The combined effects of L-theanine and caffeine on cognitive performance and mood

Gail N. Owen[1], Holly Parnell[1], Eveline A. De Bruin[2], Jane A. Rycroft[1]

[1]Unilever Research and Development, Colworth House, Sharnbrook, Bedford, UK
[2]Unilever Food and Health Research Institute, Unilever R&D Vlaardingen, The Netherlands

The aim of this study was to compare 50 mg caffeine, with and without 100 mg L-theanine, on cognition and mood in healthy volunteers. The effects of these treatments on word recognition, rapid visual information processing, critical flicker fusion threshold, attention switching and mood were compared to placebo in 27 participants. Performance was measured at baseline and again 60 min and 90 min after each treatment (separated by a 7-day washout). Caffeine improved subjective alertness at 60 min and accuracy on the attention-switching task at 90 min. The L-theanine and caffeine combination improved both speed and accuracy of performance of the attention-switching task at 60 min, and reduced susceptibility to distracting information in the memory task at both 60 min and 90 min. These results replicate previous evidence which suggests that L-theanine and caffeine in combination are beneficial for improving performance on cognitively demanding tasks.

Keywords: L-theanine, caffeine, cognitive performance, mood

Introduction

Black tea is traditionally associated with relaxation and refreshment. The active components in tea associated with this revitalising effect are caffeine and L-theanine. Caffeine is a well-known stimulant that makes up 2–5% of the dry weight of tea leaves; a typical serving of black tea contains around 40 mg caffeine depending on the blend and brewing procedure.[1] Peak plasma concentrations are attained 30–120 min after consumption.[2] Caffeine crosses easily through the blood–brain barrier and, once in the brain, it indirectly increases neurotransmitter activity by blocking the inhibitory actions of the neuromodulator adenosine.[1]

Improvements in cognitive function, and in attention specifically, have been shown at doses equal to and lower than the amount of caffeine typically found in a single serving of tea.[3,4] Caffeine is also associated with changes in subjective state, including increased feelings of well-being, energy, motivation, self confidence, alertness and concentration.[5] Direct comparisons of coffee and tea containing the same dose of caffeine indicate that both produce similar alerting effects.[6] However, tea produces more consistent levels of arousal during the day and is less likely to subsequently disrupt sleep.[7]

Theanine (γ-*N*-ethylglutamine) is one of the predominant amino acids found in species of the tea plant *Camellia* and constitutes 1–2% of the dry weight of tea,[8–10] which corresponds to up to 60 mg per 200-ml serving. Within tea, the predominant form of theanine is the L isomer (around 98% of total theanine).

L-theanine crosses the blood–brain barrier within 30 min and increases activity in the alpha frequency band of the electroencephalogram (EEG) not only at a high dose (200 mg)[11,12] but also at a lower dose (50 mg),[13] suggesting that L-theanine even in low amounts (such as present in 2–3 cups of tea) contributes to a relaxed, yet alert, feeling at rest. Furthermore, a recent study has shown that L-theanine improves attention-related

Correspondence to: Eveline A. de Bruin, Unilever Food and Health Research Institute, PO Box 114, 3130 AC Vlaardingen, The Netherlands.
Tel: +31 10 460 5831; Fax: +31 10 460 5794;
E-mail: Eveline-de.Bruin@unilever.com
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anticipatory alpha during an attention task, indicating improvement of the ability to focus attention when mentally active.[14]

Despite the common co-consumption of caffeine and L-theanine in tea, there is virtually no research that explicitly examines the interaction of the two substances. In a recent study, a combined dose of 150 mg caffeine and 250 mg L-theanine significantly improved simple reaction time but also speed of numeric working memory and word recognition, and accuracy of sentence verification in the absence of any effect of caffeine or L-theanine alone.[15] Furthermore, alertness and tiredness were also improved by L-theanine and caffeine in combination. In another study investigating EEG during the execution of an attention task, 50 mg caffeine and 100 mg L-theanine in combination desynchronised tonic alpha and improved accuracy at the behavioural level, whereas caffeine or L-theanine alone did not.[16] Additionally, the caffeine+L-theanine combination reversed a reduction in the alpha attention effect seen after ingestion of caffeine alone.

The aim of the current study was to explore further the combined effects of L-theanine and caffeine on cognitive function. As caffeine and L-theanine are usually consumed together in tea, the vehicle used for administration was a tea-based soft drink (iced tea). The combined effects of L-theanine+caffeine, and caffeine alone were compared to placebo using tests of attention, arousal, memory and mood.

Subjects and methods

Participants

The ethics committee at Unilever Research and Development, Colworth House, Bedford, UK approved the study and participants gave written informed consent. A power calculation based on the results of a simple reaction-time task in a pilot study (unpublished data) suggested that a study of 27 participants should have sufficient power to detect a difference in change in mean reaction time from baseline of ~20 ms between treatments with 80% certainty at a significance level of 5%. Therefore, a total of 27 participants (13 females, 14 males) were recruited from employees at Unilever. They had a mean age of 28.3 ± 5.34 years and a mean body mass index (BMI) of 25.8 ± 4.65 kg/m^2. All reported being free of neurological disorders, were in general good health, and had an average State-Trait Anxiety Inventory (STAI) score of 35.4 ± 5.74. All participants were regular caffeine consumers (9.6 ± 5.51 servings of caffeine per week). Additionally, they were free from over-the-counter or prescribed medication (with the exception of the contraceptive pill).

Treatment

L-theanine and caffeine solutions were prepared by adding 100 mg L-theanine and/or 35 mg caffeine to 250 ml Lipton Iced Tea (LIT). LIT contains 15 mg caffeine from tea powder (thus total caffeine was 50 mg/serving). L-theanine was administered in a synthetic form of pure L-theanine known as Suntheanine™ (Taiyo Kagaku Co. Ltd, Yokkaichi, Japan), an odourless, white, crystal powder with a slightly sweet taste. L-theanine and caffeine were added immediately before serving. The placebo solution was 250 ml LIT with tea powder (including 15 mg caffeine) removed.

Design

A cross-over, double-blind, randomised, placebo-controlled design was used. Participants completed a series of three test sessions and consumed one of the three drinks during each session – placebo, caffeine, L-theanine+caffeine. Treatments were allocated using a Latin square design such that the order of treatments was counterbalanced across participants. Sessions were separated by at least 7 days. Each session was standardised according to the procedures below and lasted approximately 2 h.

Procedures

Prior to the start of the study, participants were given the opportunity to familiarise themselves with the tests of cognition and mood to be used during the study. On the day of the experiment, they arrived at the laboratory having consumed their normal breakfast and having abstained, from 9 p.m. the previous night, from caffeinated and alcoholic beverages, caffeinated foods and medications, and mushrooms (the edible Bay bolete contains L-theanine). On arrival, they completed baseline tests of mood and cognitive performance in the order shown below. Following completion of the baseline test battery, they were given 5 min in which to consume the test drink. Further test batteries were complete 60 min and 90 min from the end of the 5-min ingestion period. When the participants were not being tested, they were allowed to read quietly.

Test battery

The test battery consisted of a number of tests of mood and cognition. The choice of tests was based on the study by Haskell *et al*.[15] and a pilot study (unpublished results). The tests took about 14 min to complete in total and were always performed in the

same order. They are described below in the order in which they appeared in the test battery.

Word recognition

At the start of the test battery, 15 words matched for frequency and concreteness were presented in sequence for 1 s each with a 1-s ISI. At the end of the test battery, these original words (old words) plus 15 distracter words (new words) were presented, one at time, in randomised order. For each word, the participant had to indicate whether it was in the original list by pressing a designated key as quickly as possible. Mean reaction times and accuracy were recorded. The total duration of word presentation was 30 s and the duration of the recognition test was 1 min.

Rapid visual information processing (RVIP)

A single digit (between 1 and 9) was presented in the centre of a computer screen in a continuous stream. Each digit was displayed for 600 ms with no interval between stimuli. The participant was required to press a designated key as soon as possible following a sequence of either three consecutive odd or even numbers. The task continued for 4 min and a target occurred on 12.5% presentations. Mean reaction times, the number of correct responses, missed targets and false alarms were recorded.

Critical flicker fusion test (CFFT)

Participants were required to discriminate flicker from fusion, and *vice versa*, in a set of light-emitting diodes. The diodes were held at a constant fixation distance. Individual thresholds are determined by three ascending (flicker to fusion) and three descending (fusion to flicker) scales. The mean of these three ascending and descending presentations gives the threshold frequency in Hertz. The duration of the task was 2 min.

Attention switching

Participants were presented simultaneously with a letter (A, E, G, I, K, M, R or U) and number (2, 3, 4, 5, 6, 7, 8 or 9) one either side of, and at a set distance from, a central fixation. The position of the letter and number varied randomly between trials but with equal probability that either could occur to the left or right of the fixation point. The number and letter were chosen at random with the only constraint being that neither had appeared in the preceding trial.

The stimuli appeared together for 1 s in either a red or purple font. The colour of the font changed in a fixed sequence every fourth presentation (*i.e.* three red followed by three purple and so forth). As in most task-switching tests, the participant was required to perform a different task depending on the characteristics of the stimulus pair for each trial. When the letter and number appeared in red, the participant was required to attend to the letter and press a designated key only if the letter was a vowel. When the two items appeared in purple, participants responded only if the number was even. Despite the predictable pattern of task switching, performance of such tasks is typically poorer on trials when a switch is required compared to performance when no switch is required.[17,18]

In the current study, the test duration was 5 min and average response time, number of correct responses, number of missed targets and numbers of false alarms were recorded. Performance for the trials requiring a switch (*i.e.* first stimulus pair to appear in either red or purple following three successive stimuli in the alternative colour) were also analysed separately.

Bond–Lader mood questionnaire

Mood state was measured using the Bond–Lader analogue mood rating scale.[19] The scale comprises 16 visual analogue scales (VAS) anchored by adjective pairs (*e.g.* tense/relaxed). Participants place a vertical mark on the VAS to represent how they are feeling in relation to the dimension defined by the adjectives. By combining scores for each adjective pair, it is possible to measure three distinct mood factors – alertness, contentedness, and calmness.

Data analysis

Scores for cognitive performance and mood at 60 min and 90 min post-ingestion were calculated as change from baseline. The data were subjected to a mixed model analysis of variance with 'participant' as a random effect and 'treatment', 'gender', 'BMI' and 'STAI anxiety score' as fixed effects. Omnibus tests of the main effects of treatment were followed by planned comparisons, using Tukey–Kramer tests to assess the efficacy of the individual treatments in comparison with the placebo.

Results

Word recognition

There was no evidence of a treatment effect for response time for either new or old words. Average response time did decrease significantly between 60 min and 90 min, but this occurred across all treatments.

There were no treatment effects for the number of correctly identified original (old) words, but there was

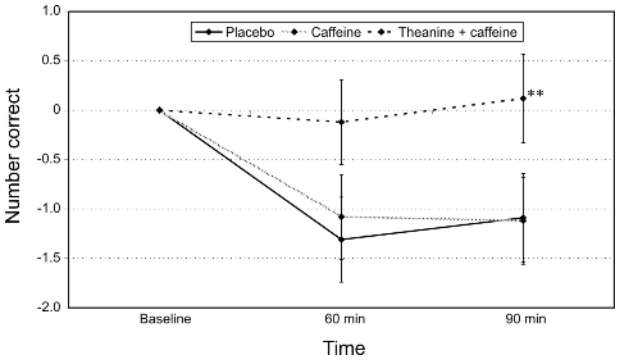

Figure 1 Change in number of correctly identified 'new' words in the word recognition task

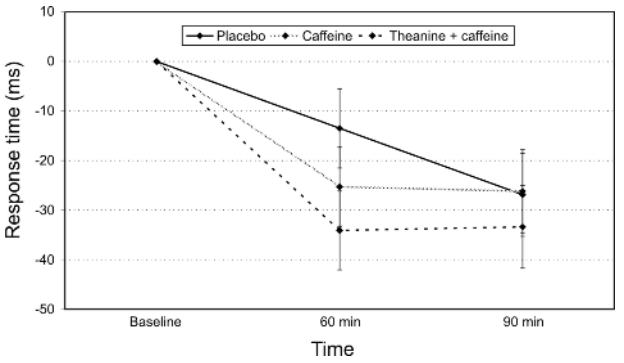

Figure 2 Change in response time in the attention-switching task

a main effect of treatment for the number of correctly identified new words ($F(2,108) = 6.14$; $P < 0.01$). In the L-theanine+caffeine condition only, the number of correctly identified words was greater at 60 min and 90 min than at baseline (Fig. 1).

Rapid visual information processing (RVIP)

There were no significant treatment effects for this task. The number of false alarms decreased at 60 min and 90 min but there were no significant differences between treatments.

Critical flicker fusion test (CFFT)

There was a significant decrease in arousal between 60 min and 90 min across all treatments. Although there were no significant treatment effects, the decrease in arousal at 60 min appeared to be slightly reduced in both the caffeine and the L-theanine+caffeine conditions.

Attention switching

There was a decrease in response time in all conditions that may be indicative of a practice effect (Fig. 2). There were no treatment effects although response times were faster in both the L-theanine+caffeine and caffeine conditions at 60 min but not 90 min.

There was a treatment by time interaction for the number of correct responses ($F(2,115) = 49.34$; $P < 0.001$). A direct comparison of L-theanine+caffeine and caffeine showed that the number correct increased significantly in the L-theanine+caffeine condition after 60 min ($P < 0.001$), and at 90 min for the caffeine only condition ($P < 0.01$; Fig. 3).

The response time for trials requiring a switch followed the same pattern as that seen for overall performance of this task and the response time decreased relative to baseline in all conditions. Although there were no significant treatment effects, response times were fastest in the L-theanine+caffeine condition.

Bond–Lader mood questionnaire

There was a main effect of treatment for the composite alertness ($F(2, 120) = 4.08$; $P < 0.05$). Alertness increased in the caffeine condition relative to placebo ($P < 0.01$). L-theanine+caffeine also appeared to increase alertness compared to placebo, but this was not

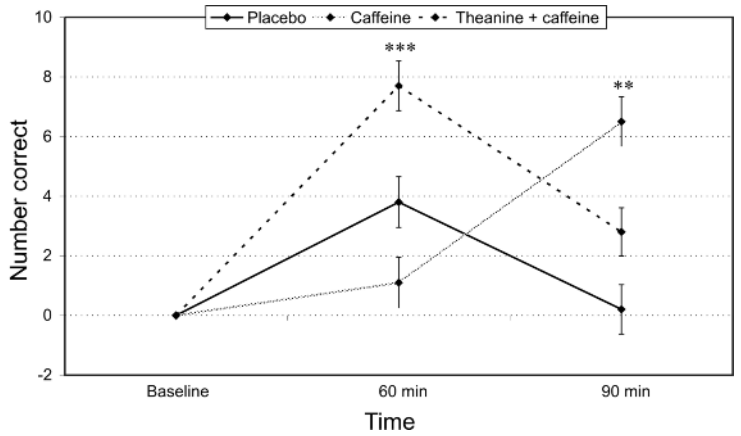

Figure 3 Change in the number of correct responses in the attention-switching task

statistically significant. There was a decrease in calmness between 60 min and 90 min post-ingestion, but this was common to all treatments. There were no treatment or co-variate effects for the contentedness scale.

Discussion

Both the caffeine and L-theanine+caffeine treatments produced significant changes in performance relative to placebo. After caffeine ingestion, response speed and accuracy improved in the attention-switching task, at 60 min and 90 min, respectively. The L-theanine and caffeine combination also produced faster responses and improved accuracy during this task but this occurred simultaneously after 60 min. The combined treatment also led to an increase in the number of correctly identified new words in the word recognition task. This effect was significant relative to placebo at both 60 min and 90 min post-ingestion. RVIP performance was not significantly altered by either treatment and, although caffeine increased subjective ratings of alertness at 60 min, there was no change in arousal as measured by CFFT.

The task most significantly affected by both treatments was the attention switching task. Given that this was the most cognitively demanding task of the test battery, this is not entirely unexpected. What was interesting is that although both caffeine and the L-theanine+caffeine combination improved response speed and accuracy, it was only the latter that improved them simultaneously. L-theanine is known to improve attention-related anticipatory alpha[14] and caffeine, as demonstrated in the current study, can increase alertness. Thus, the combination was associated with an improved ability to focus attention, whilst caffeine alone lead to an improvement in basic response speed or accuracy but not both.

In the word recognition task, response speed for correctly recognised words (old words) tended to improve after 60 min but returned to baseline by 90 min. This effect was not significant and did not vary across treatments. There was also no treatment effect with regard to the number of correctly identified old words. However, whilst there was a decrease in the number of correctly rejected distracter (new) words post-treatment in the placebo and caffeine conditions, there was no impairment in the L-theanine+caffeine treatment. Participants were, therefore, less susceptible to distraction following ingestion of L-theanine and caffeine in combination.

There was no effect of either treatment on RVIP although improved RVIP accuracy has been found previously for both caffeine and L-theanine+caffeine combined.[15] However, in this study, the doses used were significantly higher (150 mg caffeine, 250 mg L-theanine) than those in the current study. The current absence of effects on RVIP in combination with the tendency for both caffeine and L-theanine+caffeine to protect against the decline in arousal (as measured by CFFT) seen in the placebo condition suggest that the current study may have been underpowered for demonstrating these effects.

Both caffeine and L-theanine+caffeine increased subjective alertness relative to placebo, although the latter was non-significant. Improvements in objectively measured attention without concurrent changes in subjective alertness are common and have been reported for other studies as well (see, for example, Hindmarch *et al.*[7] and Lieberman *et al.*[20]). In the current study, this may be explained by the 'dampening' effect of L-theanine on caffeine as previously suggested. For example, one study showed an inhibitory effect of L-theanine on caffeine stimulation as evaluated by EEG in the rat when the dose of L-theanine was five times that of caffeine, but not when the level of L-theanine was less than that of caffeine.[21] A human study recently found an interactive effect of a combination of caffeine and L-theanine indicating that L-theanine antagonised the caffeine-induced rise in blood pressure but had no significant effects on blood pressure when ingested alone.[22]

Conclusions

This study represents a systematic assessment of L-theanine and caffeine interactions and replicates previous work which suggests a synergy between these two tea components. In particular, L-theanine and caffeine in combination appear to improve, significantly, aspects of memory and attention to a greater extent than caffeine alone. The levels of L-theanine and caffeine used here are higher than those found in real tea beverages and also in the reverse ratio as in tea the caffeine level is typically higher than that of L-theanine (approximately 2:1). Further studies should therefore be aimed at establishing a full dose-range effect for L-theanine and caffeine in combination.

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