine appears to decrease diverting attention to internal as well as external distractors, thereby allowing an individual to more efficiently attend to target stimuli. Therefore, oral intake of L-theanine supplements and beverages that contain L-theanine as an ingredient at comparable doses (ie, 200 mg) may improve target discrimination and therefore the performance of day-to-day activities as well as skilled tasks that require vigilance and target discrimination. It should be noted however that L-theanine has been shown to have a vasodilator action in the peripheral circulation, possibly via increasing the production of nitric oxide in the vascular intima [48,49]. If this effect exists in cerebral circulation as well, L-theanine may be increasing the BOLD responses irrespective of the status of the task, possibly increasing the baseline BOLD activations and suppressing the BOLD contrast ratio. Future fMRI studies controlling for the vascular effects of L-theanine are needed to fully understand the effects of L-theanine on brain regions that regulate visual attention. Furthermore, more evidence coming from studies that examine the effects of L-theanine on specific day-to-day tasks as well as skilled tasks is required before making recommendations regarding the ability to translate our preliminary finding to practice.

Analysis of fMRI data did not reveal significant differences in BOLD responses in the caffeine condition compared with the placebo. However, the improvement in reaction times to target stimuli in the caffeine condition approached significance as compared with the placebo. This lack of significant findings may have been due to several reasons. First, our study was powered to detect significant improvements in neural resource allocation for attention in L-theanine vs placebo comparison. Second, caffeine is a well-known cerebral as well as peripheral vasoconstrictor [48]. As a result, caffeine has been shown to suppress the baseline fMRI BOLD responses [48]. Therefore, an adequately powered study needs to be conducted controlling for the vascular effects of caffeine to fully understand and appreciate the neurophysiological effects of caffeine on attention-related neural resources while an individual is engaged in a task that requires stimulus discrimination.

We also observed that the L-theanine–caffeine combination appears to result in faster visual color stimulus discrimination reaction times, possibly via decreasing mind wandering when target stimuli are presented and decreasing early processing of distractor stimuli. Therefore, as with L-theanine, the L-theanine–caffeine combination also appears to improve the efficiency of neural resource allocation to process target stimuli. Because P300 event-related potentials seem to decrease with mind wandering [50], it is likely that the action of the L-theanine–caffeine combination on the default mode network in the brain on reducing mind wandering may be resulting in increased P300 amplitudes. Therefore, the finding that the L-theanine–caffeine combination was associated with decreased BOLD responses in the posterior cingulate cortex and the precuneus is consistent with the increased P300 amplitudes we observed previously in response to administration of the L-theanine–caffeine combination [15]. Similarly, Kelly et al [51] demonstrated that administration of a combination of L-theanine and caffeine seems to suppress background electroencephalographic α activity, supporting our conclusion. Therefore, our findings suggest that oral intake of a combination of 200 mg of L-theanine and 160 mg of caffeine as supplements or as ingredients in food/beverages may be associated with enhanced performance in day-to-day tasks as

well as skilled tasks that require target discrimination. However, because caffeine has a vasopressor effect on cerebral circulation [52] and as L-theanine may be counteracting this effect [48,49], it is essential to examine these potential mechanisms in a study that controls for the effects of L-theanine and caffeine on cerebral blood flow.

Factorial analysis of fMRI data provided additional support regarding the specific actions of L-theanine, caffeine, and their combination. Both L-theanine and caffeine factors significantly decreased fMRI BOLD responses in the right precuneus (a brain region in the default mode network that is involved in mind wandering [23-25]) when processing target stimuli, and the L-theanine × caffeine interaction was also significant, suggesting that these substances seem to have a synergistic effect in decreasing mind wandering when processing target stimuli. When processing target stimuli as compared with distractor stimuli, L-theanine and caffeine factors showed a significant interaction in the left posterior cingulate cortex, which also shows increased activity during mind wandering [23-25], supporting the fact that the actions of L-theanine and caffeine may be synergistic at least at a neurophysiological level. However, only L-theanine was associated with decreasing fMRI BOLD responses in visual information processing regions to distractor stimuli, indicating that this may be a unique action of L-theanine. Therefore, whereas L-theanine and caffeine appear to act via similar mechanisms showing synergistic effects, L-theanine also appear to act via a mechanism that is independent of the action of caffeine to enhance performance in target stimulus discrimination.

This makes intuitive sense based on the known actions of these compounds on neurotransmitters in the brain. L-Theanine is thought to exert cognitive effects by increasing release of dopamine in the brain [53,54] and reducing the release of serotonin, noradrenaline, and possibly GABA [55,56]. L-Theanine also functions as a glutamate reuptake inhibitor and a partial antagonist of glutamate [57,58]. Caffeine is thought to exert its cognitive effects primarily by competitive inhibition of adenosine receptors A1 and A2A in the brain, resulting in an increased release of dopamine, serotonin, glutamate, and noradrenaline [59,60]. Thus, both L-theanine and caffeine increase dopamine levels in the brain. However, L-theanine decreases serotonin and noradrenaline that are increased by caffeine. Moreover, L-theanine decreases GABA levels in the brain, whereas caffeine increases glutamate levels. Together, these different mechanisms of action and their interactions may have given rise to the distinct patterns of BOLD responses observed with L-theanine and possibly the L-theanine–caffeine combination.

Being a pilot investigation, the current study has some limitations. First, because of the novelty of using fMRI to examine the differential effects of L-theanine and caffeine on the brain, the sample size was determined using the effect sizes for increases in auditory P300 event-related potentials in a previous study. Therefore, our study may have been underpowered to detect changes in BOLD responses in some brain regions. It does however provide valuable data upon which future research can more accurately determine sample size. Second, caffeine is well known to affect cerebral circulation. The effects of L-theanine on human cerebral circulation are not fully understood. Because BOLD fMRI responses depend entirely on changes in regional blood oxygen levels, it is not completely clear whether our observations are due to underlying neural responses or due to vascular responses that are independent of the changes in neural activations [48,49,52]. Third, our sample was limited to male subjects to avoid the menstrual cyclical variability in reaction times in female subjects. This limits the generalizability of our findings. Finally, owing to being a pilot study with a limited sample size, fMRI data were analyzed and thresholded using a Gaussian random field theory–based approach (an a priori decision). Given that thresholding fMRI statistical images using a Gaussian random field theory–based approach has been shown recently to result in higher family-wise error rates than expected, our findings need to be confirmed in a study that is sufficiently powered to use permutation-based thresholding approaches that are robust to the caveats in conventional cluster-wise thresholding options [61]. However, it should be noted that inflation of type I error rates in Gaussian random field theory–based approaches is mainly due to identifying small, near-threshold clusters as significant [61]. All clusters we observed in the current study were larger than 1500 voxels, and most had cluster-wise P values lower than $P < .001$. Thus, despite acknowledged limitations of Gaussian random field theory, our clusters are likely sufficiently improbable to have arisen solely owing to chance. Still, future studies building off of this work will want to explicitly evaluate whether our observed clusters also appear in larger, confirmatory samples.

In conclusion, our hypothesis that L-theanine and caffeine may be improving the speed of stimulus discrimination by the human brain, via increased allocation of attention-related neural resources to process target stimuli and decreased deviation of attention-related neural resources to both internal and external distractors, was confirmed. Our results also indicated that, in addition to the hypothesized additive effects, L-theanine and caffeine may have synergistic effects in decreasing deviation of attention to internal distractors (ie, mind wandering). Therefore, our study provides preliminary evidence to suggest that oral consumption of 200 mg of L-theanine alone or in combination with 160 mg of caffeine may improve performance in tasks that require stimulus discrimination. Thus, our study provides mechanistic evidence to support the fact the food, beverages, and nutritional supplements containing L-theanine and the L-theanine–caffeine combination may enhance stimulus discrimination. However, more evidence is required before these preliminary findings can be translated to clinical practice.

Acknowledgment

The work was supported by the Neuroimaging Seed Grant of the College of Human Sciences, Texas Tech University, Lubbock, TX, USA. The authors do not have any potential conflicts of interest directly related to the production of this manuscript to declare. Dr Binks wishes to report that he received educational funding from The Coca Cola Company (2015) and research funding from Nestlé Health Science Inc (2016). He also has an ongoing nonfinancial relationship with International Food Information Council (Scientific Advisory Board Volunteer).

Appendix A. Supplemental materials

Supplemental materials to this article can be found online at https://doi.org/10.1016/j.nutres.2017.11.002.

REFERENCES

[1] Camfield DA, Stough C, Farrimond J, Scholey AB. Acute effects of tea constituents L-theanine, caffeine, and epigallocatechin gallate on cognitive function and mood: a systematic review and meta-analysis. Nutr Rev 2014;72:507–22. https://doi.org/10.1111/nure.12120.

[2] McCusker RR, Goldberger BA, Cone EJ. Caffeine content of energy drinks, carbonated sodas, and other beverages. J Anal Toxicol 2006;30:112–4. https://doi.org/10.1093/jat/30.2.112.

[3] FDA. Determination of the GRAS status of suntheanine L-theanine for use in food. Office of Premarket Approval, US Food and Drug Administration; 2006.

[4] FDA. Agency response letter GRAS notice no. GRN 000209: Office of Food Additive Safety, U. S. Food and Drug Administration. [accessed 15 September 2017]. Available from https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153810.htm; 2007.

[5] FDA. Food additive status list: U.S. Food and Drug Administration. [accessed 15 September 2017]. Available from https://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm091048.htm#ftnL; 2014.

[6] Unno T, Suzuki Y, Kakuda T, Hayakawa T, Tsuge H. Metabolism of theanine, gamma-glutamylethylamide, in rats. J Agric Food Chem 1999;47:1593–6. https://doi.org/10.1021/jf981113t.

[7] Juneja LR, Chu D-C, Okubo T, Nagato Y, Yokogoshi H. L-Theanine—a unique amino acid of green tea and its relaxation effect in humans. Trends Food Sci Technol 1999;10:199–204. https://doi.org/10.1016/S0924-2244(99)00044-8.

[8] Terashima T, Takido J, Yokogoshi H. Time-dependent changes of amino acids in the serum, liver, brain and urine of rats administered with theanine. Biosci Biotechnol Biochem 1999;63:615–8. https://doi.org/10.1271/bbb.63.615.

[9] Carrillo JA, Benitez J. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. Clin Pharmacokinet 2000;39:127–53. https://doi.org/10.2165/00003088-200039020-00004.

[10] Mandel H. Update on caffeine consumption, disposition and action. Food Chem Toxicol 2002;40:1231–4. https://doi.org/10.1016/S0278-6915(02)00093-5.

[11] Beach CA, Mays DC, Guiler RC, Jacober CH, Gerber N. Inhibition of elimination of caffeine by disulfiram in normal subjects and recovering alcoholics. Clin Pharmacol Ther 1986;39:265–70. https://doi.org/10.1016/S0278-6915(02)00093-5.

[12] Carrillo JA, Herráiz AG, Ramos SI, Gervasini G, Vizcaíno S, Benítez J. Role of the smoking-induced cytochrome P450 (CYP) 1A2 and polymorphic CYP2D6 in steady-state concentration of olanzapine. J Clin Psychopharmacol 2003;23:119–27. https://doi.org/10.1097/00004714-200304000-00003.

[13] Lane J, Steege J, Rupp S, Kuhn C. Menstrual cycle effects on caffeine elimination in the human female. Eur J Clin Pharmacol 1992;43:543–6.

[14] Polich J. Updating P300: an integrative theory of P3a and P3b. Clin Neuropsychol 2007;118:2128–48. https://doi.org/10.1016/j.clinph.2007.04.019.

[15] Kahathuduwa CN, Dassanayake TL, Amarakoon AT, Weerasinghe VS. Acute effects of theanine, caffeine and theanine-caffeine combination on attention. Nutr Neurosci 2016:1–9. https://doi.org/10.1080/1028415X.2016.1144845.

[16] Kahathuduwa C, Weerasinghe V, Dassanayake T, Amarakoon T, Binks M. High doses of theanine improve visual stimulus discrimination in a dose-dependent manner. FASEB J 2016;30 [1284.3].

[17] Kahathuduwa C, Weerasinghe V, Amarakoon T, Dassanayake T. Synergistic effect of theanine and caffeine on visual reaction time, evoked potentials and cognitive event related potentials. ACNS annual meeting and courses February 3-8, 2015 JW Marriott Houston Houston, Texas; 2015. https://doi.org/10.1097/WNP.0000000000000175.

[18] Higashiyama A, Htay HH, Ozeki M, Juneja LR, Kapoor MP. Effects of L-theanine on attention and reaction time response. J Funct Foods 2011;3:171–8. https://doi.org/10.1016/j.jff.2011.03.009.

[19] Kawamura N, Maeda H, Nakamura J, Morita K, Nakazawa Y. Effects of caffeine on event-related potentials: comparison of oddball with single-tone paradigms. Psychiatry Clin Neurosci 1996;50:217–21. https://doi.org/10.1111/j.1440-1819.1996.tb02745.x.

[20] Deslandes AC, Veiga H, Cagy M, Piedade R, Pompeu F, Ribeiro P. Effects of caffeine on visual evoked potential (P300) and neuromotor performance. Arq Neuropsiquiatr 2004;62:385–90. https://doi.org/10.1590/S0004-282X2004000300002.

[21] Johnson EN, Mullen KT. Color in the cortex. Human Color Vision: Springer; 2016; 189–217. https://doi.org/10.1007/978-3-319-44978-4_7.

[22] Eštočinová J, Gerfo EL, Della Libera C, Chelazzi L, Santandrea E. Augmenting distractor filtering via transcranial magnetic stimulation of the lateral occipital cortex. Cortex 2016;84:63–79. https://doi.org/10.1016/j.cortex.2016.08.012.

[23] Raichle ME, Snyder AZ. A default mode of brain function: a brief history of an evolving idea. Neuroimage 2007;37:1083–90. https://doi.org/10.1016/j.neuroimage.2007.02.041.

[24] Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 2005;102:9673–8. https://doi.org/10.1073/pnas.0504136102.

[25] Mason MF, Norton MI, Van Horn JD, Wegner DM, Grafton ST, Macrae CN. Wandering minds: the default network and stimulus-independent thought. Science 2007;315:393–5. https://doi.org/10.1126/science.1131295.

[26] van Veen V, Cohen JD, Botvinick MM, Stenger VA, Carter CS. Anterior cingulate cortex, conflict monitoring, and levels of processing. Neuroimage 2001;14:1302–8. https://doi.org/10.1006/nimg.2001.0923.

[27] Botvinick M, Nystrom LE, Fissell K, Carter CS, Cohen JD. Conflict monitoring versus selection-for-action in anterior cingulate cortex. Nature 1999;402:179–81. https://doi.org/10.1038/46035.

[28] Poldrack RA, Mumford JA, Nichols TE. Handbook of functional MRI data analysis: Cambridge University press; 2011.

[29] WHO. World Medical Association declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ 2001;79:373–4 Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2566407/pdf/11357217.pdf.

[30] Deichmann R, Gottfried JA, Hutton C, Turner R. Optimized EPI for fMRI studies of the orbitofrontal cortex. Neuroimage 2003;19:430–41. https://doi.org/10.1016/S1053-8119(03)00073-9.

[31] Rorden C, Brett M. MRIcro. Availabile from http://www.sph.sc.edu/comd/rorden/mricro.html; 2005.

[32] Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 1999;9:179–94. https://doi.org/10.1006/nimg.1998.0395.

[33] Fischl B, Salat DH, van der Kouwe AJ, Makris N, Ségonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. Neuroimage 2004;23(Suppl. 1):S69–84. https://doi.org/10.1016/j.neuroimage.2004.07.016.

[34] Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002;17:825–41. https://doi.org/10.1006/nimg.2002.1132.
[35] Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17:143–55. https://doi.org/10.1002/hbm.10062.
[36] Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Med Image Anal 2001;5:143–56. https://doi.org/10.1016/S1361-8415(01)00036-6.
[37] Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage 2001;14:1370–86. https://doi.org/10.1006/nimg.2001.0931.
[38] Siegel JS, Power JD, Dubis JW, Vogel AC, Church JA, Schlaggar BL, et al. Statistical improvements in functional magnetic resonance imaging analyses produced by censoring high-motion data points. Hum Brain Mapp 2014;35:1981–96. https://doi.org/10.1002/hbm.22307.
[39] Woolrich MW, Behrens TE, Beckmann CF, Jenkinson M, Smith SM. Multilevel linear modelling for FMRI group analysis using Bayesian inference. Neuroimage 2004;21:1732–47. https://doi.org/10.1016/j.neuroimage.2003.12.023.
[40] Woolrich M. Robust group analysis using outlier inference. Neuroimage 2008;41:286–301. https://doi.org/10.1016/j.neuroimage.2008.02.042.
[41] Beckmann CF, Jenkinson M, Smith SM. General multilevel linear modeling for group analysis in FMRI. Neuroimage 2003;20:1052–63. https://doi.org/10.1016/S1053-8119(03)00435-X.
[42] Hampshire A, Chamberlain SR, Monti MM, Duncan J, Owen AM. The role of the right inferior frontal gyrus: inhibition and attentional control. Neuroimage 2010;50:1313–9. https://doi.org/10.1016/j.neuroimage.2009.12.109.
[43] Sturm W, De Simone A, Krause B, Specht K, Hesselmann V, Radermacher I, et al. Functional anatomy of intrinsic alertness: evidencefor a fronto-parietal-thalamic-brainstem network in theright hemisphere. Neuropsychologia 1999;37:797–805. https://doi.org/10.1016/S0028-3932(98)00141-9.
[44] Paus T, Petrides M, Evans AC, Meyer E. Role of the human anterior cingulate cortex in the control of oculomotor, manual, and speech responses: a positron emission tomography study. J Neurophysiol 1993;70:453–69.
[45] Banich MT, Milham MP, Jacobson BL, Webb A, Wszalek T, Cohen NJ, et al. Attentional selection and the processing of task-irrelevant information: insights from fMRI examinations of the Stroop task. Prog Brain Res 2001;134:459–70. https://doi.org/10.1016/S0079-6123(01)34030-X.
[46] Gomez-Ramirez M, Higgins BA, Rycroft JA, Owen GN, Mahoney J, Shpaner M, et al. The deployment of intersensory selective attention: a high-density electrical mapping study of the effects of theanine. Clin Neuropharmacol 2007;30:25–38. https://doi.org/10.1097/01.WNF.0000240940.13876.17.
[47] Gomez-Ramirez M, Kelly SP, Montesi JL, Foxe JJ. The effects of L-theanine on alpha-band oscillatory brain activity during a visuo-spatial attention task. Brain Topogr 2009;22:44–51. https://doi.org/10.1007/s10548-008-0068-z.
[48] Yoto A, Motoki M, Murao S, Yokogoshi H. Effects of L-theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. J Physiol Anthropol 2012;31:28. https://doi.org/10.1186/1880-6805-31-28.
[49] Siamwala JH, Dias PM, Majumder S, Joshi MK, Sinkar VP, Banerjee G, et al. L-Theanine promotes nitric oxide production in endothelial cells through eNOS phosphorylation. J Nutr Biochem 2013;24:595–605. https://doi.org/10.1016/j.jnutbio.2012.02.016.
[50] Smallwood J, Beach E, Schooler JW, Handy TC. Going AWOL in the brain: mind wandering reduces cortical analysis of external events. J Cogn Neurosci 2008;20:458–69. https://doi.org/10.1162/jocn.2008.20037.
[51] Kelly SP, Gomez-Ramirez M, Montesi JL, Foxe JJ. L-Theanine and caffeine in combination affect human cognition as evidenced by oscillatory alpha-band activity and attention task performance. J Nutr 2008;138:1572S–7S.
[52] Mulderink TA, Gitelman DR, Mesulam MM, Parrish TB. On the use of caffeine as a contrast booster for BOLD fMRI studies. Neuroimage 2002;15:37–44. https://doi.org/10.1006/nimg.2001.0973.
[53] Yamada T, Terashima T, Okubo T, Juneja LR, Yokogoshi H. Effects of theanine, r-glutamylethylamide, on neurotransmitter release and its relationship with glutamic acid neurotransmission. Nutr Neurosci 2005;8:219–26. https://doi.org/10.1080/10284150500170799.
[54] Yokogoshi H, Kobayashi M, Mochizuki M, Terashima T. Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. Neurochem Res 1998;23:667–73. https://doi.org/10.1023/A:1022490806093.
[55] Kimura R, Murata T. Influence of alkylamides of glutamic acid and related compounds on the central nervous system. IV. Effect of theanine on adenosine 3′, 5′-monophosphate formation in rat cerebral cortex. Chem Pharm Bull 1980;28:664–6. https://doi.org/10.1248/cpb.28.664.
[56] Kimura R, Murata T. Effect of theanine on norepinephrine and serotonin levels in rat brain. Chem Pharm Bull(Tokyo) 1986;34:3053–7. https://doi.org/10.1248/cpb.34.3053.
[57] Kakuda T, Nozawa A, Unno T, Okamura N, Okai O. Inhibiting effects of theanine on caffeine stimulation evaluated by EEG in the rat. Biosci Biotechnol Biochem 2000;64:287–93. https://doi.org/10.1271/bbb.64.287.
[58] Nathan PJ, Lu K, Gray M, Oliver C. The neuropharmacology of L-theanine (N-ethyl-L-glutamine) a possible neuroprotective and cognitive enhancing agent. J Herb Pharmacother 2006;6:21–30. https://doi.org/10.1080/J157v06n02_02.
[59] Benowitz NL. Clinical pharmacology of caffeine. Annu Rev Med 1990;41:277–88.
[60] Solinas M, Ferré S, You ZB, Karcz-Kubicha M, Popoli P, Goldberg SR. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. J Neurosci 2002;22:6321–4.
[61] Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A 2016;113:7900–5. https://doi.org/10.1073/pnas.1602413113.