Research article

The combination of L-theanine and caffeine improves cognitive performance and increases subjective alertness

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The non-proteinic amino acid L-theanine and caffeine, a methylxanthine derivative, are naturally occurring ingredients in tea. The present study investigated the effect of a combination of 97 mg L-theanine and 40 mg caffeine as compared to placebo treatment on cognitive performance, alertness, blood pressure, and heart rate in a sample of young adults (n = 44). Cognitive performance, self-reported mood, blood pressure, and heart rate were measured before L-theanine and caffeine administration (*i.e.* at baseline) and 20 min and 70 min thereafter. The combination of moderate levels of L-theanine and caffeine significantly improved accuracy during task switching and self-reported alertness (both $P < 0.01$) and reduced self-reported tiredness ($P < 0.05$). There were no significant effects on other cognitive tasks, such as visual search, choice reaction times, or mental rotation. The present results suggest that 97 mg of L-theanine in combination with 40 mg of caffeine helps to focus attention during a demanding cognitive task.

Keywords: caffeine, theanine, attention, alertness

Introduction

Traditionally, tea consumption has been associated with mental clarity.[1] This effect has been attributed to the non-proteinic amino acid L-theanine and caffeine, a methylxanthine derivative, which are naturally occurring ingredients in tea. Both have been associated with behavioural and physiological effects (for a review, see Bryan[2]). The psychostimulant effects of caffeine have been frequently demonstrated (for a review, see Ruxton[3]). Specifically, caffeine has been shown to increase self-reported alertness, improve mood, and enhance psychomotor and cognitive performance.[4,5] Furthermore, studies investigating the effects of caffeine on different aspects of attention have provided support for enhanced vigilance,[6] faster reaction times,[7] and narrowing of the focus of attention.[8]

L-Theanine has been demonstrated to influence resting-state cortical activity as measured by the electroencephalogram (EEG). For example, Ito *et al.*[9] showed that 200 mg of L-theanine increased power in the alpha frequency in higher anxiety individuals (see also Song *et al.*[10]). This finding has recently been replicated and extended by Nobre *et al.*[11] who showed that the normal increase of alpha power during prolonged rest is facilitated by intake of 50 mg of L-theanine irrespective of anxiety status.

Interestingly, higher levels of resting state alpha power are thought to be associated with superior attentional capacities.[12] That is why the aforementioned resting state EEG findings have inspired two EEG studies directed at the specific effect of L-theanine on

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the brain's attentional circuitry during the execution of demanding cognitive tasks.[13,14]

Specifically, Gomez-Ramirez *et al*.[13] used an intersensory attention cueing task and found that 250 mg of L-theanine increased the difference in phasic (task-induced) alpha band activity between attending to auditory or visual stimuli whilst suppressing information from the other modality, and at the same time synchronised tonic (background) alpha activity.[15] Both increased task-induced alpha and increased background alpha synchronisation during the task indicate more efficient attentional processing. The latter finding seemingly contradicts prior studies. However, it is important to keep in mind that tonic activity was measured while participants were engaged in a highly demanding task in this study, rather than in a passive resting state. In a follow-up study, Gomez-Ramirez *et al*.[14] replicated the latter effect in a visuospatial task.

In contrast to the robust relationship between L-theanine and power in the alpha band of the EEG, relatively few beneficial effects of L-theanine in isolation have been found in studies using behavioural measures of alertness and cognitive performance.[16,17] However, L-theanine is virtually only found in tea. Therefore, theanine is commonly consumed in combination with caffeine rather than in isolation. Nevertheless, only few studies have focused on the interactive effects of L-theanine and caffeine on mood and cognitive performance, comparing their effects alone and in combination. Owen *et al*.[18] used moderate doses of 100 mg of L-theanine and 50 mg of caffeine and found that both caffeine alone and the combination of L-theanine and caffeine improved accuracy on an attention-switching task and reduced distractibility in a memory task compared to placebo. Likewise, Haskell *et al*.[16] examined the effects of 250 mg of L-theanine and 150 mg of caffeine and found a significant positive L-theanine by caffeine interaction on delayed word recognition reaction time. L-Theanine consumed in isolation was associated with reduced performance on the serial sevens task and increased headache ratings. Further investigation of the main effects showed that both caffeine alone and the combination of L-theanine and caffeine improved accuracy on a rapid information processing task and decreased self-reported mental fatigue. The combination of L-theanine and caffeine also led to faster simple reaction times and numeric working memory reaction time, improved sentence verification accuracy, increased alertness and decreased tired ratings compared to placebo while caffeine alone did not.[16]

A recent study by Kelly *et al*.[19] extended the findings of both Owen *et al*.[18] and Gomez-Ramirez *et al*.[13] by exploring whether the beneficial effects of L-theanine and caffeine on attention could also be found at lower doses (*i.e*. 100 mg L-theanine and 50 mg caffeine) using both behavioural measures and EEG. No behavioural or electrophysiological effects were observed for caffeine or L-theanine alone, but the combination of L-theanine and caffeine was shown to improve performance on a visuospatial cueing task and to modulate alpha brain activity. Both findings suggest enhanced preparatory attentional deployment as compared to the placebo condition.[20] Thus, both behavioural and neuro-imaging studies seem to indicate that L-theanine and caffeine together may have greater positive effects on cognitive performance and attention in particular than either ingredient alone. Likewise, a recent fMRI study compared a tea base with L-theanine (135 mg) in combination with caffeine (350 mg) or without the latter two (L-theanine, 6 mg; caffeine, 21 mg). The active treatment modulated activation in the executive control network,[21] including increased activation of the ventrolateral prefrontal cortex, which is involved in inhibition. Activity in the alerting/orienting network was also influenced including increased activity at the border of the dACC and supplementary motor area, precentral gyrus, the left lateral occipital cortex, superior portions of the middle frontal gyrus bilaterally, and the inferior frontal gyrus bilaterally.[22]

The aim of the present study was to investigate further the cognitive and subjective effects of L-theanine in combination with caffeine. In line with previous research, it was hypothesized that the combination of L-theanine and caffeine would improve attention especially on demanding cognitive tasks[13] such as the attention switching task. To investigate the specificity of possible findings, a choice-reaction-time, an egocentric mental-rotation task, and a visual-search task were added to the design. These tasks have been shown to be sensitive to the cognitive effects of caffeine;[8,23,24] however, most studies used higher levels than the ones found in the present study or found in regular tea for that matter. Moreover, it was hypothesized that the combination of L-theanine and caffeine would improve self-reported alertness and would reduce experienced task demands.

Subjects and methods

Participants

Forty-four (28 female) young adults aged between 18–34 years (mean 21.2 years; SD, 3.2 years) took part in the study. Their mean habitual daily caffeine

consumption was 173.0 mg (SD 86.8 mg). Nearly half this caffeine intake was from tea (mean, 79.4 mg/day; SD, 51.7 mg/day). Estimated mean daily L-theanine intake for this sample was 39.7 mg (SD, 25.9 mg). Participants were all students at the University of Bristol, recruited through print and/or email advertisements. They were rewarded with course credit points, a combination of course credit points and money, or a monetary reward. The study was described as an investigation into the effects of a novel tea-based soft drink on attention and mood. The protocol for this study was approved by the University of Bristol, Faculty of Science Human Research Ethics Committee, and all participants provided informed consent.

Inclusion criteria were regular caffeinated tea/coffee consumption (i.e. at least five cups per week), non-smoking, regular breakfast consumption, normal or corrected-to-normal vision, and a body mass index (BMI) between 20–30 kg/m^2. Exclusion criteria were allergies to caffeine, artificial sweeteners or herbal supplements, colour blindness, dyslexia, pregnancy, breast-feeding, or medication use with the exception of the contraceptive pill. Participants reported that they were in good health and free from recreational drugs.

Cognitive tasks

Choice-reaction-time task

This task was based on the two-choice reaction time task developed by Eriksen and Eriksen[25] (see also Broadbent *et al*.[26]). On each trial, three warning crosses were presented on the monitor for 500 ms and then replaced by a target letter (A or B). This target was either presented alone or accompanied by distracter stimuli on both sides. The distracters were stars, letters the same as the target or letters different to the target letter that were positioned either near or far from the target. Participants were required to indicate as quickly and accurately as possible whether the target was an A or B by means of a key press. Ten continuous blocks of 16 trials were completed. Trials were self-paced. The order of these trials was randomized. The dependent variables were mean reaction time and accuracy. A practice block of 32 trials was completed during the training session.

Visual-search task

This task was based on the feature search task developed by Treisman and Gelade.[27] On each trial, participants were required to detect the presence or absence of a target arrow (upward facing arrow) amongst a visual display of 16 arrows. The distracter arrows either all faced the same direction or different directions. An upward arrow was present on some trials and absent on others. Participants were required to indicate the presence or absence of the target stimulus as quickly and accurately as possible by key press. Stimuli were presented for 1200 ms and trials were terminated on response. Trials were spaced 300 ms from each other. 320 trials were completed. Reaction times and correct responses were measured. A practice block of 40 trials was completed during the training session.

Egocentric mental-rotation task

This task was based on the mental own-body transformation task developed by Parsons[28] and Zacks *et al*.[29] Left–right judgments about the position of a target shape (black square) located on the hand or foot of a schematic human figure were required after having imagined being in the body position of the figure. A number of possible shapes were located on the body, including a black square (BS), black circle (BC), white circle (WC) and/or white square (WS). There were 5 different shape combinations, three of which required a left/right response (*i.e*. BS–WS, BS–WC, BS–WS) and two that required no response (*i.e*. BC–WC, BC–WS). The schematic figure could be facing to the front or backwards, the right way up or upside down. On each trial, a fixation cross was presented for 300 ms followed by the stimulus, which was presented for 2000 ms being terminated on response. Next, they were presented with a blank screen for 1000 ms during which participants could still respond. Two continuous blocks of 96 trials presented in randomized order were completed. Reaction times and correct responses were quantified. A practice block of 42 trials was completed during the training session.

Attention-switching task

This task was based on the methods described by Rogers and Monsell.[30] On each trial, a pair of characters consisting of a letter and a number was presented on the screen simultaneously. The letters used were drawn from a set of letters containing four vowels (A, E, I, U) and four consonants (G, K, M, R) and the numbers were drawn from a set containing four even numbers (2, 4, 6, 8) and four odd numbers (3, 5, 7, 9). The letter and number were drawn at random with the only constraint being that neither the letter nor the number was repeated with respect to the previous trial. Numbers and letters were presented 1° to the left or right of the central fixation point. Each set of character pairs was displayed on the screen for 1000 ms. Stimuli were coloured and alternated

between three red and three purple sets. Participants were instructed to respond by pressing the space bar on the keyboard as quickly as possible to even numbers only if the stimulus colour was purple and respond to vowels only if stimuli were red. Participants completed four blocks of 144 trials per session. Performance was evaluated in terms of reaction times and hits. Four practice blocks of 42 trials were completed during the training session.

Self-report measure

Task demand rating scale

This scale was used in previous research on caffeine.[8] On completing the test battery, participants rated how difficult, effortful, and tiring they experienced the tasks on 9-point scales (anchors: 1, not at all and 9, extremely).

Bond–Lader visual analogue mood scale[31]

This mood scale comprises 16 bipolar items. Each item consisted of an adjective pair (*e.g*. tense/relaxed) which serve as anchors for a 100 mm visual-analogue (VAS) scale on which participants are required to indicted how they feel. Three factors can be derived from the individual scores: alertness, calmness and contentedness.

Cognitive tasks and self-report measures were completed in the order in which they are presented and took about 30 min to complete.

Physiological measures

Systolic and diastolic blood pressure (BP) and heart rate (HR) were measured using the Omron 711 Intellisense Blood Pressure Monitor (Omron Healthcare, West Sussex, UK). BP and HR served as physiological measures of arousal. Participants had been seated for 5 min before the measurements were taken. As a minimum of two readings is required to obtain a reliable estimate of BP,[32] three readings were taken at each time point. The average of these readings was used in the statistical analysis.

Design

The study employed a randomized, placebo-controlled, double-blind, within-subjects design, in which participants received a drink with L-theanine and caffeine on one occasion and a matched placebo drink on the other occasion (treatment order was counterbalanced between participants). Participants attend a practice session to familiarize themselves with the test battery and to limit the influence of trainings effects. Experimental sessions were conducted at least 6 days but no more than 14 days apart. The order of conditions was balanced.

Treatment

The experimental treatment consisted of 40 mg of caffeine and 97 mg of L-theanine in a tea-based soft drink. This drink was made by dissolving powder containing tea, sweeteners, and lemon flavour into 500 ml mineral water (powders supplied by Lipton Institute of Tea, Unilever R&D, Colworth, UK). The iced tea powder contained colourings and tea flavourings, as well as sweeteners (sucralose) and lemon flavour to mask the taste of caffeine. The placebo drink matched the active drink except for the absence of L-theanine and caffeine. No interactions between the ingredients, which are present in the ice tea powder and L-theanine and/or caffeine, are expected. Participants consumed the drinks within 10 min.

L-Theanine is nearly universally consumed in combination with caffeine with a regular cup of tea containing 35–61 mg of caffeine and 4.5–22.5 mg of L-theanine. As we were specifically interested in the role L-theanine and in line with prior studies,[18,33] we increased the amount of theanine relative to caffeine to amplify possible effects of L-theanine.

Procedure

On each test day, participants arrived at 9 am having abstained from caffeine, L-theanine and alcohol for a minimum of 12 h. Participants were tested in groups with the experimenter present and each participant being visually isolated from the others. Participants were not allowed to consume anything other than the test drinks until completion of the test day. First, BP and HR were measured and participants completed all cognitive tasks and questionnaires (*i.e*. baseline measurement; duration 30 min). Second, participants consumed the drink within 10 min, which was followed by a 10 min break. Third, participants completed all tasks and questionnaires again and HR and BP were measured starting at 20 min post-drink. After 20 min break, BP and HR were measured and all tasks and questionnaires were completed for a third time, followed by a BP and HR measurement (*i.e*. starting at 70 min post-drink). On completion of the study, participants were thanked for their participation and debriefed.

Statistical analysis

Changes from pre-treatment baseline in blood pressure and heart rate, cognitive performance, and subjective measures were analyzed using an analysis of variance-based mixed model, with subjects modelled

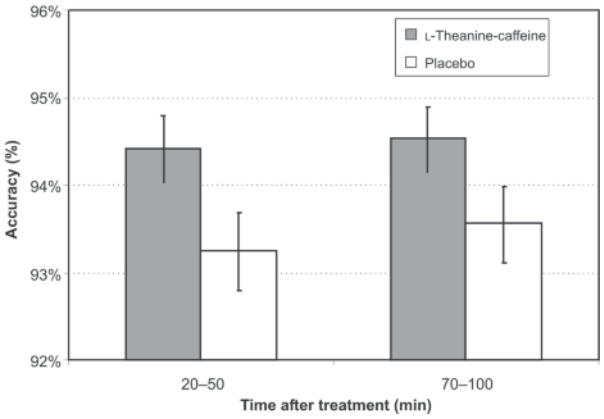

Figure 1 Accuracy on the attention switching task (mean ± SE). Main effect of drink ($P < 0.01$)

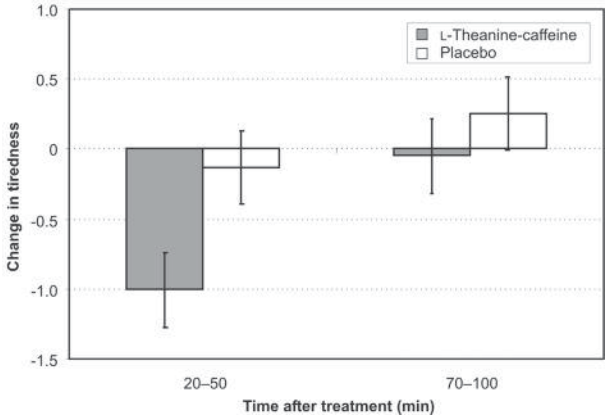

Figure 2 Change in self-reported tiredness on the task demand scale (mean ± SE). Main effect of drink ($P < 0.05$)

as a random effect and a repeated-measures co-variance structure to accommodate likely correlation between subsequent assessments taken from the same individual with drink (experimental vs placebo) and time (20 min post-drink vs 70 min post-drink) as within-subjects factors. The proportion of correct responses on the attention switching task was analyzed with a logistic regression-based generalized linear mixed model, with aforementioned within-subject factors. For the switch task, trial type (1st, 2nd, 3rd trial after a switch between task sets) and response type (correct hit, correct withhold) were used as additional within-subject factors. For all tasks, baseline scores were employed as co-variates and order of testing was entered as factor. Standard error and denominator degrees of freedom were estimated by the Kenward–Roger method. Statistical tests were all two-sided with alpha = 0.05. All analyses were conducted using the SAS statistical package.

Results

Cognitive performance

Attention-switching task

A main effect was found for L-theanine in combination with caffeine significantly improving accuracy as compared to placebo (see Fig. 1; $F_{(1,44.73)} = 13.55$; $P < 0.01$). Interactions of treatment with switch type and response type were not statistically significant (all $F < 1$, all $P > 0.1$). Importantly, improved accuracy was not the result of a trade-off between speed and accuracy, as there was no evidence for a main or interaction effect of treatment on reaction time (main effect – $F_{(1,469)} = 0.54$, $P > 0.05$; interaction – $F_{(2,469)} = 2.63$, $P > 0.05$). To investigate whether our findings are likely to be fuelled by the relief of caffeine withdrawal, we re-ran the statistical analysis, while including either habitual caffeine or tea consumption in the model. The critical interactions between treatment and habitual caffeine or habitual tea consumption or any higher order interactions including both terms were non-significant (all $F < 1$, all $P > 0.1$).

Choice-reaction-time task

There was no evidence of a main or interaction effect of treatment on reaction time (all $F < 2.4$, all $P > 0.12$). However, there was a significant drink by time interaction for accuracy ($F_{(1,86.18)} = 4.94$; $P < 0.04$) indicating a switch in relative accuracy in the two conditions between sessions. This interaction is, however, qualified by the fact that post-hoc tests did not reveal any differences between conditions ($P > 0.05$).

Visual-search task and egocentric mental-rotation task

For the visual-search task and the egocentric mental-rotation task, reaction times and accuracy were not affected by treatment (all $F < 1.59$, all $P > 0.21$).

Self-report measures

Task demand

No main or interactive treatment effects emerged for the effort and difficult subscales of self-reported task demand (all $F < 1.98$, all $P > 0.16$). However, self-reported tiredness increased during the course of the test day as evidenced by a main effect of time ($F_{(1,86)} = 13.27$; $P < 0.01$). Moreover, the experimental manipulation significantly reduced self-reported tiredness as compared to placebo as demonstrated by a main effect of treatment ($F_{(1,38.8)} = 5.13$; $P < 0.05$; see Fig. 2).

Bond–Lader visual analogue mood scales

A main effect emerged for experimental treatment significantly increasing self-reported alertness as compared to placebo ($F(1,36.5)$ = 10.3; $P < 0.01$; see Fig. 3). There was no evidence for a treatment-related main or interaction effect self-reported calmness or contentedness (all $F < 1$, all $P > 0.39$).

Physiological measures

Table 1 presents mean changes in blood pressure and heart rate for both treatments and time point separately. A main effect of treatment on systolic blood pressure (SBP) was found with SBP being raised by the treatment as compared to placebo treatment ($F_{(1,41.4)} = 8.08$; $P < 0.01$). A similar trend into the same direction toward a main effect of treatment on diastolic blood pressure (DBP) was also found ($F_{(1,36.7)} = 2.93$; $P = 0.096$). Moreover, significant drink by time interaction was found for HR ($F_{(1,86)} = 4.10$; $P < 0.05$). This interaction is due to a reversal in HR change between the two conditions between time points. This interaction is, however, qualified by the fact that post-hoc tests did not reveal any differences between conditions ($P > 0.05$).

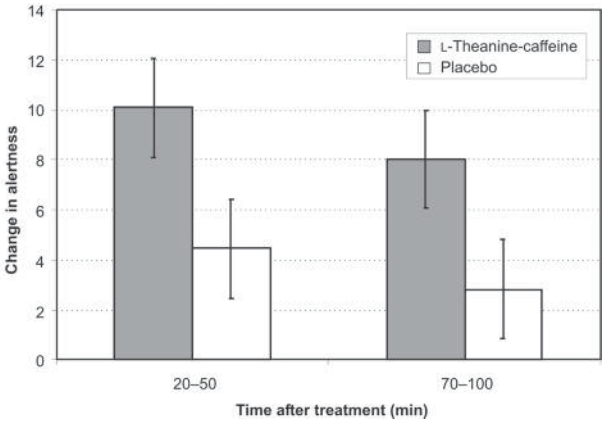

Figure 3 Change in self-reported alertness on the Bond–Lader visual analogue mood scales (mean ± SE). Main effect of drink ($P < 0.01$)

Discussion

The main findings of the present study can be summarized as follows. First, the combination of moderate levels of L-theanine and caffeine improved task switching accuracy as compared to placebo treatment in the context of comparable response speed. This effect was specific in that other cognitive tasks, such as visual search, choice reaction times, and mental rotation were not affected. Second, the combination of L-theanine and caffeine also increased self-reported alertness and reduced task-induced fatigue. Third, this increase in alertness was accompanied by a slight increase in systolic blood pressure, but not, to a demonstrable extent, in diastolic blood pressure or heart rate.

Task switching performance accuracy was specifically improved by our treatment. Importantly, response speed was not negatively affected by our treatment demonstrating that our findings cannot be accounted for by a speed–accuracy trade-off. Other cognitive tests, such as choice reaction times, remained unaffected. This specificity agrees with the findings of Owen *et al.*,[18] who showed a specific beneficial effect of the combination of L-theanine and caffeine on task switching and critical flicker fusion threshold, but not on rapid visual information processing or word recognition. It also corresponds with those of Kelly *et al.*,[19] who demonstrated positive effects of a combination of 100 mg L-theanine and 50 mg caffeine on performance on a visuospatial cueing task. On the other hand, the lack of improvements in response speed is in contrast with findings of Haskell *et al.*[16] Together, this suggests that the effects of the L-theanine and caffeine combination are most prominent in the attention domain, and that L-theanine and caffeine combined effects are best demonstrated with relatively challenging tests of attention. We would like to note that this is in sharp contrast to findings on the cognitive effects of caffeine only, where one consistently finds increased

Table 1 Changes in systolic and diastolic blood pressure and heart rate

Treatment	Time after treatment (min)	Systolic blood pressure Mean	SE	Diastolic blood pressure Mean	SE	Heart rate Mean	SE
L-Theanine-caffeine							
	20	1.78	0.83	2.42	0.62	–14.11	0.90
	70	1.31	0.83	2.32	0.62	–12.63	0.90
Placebo							
	20	–1.16	0.83	1.33	0.62	–13.17	0.90
	70	–0.90	0.83	1.20	0.62	–13.30	0.90

Common variance across treatment groups, resulting in identical standard errors, is assumed by the model fitted.

performance after caffeine on simple,[34] but not complex tasks.[4,35] Nevertheless, the current study did not include a treatment condition with caffeine only. Therefore, we cannot completely rule out the possibility that our pattern of findings could also be obtained from caffeine administered in isolation.

In line with findings of improved cognitive performance in terms of task switching performance, self-reported alertness was also increased and perceived fatigue was reduced by the combination of L-theanine and caffeine. This finding is in line with Haskell *et al*.,[16] who found increased alertness and decreased tiredness following a combination of 250 mg L-theanine and 150 mg caffeine. A similar effect was also evident in a study by Owen *et al*.,[18] who used more moderate doses of 100 mg of L-theanine and 50 mg of caffeine.

Nevertheless, some authors argue that the psychostimulant effects of caffeine actually represent reversal of withdrawal effects.[36,37] This interpretation of caffeine effects stems from the fact that most studies investigate habitual consumers who are asked to abstain from caffeine for a given time. To test the merits of this assumption regarding the effects of caffeine, Haskell *et al*.[7] compared habitual caffeine consumers to non-consumers after overnight caffeine withdrawal. The idea is that, if alleviation of withdrawal symptoms underlies the psychostimulant effects of caffeine, habitual consumers would be most likely to show beneficial effects on cognitive tasks. However, in contrast to this prediction, performance in terms of faster reaction times was increased to the same extent in both habitual consumers and non-consumers. Thus, in general, it seems that psychostimulant effects of caffeine cannot be attributed solely to the reversal of withdrawal effects (see also Christopher *et al*.[38]). More specifically, in the context of the present study, no benefit was apparent for cognitive tasks other than the switch task. If effects were fuelled by withdrawal, one would be expected positive findings on other tasks, too. Moreover, participants with higher habitual levels of caffeine consumption did not appear to be specifically affected by withdrawal symptoms, as evidenced by the absence of a moderating effect of caffeine consumption on task performance. Taken together, the specific pattern of findings is difficult to explain as the result of caffeine withdrawal.

The drink with theanine and caffeine also slightly increased systolic blood pressure as compared to the control drink. The present blood pressure findings (*i.e*. increase of 2.94 mmHg and 2.20 mmHg as compared to placebo at 20–50 min and 70–100 min, respectively) are of slightly smaller magnitude than the ones reported by Quinlan *et al*.,[39] who found that both one cup of tea or coffee significantly increased systolic blood pressure by 4.1–4.5 mmHg as compared to hot water 10–60 min after ingestion. Of course, caffeine is a stimulant and well-known to lead to an acute increase in blood pressure even at dietary levels.[40] In contrast, L-theanine has been demonstrated to lower blood pressure in rats[41,42] and to counteract the blood-pressure-increasing effects of caffeine at high doses in humans (200 mg L-theanine and 250 mg caffeine).[17] However, due to the fact that the present study did not include a caffeine-only condition, it is impossible to discern whether the seemingly smaller blood pressure effects in the present study are due to the addition of theanine. Moreover, despite the acute effect of tea on blood pressure, meta-analyses suggest that long-term intake of black or green tea does not increase blood pressure chronically,[43] and may even reduce stroke incidence.[44]

Two limitations of the present study deserve discussion. First, in the context of the present study, we assumed an interaction between L-theanine and caffeine based on previous studies[16–19] and chose not to include a L-theanine- and caffeine-only condition. Therefore, the design of the current study precludes attribution of the effects to L-theanine or caffeine and conclusions about the interaction between the two ingredients *per se*. Moreover, the treatment contained higher levels of L-theanine, in combination with the same amount caffeine which is naturally present in tea to amplify possible effects of L-theanine. In order to extend the current conclusions to tea, future studies should employ a full dose-range effect of L-theanine and caffeine.

Conclusions

The present study clearly demonstrates attentional benefits after consumption of 97 mg L-theanine together with 40 mg caffeine Moreover, L-theanine and caffeine increased self-reported alertness and decreased feelings of tiredness. Taken together with previous studies, these findings indicate that L-theanine in combination with caffeine may help to focus attention during cognitively demanding tasks.

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