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The effects of L-theanine, caffeine and their combination on cognition and mood

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Abstract

L-Theanine is an amino acid found naturally in tea. Despite the common consumption of L-theanine, predominantly in combination with caffeine in the form of tea, only one study to date has examined the cognitive effects of this substance alone, and none have examined its effects when combined with caffeine. The present randomised, placebo-controlled, double-blind, balanced crossover study investigated the acute cognitive and mood effects of L-theanine (250 mg), and caffeine (150 mg), in isolation and in combination. Salivary caffeine levels were co-monitored. L-Theanine increased 'headache' ratings and decreased correct serial seven subtractions. Caffeine led to faster digit vigilance reaction time, improved Rapid Visual Information Processing (RVIP) accuracy and attenuated increases in self-reported 'mental fatigue'. In addition to improving RVIP accuracy and 'mental fatigue' ratings, the combination also led to faster simple reaction time, faster numeric working memory reaction time and improved sentence verification accuracy. 'Headache' and 'tired' ratings were reduced and 'alert' ratings increased. There was also a significant positive caffeine × L-theanine interaction on delayed word recognition reaction time. These results suggest that beverages containing L-theanine and caffeine may have a different pharmacological profile to those containing caffeine alone.

Keywords: L-Theanine; Caffeine; Tea; Cognition; Mood; Memory; Attention; Humans

1. Introduction

Behavioural effects of caffeine, particularly in the form of caffeinated coffee, have received considerable attention in the literature. Numerous studies have highlighted the beneficial effects of caffeine on cognition and mood (see Smith, 2002). The most commonly reported being increases in ratings of 'alertness' (Rogers et al., 2003), faster reaction times, and improved sustained attention (Lieberman et al., 1987; Richardson et al., 1995). A detailed description of the pharmacological actions of caffeine is beyond the scope of this paper, the mechanisms underlying these effects have, however, been discussed in depth elsewhere (for review see Fredholm et al., 1999). Although caffeine is rarely consumed in isolation, far less is known about the behavioural effects of caffeine when combined with other concomitant phytonutrients. Of particular interest are the effects of caffeine when consumed in the form of tea, given that, with the exception of water, it is the most widely consumed beverage in the world (Gilbert, 1984).

Studies that have considered effects of tea have found that, although the majority of effects can be explained by caffeine, some differences exist between tea and coffee, even when the caffeine level is matched. Quinlan et al. (1997) found that tea, containing 100 mg caffeine, had significantly greater effects than coffee with a matched caffeine level, in raising skin temperature. Hindmarch et al. (2000) also found that tea significantly increased critical flicker fusion threshold when compared with coffee, despite both beverages containing 75 mg caffeine. Coffee also significantly speeded recognition reaction time over that of tea at the same dose. Steptoe et al. (2007) considered the effects of chronic tea administration, containing 72 mg caffeine, on acute stress responses as compared to a control matched for caffeine level. They found that tea was able to reduce platelet activation and cortisol levels in response to stress, as well as increase 'relaxation' ratings relative to control.

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The finding of decreased cortisol levels is particularly interesting, as caffeine has been shown to increase cortisol levels, especially in response to stress (Lovallo et al., 2006). This suggests that components within tea were actually able to produce an opposing effect to that seen with caffeine.

A possible candidate for the modulation of caffeine effects, evinced in the form of tea, is the amino acid, L-theanine (γ-glutamylethylamide). Animal studies have shown that L-theanine is able to inhibit the stimulation produced by caffeine, as evaluated by EEG (Kakuda et al., 2000), and measures of spontaneous activity (Kimura and Murata, 1971). In line with these findings, L-theanine has been used historically as a relaxing agent. However, its pharmacology is relatively unknown and human research in this area is by no means conclusive. Lu et al. (2004) studied the subjective mood effects of L-theanine and found that 200 mg L-theanine was able to increase 'tranquil' ratings, as measured by the 'tranquil-troubled' item of the Bond–Lader visual analogue scales (Bond and Lader, 1974). However, this finding was only evident in rested participants and was not replicated when participants were under conditions of increased anxiety. Conversely, Kimura et al. (2007) found reduced heart rate and salivary IgA responses to acute stress following 200 mg L-theanine. In addition, 'state anxiety' (Spielberger, 1977) and subjective perceived 'stress' ratings were also reduced following L-theanine as compared to placebo. Similarly, Kobayashi et al. (1998) found that 200 mg L-theanine, but not 50 mg, led to increased alpha (α) waves when administered to resting participants, which the authors suggest is indicative of relaxation without drowsiness. However, Gomez-Ramirez et al. (2007) found evidence of a decrease in alpha activity following 250 mg L-theanine when measured during performance of a highly demanding attention task. This study also represents the only investigation to date of cognitive effects of L-theanine in humans, and this decrease in alpha activity was associated with a slowing of reaction time on an auditory attention task.

Clearly further research is needed to explore the cognitive effects of L-theanine and to clarify some of the mood effects. Given the evidence from animal studies for an interaction between caffeine and L-theanine, and the fact that the vast majority of L-theanine consumption is in the form of tea, it is important to assess the effects of L-theanine alone and in combination with caffeine. The current randomised, placebo-controlled, double-blind, balanced crossover study investigated the cognitive and mood effects of administration of the two agents both alone [L-theanine (250 mg), caffeine (150 mg)] and in combination (250 mg/150 mg). The tasks selected included attentional tasks as well as specific semantic memory and semantic reasoning tasks, which are known to be sensitive to caffeine (e.g. Haskell et al., 2005; Warburton, 1995; Smith et al., 1994, 1999). Additional secondary memory tasks were also included, which although not typically sensitive to caffeine, have been shown to be improved by caffeine when combined in the form of guaraná (Haskell et al., 2007). Furthermore, this extensive range of tasks also allowed a cognitive profile for L-theanine to be produced, which is essential given the lack of evidence with regards the effects of this substance.

2. Methods

2.1. Initial screening

Prior to participation in the study volunteers signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and free from social drugs and medication with the exception of the contraceptive pill. Habitual smokers were excluded from the study.

2.2. Participants

Participants were informed that the study investigated the cognitive and mood effects of a commercially available fruit drink containing active components (one of which may be caffeine). Twenty-four participants completed the experiment (9 male and 15 female, mean age 21.3 years, S.E.M. 0.83, range 18–34 years). All were undergraduate volunteers. Participants abstained from caffeine and alcohol for a minimum of 12 h prior to the first testing session and throughout the morning until the final testing session was completed. The study was approved by the Northumbria University Division of Psychology Ethics Committee, and was carried out in accordance with the Declaration of Helsinki.

2.3. Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt, Leicester, UK). Samples were taken immediately prior to baseline assessment in order to confirm compliance to overnight abstinence and immediately prior to both post-treatment assessments to confirm effective caffeine absorption. The saliva samples were immediately frozen at −20 °C until thawing for in-house batch analysis using the Emit system (Syva, Palo Alto, USA). This is an enzyme immunoassay intended to measure caffeine as a metabolite and is based on competition for antibody binding sites between caffeine and an enzyme labelled drug.

2.4. Assessment

A tailored version of the Cognitive Drug Research battery (CDR Ltd., Goring-on-Thames, UK) was used. The CDR computerised assessment battery has been used in hundreds of European and North American drug trials, and has been shown to be sensitive to acute cognitive improvements as well as impairments with a wide variety of substances (e.g. Scholey et al., 1999; Kennedy et al., 2002, 2003) including caffeine (Scholey and Kennedy, 2004; Haskell et al., 2005).

The selection of computer-controlled tasks from the system was administered with parallel forms of the tests being presented at each testing session. Presentation was via laptop computers. All responses were recorded via two-button (Yes/No) response boxes with the exception of the written word recall task. A full description of the tasks used can be found in Kennedy et al. (2003). Additionally, a logical reasoning task was included as well as a sentence verification task and serial subtractions. Mood scales employed were the Bond–Lader visual analogue scales (Bond and Lader, 1974) and the Caffeine Research Visual Analogue Scales (CRVAS) (described in Haskell et al., 2005).

2.5. Treatments

Participants received four drinks containing: (1) 0 mg caffeine plus 0 mg L-theanine (placebo); (2) 150 mg of caffeine; (3) 250 mg L-theanine and (4) 150 mg caffeine plus 250 mg L-theanine on separate occasions. In each case the treatment was presented in a 250 ml modified Peach Lite Lipton Ice Tea drink. During initial preparation all tea powder (including L-theanine and caffeine) was removed from the drink and the sweetener levels adjusted to mask the bitter taste of the high dose of added caffeine so that no discernible taste difference could be detected between the drinks. The drink also contained the following: trisodium citrate; peach flavour; peach juice; malic acid; aspartame; acesulfame K and ascorbic acid. Ten minutes was allowed for drink consumption. These drinks were prepared off-site and assigned a treatment code by a disinterested third party.

2.6. Procedure

Each participant was required to attend a total of 5 study days that were conducted 7 days apart to ensure a sufficient wash out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival at their first session on the first day, participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the 4 active days of the study.

The first day involved completion of the test battery four times in order to control for practice effects and to allow familiarisation with the test battery and procedure on subsequent visits. The practice day data were not included in any analyses.

Each of the four active study days comprised three identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day, the second took place 30 min post-drink and the final session took place 90 min post-drink.

Each testing session lasted approximately 30 min and comprised producing a saliva sample, completion of the CDR test battery, a sentence verification task, serial subtractions (threes and sevens) and visual analogue mood scales.

2.7. Statistics

Prior to the primary statistical analysis, separate, one way, repeated measures ANOVAs of pre-dose baseline data were conducted to ascertain any chance baseline differences in performance across study days prior to the treatments.

Scores on the individual task outcomes were analysed as 'change from baseline' using Minitab 14.0.1.

A 2 × 2 × 2 repeated measures ANOVA (General Linear Model) was carried out on the 'change from baseline' data, with terms fitted to the model for caffeine (present, absent), L-theanine (present, absent) and assessment (30 min, 90 min). This analysis adequately describes the main effect of caffeine across the two caffeine containing drinks, the main effect of L-theanine across the two L-theanine containing drinks, and any interaction between the two, but does not explore the effects of the individual drinks in comparison to placebo. Therefore, any measures that generated a significant main or interaction effect were further explored using Student's paired *t*-tests comparing the relevant active treatment(s) to placebo. To ensure the overall Type I error protection level all testing was two-tailed. In addition, effect sizes were calculated using Cohen's *d*.

3. Results

3.1. Salivary caffeine levels

Salivary analysis confirmed compliance with overnight abstinence, mean baseline values were 0.42 μg/ml (levels just below 1 μg/ml have been reported for overnight caffeine abstinence—Evans and Griffiths, 1999). Two datasets from time points other than baseline were unusable and were excluded from any analyses. Analysis of post-treatment salivary caffeine levels revealed a main effect of caffeine [$F(1, 21) = 113, p < 0.0001$], with significantly higher salivary caffeine levels following caffeine administration [$t(21) = 9.30$, $p < 0.0001$, $d = 2.5$] and following the combination [$t(21) = 8.28$, $p < 0.0001$, $d = 2.2$], see Fig. 1.

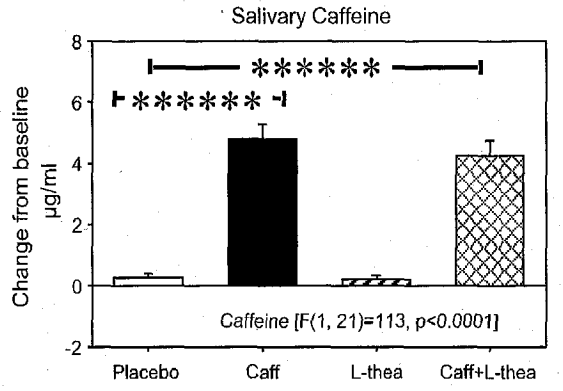

Fig. 1. Mean change from baseline salivary caffeine levels are presented following placebo, 150 mg caffeine, 250 mg L-theanine, and a combination of 150 mg caffeine plus 250 mg L-theanine, with more positive values representing higher levels. Significant treatment effects compared with placebo are indicated (******$p < 0.0001$). Main effect of caffeine from ANOVA is indicated.

3.2. Baseline scores

Mean pre-dose baseline scores and change from baseline scores, for each condition on each outcome measure are presented in Tables 1 and 2. There were significant baseline differences in accuracy of spatial memory ($F(3, 69) = 2.82$, $p < 0.05$) and ratings of 'content' [$F(3, 69) = 3.00$, $p < 0.05$].

3.3. Post-treatment CDR scores

Only those measures that generated significant main, or interaction effects, are reported below.

3.3.1. Simple reaction time

There was a significant main effect of caffeine on simple reaction time [$F(1, 23) = 5.67$, $p < 0.05$]. However, comparisons of both of the individual caffeine bearing drinks to placebo showed that this reached significance only following caffeine plus L-theanine [$t(23) = 2.67$, $p < 0.05$, $d = 0.7$], see Fig. 2a.

3.3.2. Digit vigilance reaction time

Digit vigilance reaction time showed a significant main effect of caffeine [$F(1, 23) = 6.67$, $p < 0.05$]. Comparisons of both caffeine bearing treatments to placebo revealed that reaction time was significantly faster only following caffeine alone [$t(23) = 2.33$, $p < 0.05$, $d = 0.7$], see Fig. 2b.

3.3.3. Rapid Visual Information Processing (RVIP)

There was a significant main effect of caffeine on accuracy of the RVIP task [$F(1, 23) = 28.31$, $p < 0.0001$]. This emerged as a significant improvement following both the caffeine drink [$t(23) = 3.23$, $p < 0.005$, $d = 0.9$] and the caffeine/L-theanine combination [$t(23) = 4.35$, $p < 0.001$, $d = 1.0$], see Fig. 2c.

3.3.4. Spatial memory accuracy (SI)

Due to significant baseline differences in spatial working memory baseline scores were included as a covariate in an ANCOVA performed on this measure. No significant differences remained following this analysis.

3.3.5. Numeric working memory reaction time

Numeric working reaction time showed a significant main effect of caffeine [$F(1, 23) = 7.39$, $p < 0.05$]. *t*-Tests comparing both caffeinated treatments to placebo revealed that this effect was restricted to a significant improvement following caffeine plus L-theanine [$t(23) = 2.26$, $p < 0.05$, $d = 0.5$], see Fig. 2d.

Table 1
Baseline and change from baseline scores for each cognitive measure for each treatment condition

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score	
			30 min	90 min
Immediate word recall accuracy (%)	Placebo	51.4 ± 4.00	−3.47 ± 3.95	−5.14 ± 3.05
	Caffeine	47.6 ± 3.70	−3.06 ± 2.98	−5.56 ± 3.46
	L-Theanine	47.9 ± 4.06	−5.14 ± 2.56	−2.64 ± 2.51
	Caff + L-thea	47.6 ± 3.28	−3.33 ± 2.90	−0.28 ± 3.69
Simple reaction time (ms)	Placebo	294 ± 7.57	17.4 ± 7.20	31.0 ± 10.2
	Caffeine	296 ± 7.62	4.26 ± 6.18	6.76 ± 9.86
	L-Theanine	296 ± 7.94	8.27 ± 5.98	23.5 ± 8.16
	Caff + L-thea	296 ± 7.77	−0.98 ± 5.99	−1.12 ± 6.53
Digit vigilance accuracy (%)	Placebo	92.7 ± 1.42	0.56 ± 0.79	−3.98 ± 1.29
	Caffeine	92.7 ± 1.41	−0.74 ± 1.58	−0.37 ± 1.74
	L-Theanine	93.1 ± 1.43	−2.04 ± 1.91	−3.80 ± 1.61
	Caff + L-thea	94.8 ± 1.68	−2.50 ± 1.31	−2.69 ± 1.38
Digit vigilance reaction time (ms)	Placebo	440 ± 6.51	20.0 ± 7.06	21.8 ± 6.97
	Caffeine	443 ± 9.73	1.68 ± 5.65	−1.88 ± 8.66
	L-Theanine	433 ± 7.61	19.2 ± 7.15	24.6 ± 6.94
	Caff + L-thea	436 ± 8.72	2.50 ± 8.35	8.93 ± 6.39
Digit vigilance false alarms (number)	Placebo	1.25 ± 0.23	−0.17 ± 0.27	0.38 ± 0.31
	Caffeine	1.71 ± 0.34	−0.92 ± 0.38	0.13 ± 0.31
	L-Theanine	1.42 ± 0.25	0.17 ± 0.35	0.04 ± 0.36
	Caff + L-thea	1.33 ± 0.38	−0.13 ± 0.37	0.25 ± 0.30
Choice reaction accuracy (%)	Placebo	94.8 ± 0.79	1.67 ± 0.66	−0.50 ± 0.79
	Caffeine	95.3 ± 0.67	−0.25 ± 0.89	0.33 ± 0.70
	L-Theanine	95.2 ± 0.84	−0.17 ± 0.68	−1.33 ± 0.80
	Caff + L-thea	95.1 ± 0.82	0.25 ± 0.67	0.17 ± 0.70
Choice reaction time (ms)	Placebo	434 ± 9.97	20.7 ± 9.19	17.8 ± 9.68
	Caffeine	444 ± 15.1	−13.6 ± 11.8	−16.6 ± 9.51
	L-Theanine	442 ± 14.9	−5.43 ± 10.8	−6.12 ± 12.0
	Caff + L-thea	441 ± 12.6	1.94 ± 11.1	4.76 ± 11.8
RVIP accuracy (%)	Placebo	59.5 ± 5.05	−6.38 ± 1.81	−6.64 ± 2.50
	Caffeine	55.2 ± 4.87	−1.30 ± 2.50	8.20 ± 2.70
	L-Theanine	57.4 ± 4.91	−4.69 ± 2.44	−4.69 ± 2.36
	Caff + L-thea	56.6 ± 4.27	3.52 ± 2.40	4.82 ± 2.66
RVIP reaction time (ms)	Placebo	502 ± 18.6	4.57 ± 15.6	7.26 ± 19.1
	Caffeine	485 ± 12.4	−17.6 ± 11.3	−10.2 ± 14.7
	L-Theanine	505 ± 17.0	19.2 ± 16.6	−4.95 ± 10.7
	Caff + L-thea	524 ± 20.5	−36.1 ± 15.9	−25.9 ± 19.2
RVIP false alarms (number)	Placebo	0.83 ± 0.19	0.38 ± 0.32	0.50 ± 0.32
	Caffeine	1.33 ± 0.37	−0.17 ± 0.29	−0.08 ± 0.46
	L-Theanine	1.00 ± 0.23	0.17 ± 0.21	0.29 ± 0.20
	Caff + L-thea	1.46 ± 0.32	0.38 ± 0.37	0.33 ± 0.32
Spatial memory (sensitivity index)	Placebo	0.95 ± 0.01	−0.02 ± 0.01	−0.12 ± 0.04
	Caffeine	0.86 ± 0.05	0.09 ± 0.05	0.07 ± 0.05
	L-Theanine	0.95 ± 0.02	−0.05 ± 0.03	−0.05 ± 0.03
	Caff + L-thea	0.94 ± 0.01	0.00 ± 0.02	−0.09 ± 0.05
Spatial memory reaction time (ms)	Placebo	551 ± 28.7	−3.18 ± 27.9	15.2 ± 15.1
	Caffeine	540 ± 19.7	−9.75 ± 10.7	−30.1 ± 9.89
	L-Theanine	550 ± 24.8	−23.3 ± 21.4	5.96 ± 29.9
	Caff + L-thea	555 ± 23.2	−28.0 ± 15.3	−28.7 ± 15.6
Logical reasoning accuracy (%)	Placebo	74.1 ± 4.36	−4.69 ± 2.32	−1.04 ± 1.96
	Caffeine	73.4 ± 4.60	−1.74 ± 1.50	1.56 ± 1.39
	L-Theanine	71.9 ± 5.24	0.35 ± 1.82	−0.17 ± 1.67
	Caff + L-thea	73.8 ± 5.20	−1.22 ± 2.12	−2.43 ± 1.84
Logical reasoning reaction time (ms)	Placebo	2736 ± 196	21.5 ± 155	−184 ± 235
	Caffeine	2442 ± 153	−16.1 ± 99.4	−126 ± 81.8
	L-Theanine	2557 ± 183	−157 ± 127	−223 ± 111
	Caff + L-thea	2615 ± 222	−30.4 ± 124	−202 ± 127

Table 1 (*Continued*)

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score	
			30 min	90 min
Numeric working memory (sensitivity index)	Placebo	0.90 ± 0.02	−0.01 ± 0.01	−0.02 ± 0.01
	Caffeine	0.91 ± 0.01	0.00 ± 0.01	−0.02 ± 0.02
	L-Theanine	0.90 ± 0.02	0.00 ± 0.02	0.00 ± 0.02
	Caff + L-thea	0.92 ± 0.01	0.00 ± 0.01	−0.02 ± 0.02
Numeric working memory reaction time (ms)	Placebo	571 ± 18.5	−13.7 ± 8.71	3.66 ± 14.0
	Caffeine	564 ± 16.6	−36.3 ± 11.9	−27.3 ± 11.3
	L-Theanine	555 ± 18.5	17.1 ± 14.7	8.36 ± 11.0
	Caff + L-thea	562 ± 15.1	−33.4 ± 7.97	−21.8 ± 7.64
Delayed word recall accuracy (%)	Placebo	39.2 ± 3.68	−6.67 ± 3.38	−13.3 ± 2.95
	Caffeine	35.8 ± 3.08	−9.72 ± 3.43	−9.58 ± 3.57
	L-Theanine	35.6 ± 3.08	−8.89 ± 3.29	−10.8 ± 3.36
	Caff + L-thea	36.5 ± 2.92	−12.8 ± 3.89	−7.08 ± 3.63
Delayed word recognition (sensitivity index)	Placebo	0.67 ± 0.04	−0.07 ± 0.04	−0.08 ± 0.04
	Caffeine	0.65 ± 0.05	−0.05 ± 0.05	−0.08 ± 0.04
	L-Theanine	0.64 ± 0.05	−0.12 ± 0.05	−0.03 ± 0.03
	Caff + L-thea	0.68 ± 0.04	−0.07 ± 0.05	−0.07 ± 0.05
Delayed word recognition reaction time (ms)	Placebo	720 ± 33.5	4.83 ± 29.0	−11.2 ± 15.1
	Caffeine	694 ± 21.5	−1.73 ± 17.4	−14.7 ± 26.2
	L-Theanine	689 ± 22.1	13.9 ± 17.6	31.2 ± 21.6
	Caff + L-thea	755 ± 34.2	−83.6 ± 37.3	−86.6 ± 39.8
Delayed picture recognition (sensitivity index)	Placebo	0.64 ± 0.04	0.02 ± 0.04	−0.04 ± 0.04
	Caffeine	0.64 ± 0.05	−0.04 ± 0.06	0.02 ± 0.04
	L-Theanine	0.64 ± 0.05	−0.03 ± 0.04	−0.07 ± 0.04
	Caff + L-thea	0.65 ± 0.05	−0.05 ± 0.05	−0.04 ± 0.06
Delayed picture recognition reaction time (ms)	Placebo	781 ± 26.1	7.32 ± 16.7	38.7 ± 24.2
	Caffeine	788 ± 24.9	−9.84 ± 20.3	−22.8 ± 16.9
	L-Theanine	809 ± 36.2	−13.9 ± 27.2	−21.1 ± 28.3
	Caff + L-thea	793 ± 23.3	−9.97 ± 19.8	−27.5 ± 20.4
Sentence verification accuracy (%)	Placebo	96.5 ± 1.25	−1.52 ± 1.24	−1.36 ± 1.11
	Caffeine	95.6 ± 1.08	0.30 ± 1.22	0.15 ± 1.06
	L-Theanine	96.4 ± 1.03	0.15 ± 1.15	−2.12 ± 0.97
	Caff + L-thea	96.1 ± 1.04	−0.15 ± 1.06	2.42 ± 1.03
Sentence verification reaction time (ms)	Placebo	1345 ± 82.2	−79.7 ± 74.9	−70.8 ± 41.8
	Caffeine	1306 ± 45.4	−57.8 ± 32.8	−84.1 ± 37.4
	L-Theanine	1366 ± 64.9	−179 ± 51.0	−123 ± 60.9
	Caff + L-thea	1267 ± 63.4	−9.55 ± 50.3	−87.0 ± 36.6
Serial three subtractions correct (number)	Placebo	38.0 ± 2.34	2.64 ± 1.45	4.05 ± 1.83
	Caffeine	39.7 ± 2.56	4.41 ± 1.28	6.36 ± 0.95
	L-Theanine	40.5 ± 2.53	1.64 ± 1.58	2.32 ± 1.68
	Caff + L-thea	39.8 ± 2.64	5.55 ± 1.39	5.77 ± 1.57
Serial three subtractions errors (number)	Placebo	4.18 ± 0.46	0.82 ± 0.54	−0.23 ± 0.36
	Caffeine	4.55 ± 0.75	−0.64 ± 0.72	−0.77 ± 0.61
	L-Theanine	3.82 ± 0.56	0.09 ± 0.51	−0.32 ± 0.73
	Caff + L-thea	3.77 ± 0.50	−0.32 ± 0.46	−0.59 ± 0.46
Serial seven subtractions correct (number)	Placebo	22.1 ± 2.16	0.50 ± 0.91	3.32 ± 1.15
	Caffeine	22.6 ± 2.02	1.82 ± 0.90	4.64 ± 1.13
	L-Theanine	22.4 ± 2.08	1.41 ± 1.00	−0.86 ± 1.25
	Caff + L-thea	23.4 ± 2.04	1.41 ± 0.98	2.32 ± 1.23
Serial seven subtractions errors (number)	Placebo	2.95 ± 0.41	0.27 ± 0.46	0.32 ± 0.51
	Caffeine	4.00 ± 0.62	0.59 ± 0.79	−0.14 ± 0.80
	L-Theanine	4.00 ± 0.69	−0.86 ± 0.67	0.36 ± 0.68
	Caff + L-thea	3.68 ± 0.43	−0.45 ± 0.46	0.05 ± 0.58

Means ± S.E.M. are presented.

Table 2
Baseline and change from baseline scores for mood for each treatment condition

Measure	Treatment	Pre-dose baseline score	Post-dose change from baseline score	
			30 min	90 min
Bond–Lader factors				
Alert	Placebo	50.0 ± 3.72	−2.70 ± 2.78	−2.95 ± 3.82
	Caffeine	55.4 ± 2.70	0.11 ± 2.25	−0.07 ± 1.67
	L-Theanine	47.2 ± 2.93	−3.63 ± 3.04	−3.19 ± 2.84
	Caff + L-thea	53.4 ± 3.47	6.24 ± 1.82	5.03 ± 2.52
Content	Placebo	60.5 ± 3.03	−2.45 ± 1.31	−1.06 ± 1.78
	Caffeine	59.5 ± 3.04	−0.22 ± 1.46	0.40 ± 1.38
	L-Theanine	53.1 ± 3.47	1.41 ± 1.65	3.15 ± 2.00
	Caff + L-thea	62.8 ± 3.27	1.27 ± 2.13	2.74 ± 2.23
Calm	Placebo	62.2 ± 3.36	−4.40 ± 2.74	−4.85 ± 2.56
	Caffeine	61.5 ± 2.79	−3.58 ± 3.22	−4.96 ± 3.22
	L-Theanine	57.9 ± 3.10	0.81 ± 2.97	−2.44 ± 2.31
	Caff + L-thea	63.1 ± 2.94	−10.2 ± 3.30	−9.00 ± 2.95
Caffeine Research VAS				
Relaxed	Placebo	62.9 ± 4.17	−6.13 ± 3.52	−10.8 ± 5.26
	Caffeine	60.7 ± 4.36	−6.43 ± 4.60	−6.22 ± 4.96
	L-Theanine	53.6 ± 3.86	7.26 ± 3.28	−5.00 ± 4.77
	Caff + L-thea	61.0 ± 3.85	−7.35 ± 5.34	−6.57 ± 5.06
Alert	Placebo	45.8 ± 4.94	0.91 ± 4.90	8.13 ± 4.87
	Caffeine	56.0 ± 4.01	4.09 ± 2.97	6.52 ± 4.86
	L-Theanine	48.5 ± 4.73	−6.17 ± 4.98	0.57 ± 4.36
	Caff + L-thea	51.3 ± 4.59	10.2 ± 4.66	12.0 ± 4.12
Jittery	Placebo	23.7 ± 4.53	4.09 ± 2.90	10.7 ± 4.74
	Caffeine	23.6 ± 3.52	14.2 ± 5.64	11.4 ± 5.80
	L-Theanine	24.6 ± 4.76	4.30 ± 3.57	5.48 ± 4.67
	Caff + L-thea	27.4 ± 5.30	6.65 ± 4.70	7.43 ± 4.94
Tired	Placebo	55.0 ± 5.83	−1.22 ± 5.95	−3.39 ± 6.83
	Caffeine	51.7 ± 4.10	−17.7 ± 4.05	−16.1 ± 4.38
	L-Theanine	54.1 ± 5.35	7.22 ± 5.93	−1.13 ± 5.11
	Caff + L-thea	56.7 ± 4.69	−18.7 ± 4.69	−22.5 ± 3.90
Tense	Placebo	28.9 ± 4.81	3.35 ± 4.66	11.1 ± 5.04
	Caffeine	33.7 ± 4.67	5.22 ± 4.54	6.57 ± 4.67
	L-Theanine	38.4 ± 5.44	−4.96 ± 3.39	0.52 ± 3.50
	Caff + L-thea	30.4 ± 5.06	7.13 ± 5.92	0.57 ± 4.42
Headache	Placebo	17.7 ± 4.74	3.09 ± 1.86	2.65 ± 1.97
	Caffeine	18.7 ± 4.41	−2.22 ± 1.91	−3.13 ± 2.76
	L-Theanine	19.1 ± 4.86	5.57 ± 1.86	10.0 ± 3.26
	Caff + L-thea	17.5 ± 4.33	0.00 ± 1.92	−4.04 ± 3.01
Overall mood	Placebo	61.9 ± 3.89	−1.09 ± 2.70	2.26 ± 2.60
	Caffeine	63.2 ± 4.60	2.13 ± 4.49	1.00 ± 2.90
	L-Theanine	53.4 ± 4.13	−1.35 ± 2.62	1.00 ± 2.61
	Caff + L-thea	60.4 ± 3.99	6.96 ± 2.96	8.83 ± 2.89
Mental fatigue	Placebo	38.8 ± 5.05	11.0 ± 2.93	4.87 ± 4.13
	Caffeine	36.7 ± 3.99	−2.74 ± 4.51	−4.17 ± 4.62
	L-Theanine	44.5 ± 4.81	1.65 ± 5.89	5.35 ± 4.64
	Caff + L-thea	42.1 ± 4.21	−2.87 ± 3.75	−5.22 ± 3.78

Means ± S.E.M. are presented.

3.3.6. Delayed word recognition reaction time

There was a significant main effect of caffeine on delayed word recognition reaction time [$F(1, 23) = 4.48$, $p < 0.05$]. Comparisons of the caffeine bearing treatments to placebo revealed that there was a trend towards significantly speeded word recognition reaction time following the combination [$t(23) = 1.93$, $p = 0.07$]. There was also a positive caffeine × L-theanine interaction on this measure [$F(1, 23) = 5.61$, $p < 0.05$], see Fig. 2f.

3.4. Post-treatment scores on other cognitive measures

Due to a data capture error with 2 datasets these analyses look at only 22 participants.

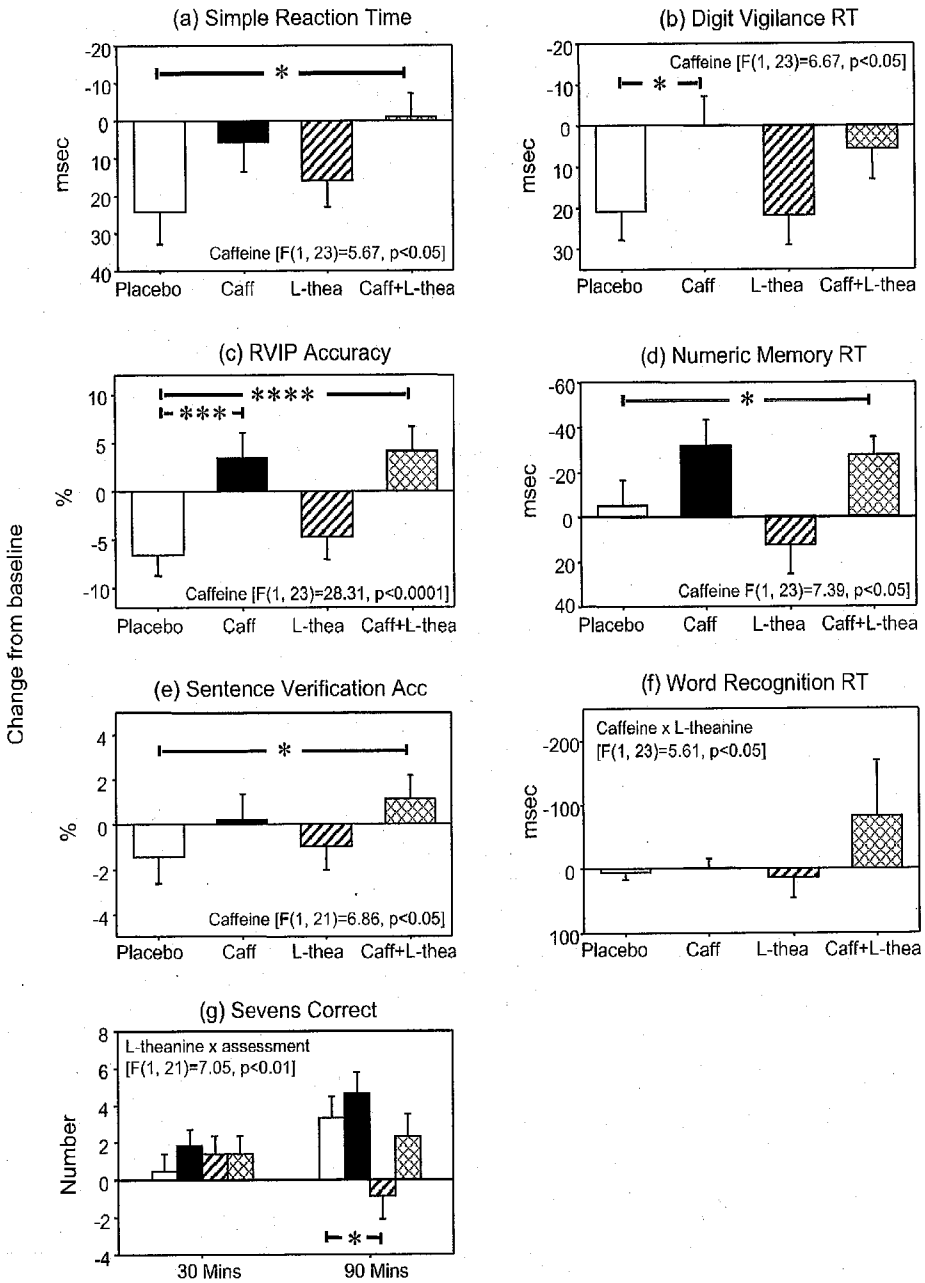

Fig. 2. Mean change from baseline scores on cognitive measures are presented following placebo, 150 mg caffeine, 250 mg L-theanine, and a combination of 150 mg caffeine plus 250 mg L-theanine, with more positive values representing higher levels. Treatment effects are shown for (a) simple reaction time, and (b) digit vigilance reaction time, (c) RVIP accuracy, (d) speed of numeric working memory, (e) accuracy of sentence verification. Main effect of caffeine from relevant ANOVA is indicated for each task. A caffeine × L-theanine interaction is shown for (f) delayed word recognition reaction time, and an assessment × L-theanine effect for (g) correct serial seven subtractions ($^{*}p < 0.05$; $^{***}p < 0.005$; $^{****}p < 0.001$).

3.4.1. Sentence verification accuracy

There was a significant main effect of caffeine on the accuracy of the sentence verification task [$F(1, 21) = 6.86$, $p < 0.05$]. *t*-Tests comparing the treatments to placebo revealed that only caffeine plus L-theanine evinced an individual significant effect [$t(21) = 2.38$, $p < 0.05$, $d = 0.5$], see Fig. 2e.

3.4.2. Serial sevens subtraction task

There was a significant L-theanine × assessment interaction on the number of correct serial seven subtractions [$F(1, 21) = 7.05$, $p < 0.01$]. Comparisons of the individual drinks to placebo showed that L-theanine alone significantly impaired performance on this measure at 90 min [$t(21) = 2.70$, $p < 0.05$, $d = 0.8$], see Fig. 2g.

3.5. Post-treatment subjective mood ratings

3.5.1. Bond–Lader 'alert'

There was a significant main effect of caffeine on ratings on the Bond–Lader visual analogue scales 'alert' factor [$F(1, 23) = 8.06$, $p < 0.01$]. Comparisons to placebo showed that this was only evinced as a significant improvement following caffeine plus L-theanine [$t(23) = 2.88$, $p < 0.01$, $d = 0.7$], see Fig. 3a.

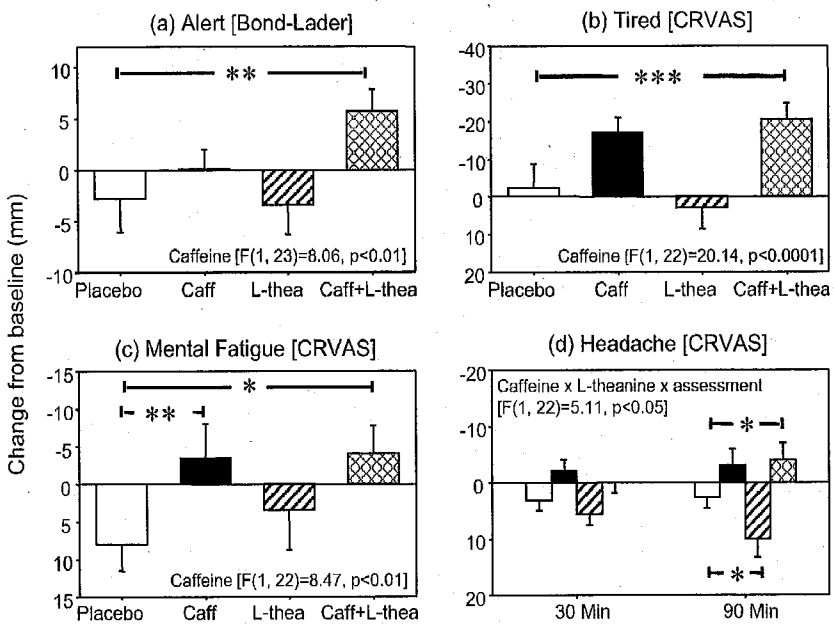

Fig. 3. Mean change from baseline scores on subjective mood measures are presented following placebo, 150 mg caffeine, 250 mg L-theanine, and a combination of 150 mg caffeine plus 250 mg L-theanine, with more positive values representing higher ratings. Treatment effects are shown for (a) Bond–Lader 'alert', and (b) Caffeine Research VAS 'tired'. Main effect of caffeine from relevant ANOVA is indicated for each rating. Caffeine × L-theanine × assessment effects are shown for (c) Caffeine Research 'headache' ratings ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$).

There were a number of treatment effects on the Caffeine Research Visual Analogue Scales. Due to a data capture error with 1 dataset these analyses looks at only 23 participants.

3.5.2. 'Tired'

There was a significant main effect of caffeine on 'tired' ratings [$F(1, 22) = 20.14$, $p < 0.0001$]. Comparison of the caffeinated drinks to placebo showed that caffeine plus L-theanine significantly decreased these ratings [$t(22) = 3.46$, $p < 0.005$, $d = 0.8$], see Fig. 3b.

3.5.3. 'Headache'

There was also a significant main effect of caffeine on 'headache' ratings [$F(1, 22) = 14.3$, $p < 0.001$] and a significant caffeine × L-theanine × assessment interaction [$F(1, 22) = 5.11$, $p < 0.05$]. Paired t-tests revealed a significant decrease in 'headache' ratings following the combination [$t(22) = 2.08$, $p < 0.05$, $d = 0.6$] and a significant increase following L-theanine [$t(22) = 2.26$, $p < 0.05$, $d = 0.6$], at 90 min, see Fig. 3d.

3.5.4. 'Mental fatigue'

There was a significant main effect of caffeine on 'mental fatigue' [$F(1, 22) = 8.47$, $p < 0.01$]. Comparison between drinks showed that there was a significant decrease following caffeine [$t(22) = 2.83$, $p < 0.01$, $d = 0.6$], and the caffeine/L-theanine combination [$t(22) = 2.49$, $p < 0.05$, $d = 0.7$], see Fig. 3c.

4. Discussion

This study represents the first systematic assessment of the neurocognitive effects of L-theanine. L-Theanine alone had relatively few effects. However, there is evidence of some effects of caffeine when combined with L-theanine, not seen with either treatment in isolation.

L-Theanine was associated with a detrimental effect on performance of serial sevens (Fig. 2g) and an increase in 'headache' ratings (Fig. 3c). The impairment to serial sevens, a largely attentional task, is supportive of findings from Gomez-Ramirez et al. (2007) showing impairment to reaction time on an auditory attention task. It is interesting to note that the effects of L-theanine were not evident until 90 min post-administration. The lack of effects on subjective 'calm' or 'relaxed' ratings in the current study is not supportive of the literature. Specifically, the data do not support the findings from Kimura et al. (2007) of reduced subjective and physiological responses to acute stress following L-theanine. Lu et al. (2004) also found improvements on the 'tranquil-troubled' subscale of the Bond–Lader, and previous EEG findings show that L-theanine can increase α-wave activity in the parietal and occipital regions of the brain (Kobayashi et al., 1998). A finding that, the authors suggest, is indicative of increased relaxation without increased drowsiness. In light of the purported effects of L-theanine on subjective measures related to 'calmness', paired t-tests were carried out examining the effects of L-theanine on these measures alone. Interestingly, L-theanine significantly increased 'relaxed' when compared to placebo at 30 min post-administration. (However, given the absence of a main effect or interaction effect of L-theanine on this measure, we have not explored this finding further here.) It should be noted that Gomez-Ramirez et al. (2007) found that L-theanine actually decreased alpha levels when measured during performance of a demanding attentional task. This suggests that the effects of L-theanine on alpha activity may be moderated by the task being performed and this may explain the lack of effects on subjective mood ratings in the

current study. It is unfortunate that no such record was made in the Gomez-Ramirez et al. (2007) study, as this would have allowed comparison between the two.

Caffeine alone improved speed of digit vigilance reaction time (Fig. 2b) and accuracy of Rapid Visual Information Processing (see Fig. 2c), and attenuated increases in self-reported 'mental fatigue' ratings. The improvements to digit vigilance reaction time and 'mental fatigue' ratings replicate those reported previously for 150 mg caffeine, using the same battery in habitual consumers and habitual non-consumers of caffeine (Haskell et al., 2005). It has been suggested that caffeine merely reverses the negative effects of caffeine withdrawal (Rogers et al., 2003) so, given that the participants in the current study were overnight caffeine withdrawn (as confirmed by analysis of salivary caffeine levels), it is unfortunate that no record was taken of habitual caffeine use, as this would have added to the debate in this area and also allowed comparison with the findings of Haskell et al. (2005) of similar responses to caffeine in consumers and non-consumers of caffeine. Of particular interest in the current study would be a record of habitual caffeine consumption, with specific reference to the source of caffeine, as this would allow exploration of any differential effects of caffeine in relation to regular caffeine source. It would also be interesting in the case of L-theanine to establish whether these effects differ with regard to habitual tea use. Related to habitual consumption is the possibility that, despite the removal of all tea powder, a placebo response to iced tea may have been elicited in some participants and not in others as a result of previous use.

Turning to the L-theanine–caffeine combination, there were a number of measures that were sensitive to this treatment. Simple reaction time (Fig. 2a), accuracy of RVIP (Fig. 2c), numeric working memory reaction time (Fig. 2d), delayed word recognition reaction time (Fig. 2f) and accuracy of sentence verification (Fig. 2e) were all improved by the combination. In addition, Bond–Lader 'alert' ratings (Fig. 3a) were increased, 'tired' (Fig. 3b) and 'mental' fatigue' ratings (Fig. 3c) were decreased and 'headache' ratings were reduced (Fig. 3d). The only effect of caffeine not evident following the combination was an improvement to digit vigilance reaction time. Given that L-theanine is a 'relaxant,' and the effects of caffeine are usually attributed to its 'stimulant' properties, it is not clear why the addition of L-theanine should ameliorate the effects of caffeine. However, it is interesting to note, that the only task to show an attenuation of the effects of caffeine when combined with L-theanine, was a sustained attention task that has previously been shown to be extremely sensitive to caffeine. In contrast, greater potentiation was observed on memory measures, which tend to be unaffected by caffeine on the whole.

The greater effects of the combination than caffeine alone on increased 'alert' ratings and reduced 'tired' ratings contradict those of Kakuda et al. (2000) who reported an inhibitory effect of L-theanine on caffeine's stimulatory properties in rats. However, the doses used are far lower than those employed previously, and it is not clear whether comparable effects would be seen in humans in the latter study. Furthermore, it may be simplistic to consider psychopharmacological effects in terms of straightforward stimulant or depressant effects. Firstly such effects are dose-dependent, classic CNS depressants such as alcohol may have stimulant effects at low doses (e.g. Scholey and Fowles, 2002). Similarly, caffeine at very high doses (outside the range used here) inhibits cognitive function and we have some evidence of functional impairment from very low doses (Haskell et al., in press). Thus mutual behavioural antagonism between depressant and stimulant drugs may be dose-dependent (e.g. Smith et al., 2003; Mackay et al., 2002). In addition, even when such effects manifest themselves at the behavioural level one should not simply assume that the two substances share common neurochemical targets.

In the context of the current study the mechanisms underlying the reported effects are not known. At the neurochemical level, both caffeine and L-theanine have been shown to, directly or indirectly, affect several neurotransmitter systems. Common targets may include dopamine, serotonin, glutamate and GABA. However, to the best of our knowledge, the effects of L-theanine and caffeine in combination have not been studied at the receptor level. There may be psychopharmacological interactions between caffeine and L-theanine, but without further work delineating the mechanisms of L-theanine's psychoactive properties this remains a matter of conjecture. It is clear from analysis of salivary caffeine levels that the reason for the difference in effects of caffeine and the combination, in favour of the latter, are not as a result of increased caffeine absorption. However, the greater effect size for increased salivary caffeine following caffeine may be indicative of greater absorption of caffeine from extracellular fluids following the combination. It is also interesting to note that, with the exception of reduced 'headache' ratings, the effects of the caffeine–L-theanine combination and caffeine in isolation are all apparent at both 30 and 90 min post-administration.

The levels of L-theanine and caffeine used here are higher than those found in tea beverages, which are typically in the region of 40 mg caffeine and 20 mg L-theanine. In this initial study it was felt that the use of known psychoactive doses was important in order to establish a neurocognitive profile for L-theanine and to examine effects of a caffeine–L-theanine combination. Further studies should be aimed at a full dose-ranging study for L-theanine, particularly as at least one study has reported mild psychostimulant effects in rats following lower rather than higher doses of L-theanine (Kakuda et al., 2000). Additionally, the next stage of studies into L-theanine–caffeine combinations should include examination of the effects of everyday doses both in isolation and in combination at levels and ratios found in real tea beverages. It is also clear that teas contain a host of potentially psychoactive ingredients, which, as well as L-theanine and caffeine, include catechins, tannins and saponins amongst others. Additionally, green tea has been reported to have anti-cholinesterase properties (Okello et al., 2004), although the components responsible for this property are at present unknown.

Further research should examine the effects of L-theanine on behaviour, on its own and in combination with the other components of tea.

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