EFFECT OF L-THEANINE ON SELECTIVE ATTENTION IN A TRAFFIC-RELATED REACTION TASK IN SLEEP-DEPRIVED YOUNG ADULTS: A DOUBLE-BLIND PLACEBO-CONTROLLED, CROSSOVER STUDY

Group 10

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Table of Contents

List of tables and figures

List of abbreviations

ADHD	- Attention deficit hyperactivity disorder
ANOVA	- Analysis of variance
CI	- Confidence interval
CLINCON	- Clinical Neurophysiology and Cognitive Neuroscience
DMN	- Default mode network
EEG	- Electroencephalogram
ERP	- Event related potentials
FDA	- Food and Drug Administration
fMRI	- functional magnetic resonance imaging
IQR	- Inter-quartile range
MEG	- Magnetoencephalography
NIH	- National Institute of Health
PC	- Personal computer
SD	- Standard deviation
SPSS	- Statistical Package for the Social Sciences
VAS	- Visual analogue scale

List of annexes

Abstract

Background: L-theanine is a non-protein-forming amino acid naturally found in tea leaves (*Camelia sinensis*). Limited evidence suggests that L-theanine improves attention. Sleep deprivation is known to impair attention, making psychomotor reactions delayed and more erratic, and potentially affecting the performance and safety of automobile driving.

Objectives: We aimed to determine whether L-theanine improves neuro-behavioural measures of selective attention in acutely sleep-deprived healthy adults in a traffic-based recognition reaction time task.

Methods: In a double-blind, placebo-controlled, counterbalanced, two-way crossover experiment we compared the effects of a single 200-mg dose of L-theanine with a placebo (150 ml of distilled water) on a traffic-scene-based visual recognition reaction time task, in 24 healthy volunteers aged 20-25 years (13 males), who were deprived of sleep the night before testing. Their task was to respond to imminent traffic accident scenes presented on a computer screen as fast as possible by pressing a response button while ignoring safe scenes. They were tested pre-dose and 45 minutes post-dose, with each treatment administered one week apart. Number of hits (i.e. responses to imminent accident scenes) and false alarms (i.e. responses to safe scenes); and hit reaction time of the task were considered the main outcome measures.

Results: Eighty-eight percent of the test sessions showed 100% hit rate, and all sessions had a hit rate more than 90%. In pre-dose vs. post-dose comparison, hit rates were similar between two treatments. However, L-theanine significantly reduced the number of false alarms ($p = 0.014$), whereas placebo did not ($p > 0.05$). L-theanine reduced reaction time to imminent accident scenes by 38.65 ms ($p = 0.007$) and placebo by 19.08 ms ($p = 0.016$), thus L-theanine showing a 20-ms advantage over placebo.

Conclusions: Our results indicate L-theanine improves selective visual attention by increasing the speed of information processing and improving target-distractor discriminability in sleep-deprived healthy volunteers. It is consistent with functional magnetic resonance imaging changes reported in previous studies, where equivalent doses of L-theanine were found to suppress cortical processing of distractors and reduce the activity of the default mode network of the brain (that is implicated in mind-wandering) during visual selective attention tasks. Recording of brain electrophysiology during the same visual attention task in future experiments will provide concurrent neurophysiological evidence for the attentional effects we observed with L-theanine.

1. Introduction

1.1. Background

L-Theanine, also known as gamma-glutamylethylamide, is a non-protein forming amino acid with a chemical structure similar to that of glutamic acid (Nathan et al., 2006). It is found naturally in tea leaves, and this happens to be the only significant dietary source of L-theanine. A cup of tea including both green and black tea, contains 4.5-22.5 mg of L-theanine (Einöther and Martens, 2013).

L-theanine is absorbed from the small intestine and shows a peak plasma concentration one hour after ingestion in rat brains (Terashima et al., 1999). It has been shown to cross the blood brain barrier and show EEG changes 40 minutes after oral administration (Juneja et al., 1999; Nobre et al., 2008) although it reached maximum concentrations within the brain after 5 hours. L-Theanine, is completely cleared from plasma within 24 hours (Terashima et al., 1999).

L-theanine has a structure similar to glutamic acid. It binds to subtypes of glutamate receptors, but with relatively low affinity. It has been shown to increase dopamine concentration in the rat brain in a dose dependent manner (Yokogoshi et al., 1998). Animal studies have reported a complex range of neurochemical actions following L-theanine administration, including inhibiting glutamate reuptake (Sugiyama and Sadzuka, 2003), increasing brain GABA (Kimura and Murata, 1971) and striatum dopamine and glycine concentrations (Yamada et al., 2008) while serotonin levels were reported to decrease globally with region-specific increases in the striatum, hippocampus and hypothalamus (Yokogoshi et al., 1998).

Tea has been traditionally consumed as a beverage, and it is believed that it has certain relaxing and calming effects. These effects were attributed to one of its components, L-theanine. Production of L-theanine in purified form was carried out for use as a nutritional supplement and for research. Subsequently, the effects of L-theanine alone as a relaxant were proved to a certain extent. Lu et al. (2004) studied the subjective mood effects of L-theanine and found that 200 mg of 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. Later, in 2007, it was shown that a single 200 mg dose of L-theanine reduced acute stress responses (i.e. subjective perception, heart rate and salivary IgA levels) induced by a mental arithmetic task (Kimura et al., 2007). It was further proved that a 250 mg dose slowed reaction time on a visual probe task indicating reduced anxiety (Rogers et al., 2008). In a recent systematic review, Williams et al. (2020) suggested that doses of 200-400 mg of L-theanine may exhibit certain stress-relieving effects.

L-theanine is safe even in very high doses. Animal studies show that rats (Borzelleca et al., 2006) and mice (Fujii and Inai, 2008) fed with extremely high doses (up to 4 g per kilogram body weight) of L-theanine for 13 weeks do not develop significant adverse effects. Similarly, 8-12-year-old boys with attention deficit hyperactivity disorder (ADHD) have tolerated 400 mg of L-theanine per day for a period of 6 weeks, with no adverse effects (Lyon et al., 2011). Similarly, Sarris et al. (2019), found greater sleep satisfaction on self-report among patients with

generalised anxiety disorder following 450-900 mg doses of L-theanine, but no significant adverse effects. In 2006, L-theanine was given FDA approval in the United States, and is currently available as a supplement in the market (FDA, 2006a, b). Certain types of supplements and beverages are now being enriched with L-theanine (Daou et al., 2019; Gibson et al., 2020; Zaragoza et al., 2019).

1.2. Effects of L-theanine on cognitive function: a literature review

The experimental studies conducted hitherto on the acute cognitive effects of L-theanine, both alone, and in combination with caffeine (another constituent of tea and coffee) are summarised in Table 1. A general trend observed in these experimental studies was that L-theanine was administered in relatively high doses – perhaps due to its general safety in humans – with doses ranging from 50 mg to 400 mg. The participants of most of these studies were healthy young individuals between the ages of 20 and 40 years, perhaps because potential interference from confounding factors is far less in that group of participants when compared with more extreme age groups.

However, the studies were heterogeneous in two main aspects: 1) the variability in cognitive domains tested and 2) the variability in techniques used to assess these domains (Table 1).

Researchers have investigated the effect of L-theanine on different cognitive domains including attention, memory encoding, working memory, judgment and evaluation, reasoning, problem-solving, and decision making. For example, Kelly et al. (2008), White et al. (2016), Dodd et al. (2015), Rogers and Smith (2008) and Foxe et al. (2012) experimented on the effect of L-theanine on selective attention; whereas White et al. (2016) examined the effects on mathematical processing, psychomotor tracking and memory (Table 1).

Effects of L-theanine on cognition have been tested using subjective ratings, behavioural testing, electrophysiology, and more recently, neuroimaging. Assessment of mood, anxiety, stress levels and fatigue were measured with subjective rating scales in certain studies such as those conducted by Giles et al. (2017), Rogers et al. (2008) and White et al. (2016); whereas behavioural tests were conducted by Gomez-Ramirez et al. (2007; 2009) and Haskell et al. (2008). Electrophysiological modalities such as EEG (Dassanayake et al., 2020; Foxe et al., 2012; Gomez-Ramirez et al., 2007; Gomez-Ramirez et al., 2009; Kahathuduwa et al., 2017; Kelly et al., 2008), MEG (White et al., 2016), and functional magnetic resonance imaging (fMRI) techniques (Kahathuduwa et al., 2018; Kahathuduwa et al., 2020) were particularly used to test selective attentional mechanisms of the brain.

Table 1. Summary of experimental studies that determined the acute effects of L-theanine on cognitive functions.

Study	Design	Participants	Treatments	Outcome measures and results	Comments
(Gomez-Ramirez et al., 2007)	Double blind, placebo-controlled, counter-balanced experiment	15 healthy volunteers	1. 250 mg L-theanine in 200 ml water 2. Placebo (200 ml of water)	Reaction time in intersensory (auditory and visual) attention task: Slower after L-theanine treatment than placebo for both the unisensory – auditory and multisensory-auditory stimulus types. Alpha EEG activity: Attention related increase in anticipatory (i.e. phasic) alpha power and decrease in background (tonic) alpha power.	
(Kelly et al., 2008)	Single-blind, placebo-controlled, 4-way, crossover trial	16 healthy volunteers (5 females); age 21-40 years; with low habitual tea (3.7 cups/week) and coffee (3.8 cups/week) consumption	1. 100 mg of L-theanine 2. 50 mg of caffeine 3. L-theanine and caffeine combination 4. Placebo (100 ml distilled water)	Theanine: no effect on hit rate or stimulus discrimination or EEG alpha power Caffeine: significantly improved stimulus discrimination Theanine-caffeine combination: improved hit rate and stimulus discrimination	Single blinding may introduce a bias. The order effect superseded that arising from treatment.
(Rogers et al., 2008)	Randomized, double-blind placebo-controlled trial.	48 healthy volunteers divided into 4 groups; age 18-28 years	1. 200 mg of L-theanine 2. 250 mg of caffeine 3. L-theanine and caffeine combination 4. Placebo (distilled water)	Reaction time: L-Theanine slowed reaction time on the visual probe task whereas caffeine made this faster, but this effect was not significant.	

Table 1. Continued.

Study	Design	Participants	Treatments	Outcome measures and results	Comments
(Haskell et al., 2008)	Randomized, placebo-controlled, double-blind, counterbalanced, crossover study	24 healthy volunteers; age 18-34 years	1. 200 mg of L-theanine 2. 250 mg of caffeine 3. L-theanine and caffeine combination 4. Placebo (Peach Lite Lipton iced tea)	Reaction time and accuracy: Caffeine & L-theanine improved the simple reaction time, accuracy of rapid visual information processing, numeric working memory reaction time, delayed word recognition reaction time and accuracy of sentence verification.	
(Gomez-Ramirez et al., 2009)	Double-blind, placebo-controlled, counterbalanced experiment.	13 healthy volunteers (9 females) mean age: 23.5 years (SD = 3.25 years)	1. 250 mg L-theanine in 200 ml water 2. Placebo (200 ml of water)	Behavioural Performance: No effects of treatment on attention and no interaction of treatment on attention. Tonic alpha band activity: Reduction over posterior regions of right hemisphere with L-theanine compared to placebo. Phasic EEG alpha band activity: Directing attention to the left visual field evoked significantly greater alpha band activity over the left hemisphere than directing attention to the right visual field.	
(Higashiyama et al., 2011)	Double-blind, Placebo-controlled, counterbalanced, crossover study	18 healthy male volunteers; age 18-20 years	1. L-Theanine 200 mg in 100 ml of water 2. Placebo (100 ml water)	Attention and reaction time in an auditory task: L-Theanine enhanced reaction time only in high-anxiety participants and no effect in low-anxiety participants.	

Table 1. Continued.

Study	Design	Participants	Treatments	Outcome measures and results	Comments
(Foxe et al., 2012)	Double-blind, placebo-controlled, 4-way, crossover trial	27 healthy volunteers (8 females); age 18-40 years.	1. 100 mg L-theanine 2. 50 mg caffeine 3. L-theanine and caffeine combination 4. Placebo (200 ml water)	Sustained visual attention to response task: L-Theanine significantly decreased omission errors by 36% and decreased commission errors by 23% relative to placebo. No main or interaction effects involving theanine, caffeine or the combination on reaction time.	
(Dodd et al., 2015)	Double-blind, placebo-controlled, counterbalanced, crossover study	24 healthy volunteers (10 males); age 18-35 years	1. 50 mg L-theanine 2. 75 mg of caffeine 3. L-Theanine and caffeine combination 4. Placebo	Performance visual choice reaction task: No effect of L-theanine on reaction time. Caffeine decreased reaction time. L-Theanine co-administration with caffeine abolished this effect.	
(White et al., 2016)	Randomized, placebo-controlled, double-blind, crossover study	36 healthy volunteers; age 18–40 years	1. L-Theanine (200 mg) 2. Placebo 3. L-Theanine (200 mg) 4. Placebo	Mood response to cognitive stress: Subjective stress response to a multitasking cognitive stressor, was significantly reduced one hour after administration of L-theanine. Resting state MEG alpha oscillatory activity: Significantly greater in posterior scalp sites after L-theanine compared to placebo.	Change in resting state alpha oscillatory activity was not correlated with the change in subjective stress response or the cortisol response.

Table 1. Continued.

Study	Design	Participants	Treatments	Outcome measures and results	Comments
(Giles et al., 2017)	Double-blind, counterbalanced, 4-way, crossover study	36 healthy volunteers (12 males); mean age 19.3, SD = 1.7 years	1. 200 mg L-theanine 2. 200 mg caffeine 3. L-theanine and caffeine combination 4. Placebo All treatments as capsules	Global vs. local processing in a hierarchical shape: Global processing increased with caffeine than placebo, no significant difference in global and local processing between combination treatment and placebo. Caffeine accentuated global processing and L-theanine accentuated the local processing. Visual attention: Caffeine or L-theanine didn't affect lower order visual attention following an emotionally arousing experience.	
(Kahathuduwa et al., 2017)	Double-blind, placebo-controlled, 5-way, counterbalanced, crossover study.	20 healthy males; age 21-23 years	1. L-Theanine (200 mg) 2. Caffeine (160 mg) 3. L-Theanine and caffeine combination 4. Black tea (150 ml) 5. Placebo (distilled water 150 ml)	Recognition visual reaction time: Significantly improved by L-theanine, caffeine and their combination but not by tea or placebo. Simple visual reaction time: Did not show significant inter-treatment difference. Auditory event-related potentials: L-Theanine, caffeine and the combination elicited lager mean peak to peak N2-P300 ERP amplitude than placebo.	Auditory event-related potentials were measured only post-dose. A pre-post comparison cannot be made.

Table 1. Continued.

Study	Design	Participants	Treatments	Outcome measures and results	Comments
(Kahathuduwa et al., 2018)	Double-blind, placebo controlled, repeated-measures, counterbalanced, 4-way crossover trial	9 adult males volunteers; age 18-60 years	1. L-Theanine (200 mg) 2. Caffeine (160 mg) 3. L-Theanine (200 mg) and caffeine (160 mg) 4. Placebo (200 ml of distilled water)	Reaction time of visual attention task: L-Theanine and the combination resulted in faster visual colour stimulus discrimination reaction times. Functional MRI activity: L-Theanine decreased fMRI responses to distractor stimuli in brain regions that regulate visual attention (i.e., decreased neural resource allocation for distractors) L-Theanine and L-theanine-caffeine combination decreased fMRI responses to targets compared to distractors in brain areas involved in mind wandering.	
(Dassanayake et al., 2020)	Double-blind, placebo-controlled, 4-way crossover study	28 healthy young adults (17 males); age range: 24-37 years	1. 100 mg L-theanine 2. 200 mg L-theanine 3. 400 mg L-theanine 4. Placebo (150 ml of distilled water)	Primary: Behavioural measures: None of the doses significantly improved mean reaction time to target tones compared to the placebo. ERP measures: All doses of L-theanine reduced mean P3b latency linearly, but a significant effect was observed only with 400 mg (i.e. L-theanine in high doses enhanced attentional processing in a dose-dependent manner. Secondary Latencies & amplitudes of pre- or early attentive processing components: N1 latency at CZ scalp site was delayed with 100 mg of L-theanine but not with higher doses. VAS Alertness score: Subjective self-assessment of level of alertness was not affected by any of the doses.	Authors speculate that the task demands of the auditory two-tone attention task was relatively low leading to a "ceiling effect" behaviourally.

Table 1. Continued.

Study	Design	Participants	Treatments	Outcome measures and results	Comments
(Kahathuduwa et al., 2020)	Randomized, placebo-controlled four-way repeated measures crossover trial.	6 male children with ADHD; age 8-17 years	1. 2.5 mg/kg body weight of L-theanine (range 70-263 mg) 2. 2.0 mg/kg body weight of caffeine (range 56-210 mg) 3. Combination of 2.5 mg/kg body weight of L-theanine and 2.0 mg/kg body weight of caffeine 4. Placebo (100 ml of distilled water)	Sustained attention in an auditory task: L-theanine did not improve task accuracy. L-theanine and caffeine was associated with task-related decrease in reactivity of a brain network associated with mind wandering (default mode network). Inhibitory control: Caffeine worsened and L-theanine had a trend of worsening inhibitory control (increased stop signal reaction time). L-Theanine and caffeine combination showed a trend of improvement of inhibitory control. Overall cognition: L-theanine improved total cognition composite in NIH cognition toolbox vs. placebo L-theanine and caffeine combination improved total cognition composite.	

1.2.1. Effects of L-theanine on selective attention

The literature reviewed hitherto indicates that – among different cognitive domains – selective attention is the most extensively studied. Selective attention is the ability to direct attention to the task-relevant stimulus while ignoring the distractors in an environment with competing stimuli (Lezak et al., 2004). Alpha EEG rhythm is sensitive to overall attentional states. When focusing attention selectively to a certain task; anticipatory (i.e. phasic) alpha power increases and background (tonic) alpha power decreases. Gomez-Ramirez et al. (2007) found that, in an inter-sensory attention task, parieto-occipital alpha power is increased more for visual stimuli than to auditory stimuli by a dose 250 mg of L-theanine. Corroborating these findings in a functional MRI study, Kahathuduwa et al. (2018) also found a similar dose of L-theanine improves visual processing in visual association areas of the brain, while decreasing the activation of some brain areas that constitute the default mode network, that is associated with mind wandering. A more recent study conducted by the same authors replicated these findings in group of children with ADHD who received L-theanine (Kahathuduwa et al., 2020). The study further went on to show that compared to a placebo, L-theanine significantly improved total cognition composite and sustained attention in these children. Some recent electrophysiological evidence suggests that auditory attentional processing is enhanced by high doses (100-400 mg) of L-theanine in a dose-dependent manner in healthy young adults (Dassanayake et al., 2020).

Different modalities have been considered in order to measure selective attention; but in most studies, either auditory or visual selective attention has been tested. In certain studies, both visual and auditory stimuli were used in indexing reaction time and accuracy to assess selective attention. For example, Higashiyama et al. (2011) used numbers ranging from 1 to 9 as visual cues used to test recognition reaction time, whereas tones of varying frequency were used as auditory stimuli. Gomez-Ramirez et al. (2007) used both visual and auditory stimuli in an intersensory task.

Visual selective attention paradigms have been used more than auditory selective attention paradigms. The visual stimuli used varied among different research studies. The series of studies conducted by Kahathuduwa et.al. (Kahathuduwa et al., 2016; Kahathuduwa et al., 2017; Kahathuduwa et al., 2018), presented flashes of red and white on a computer screen as distractors and targets, respectively. Dodd (2015) conducted a study where upward pointing arrows were used in order to assess simple reaction time. In Kelly et al. (2008) and Gomez Ramirez et al. (2009) studies, arrows embedded in circles of the colours red and green were used as cues whereas crosses appearing at the periphery of the visual field were used.

When considering the above task paradigms, the general trend was to use abstract visual stimuli rather than visual scenes from real-life. Therefore, to improve the ecological validity of the present research study, we considered incorporating more real-life-based stimulus paradigms in the assessment of the effects of L-theanine on selective attention. Given that in the contemporary society driving is one of the main daily activities that heavily relies on selective attention–particularly under time constraints–we adopted a driving scenario-based reaction time task in this study. The task we used was a computerised visual stimulus recognition task originally developed and tested by Martin et al. (1992). This task presented pictures of safe

driving scenes and imminent accident scenes (as seen from the driving seat of a car) which were used as distractor and target stimuli, respectively.

It is also noteworthy that all previous experimental studies on the cognitive effects of L-theanine on healthy adult test groups were on well-rested, healthy individuals who received an adequate sleep. However, among healthy adults, sleep deprivation is perhaps the most common situation that impairs attention and reduces reaction accuracy and delays reaction time. In the modern society, it is also not uncommon for sleep-deprived individuals to engage in real-life attentional tasks (such as driving and operating machinery) that demand high accuracy and fast psychomotor reactions.

1.3. Research hypothesis and objectives

Given that L-theanine has not shown unequivocally to improve attention in relatively easy tasks when administered to healthy volunteers under optimal conditions, we aimed to test the effects of L-theanine on participants in a relatively compromised state, i.e. under sleep deprivation; using a more demanding traffic-scene related attentional task originally developed and used by Martin et al. (1992), hypothesizing that those experimental conditions unravel any beneficial effects of L-theanine on selective attention. Specifically, we hypothesized that a high-dose of orally administered L-theanine, compared to a placebo (distilled water), will increase accuracy and reduce reaction time in acutely sleep-deprived, otherwise healthy young individuals.

Consequently, the objective of the present study was to determine whether a single 200 mg dose of orally administered L-theanine–compared to a placebo–acutely improves the accuracy and reaction time, in a traffic-scene-related visual recognition task in acutely sleep-deprived healthy adults.

2. Methodology

2.1. Study design

This study was a double-blind, placebo-controlled, counterbalanced, two-way crossover experiment that compared the effects of a single 200-mg dose of L-theanine (dissolved in 150 ml of distilled water) with a placebo (150 ml of distilled water) on a traffic-scene-based recognition visual reaction time task, in healthy sleep-deprived young adult volunteers. The treatment order was counterbalanced so that half of the sample received the active treatment first and the placebo second, and the other half received the treatments in the reverse order. Reaction time was assessed before (i.e. *pre-dose*) and after (i.e. *post-dose*) each treatment (Figure 1). The two treatments were administered one week apart.

2.2. Study Setting

The study was carried out in the Clinical Neurophysiology and Cognitive Neuroscience (CLINCON) Laboratory at the Department of Physiology, Faculty of Medicine, University of Peradeniya. The CLINCON had the necessary testing equipment, setting and was accessible to the researchers and the participants.

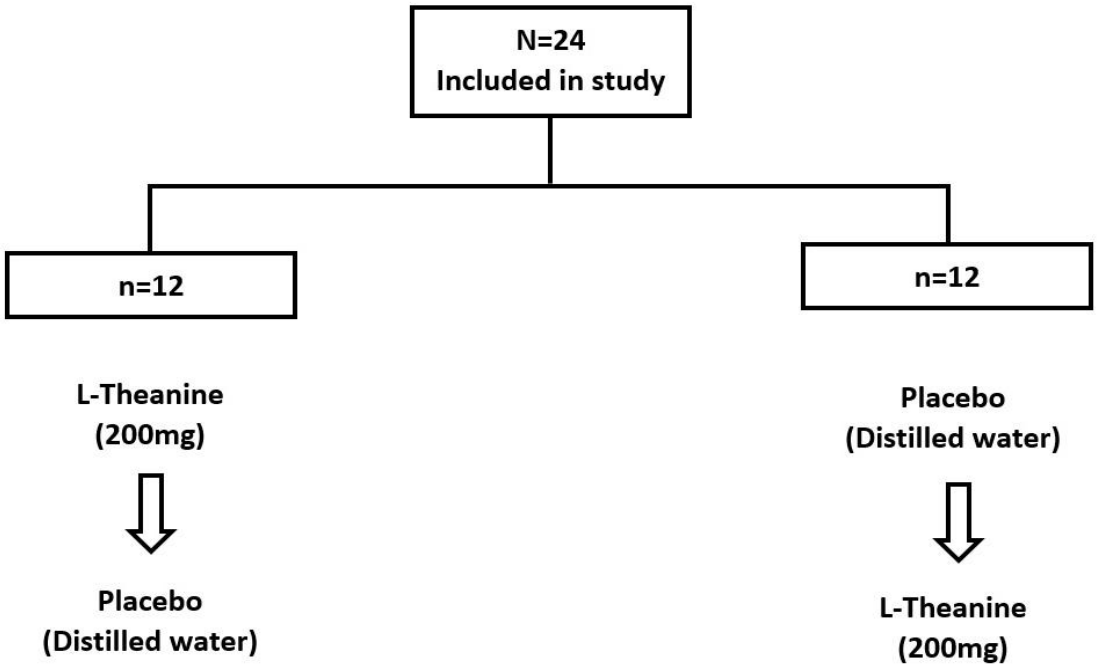

Figure 1. Study design: treatment crossover and counterbalancing

2.3. Participants

Participants were 24 healthy, young adult volunteers between the ages of 20-25 years, without a significant past medical history. They had normal, or corrected-to-normal vision, and normal hearing and were capable of staying awake for 24 hours without significant medical consequences.

We excluded the individuals who consumed more than 5 cups of tea, coffee or other caffeinated drinks per day; those who had alcohol dependence or abuse; those who smoked or consumed nicotine in any form, and those who used recreational psychoactive drugs. Those with learning difficulties, colour blindness, previous history of head trauma, epilepsy, strokes and any other serious neurological or psychiatric illnesses, or those who had taken treatment for such illnesses were also excluded. Those who had undergone a stressful or traumatic experience in the recent past were also excluded.

The eligible participants were selected based on all inclusion and exclusion criteria mentioned above by filling in an interviewer-administered data sheet a week prior to experimentation.

2.4. Ethics

The study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013) and was approved by the Ethics Review Committee of the Faculty of Medicine, University of Peradeniya. Informed written consent was obtained from all the participants before the experiment was conducted. Participants were assigned a participant number (which appeared in the consent form that carried their names) and only this number appeared in the data sheet and computerized test results. The participants had the right to withdraw from this experiment if they wished at any point of time. In order to ensure that routine tasks were not affected due to acute sleep deprivation, we took steps to ensure that participants did not engage in risk activities such as riding on a motorcycle on that day.

2.5. Sample size and sampling

Twenty-four participants were required to observe an effect size (*Cohen's d*) of 0.6, with an alpha error of 0.05 and a power of 80%.

Effect size (*Cohen's d*) = $m_a - m_b / SD$

where,

m_a = pre- vs post-dose reaction time difference with L-theanine treatment,

m_b = pre- vs post-dose reaction time difference with placebo treatment and

SD = standard deviation of pre-post differences across treatment conditions.

The sample size was calculated using a standard sample size calculation formula using the PS Sample Size Calculation™ software. (Producer: William D. Dupont and Walton D. Plummer, Jr. available at https://ps-power-and-sample-size-calculation.software.informer.com/3.1/). Since the present study is a crossover study the eligible participants were consecutively sampled adopting a convenient sampling method.

2.6. Training of test administrators

All test administrators were members of Group 10 assigned for the course module MED4233 (Communication, Learning and Research -5) of the Faculty of Medicine, University of Peradeniya, Sri Lanka. All members of the research team were allocated a specific training period to interview participants and to administer the test. They were trained by a Professor in Neurophysiology who has experience in the testing techniques and research track-record on L-theanine.

2.7. Treatments

The active treatment was a 200 mg dose of L-theanine. L-theanine was obtained as a commercial preparation in its pure form and was stored in airtight packaging prior to usage. It was measured out with a digital micro-scale having a sensitivity of 1 mg. The dose of L-theanine was dissolved in 150 ml of distilled water measured using a standard measuring cylinder and was given to participants in a drinking glass. The placebo treatment was 150 ml of distilled water, and was also administered in the same way. Dissolved L-theanine is colourless, tasteless and odourless, and therefore cannot be distinguished from distilled water. The treatments were prepared and administered by a different member of the research team, and thus the test administrator was also blind to the treatment the participants received on each day of testing.

2.8. Test description

The main cognitive domain evaluated in this experimental study is selective visual attention. It was tested by employing a traffic-scene-related visual recognition reaction time task originally developed by Martin et al. (1992). Visual stimuli were presented, and the responses were registered using the Presentation® (Neurobehavioral Systems, Inc. Albany CA) software on a Windows™ based personal computer. The stimuli were presented on a standard 14.5" PC screen with a resolution of 1024 by 768 pixels, placed at the eye-level of the participant sitting 60 cm from the screen. The participants were asked to fixate their eyes on a fixation cross at the centre of the screen in between stimuli, each visual stimulus was a 384 by 256-pixel picture of a traffic scene, as a car driver would see through the windshield (Figure 2), and it flashed and lasted on the centre of the screen for 200 ms. The stimuli were presented with an inter-stimulus interval of 1600 ms. There was a 20% probability for a given stimulus to be an imminent accident scene (i.e. *target stimulus*) and 80% probability for it to be a safe scene (i.e. *standard stimulus*). The task of the participants was to press the response button with the index finger of

their dominant hand for the targets while ignoring the standard stimuli. A total of 300 stimuli were presented throughout the task in 4 blocks, each block consisting of 15 targets and 60 standards randomly intermixed with the block. The first task block was a practice session. Each task block was run for about 2 minutes 15 seconds and the whole task took about 10 minutes. The latter three blocks (that presented a total of 45 targets and 180 standard stimuli) were assessed to obtain the outcome measures.

Figure 2. Traffic task stimuli and paradigm:
a. A sample of a safe scene; b. A sample of an imminent accident scene; c. A sample stretch of the task paradigm.

At the end of the task, the software reported the following outcome measures (*see also* Figure 3):

1. Hits: correct responses to targets (i.e. *imminent accident scenes*)
2. Misses: missed responses to targets
3. False alarms: active responses made for safe scenes
4. Mean hit reaction time: Time from onset of the target stimulus to response

The first 3 outcomes measured the accuracy of task performance, while reaction time measured the speed.

	Target (Imminent accident scene)	Standard (Safe scene)
Response	Hit	False alarm
No response	Miss	Correct rejection

Figure 3. Definition of accuracy measures of the visual attention task based on response-stimulus parameters.

2.9. Testing protocol

The participants were asked to strictly adhere to the following test preparation instructions given to them:

1) Sleep at least 6 hours per night for 7 days before the experiment. This was to ensure that we eliminated chronic sleep deprivation among participants.

2) To avoid consumption of alcohol within 48 hours prior to experimentation (We assumed that alcohol is completely removed from the body within 48 hours even if a participant was an occasional user).

3) To have only water as a beverage 12 hours prior to experimentation (in order to rule out the confounding effects of other caffeinated beverages).

The participants were tested on a day of the week that they chose to minimize the study participation interfering with the routine tasks of the participants. The test preparation timeline is illustrated in Figure 4. On the day prior to testing, the participants were allowed to engage in routine tasks and were instructed to have a 2-hour sleep after their lunch. On the following day, participants were asked to consume only a light breakfast, half an hour prior to arrival at the laboratory. One of the researchers maintained frequent contact with the participants during the preparation period to ensure that the participants would stick to the above routine.

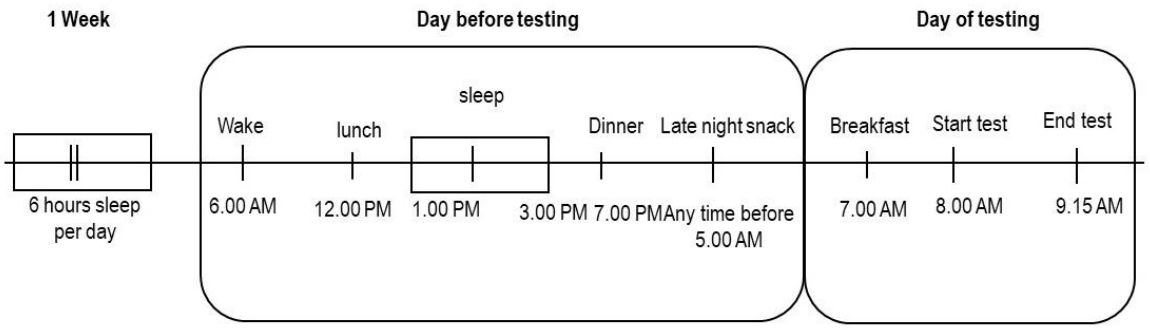

Figure 4. Test preparation timeline.
(Note: Due to time constraints available to carry out the project, half of the participants were tested at 8.00 am and the other half were tested at 10.00 am. The former subgroup had their pre-test day lunch at 12.00 pm and slept from 1-3 pm. The other half took their lunch at 2.00 pm and slept from 3-5 pm.)

The testing protocol of each day of testing is illustrated in Figure 5. Participants arrived at the laboratory 15 minutes in advance to the commencement of the trial. For a 10-minute period, while the participants were seated in front of the PC, a test administrator filled in the data sheet with the information relevant to the participants, in order to ensure that they had adhered to procedural and preparatory guidelines, and that they were still eligible to take part in the study. Then the traffic task was introduced and explained in their native language or any language they were most comfortable with. Then, they engaged in the task described in 2.7. above. Both speed and accuracy of performing the task was emphasized to the participants to optimize outcomes. This was the pre-dose assessment. Once the pre-dose task was completed (duration about 10 minutes), participants were given the treatment prepared for the day according to the assignment of the participant in the counterbalancing subgroup (*as per* Figure 1). They were then made to sit resting for 40 minutes in the laboratory while watching a non-stimulating documentary of a genre they preferred (in order to prevent them getting bored, as done routinely in this type of experiments). Forty-five minutes after they finished drinking the treatment, they repeated the traffic task (i.e. *post-dose test*), that was identical to the pre-dose task.

On completion of the post-dose test, the participants were given the next appointment date and the preparation instructions; and were allowed to leave the laboratory. The preparation and the testing protocol on the second day were identical to that of the first, except that the participants were getting the other treatment.

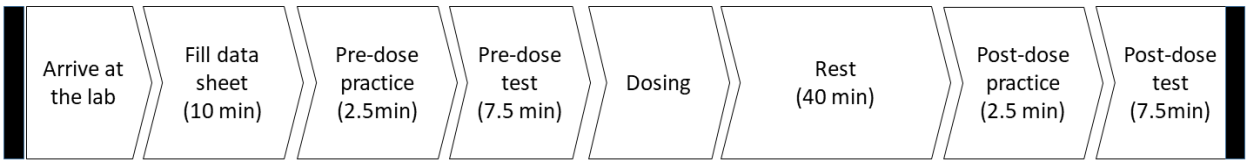

Figure 5. Testing and the treatment protocol for a given day of testing.

2.10. Variables

In this experiment, participants were given both L-theanine solution and placebo in a crossover design and were tested pre- and post-dose. Thus, the independent variables were two categorical variables:

1. Treatment: L-theanine vs. placebo
2. Time of testing: pre-dose vs. post-dose

They have an orthogonal relationship with one another as follows:

		Treatment	
		L-theanine	Placebo
Time	Pre-dose		
	Post-dose		

As defined under 2.7 above, the dependent variables were reaction time and measures of accuracy (*viz.* number of hits and false alarms).

2.11. Data analysis

Anonymised demographic data, treatment conditions and outcome measures were entered into MS Excel, and then IBM SPSS (IBM Corporation, NY, USA) spreadsheets. The distributions of hit and false alarm counts are presented in histograms. False alarm counts are also presented in box and whisker plots. These accuracy measures had skewed distributions and thus were summarised as median and interquartile ranges (IQR); and were analysed using Wilcoxon Signed Rank Test.

Hit reaction time data are presented as means and SD values. A treatment (L-theanine and placebo) × time (pre-dose and post-dose) two-way within-subject analysis of variance (ANOVA) was conducted to analyse the combined treatment and time effects on hit reaction time. Subsequent pairwise pre- vs. post-dose comparisons for each treatment arm were

conducted with paired t-tests. As a subsidiary analysis, inter-session correlation of hit reaction times were explored by calculating Pearson correlation coefficients for hit reaction times among four testing sessions.

Significance level was set at a cut-off *p* value of 0.05 for all statistical tests. Statistical analyses were carried out using IBM SPSS software (IBM Corporation, NY, USA).

3. Results

3.1. Sample characteristics

Of the 24 participants tested, the software-generated traffic task data from one participant had not been recorded, and this was considered data missing-completely-at-random. Consequently, data of the remaining 23 healthy young adults (13 males) of the age range 21 to 26 (mean = 24.3, SD = 1.146) years were analysed.

Of them, 21 were right-handed and two were left-handed on self-report, and they used the same hand to press the response button during testing. Eleven participants were frequent computer users, 11 occasional users, and one was classified as having used a computer at least once. The treatment order was counterbalanced so that 12 participants received the placebo first and L-theanine second, and 11 received the treatments in the reverse order.

3.2. Accuracy data

Hits (correct responses to targets i.e. imminent accident scenes) and false alarms (active responses made for safe scenes) were recorded before and after each dose of placebo and L-theanine. Hits were counted when the participants pressed the response button for accident scenes. Histograms of the hits for all four testing sessions are shown in in Figure 6. A hit rate of more than 90% was observed among the participants in the traffic-task. Of the 23 participants, a hit count of 45 (i.e. 100%) was observed in 19 participants, 44 in two, 43 in one and 41 in one during the pre-dose testing with the active treatment. During the post-dose testing with the same treatment, a hit count of 45 (100%) was observed in 20 participants, 44 in two and 43 in one. The pre-dose results with placebo showed a hit count of 45 in 21 participants and 44 in two. The placebo post-dose results were exactly the same. Overall, 100% hit counts were observed in 81 out of the total 92 (i.e. 88%) testing sessions in all four days. As the data was highly skewed to the left, hit counts were analysed using Wilcoxon Signed Ranks Test. The median hit count in all four testing sessions was 45. Pre-dose vs. post-dose hit counts were not significantly different in the L-theanine arm ($p = 0.496$) or the placebo arm ($p = 1.00$).

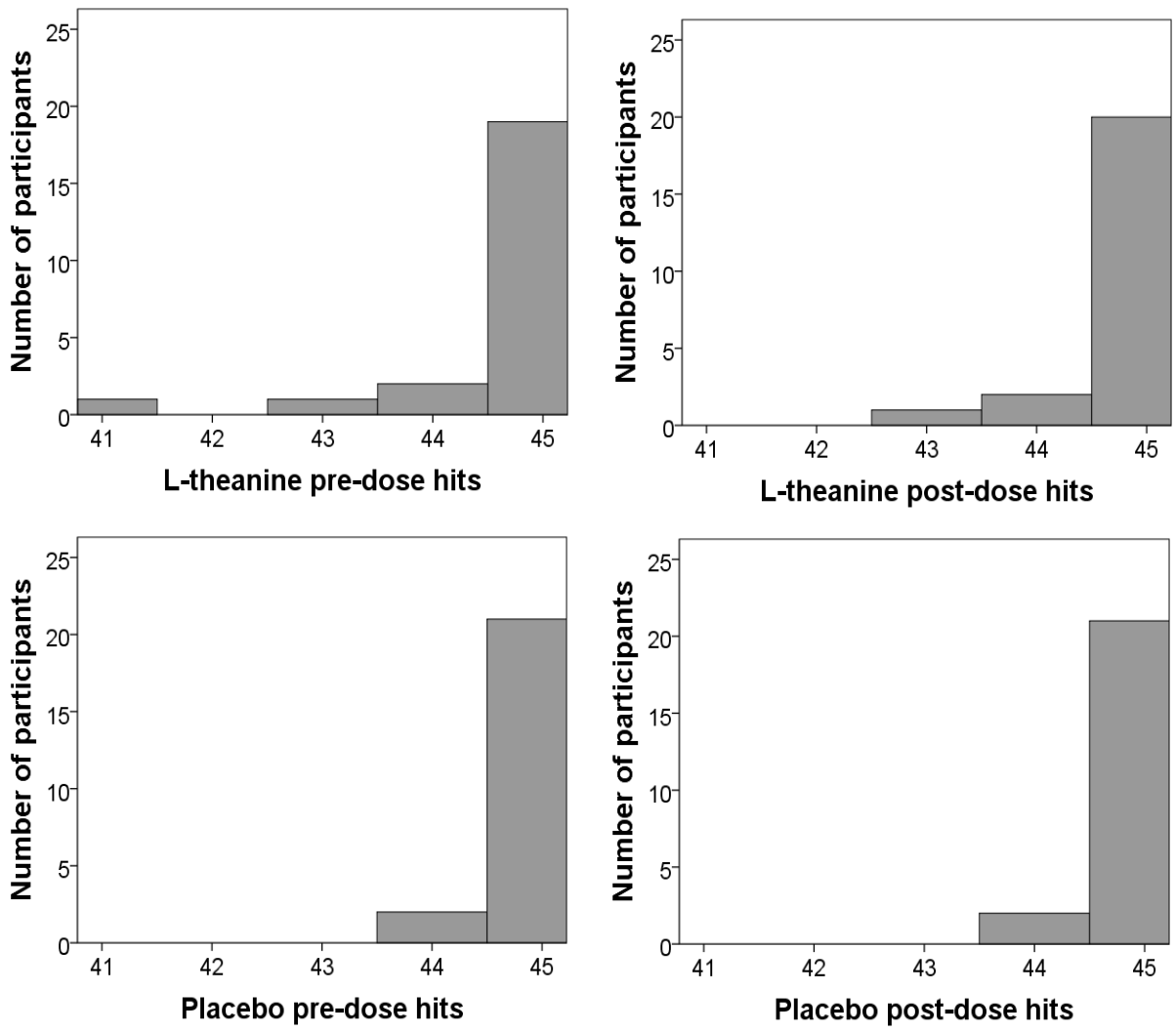

Figure 6. Distribution of hit counts observed before and after treatment with L-theanine and placebo.

False alarms were counted when the participants pressed the response button for non-accident scenes. The histograms of all four sessions are shown in Figure 7, and the box and whisker plot depicting the distribution of false-alarm data is shown in Figure 8. The median number of false alarms for pre-dose test was 3 (inter-quartile range = 6), for post-dose was 2 (inter-quartile range = 5) when participants had L-theanine, while it was 4 (inter-quartile range = 5) for pre-dose test and 2 (inter-quartile range = 7) for post-dose test when participants had placebo. As the distributions were all highly skewed to the right, the data was analysed with Wilcoxon Signed Ranks Test. A significant reduction of the false-alarm count was observed with L-theanine ($p = 0.014$) but not with placebo ($p = 0.073$).

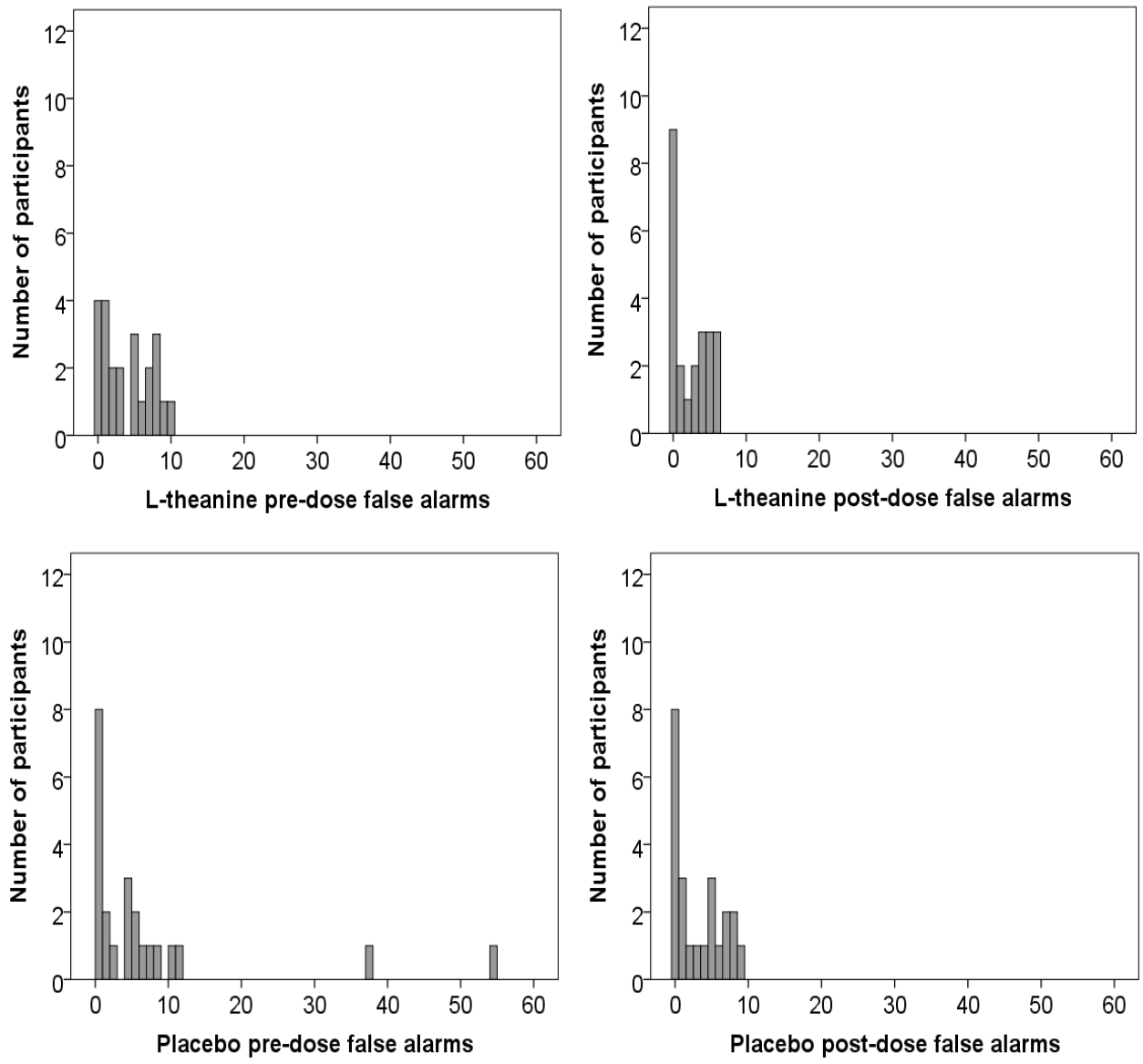

Figure 7. Distribution of false alarms observed before and after treatment with L-theanine and placebo.

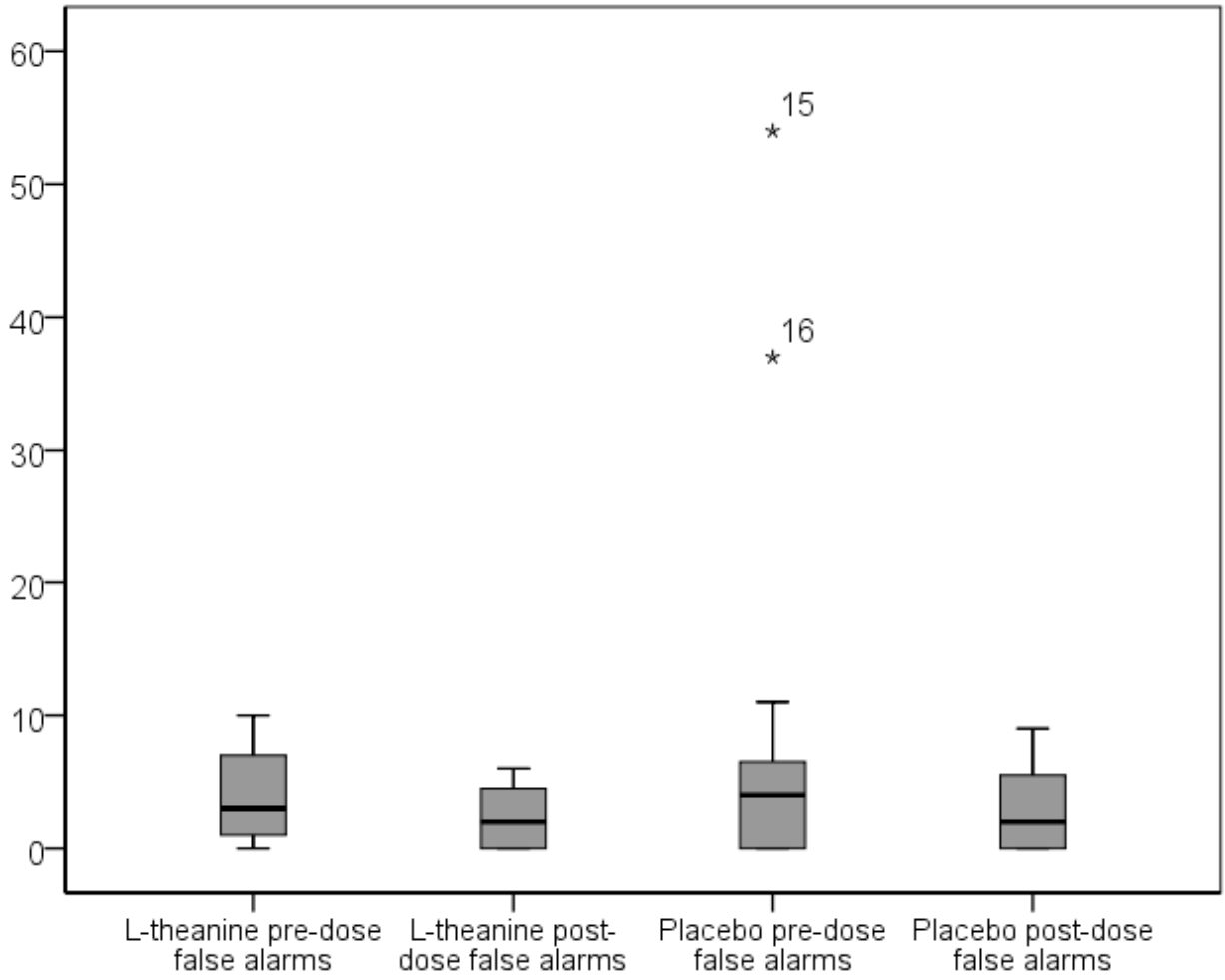

Figure 8. Box-and-whisker plots of false alarms observed among participants before and after treatment with L-theanine and placebo.

3.3. Hit reaction time

For each session of each participant, hit reaction time was measured averaging all 45 trials. Figure 9 shows a sample of trial-by-trial hit reaction times for all 45 trials of a participant in one of the testing sessions (placebo treatment, post-dose session).

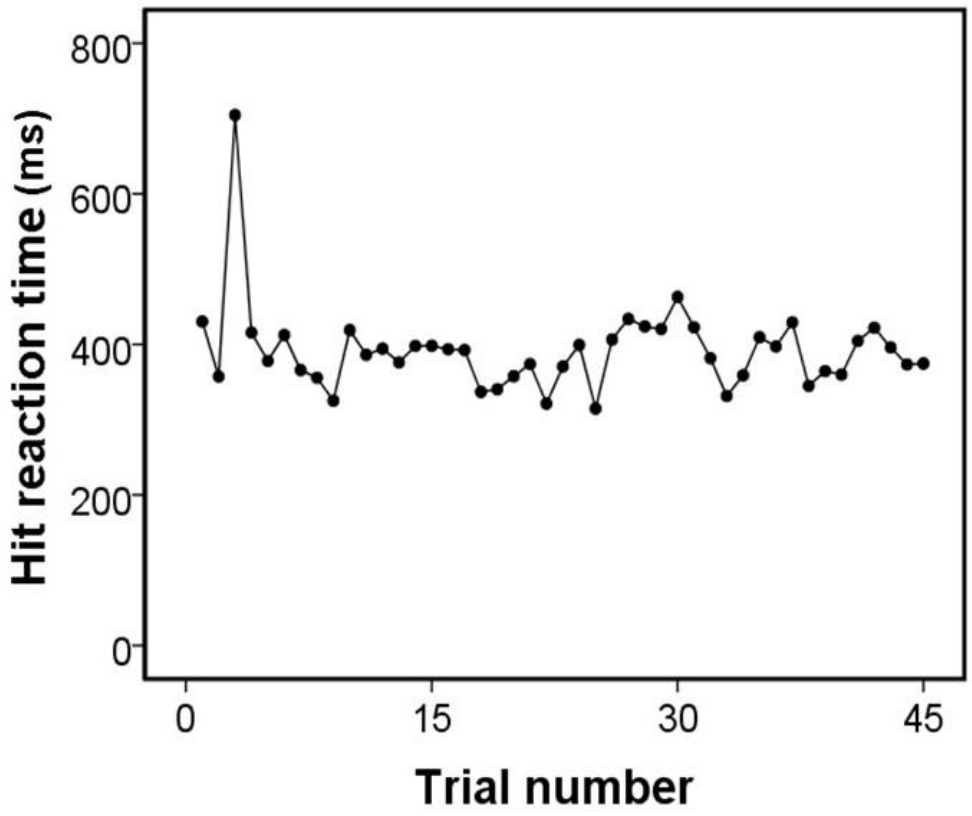

Figure 9. Trial-by-trial hit reaction times of all 45 trials of a participant in the post-placebo treatment testing session.

Treatment (L-theanine and placebo) and time (pre-dose and post-dose) effects on reaction time analysed with two-way ANOVA are summarised in Table 2. There was a main effect on time ($F_{(1,22)} = 12.92$, $p = 0.002$) indicating there was an improvement of hit reaction time from pre-dose session to post-dose session averaging across treatment conditions.

Table 2. Summary of results of two-way ANOVA on treatment (L-theanine and placebo) and time (pre-dose and post-dose) effects on reaction time.

Independent variables	**F**	**degrees of freedom (df)**	***P* value**	**Observed power**
Time	12.922	1,22	0.002	0.93
Treatment	2.835	1,22	0.106	0.364
Treatment x time	2.104	1,22	0.161	0.284

There was a trend for treatment × time interaction (Figure 10) suggesting the reduction in reaction time caused by L-theanine was greater than the reduction in reaction time caused by placebo, but this was not statistically significant ($F_{(1,22)} = 2.104$, $p = 0.161$). However, the

observed power to test this interaction was very low (0.284), suggesting high probability of type II error (i.e. not observing a significant treatment × time interaction in the sample despite the population having a significant interaction).

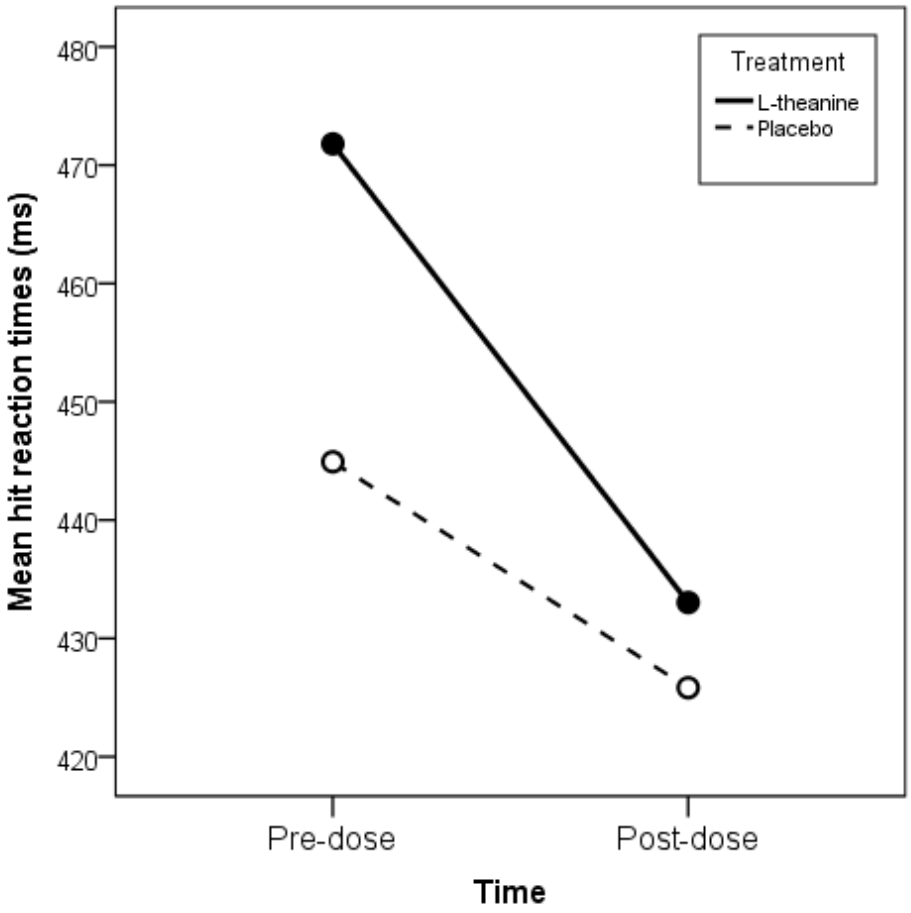

Figure 10. Treatment × time two-way ANOVA marginal means for pre-dose and post-dose hit reaction times for L-theanine and placebo treatment arms.

Two paired t-tests were carried out as there was a time main-effect, and as the treatment × time interaction approached significance. Table 3 summarises the results of paired t-tests for the pre- vs. post-dose hit reaction times for L-theanine and placebo arms. The post-dose reaction time compared to the pre-dose reaction time was 38.75 ms faster for L-theanine (CI = 11.86 – 65.65, p = 0.007). The reduction in reaction time achieved for pre- and post-dose placebo was 19.08 ms (CI = 3.94 – 34.22, p = 0.016).

Table 3. Summary of the relevant descriptive statistics and paired t-test data for L-theanine and placebo arms.

Treatment	Pre-dose hit reaction time mean (SD)	Post-dose hit reaction time mean (SD)	Mean improvement (95% CI) ms	*P* value
L-theanine	471.79 (87.49)	433.03 (46.87)	38.75 (11.86 – 65.65)	0.007
Placebo	444.91 (55.17)	425.83 (51.14)	19.08 (3.94 – 34.22)	0.016

Pre- and post-dose hit reaction times for within and across treatments had significant positive correlations with one another, showing moderate to strong correlation coefficients (Table 4). We observed the strongest correlation across treatments on the same day, where the correlation coefficient for L-theanine pre- and post-dose hit reaction times was 0.73 ($p = 0.0001$) and that for placebo was 0.786 ($p = 0.0001$).

Table 4. Correlation among for hit reaction times in the four testing sessions.

Treatment-time condition		1	2	3	4
1. L-theanine pre-dose	***r***	–			
	***p* value**				
2. L-theanine post-dose	***r***	0.730	–		
	***p* value**	< 0.0001			
3. Placebo pre-dose	***r***	0.609	0.602	–	
	***p* value**	0.002	0.002		
4. Placebo post-dose	***r***	0.516	0.584	0.786	–
	***p* value**	0.012	0.003	< 0.0001	

Note: r = Pearson correlation coefficient

4. Discussion

Even though cognitive effects of L-theanine on attention have been tested widely in healthy adults under normal conditions, only limited research has been carried out on more compromised groups of individuals: Sarris et al. (2019) investigated the effects of L-theanine as an adjunctive treatment administered over few weeks in patients with generalized anxiety disorder, whereas Kahathuduwa et al. (2020) tested the acute effects of L-theanine on a group of children with ADHD. However, to our knowledge, this is the first study that examined the acute effects of L-theanine on selective attention in a group of sleep-deprived individuals. To improve the ecological validity of the study, we administered a traffic-scene-related visual attention task rather than a task that used abstract stimuli.

4.1. Effect of L-theanine on visual selective attention

In terms of the task accuracy, nearly 90% of the test sessions showed 100% hit rate, and all sessions had a hit rate more than 90%. This indicates that the participants had a very high level of accuracy in actively responding to imminent accident scenes across sessions, so that there was not much room for further improvement of recognition accuracy of imminent accidents. In contrast, there was a significant reduction in false alarms following administration of L-theanine but not following placebo, indicating that L-theanine reduced the participants unnecessarily responding to non-accident scenes. Across the two treatment conditions, there was a significant improvement of hit reaction time after treatment (i.e. time main effect), which may be attributed to either a practice effect, placebo effect or a combination of both. We found that placebo improved reaction time by about 19 ms and L-theanine by about 39 ms, denoting that L-theanine had a 20-ms advantage over placebo. It is also interesting to note that the pre- and post-dose hit reaction times for within and across treatments had a significant positive correlation with one another indicating that the traffic task has a high test-retest reliability. This property of the task would be useful to test participants over a period of time (e.g. weeks) following repeated administration of L-theanine (as in Gibson et al., 2020; Hidese et al., 2019; Zaragoza et al., 2019), or to measure the time course of acute attentional effects following a single dose of the treatment (as in Higashiyama et al., 2011).

We found that the accuracy results of our study corroborate with the findings of previous experiments which have shown that L-theanine improves accuracy in behavioural tests (Dodd et al., 2015; Foxe et al., 2012). However, the findings for effects on recognition reaction time by L-theanine have been mixed. Previous studies based on auditory attention tasks have shown 200 mg of L-theanine to show either a delay (Gomez-Ramirez et al., 2007) or no change (Dassanayake et al., 2020; Higashiyama et al., 2011) in reaction time.

When narrowing down the comparisons to the attention tasks that tapped into processing of visual information, although some studies have shown no significant improvement in choice reaction time with abstract visual stimuli (Dodd et al., 2015), or in reaction time in a visuospatial

attention task (Kelly et al., 2008; Rogers et al., 2008), we found our visual recognition reaction time data to be more consistent with the series of studies conducted by Kahathuduwa et al. (2017; 2018; 2020) that have consistently shown improvement of visual recognition reaction time. In combination these findings suggest that the effects of L-theanine on response speed may be at least partly attributable to the sensory modalities, favouring enhancement of visual selective attention. The number of studies conducted on the acute effects of L-theanine on visual attention is rather limited, and their experimental parameters are too heterogenous to infer the specific reasons behind why we observed a significant improvement with L-theanine while some other studies have not. Nevertheless, it is noteworthy that our study is specifically different from the aforementioned studies with negative results (Dodd et al., 2015; Kelly et al., 2008; Rogers et al., 2008) because we tested participants who were sleep-deprived, thus potentially enabling room for improvement of their performance, compared to well-slept healthy individuals who may have been performing at their best even pre-dose.

We observed a concurrent improvement in accuracy (in terms of reduced false alarms) and response speed, contrary to the usual speed-accuracy trade-off expected in these types of selective attention tasks. Kahathuduwa et al., provide neurophysiological evidence corroborating our behavioural findings (Kahathuduwa et al., 2018; Kahathuduwa et al., 2020). Their fMRI findings in healthy adults indicate that L-theanine appears to decrease recruitment of neural resources to distractors while permitting cortical processing of target stimuli (Kahathuduwa et al., 2018). Those studies also revealed that L-theanine causes a task-related reduction of reactivity in the brain as areas that constitute the default mode network, which is implicated in mind-wandering. We find that our hit reaction time data can also be partly explained by this knowledge, as reduced activity of the default mode network (and thus mind wandering) will facilitate more efficient attentional deployments to targets, thereby increasing response speed. Taken together, these observations indicate that L-theanine may improve speed of attentional processing while increasing the accuracy of target-distractor discrimination.

Exploring a different avenue focusing on how the mental state of the individuals may affect the outcomes, Higashiyama et al (2011) reports that L-theanine improved reaction time in high-trait-anxiety participants while showing no improvement in low-trait-anxiety participants. Similarly, in our study, we tested healthy participants at a compromised state (when they are sleep-deprived) and interestingly, the reaction time improvement we observed is of similar magnitude to that they observed. These findings, in combination, suggest that beneficial effects of L-theanine become more evident in compromised groups of individuals.

4.2. Potential limitations and generalizability of the findings

Our experimental paradigm was adequately powered to detect main effect of time and simple effects (i.e. effects of L-theanine and placebo separately) although it was inadequate to detect a significant treatment × time interaction. We found that the observed power to detect such interaction was very low, whereby it would be necessary to increase the sample size to an impractically large number to have adequate statistical power to detect any significant treatment

× time interaction[1]. The reduction in power may be a result of increased variability of reaction time to targets, and we speculate that this increase in variability could have been caused by one or both of the following factors: 1) We used traffic scenario-based stimuli compared to abstract stimuli as used in previous experiments potentially resulting in the task being more complex and thereby requiring higher order processing, and 2) participants were acutely sleep-deprived and therefore were compromised to varying degrees in their level of alertness. However, these two factors set the foundation to our study design that intended to increase the ecological validity of the study.

To ensure the generalizability of our results, we included both male and female participants. However, there is some evidence of variability of reaction time with the phase of the menstrual cycle (Kumar et al., 2013), but since we compared pre- vs. post- effects on the same day within a duration of approximately an hour, we do not believe any such longer-term cyclical changes could affect our results.

In the contemporary society, commercial preparations of L-theanine are already being promoted with claims of beneficial effects on cognition and performance; and L-theanine also appears as a constituent of many supplements (Gibson et al., 2020; Zaragoza et al., 2019). Since these supplements have been administered to athletes who may be benefited by improvements in reaction time, it can be said that research of this nature adds to the evidence behind incorporating L-theanine into these beverages. However, a fair degree of caution is warranted as the interaction of L-theanine with other constituents has not been adequately studied and this presents avenues for future exploration.

4.3. Conclusions and future directions

In conclusion, our findings indicate that a single 200-mg dose of L-theanine acutely improves response accuracy by reducing false alarms, and has around a 20-ms advantage over placebo in improving reaction time in a traffic-based selective attention task in sleep-deprived young adults. This indicates that L-theanine concurrently improves attentional processing speed and the accuracy of responses. This may be of importance in the applicability of our findings to real-life situations such as driving, in which a reaction time advantage of the order of magnitude that we observed with improvement of response accuracy could be of significant benefit in sleep-deprived or otherwise fatigued drivers in preventing imminent traffic accidents.

In the present experiment we tested and observed only the behavioural effects (in the form of a reaction time task) of L-theanine on visual selective attention in sleep-deprived individuals. Thus, the next step could focus on concurrently recording neurophysiological measures together with behavioural testing [as has been done by Dassanayake et al. (2020) with cognitive event-related potentials], using the same experimental paradigm.

[1] Based on the observed data, we recalculated the sample size to detect a significant interaction (at an alpha error of 0.05) with a power of 0.8, and the estimated sample size was 85, which is much larger than that of any study conducted hitherto in this area of research.

Albeit limited, the research evidence hitherto suggests combination of L-theanine with caffeine producing additive or synergistic effects (Gomez-Ramirez et al., 2007; Haskell et al., 2008; Kahathuduwa et al., 2017; Kahathuduwa et al., 2018; Kelly et al., 2008). Hence, we believe that incorporating a caffeine arm to the same task paradigm may build upon our study design and bring forth further evidence on combined effects of L-theanine and caffeine.

It is known that fatigue related to sleep-deprivation is a common cause of road traffic accidents particularly in long-haul drivers (National Academies Press, US, 2016). Therefore, it may be useful to test the effects of L-theanine on professional drivers using the same experimental design, and possibly, also to quantify effects of the treatment on subjective mental alertness (which they perceive), to further enhance the ecological validity of the experimental design and explore its applicability.

5. References

Bond, A., Lader, M., 1974. *The use of analogue scales in rating subjective feelings.* British Journal of Medical Psychology 47, 211-218.

Borzelleca, J.F., Peters, D., Hall, W., 2006. *A 13-week dietary toxicity and toxicokinetic study with l-theanine in rats.* Food Chem Toxicol 44, 1158-1166.

Daou, M., Sassi, J.M., Miller, M.W., Gonzalez, A.M., 2019. *Effects of a Multi-Ingredient Energy Supplement on Cognitive Performance and Cerebral-Cortical Activation*. Journal of dietary supplements 16, 129-140.

Dassanayake, T.L., Kahathuduwa, C.N., Weerasinghe, V.S., 2020. *L-theanine improves neurophysiological measures of attention in a dose-dependent manner: a double-blind, placebo-controlled, crossover study.* Nutritional neuroscience, 1-11.

Dodd, F.L., Kennedy, D.O., Riby, L.M., Haskell-Ramsay, C.F., 2015. *A double-blind, placebo-controlled study evaluating the effects of caffeine and L-theanine both alone and in combination on cerebral blood flow, cognition and mood.* Psychopharmacology (Berl) 232, 2563-2576.

Einöther, S.J., Martens, V.E., 2013. *Acute effects of tea consumption on attention and mood.* American Journal of Clinical Nutrition 98, 1700S-1708S.

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

FDA, 2006b. Division of Biotechnology and GRAS Notice Review (HFS-255). Office of Premarket Approval, Center for Food Safety and Applied Nutrition. *Determination of the GRAS Status of Suntheanine L-Theanine for Use in Food.*

Foxe, J.J., Morie, K.P., Laud, P.J., Rowson, M.J., de Bruin, E.A., Kelly, S.P., 2012. *Assessing the effects of caffeine and theanine on the maintenance of vigilance during a sustained attention task.* Neuropharmacology 62, 2320-2327.

Fujii, S., Inai, K., 2008. *Tumorigenicity study of l-theanine administrated orally to mice.* Food Chemistry 110, 643-646.

Gibson, N., Baker, D., Sharples, A., Braakhuis, A., 2020. *Improving Mental Performance in an Athletic Population with the Use of Ārepa*(®), *a Blackcurrant Based Nootropic Drink: A Randomized Control Trial.* Antioxidants (Basel, Switzerland) 9.

Giles, G.E., Mahoney, C.R., Brunye, T.T., Taylor, H.A., Kanarek, R.B., 2017. *Caffeine and theanine exert opposite effects on attention under emotional arousal*. Can J Physiol Pharmacol 95, 93-100.

Gomez-Ramirez, M., Higgins, B.A., Rycroft, J.A., Owen, G.N., Mahoney, J., Shpaner, M., Foxe, J.J., 2007. *The deployment of intersensory selective attention: a high-density electrical mapping study of the effects of theanine.* Clinical Neuropharmacology 30, 25-38.

Gomez-Ramirez, M., Kelly, S.P., Montesi, J.L., Foxe, J.J., 2009. *The effects of L-theanine on alpha-band oscillatory brain activity during a visuo-spatial attention task.* Brain Topogr 22, 44-51.

Haskell, C.F., Kennedy, D.O., Milne, A.L., Wesnes, K.A., Scholey, A.B., 2008. *The effects of L-theanine, caffeine and their combination on cognition and mood.* Biol Psychol 77, 113-122.

Hidese, S., Ogawa, S., Ota, M., Ishida, I., Yasukawa, Z., Ozeki, M., Kunugi, H., 2019. *Effects of L-Theanine Administration on Stress-Related Symptoms and Cognitive Functions in Healthy Adults: A Randomized Controlled Trial.* Nutrients 11.

Higashiyama, A., Htay, H.H., Ozeki, M., Juneja, L.R., Kapoor, M.P., 2011. *Effects of l-theanine on attention and reaction time response.* Journal of Functional Foods 3, 171-178.

Juneja, L.R., Chu, D.-C., Okubo, T., Nagato, Y., Yokogoshi, H., 1999. *L-theanine—a unique amino acid of green tea and its relaxation effect in humans.* Trends in Food Science & Technology 10, 199-204.

Kahathuduwa, C., Weerasinghe, V., Dassanayake, T., Amarakoon, T., Binks, M., 2016. *High doses of theanine improve visual sStimulus discrimination in a dose-dependent manner.* The FASEB Journal 30, 1284.1283-1284.1283.

Kahathuduwa, C.N., Dassanayake, T.L., Amarakoon, A.M.T., Weerasinghe, V.S., 2017. *Acute effects of theanine, caffeine and theanine-caffeine combination on attention.* Nutritional neuroscience 20, 369-377.

Kahathuduwa, C.N., Dhanasekara, C.S., Chin, S.H., Davis, T., Weerasinghe, V.S., Dassanayake, T.L., Binks, M., 2018. *L-Theanine and caffeine improve target-specific attention to visual stimuli by decreasing mind wandering: a human functional magnetic resonance imaging study*. Nutr Res 49, 67-78.

Kahathuduwa, C.N., Wakefield, S., West, B.D., Blume, J., Dassanayake, T.L., Weerasinghe, V.S., Mastergeorge, A., 2020. *Effects of L-theanine-caffeine combination on sustained attention and inhibitory control among children with ADHD: a proof-of-concept neuroimaging RCT.* Scientific reports 10, 13072.

Kelly, S.P., Gomez-Ramirez, M., Montesi, J.L., Foxe, J.J., 2008. *L-theanine and caffeine in combination affect human cognition as evidenced by oscillatory alpha-band activity and attention task performance.* J Nutr 138, 1572S-1577S.

Kimura, K., Ozeki, M., Juneja, L.R., Ohira, H., 2007. *L-Theanine reduces psychological and physiological stress responses.* Biological psychology 74, 39-45.

Kimura, R., Murata, T., 1971. *Influence of alkylamides of glutamic acid and related compounds on the central nervous system I.* Chemical and Pharmaceutical Bulletin 19, 1257-1261.

Kumar, S., Mufti, M., Kisan, R., 2013. *Variation of reaction time in different phases of menstrual cycle.* Journal of clinical and diagnostic research : JCDR 7, 1604-1605.

Lezak, M.D., Howieson, D.B., Loring, D.W., Hannary, H.J., Fischer, J.S., 2004. *Neuropsychological assessment, 4 ed*. Oxford University Press New York.

Lu, K., Gray, M.A., Oliver, C., Liley, D.T., Harrison, B.J., Bartholomeusz, C.F., Phan, K.L., Nathan, P.J., 2004. *The acute effects of L-theanine in comparison with alprazolam on anticipatory anxiety in humans.* Human Psychopharmacology: Clinical and Experimental 19, 457-465.

Lyon, M.R., Kapoor, M.P., Juneja, L.R., 2011. *The effects of L-theanine (Suntheanine(R)) on objective sleep quality in boys with attention deficit hyperactivity disorder (ADHD): a randomized, double-blind, placebo-controlled clinical trial.* Altern Med Rev 16, 348-354.

Martin, F., Siddle, D.A., Gourley, M., Taylor, J., Dick, R., 1992. *P300 and traffic scenes: the effect of temazepam.* Biological psychology 33, 225-240.

Nathan, P.J., Lu, K., Gray, M., Oliver, C., 2006. *The neuropharmacology of L-theanine(N-ethyl-L-glutamine): a possible neuroprotective and cognitive enhancing agent.* J Herb Pharmacother 6, 21-30.

National Academies Press US, 2016. Fatigue, Hours of Service, and Highway Safety. Panel on Research Methodologies and Statistical Approaches to Understanding Driver Fatigue Factors in Motor Carrier Safety and Driver Health (Ed.). Committee on National Statistics; Board on Human-Systems Integration; Division of Behavioral and Social Sciences and Education; Transportation Research Board; National Academies of Sciences, Engineering, and Medicine. *Commercial Motor Vehicle Driver Fatigue, Long-Term Health, and Highway Safety: Research Needs.* Washington (DC), Wahington (DC).

Nobre, A.C., Rao, A., Owen, G.N., 2008. *L-theanine, a natural constituent in tea, and its effect on mental state.* Asia Pac J Clin Nutr 17 Suppl 1, 167-168.

Rogers, P.J., Smith, J.E., Heatherley, S.V., Pleydell-Pearce, C.W., 2008. *Time for tea: mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together.* Psychopharmacology 195, 569-577.

Sarris, J., Byrne, G.J., Cribb, L., Oliver, G., Murphy, J., Macdonald, P., Nazareth, S., Karamacoska, D., Galea, S., Short, A., Ee, C., Birling, Y., Menon, R., Ng, C.H., 2019. *L-theanine in the adjunctive treatment of generalized anxiety disorder: A double-blind, randomised, placebo-controlled trial.* Journal of psychiatric research 110, 31-37.

Sugiyama, T., Sadzuka, Y., 2003. *Theanine and glutamate transporter inhibitors enhance the antitumor efficacy of chemotherapeutic agents.* Biochimica et Biophysica Acta (BBA)-Reviews on Cancer 1653, 47-59.

Terashima, T., Takido, J., Yokogoshi, H., 1999. *Time-dependent changes of amino acids in the serum, liver, brain and urine of rats administered with theanine.* Bioscience, biotechnology, and biochemistry 63, 615-618.

White, D.J., de Klerk, S., Woods, W., Gondalia, S., Noonan, C., Scholey, A.B., 2016. *Anti-Stress, Behavioural and Magnetoencephalography Effects of an L-Theanine-Based Nutrient Drink: A Randomised, Double-Blind, Placebo-Controlled, Crossover Trial.* Nutrients 8.

Williams, J.L., Everett, J.M., D'Cunha, N.M., Sergi, D., Georgousopoulou, E.N., Keegan, R.J., McKune, A.J., Mellor, D.D., Anstice, N., Naumovski, N., 2020. *The Effects of Green Tea Amino Acid L-Theanine Consumption on the Ability to Manage Stress and Anxiety Levels: a Systematic Review.* Plant foods for human nutrition (Dordrecht, Netherlands) 75, 12-23.

World Medical Association, 2013. *World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.* Jama 310, 2191-2194.

Yamada, T., Terashima, T., Honma, H., Nagata, S., Okubo, T., Juneja, L.R., Yokogoshi, H., 2008. *Effects of theanine, a unique amino acid in tea leaves, on memory in a rat behavioral test.* Bioscience, biotechnology, and biochemistry 72, 1356-1359.

Yokogoshi, H., Kobayashi, M., Mochizuki, M., Terashima, T., 1998. *Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats.* Neurochemical research 23, 667-673.

Zaragoza, J., Tinsley, G., Urbina, S., Villa, K., Santos, E., Juaneza, A., Tinnin, M., Davidson, C., Mitmesser, S., Zhang, Z., Taylor, L., 2019. *Effects of acute caffeine, theanine and tyrosine supplementation on mental and physical performance in athletes.* Journal of the International Society of Sports Nutrition 16, 56.

6. Annexes