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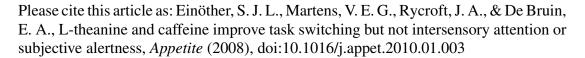
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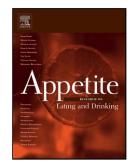
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1	L-theanine and caffeine improve task switching but not intersensory attention of
2	subjective alertness
3	
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1	<b>Abstract</b>

- 2 Tea ingredients L-theanine and caffeine have repeatedly been shown to deliver unique
- 3 cognitive benefits when consumed in combination. The current randomized, placebo-
- 4 controlled, double-blind, cross-over study compared a combination of L-theanine
- 5 (97mg) and caffeine (40mg) to a placebo on two attention tasks and a self-report
- 6 questionnaire before, and 10 and 60 minutes after consumption. The combination of L-
- 7 theanine and caffeine significantly improved attention on a switch task as compared to
- 8 the placebo, while subjective alertness and intersensory attention were not improved
- 9 significantly. The results support previous evidence that L-theanine and caffeine in
- 10 combination can improve attention.

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### 12 **Key words**

13 L-theanine; Caffeine; Attention; Alertness.

#### Introduction

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- 2 Tea is the second most widely consumed beverage in the world, only being surpassed
- 3 by water. Traditionally, tea consumption has been associated with mental clarity
- 4 (Graham, 1992). While anecdotal reports of the cognitive effects of tea are abundant,
- 5 scientists have only recently started to accumulate evidence for these effects using
- 6 cognitive tasks (e.g., Hindmarch, Quinlan, Moore, & Parkin, 1998; Hindmarch, Rigney,
- 7 Stanley, Quinlan, Rycroft, & Lane, 2000).
  - The cognitive effects of tea are commonly attributed to two of its naturally occurring components: caffeine and L-theanine (Bryan, 2008). A typical serving of black tea contains approximately 35-61mg caffeine per 200ml. Apart from a general arousing effect, caffeine has been reported to enhance cognitive performance, in particular attention (Smith, 2002). Caffeine, in doses ranging from the equivalent to a cup of tea up to 200-250mg, has been shown to improve performance on a range of attention measures, such as vigilance, visual search, choice reaction time and selective attention (Lieberman, Wurtman, Emde, Roberts, & Coviella, 1987; Fagan, Swift, & Tiplady, 1988; Lorist, Snel, Kok, & Mulder, 1996; Durlach, 1998; Smith, Sturgess, & Gallagher, 1999; Ruijter, de Ruiter, Snel, & Lorist, 2000a; Ruijter, de Ruiter, Snel, & Lorist, 2000b; Christopher, Sutherland, & Smith, 2005). Although few studies have found more pronounced effects of 500mg caffeine (Bruce, Scott, Lader, & Marks, 1986; Ruijter, Lorist, & Snel, 1999), most studies indicate that effects of caffeine on performance seem to asymptote around 200mg (Lieberman et al., 1987; Robelin & Rogers, 1998). In addition to improved performance, caffeine has been shown to affect self-reported mental state, in particular alertness (Fagan et al., 1988; Mumford, Evans, Kaminski, Preston, Sannerud, Silverman, & Griffiths, 1994; Christopher et al., 2005),

concentration (Mumford et al., 1994) and energetic arousal (Quinlan, Lane, & Aspinall,

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2 1997; Quinlan, Lane, Moore, Aspen, Rycroft, & O'Brien, 2000). 3 Beside caffeine, tea includes the nonproteinic amino acid L-theanine which is 4 found almost exclusively in tea leaves, with a typical serving of black tea containing 5 4.5-22.5mg per 200ml. The effects of L-theanine on cortical activity have been 6 investigated with electroencephalography (EEG) at rest and during task execution. L-7 theanine was found to increase EEG alpha activity at rest, indicating more relaxation 8 (Juneja, Chu, Okubi, Nagato, & Yokogoshi, 1999; Nobre, Rao, & Owen, 2008), as well 9 as background alpha desynchronisation and attention-related alpha synchronisation 10 when preparing to focus attention on specific stimuli (Gomez-Ramirez, Higgins, 11 Rycroft, Owen, Mahoney, Shpaner, & Foxe, 2007). At the behavioural level however, effects of L-theanine are equivocal. While Rogers, Smith, Heatherley, and Pleydell-12 13 Pearce (2008) reported no significant improvements following 200mg L-theanine, 14 Haskell, Kennedy Milne, Wesnes, and Scholey (2008) found that 250mg improved 15 choice reaction time and reaction times on two memory tasks. However, L-theanine also 16 decreased serial subtraction performance and self-reported alertness. 17 As tea is the most prominent source of L-theanine and it is thus nearly 18 universally consumed in combination with caffeine, studies investigating L-theanine 19 and caffeine in combination are essential to our understanding of the effects of tea. 20 Furthermore, these studies have provided evidence for an interaction between caffeine 21 and L-theanine. For example, L-theanine protects against toxic caffeine doses in the rat 22 brain in vitro (Kimura, Kurita, & Murata, 1975) and inhibits excitatory effects of 23 caffeine (Kimura et al., 1975; Kakuda, Nozawa, Unno, Okamura, & Okai, 2000). More 24 recently, evidence for this interaction emerged from human studies in which the amount

1 of L-theanine was increased relative to caffeine to amplify possible effects of L-2 theanine. Kelly, Gomez-Ramirez, Monetesi and Foxe (2008) found that 100mg L-3 theanine in combination with 50mg caffeine improved accuracy on a visuo-spatial 4 attention task and decreased background alpha activity as measured with EEG as 5 compared to placebo, indicating improved attentional processing, whereas neither L-6 theanine nor caffeine alone differed from placebo. Haskell, Kennedy, Milne, Wesnes, 7 and Scholey (2008) reported that 250mg L-theanine in combination with 150mg 8 caffeine significantly improved sentence verification, simple reaction time, numeric 9 working memory, and delayed word recognition reaction times, as compared to placebo, 10 while caffeine or L-theanine in isolation did not. Moreover, the L-theanine/caffeine 11 combination significantly increased feelings of alertness, as compared to placebo. In a 12 study by Owen, Parnell, De Bruin, and Rycroft (2008), both 100mg L-theanine and 13 50mg caffeine in combination and 50mg caffeine alone improved accuracy on a switch 14 task at 60 minutes and 90 minutes after consumption respectively. There were no 15 treatment effects on response speed. Finally, a similar L-theanine/caffeine combination 16 improved switch task accuracy, increased self-reported alertness, and reduced task-17 induced fatigue as compared to placebo (Giesbrecht et al., submitted). 18 The studies described above indicate that L-theanine and caffeine improve 19 cognitive performance, attention in particular, and that these effects are most 20 pronounced when both ingredients are combined. The current study aimed to further 21 investigate the enhancing effects of L-theanine and caffeine in combination. 22 Specifically, the aim of this study was twofold. Firstly, we wanted to replicate the effect on the switch task (Owen et al., 2008). Secondly, as the switch task assessed visual 23 24 attention, we wanted to investigate whether this effect was dependent on perceptual

1	modality by including a paradigm which required switching attention between different
2	modalities and has previously been shown to be sensitive to the effects of L-theanine
3	(Gomez-Ramirez et al., 2007). Previous studies which included L-theanine and/or
4	caffeine in isolation (Haskell et al., 2008; Kelly et al., 2008; Owen et al., 2008)
5	indicated that the L-theanine/caffeine combination had synergistic effects. Moreover, L-
6	theanine is virtually exclusively consumed in combination with caffeine. Therefore it
7	was decided to specifically compare the effects of the L-theanine/caffeine combination
8	to a placebo. In line with prior studies, we increased the amount of L-theanine, relative
9	to caffeine, to amplify possible effects of L-theanine.
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11	Methods
12	Participants
13	Twenty-nine healthy participants (11 males), aged between 18 and 45 years ( $M=30.6$
14	SD= 8.9) took part in the study. Inclusion criteria were regular caffeinated tea and/or
15	coffee consumption, non-smoking, regular breakfast consumption, and a BMI between
16	20 and 30. The participants consumed on average 11.7 ( $SD$ = 6.7) caffeinated drinks per
17	week and had a mean BMI of 25.1 (SD= 2.6). Exclusion criteria were allergies to
18	caffeine, artificial sweeteners, or herbal supplements, colour blindness, dyslexia,
19	pregnancy, breastfeeding, and use of recreational drugs and medication with the
20	exception of the contraceptive pill. The study was approved by the Unilever Colworth
21	Research Ethics Committee. Participants signed an informed consent form and were
22	paid for their participation.
23	
24	Design

1	The study employed a randomized, placebo-controlled, double-blind, crossover design
2	with drink (the active L-theanine/caffeine drink vs. a placebo) and time (cognitive test
3	battery at baseline, 10 min post-drink and 60 min post-drink) as within participant
4	factors. The order of drinks was randomized. Participants attended two test occasions,
5	separated by at least 6 and no more than 14 days, during which they consumed either
6	the active drink or the placebo and completed a test battery three times. Each test
7	occasion was preceded by a brief training session to minimize learning effects.
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9	Drinks
10	The active drink was prepared by dissolving synthetically produced L-theanine
11	(Suntheanine <sup>TM</sup> ; Taiyo Kagaku Co. Ltd., Yokkaichi, Japan), pharmaceutical grade
12	caffeine and iced tea powder into 500 ml of mineral water. The iced tea powder
13	contained colourings and tea flavourings, as well as sweeteners (sucralose) and lemon
14	flavour to mask the taste of caffeine. The placebo drink matched the active drink except
15	for the absence of L-theanine and caffeine. The active drink delivered on average 97mg
16	L-theanine and 40mg caffeine.
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18	Cognitive test battery
19	Each 40-minute session consisted of two tests of attention and a mood questionnaire.
20	Tests are described in the order in which they were performed.
21	Intersensory attention task (Foxe, Simpson, & Ahlfors, 1998). This task
22	measures the ability to selectively deploy attention to stimuli presented in different
23	sensory modalities (visual and auditory) and it was chosen to investigate whether the
24	effect of the L-theanine/caffeine combination is dependent on the visual modality as

1	opposed to a general attention effect. This intersensory attention task has been shown to
2	be sensitive to the effects of L-theanine (Gomez-Ramirez et al., 2007).
3	During each trial, a cue was presented which instructed participants to which
4	modality they would need to attend to. This cue was followed by a pair of visual stimuli
5	(Gabor patches that could differ in orientation) and/or auditory stimuli (tones that could
6	differ in pitch), with a stimulus onset asynchrony of 1200 ms. Stimuli were presented in
7	isolation (unisensory) or in combination (intersensory). Participants were required to
8	perform a discrimination task on stimuli in the cued modality. The intertrial interval
9	randomly varied between 1400 and 2400 ms. Total task duration was 30 minutes.
10	Switch task (Rogers & Monsell, 1995). The switch task measures executive
11	control, particularly the ability to shift back and forth between different task sets (i.e.,
12	task switching (Monsell, 2003)). This task has been shown to be sensitive to the effects
13	of the L-theanine/caffeine combination (Owen et al., 2008).
14	During each trial, a letter and number were simultaneously presented in red or
15	purple. The letter was randomly drawn from a set of four vowels (A, E, I, U) and four
16	consonants (G, K, M, R), the number from a set of four even (2, 4, 6, 8) and four odd
17	numbers (3, 5, 7, 9). If the letter and number stimuli were purple, participants were
18	required to press spacebar if an even number was presented; if the stimuli were red, they
19	were required to respond if a vowel was presented. In all other cases participants were
20	to withhold their response. Stimulus colours and the associated task set changed every
21	three trials. Intertrial intervals were 1700 ms. Total task duration was 10 minutes.
22	Bond-Lader visual analogue scales (Bond & Lader, 1974): Bond-Lader visual
23	analogue scales comprise of 16 bipolar items consisting of adjective pairs (e.g.

1	dimension defined by the two adjectives. These visual analogue scales have been shown
2	to posses sound psychometric properties and factor analysis has revealed three distinct
3	factors: alertness, contentedness, and calmness. Furthermore, these scales have been
4	found to be sensitive to the effects of L-theanine and caffeine in combination (Haskell et
5	al., 2008; Owen et al., 2008; Giesbrecht et al., submitted).
6	
7	Procedure
8	Participants arrived at the laboratory at 9.00am, after abstaining from caffeine-
9	containing products, alcohol, and mushrooms (as some boletes contain L-theanine) from
10	9.00pm the previous day. Compliance with restrictions was checked by means of a self-
11	report checklist. Next, participants completed a baseline measurement, followed by a 10
12	minute break during which they consumed the drink, and participated in two test
13	sessions starting 10 and 60 minutes post-drink.
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15	Analysis
16	Subjective mood scores and reaction times on the attention tests were analyzed using a 2
17	x 2 mixed model ANOVA with drink (active drink vs. placebo) and session (10-50 min
18	post-drink vs. 60-100 min post-drink) as within-subject factors. The proportion of
19	correct responses were analysed with a logistic regression-based Generalised Linear
20	Mixed Model, with aforementioned within-subject factors. For the switch task, trial type
21	(1st, 2nd, 3rd trial after a switch between task sets) and response type (correct hit, correct
22	withhold) were additional within-subject factors.
23	All analyses employed baseline scores and drink order as covariates. Statistical
24	tasts were two sided with alpha $= 0.05$ . In case of significant main or interaction effects

- 1 involving treatment, the analysis was rerun with habitual caffeine consumption as an
- 2 additional covariate. Standard error and degrees of freedom were estimated by the
- 3 Kenward-Roger method.

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#### Results

Intersensory attention task. On the unisensory auditory subtask, there was a trend for a main effect of drink on reaction times (F(1,23.2) = 3.58, p = 0.071) with participants tending to respond faster after the active drink as compared to placebo. On the intersensory visual subtask, there was a tendency for a drink by session interaction for reaction times (F(1,52) = 3.21, p = 0.079) indicating that after the active drink but not after placebo, participants tended to respond faster at 60-100 minutes post-drink.

The proportion of correct responses did not differ significantly between conditions.

Switch task. Mean switch task accuracy for both drinks 10-50 minutes and 60-100 minutes post-drink, is presented in Figure 1. There was a main effect of drink (F(1, 24.56) = 10.71, p = 0.003) indicating a higher proportion of correct responses after the active drink as compared to placebo. This effect remained significant after including habitual caffeine intake as a covariate, indicating that the improvement in number of correct responses was not simply due to relief of caffeine withdrawal. Furthermore, Figure 2 displays mean accuracy for both drinks for response type (i.e., respond vs. withhold). As can be seen, there was a significant interaction between drink and response type (F(1, 665) = 18.38, p < 0.001) indicating a more pronounced effect of the active drink on correct hits (t(38.3) = 4.52, p < 0.0001) than on correct withholds (t(29.9) = 1.41, n.s.). Worthy of note is that as the proportion of correct withholds was

- 1 not impaired by the drink, a shift in response bias can not account for the effect on 2 correct hits.
- 3 Reaction times did not differ significantly between conditions. This indicates
- 4 that the effect of the active drink on accuracy is not due to a speed/accuracy trade-off.
- 5 The drink by trial type (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> trial after a switch between task sets) interaction was
- 6 not significant, signifying that the active drink did not affect switch costs.
- 7 Bond-Lader visual analogue scales. Participants tended to feel more alert after
- 8 the placebo compared to the active drink as evidenced by a trend towards a main effect
- 9 of drink (F(1, 22.5) = 3.82, p = 0.063). Self-rated calmness and contentedness did not
- show a main effect of drink.

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#### Discussion

The main findings of the present study can be summarized as follows. First, this study provided further evidence that the L-theanine/caffeine combination improves attention on the switch task (see also Owen et al., 2008). The improvement in accuracy in combination with unaltered response speed is in line with previous studies (Kelly et al., 2008; Giesbrecht et al., submitted). Second, tentative evidence was found that the effect of the L-theanine/caffeine combination on attention is not specific to the visual modality, as we found faster responses in both the visual and auditory modality on the intersensory task after the combination as compared to placebo. Third, improvements in attention as signified by the behavioural tasks were not associated with enhanced subjective alertness. This was not in line with previous studies, where L-theanine and caffeine in combination increased subjective alertness as compared to placebo (Haskell et al., 2008; Owen et al., 2008; Giesbrecht et al., submitted).

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The finding that L-theanine and caffeine in combination clearly improved task switching while intersensory attention was only marginally improved raises questions about the specificity of the attention effect of L-theanine and caffeine. In the context of Posner's model of attention, which discriminates between three aspects of attention (i.e., alerting, orienting, and executive control; Posner & Petersen, 1990), the switch task is considered an example of a task assessing executive control as it involves response inhibition. However, as switch costs were not affected by the drink, it could be also argued that alerting (i.e. sensitivity to incoming target stimuli) was improved rather than executive control. The intersensory attention task on the other hand is considered to predominantly tap into the orienting aspect of attention as it involves the selection of specific information from multiple sensory inputs. The current results therefore seem to suggest that the L-theanine/caffeine combination improves executive control/alerting rather than orienting. To the best of our knowledge, self-reported alertness has not been characterized in terms of alerting, orienting and executive control, hence precluding interpretation of drink effects on self-reported alertness in the light of Posner's model. These interpretational ambiguities notwithstanding, evidence indicates that the L-theanine/caffeine combination influences attention. The findings cannot be accounted for by a general increase in arousal, as demonstrated by the lack of improvement in selfreported alertness. Notably, this is in contrast to the effect of caffeine in isolation. By and large there is consensus that caffeine improves basic cognitive functions (e.g., simple reaction times, vigilance) by increasing arousal via modulating adenosine receptor activation in the brain, while leaving higher-order functions unaffected (Smith et al., 2002). Although two recent studies have shown that a high dose of caffeine may improve aspects of executive control (Tieges, Snel, Kok, Wijnen, Lorist, & Richard,

1	2006; Brunye, Mahoney, Lieberman, & Taylor, In Press), the totality of the evidence is
2	equivocal. Thus, whereas caffeine predominantly yields arousing effects, a high dose of
3	L-theanine may counteract these effects, therefore allowing for improved performance
4	on higher-order tasks as employed in the current study. A case in point is that although
5	caffeine is known to increase blood pressure (James, 2004), a high dose of L-theanine
6	counteracted these arousing effects (Rogers, Smith, Heatherley, & Pleydell-Pearce,
7	2008).
8	The main limitation of the present study is that it did not include L-theanine or
9	caffeine in isolation and we therefore cannot conclude that the L-theanine/caffeine
10	combination produces greater attention benefits, than one would be able to achieve with
11	L-theanine or caffeine in isolation. Yet the robust synergistic effects of L-theanine with
12	caffeine found in previous studies make this very likely (Haskell et al., 2008; Kelly et
13	al., 2008; Owen et al., 2008).
14	In conclusion, the present study showed that the L-theanine/caffeine
15	combination improves attention as measured with the switch task, and to a lesser degree
16	with the intersensory attention task. Taken together with the previous studies, we
17	conclude that a high dose of L-theanine combined with caffeine, at the level of a single
18	cup of tea, can help to improve attention.
19	
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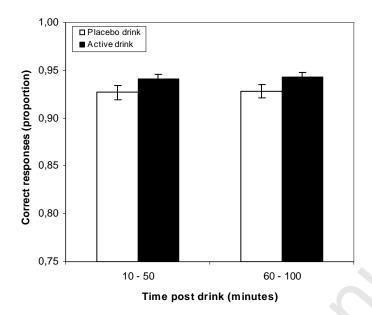
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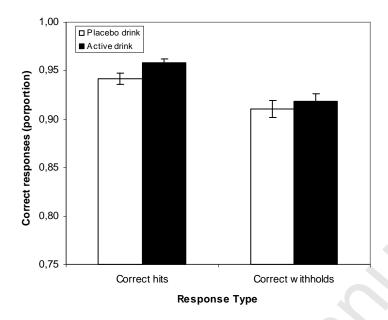
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Fig. 1 Significantly greater proportion of correct responses on switch task relative to

3 baseline after consuming the active drink than after placebo. Error bars represent

4 standard errors of the mean.



2 Fig. 2 Significantly greater proportion of correct responses on switch task after the

- 3 active drink was more marked for correct hits than for correct withholds. Error bars
- 4 represent standard errors of the mean.