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How does the tea *L*-theanine buffer stress and anxiety

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ABSTRACT

Tea (*Camellia sinensis*) is widely considered to promote feelings of calming and soothing. This effect is attributed to *L*-theanine (*L*-γ-glutamylethylamide) in tea, a non-protein amino acid mainly derived from tea leaves. As a naturally occurring structural analogue of glutamate, *L*-theanine competes for the receptors with glutamate and is able to pass the blood-brain barrier to exert its relaxation effect. This review focuses on the relaxation effect of *L*-theanine, including animal models and the latest human trials as well as the potential molecular mechanisms regarding neuron stem cells. The biological efficacy of dietary *L*-theanine in the food matrix has been further discussed in this review in relation to the physiological changes in the gastrointestinal tract and bindings of *L*-theanine with other food components.

1. Introduction

Psychological stress arises when a person perceives environmental demands tax or exceed the adaptive ability [1]. Long-term stress contributes to physical health conditions and overlaps with mental disorders such as anxiety and depression. The WHO has reported that more than 264 million people suffer from depression, which is the most common mental disorders worldwide [2]. Especially in the global population of the Coronavirus Disease (COVID-19) pandemic in 2020, nearly 7 in 10 (67%) people have experienced stress over the course of the pandemic as reported by the American Psychological Association [3]. In addition, the implications of mental stress are correlated with anxiety-related symptoms, including eating disorders, irritable bowel syndrome, and drug use disorders [2].

Phytochemicals are considered as optional adjunctive choices in relaxation and used as nutraceuticals for their potential efficacy and acceptable tolerability [4]. As the second most consumed beverage in the world after water, tea has received considerable popularity due to its favourable flavour and a variety of proposed beneficial properties [5]. The consumption of tea is widely considered to promote feelings of calmness, decreasing alertness, and alleviating stress. These anti-anxiolytic effects are believed due to *L*-theanine (*L*-γ-glutamylethylamide or 5-*N*-ethyl-glutamine), a non-protein amino acid. Apart from its benefits in protecting neurons against neurotoxic agents, improving cognition, mood, and promoting relaxation, *L*-theanine has also been reported as the major compound in providing the umami taste of tea [6]. Tea contains considerable amounts of *L*-theanine (1.0%–2.5% dry weight) in leaves. A serving size of 250 mL green tea prepared under the recommended brewing conditions provides approximately 8–30 mg *L*-theanine [7,8].

This review summarises the relaxation effects of *L*-theanine evidenced in different models alongside the underlying mechanisms. Bioavailability and subsequent studies in food matrix are also

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discussed to provide potential prospects and research reference as the bioactive efficacy is the essential theme to dive into.

2. Synthesis and bioavailability of *L*-theanine

2.1 Structure and biosynthesis

Theanine is a type of chiral compound with two isomers, *L*-form and *D*-form. Both could be synthesized chemically; However, similar to other amino acids, only the *L*-form exists predominantly in nature, especially in tea [9]. *L*-theanine has a similar structure of glutamine and glutamate (Fig. 1), which has been considered as a regulator in the neurodegeneration [5]. The analogous chemical structure to glutamate of *L*-theanine facilitates it to bind to three glutamate receptor subtypes: *N*-methyl-*D*-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA). These subtypes are excitatory neurotransmitters and are responsible for fast synaptic transmission in the central nervous system.

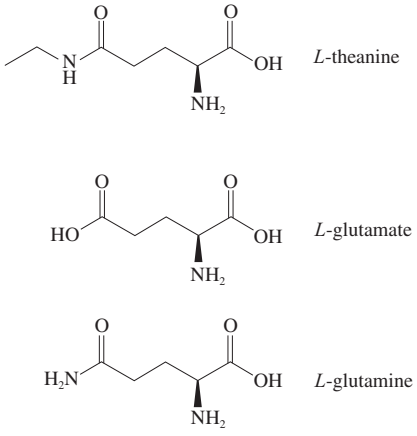

Fig. 1 Chemical structure of *L*-theanine, *L*-glutamate, and *L*-glutamine.

L-theanine ($C_7H_{14}N_2O_3$) is a white solid and transparent, colourless in water-solution. It is the main contribution to the umami taste in tea with a threshold of 24.0 mmol/L [10], binding to two G-protein-coupled receptors T1R1 and T1R3 in mammalians. When used together with monosodium glutamate or purine nucleoside inosine 5′-monophosphate, *L*-theanine exhibits synergism with these two traditional flavouring agents and leading to an enhancement of the umami taste [11]. The biosynthesis of *L*-theanine occurs in the root and derived from glutamic acid and alanine. Another synthetic pathway is the glutamic acid in the tea stem, reacting with ethylamine to produce theanine. The tea leaves then becomes the sink of *L*-theanine, which make it accessible for people to obtain this bioactive compound easily [5]. When exposed to sunlight, theanine can be hydrolysed back to ethylamine, which acts as a precursor in the subsequent synthesis of catechins [9].

2.2 Bioavailability and pharmacokinetics of L-theanine

Theanine is absorbed by the NA^+-coupled cotransporter in the brush-border membrane in the intestine and distributed to various organs.

The bioavailability of *L*-theanine remains between 47% and 54% via intake of capsules and tea, respectively, by calculating the equimolar amounts of ethylamine [7]. Scheid et al. [7] further proposed that if the remaining part of *L*-theanine in erythrocytes was considered, and using the one-compartment model, the bioavailability might be higher and reaching 72%–74%. In the study of healthy participants, 100 mg of *L*-theanine was predicted to be absorbed after a t_{lag} of 10–24 min, leading to a $C_{max,70}$ of 24–26 μmol/L.

The *L*-and *D*-enantiomers of theanine contribute to diverging physiological responses *in vivo*. *L*-theanine and *D*-form are different in the intestinal absorption. *L*-theanine is more readily absorbed with a concertation of three times higher than the *D*-form in plasma [5]. The gastrointestinal (GI) tract had a major role in inhibiting the absorption of *D*-theanine due to the stereoselectivity, that is, the active transport well distinguishes between *D*- and *L*-isomers and increases *L*-enantiomer uptake [12]. Due to the reabsorption of *L*-theanine occurred in kidneys, *D*-form has a readily urinary excretion over *L*-isomer. *L*-theanine is preferentially degraded to glutamic acid and ethylamine in the kidney, whilst *D*-theanine is rapidly eliminated with minimal metabolism and excretes in the urine route, with the fold of 10–15 times higher than *L*-form [5].

3. *L*-theanine relaxation effects

L-theanine has been proved to be able to cross the blood-brain barrier, after the ingestion from 30 min up to 5 h [5], providing the efficacy of *L*-theanine to perform its role in regulating mental health and neurotransmitters. *L*-theanine is non-mutagenic or non-carcinogenic and well-tolerated in mammal cells with an LD_{50} value of greater than 5 000 mg/kg [13]. Rodent models are widely used in the exploration of behavioural responses and cellular mechanisms. Studies of *L*-theanine conducted on rodents investigating the buffering of anxiety and stress are discussed as follows.

3.1 Rodent models

3.1.1 Neurotransmitters: γ-aminobutyric acid (GABA), dopamine, and serotonin

L-theanine exhibits pharmacological effects on brain wellness via modulating neurotransmitters, including reducing glutamate release, increasing inhibitory neurotransmitter GABA level, and enhancing glycine and dopamine release [4]. GABA and glutamate are the main excitatory and inhibitory neurotransmitters, respectively, controlling the intrinsic and extrinsic information flow in the brain. The abnormalities of glutamate and GABA are found in the brain of individuals with mood disorders [14]. Although the detailed pathways of *L*-theanine on GABA have not been fully clarified, a study has detailed the differential decrease of GABA levels in cortical and hippocampal in mice, after the treatment of 4% *L*-theanine in the drinking water for two weeks [15].

Another study has reported the accumulation of *L*-theanine into the striatum of the brain in Wistar rats which caused the dopamine to release up to 300% of the basal value [16], as well as increased glycine level involving AMPA and kainate receptors, indicating the calming effect of the *L*-theanine on the mind. Dopamine neurotransmission is related to stress, fear, and social interaction behaviours [16]. The

pathological dopaminergic neuron degeneration causes cholinergic, serotonergic and noradrenergic system dysfunction. Another study has found that *L*-theanine (2 mg/kg/day for 21 days) increased dopamine and 5-hydroxytryptamine (5-HT, also known as serotonin) in the hippocampus in the depression rat model [17]. However, there are different results regarding the effect of *L*-theanine on 5-HT. Yokogoshi et al. [18] found that the long-term administration of *L*-theanine decreased the level of 5-HT in the cerebral cortex [18]. The reason could be the differences in the mythology analysis and brain regions examined as well as the administration time. It has been proposed that *L*-theanine, may have the ability to selectively increase levels of serotonin and dopamine in certain brain areas, such as the striatum, hypothalamus, and hippocampus, however, more data and evidence are still needed to confirm this selectivity and underlying neural mechanisms [19].

3.1.2 Mouse models related to anxiety and stress

The animal models related to stress include physical (restraint or dipping into water) and psychosocial stress [20]. Chronic unpredictable mild stress rat model (CUMS) mimics chronic depression-like behaviour, consisting of several unpredictable physical stressors such as food deprivation (12 h), water deprivation (24 h), reversed light and dark (24 h), thermal stimulus (45 °C for 5 min), shaking (180 r/min for 5 min), electric shock in the foot [21]. In a recent study, rats were exposed to the CUMS for 21 days to establish the model of depression (Table 1). The oral gavage of *L*-theanine treatment (2 mg/kg) ameliorated the depressive symptoms as evidenced by the open-field test, sucrose preference test and forced swim test [17].

Confronting housing is an effective animal model to develop psychosocial stress to mimic the social conflict between two male members in mammals' chronic stress. The residence of each mouse was established in the cage by using a cage partition; confrontation was initiated by removing the partition [20]. *L*-theanine (> 5 μg/mL) in drinking water alleviated psychosocial stress in the confronting housing model by suppressing the behavioural depression including tests of tail suspension and marble lifting. The intervention of *L*-theanine reduced the adrenal hypertrophy and altered hypothalamic-pituitary-adrenal (HPA) axis, which two are sensitive markers under psychosocial stress. The adverse alterations of HPA axis improved by *L*-theanine via modulating the disorder of the diurnal rhythm of corticosterone and adrenocorticotropic hormone [20].

Unno et al. [20] further demonstrated that *L*-theanine (40 μg/mL) suppressed adrenal hypertrophy against caffeine (30 μg/mL), indicating the competitive interactions of theanine to caffeine. Caffeine is a non-selective antagonist of the adenosine receptor, which controls the uptake and release of serotonin. Considering the activity of HPA was controlled by glutamate signalling via glutamate receptors, *L*-theanine might modulate both glutamate and serotonin metabolism and suppress glutamate-induced excitation. These results provide the evidence of neurotransmitter modulation of *L*-theanine, although different doses and time were applied in studies of Unno et al. [20] and Shen et al. [17], if converting 40 μg/mL to 4.5 mg theanine/kg/day (10 days) in comparison with 2 mg/kg/day for 21 days.

The counteractive effect of *L*-theanine against caffeine has also been studied in the assessment of sleep quality. *L*-theanine decreased slow-wave sleep (SWS) induced by caffeine in male Sprague-Dawley rats [22]. Specifically, *L*-theanine at 75 mg/kg and 150 mg/kg reversed the caffeine-induced wakefulness; and lower dosages (22.5 mg/kg and 37.5 mg/kg) were able to increase the reduction of SWS by caffeine (7.5 mg/kg). These results reveal the potential of *L*-theanine as a promising sleeping aid and may be generalised to human suffering from insomnia due to caffeine consumption.

Table 1
Relaxation effects of *L*-theanine in rodent models.

Mice model	Dosage/duration	Measurements/stress models	Outcomes	Reference
Male Wistar rats	0.2 μmol/2 μL perfusion/min; and *L-trans*-PDC 0.01 μmol/2 μL perfusion/min for 1 h	Brain microdialysis	*L*-theanine perfusion caused dopamine release up to 300% of the basal value; prevented aspartic acid release and increased glycine release.	Yamada et al. [16]
Male Sprague Dawley rats, chronic unpredictable mild stress	2 mg/kg for 21 days	CUMS chronic unpredictable mild stress randomly food privation (12 h), water privation (24 h), reversed light and dark (24 h), thermal stimulus (45 °C, 5 min), shaking (180 r/min, lasting for 5 min), immersion in cold water (4 °C, 1 min), tail pinch (1 min), electric shock in the foot (10 mA electricity was given, which lasted for 10 s per time for 30 times).	*L*-theanine increased the locomotor activity open-field test ($P < 0.001$); increased sucrose consumption level sucrose preference test ($P < 0.001$); decreased immobility time in forced swim test ($P < 0.001$).	Shen et al. [17]
Posttraumatic stress disorder (PTSD) male Std-ddY mice	5, 50, 500 mg/kg; 5 days; oral	Restraint stress in a metallic cage immersed in water at 25 °C up to the clavicle for 3 h.	*L*-theanine (50–500 mg/kg) significantly prevented the decline of BrdU incorporation, and for 5 days after stress; inhibited the prolonged immobility in mice with stress in forced swimming test 14 days later. Increased total spontaneous locomotion activity was significantly ameliorated following the administration of theanine at 50 mg/kg for 14 days after stress.	Takarada et al. [34]
Wistar Kyoto rat	0.4 mg/kg/day intraperitoneal injection; 7 days	Anxiety vulnerability and refractory depression	Anxiety: longer time spent in open arms in elevated plus maze test ($P = 0.035$).	Ogawa et al. [23]
Male ddY mice	*L*-theanine (> 5–100 μg/mL) in drinking water; 2 weeks	Psychosocial stress: confronting housing	Decreased immobility time in tail suspension test ($P = 1.87 \times 10^{-5}$); no effect in marble-burying behaviour test.	Unno et al. [26]
Senescence-accelerated mice prone 10 (SAMP10) and ddY mice	6 mg/kg (20 μg/mL in normal tap water)	Psychosocial stress: confronting housing	*L*-theanine suppressed brain atrophy (volume of hippocampus and neocortex in brain).	Unno et al. [39]

Wistar Kyoto (WKY) rats have been proposed as an animal model of anxiety vulnerability and refractory depression. In the behavioural test of WKY rats, the 7-day treatment of *L*-theanine (0.4 mg/kg/day) increased hippocampal activity and exerted anxiolytic effects, as shown by the longer time spent in open arms of the elevated plus maze test compared with the saline group ($P = 0.035$), alongside with decreasing the level of glutamate in cerebrospinal fluid [23]. Although the further antidepressant effect was not observed, this might be due to the rat model used which has been considered as a refractory depression and potential insufficient dose of *L*-theanine (0.4 mg/kg/day).

Animal models benefit from reasonably simple resource access and high operability. Even though it is still important to note that rodents and other animal models could not well represent human physiological and psychological conditions. The explorations of the calming effects of *L*-theanine have been conducted in human clinical trials and several of which are described below.

3.2 Human trials

3.2.1 Stress

There are two main categories of evaluations of the severity of anxiety associated with *L*-theanine. One is subjective psychological measurements, including a variety of questionnaires and assessment tools. For instance, Profile of Mood States (POMS), Visual Analog Scale (VAS), State-Trait Anxiety Inventory (STAI), and Beck's Anxiety Inventory [24]; the other category is physiological responses. These outcomes are direct and effective, such as blood pressure, circulating hormones, salivary secretory immunoglobulin (s-IgA), heart rate (HR) and heart rate variability (HRV).

The antagonist interrelation of *L*-theanine and caffeine was studied on the human populations by Rogers et al. [6]. Healthy participants who received caffeine (250 mg) have elevated alertness and jitteriness and other prospectives of moods; *L*-theanine (200 mg) antagonised the effect of caffeine on blood pressure and slowed the overall reaction time in the visual probe task [6].

Yoto et al. [25] further analysed the effect of *L*-theanine against a lower dose of caffeine (100 mg) on physical and psychological stress [25]. In contrast to the elevation of blood pressure by caffeine of 250 mg [6], 100 mg of caffeine decreased the blood pressure in arithmetic mental task induced psychological stress. The inhibitory effect of caffeine on blood pressure has been proposed to be due to the absence of a resting period. It has been suggested that caffeine raised blood pressure by elevating the resting baseline rather than the acute blood pressure stress response, the absence of the resting period in the study by Yoto et al. [25] enabled the acute stress response to a higher level than the stress response potentiated by caffeine intake. Compared with caffeine, *L*-theanine (200 mg) had a greater inhibition effect on mental task induced blood pressure, and it decreased the Tension-Anxiety score compared with placebo, indicating the anti-anxiolytic effects of *L*-theanine. Further acute physical stress is established by applying the cold pressor test (CPT). Although neither *L*-theanine nor caffeine decreased blood pressure caused by the CPT, this could be explained by the differences in the mechanisms of blood pressure elevation caused by psychological stress and physical stress of pain [25].

Salivary amylase activity (sAA) has been considered as an efficient and non-invasive assessment to evaluate the psychological stress level [26]. Elevated sAA is a biomarker of autonomic nervous system excitation reactivity to stress. In a single-blind study on the university students ($n = 20$) during an 11-week pharmacy practice, *L*-theanine (400 mg/day) was able to alleviate the initial stress and anxiety as shown by the decreased level of pre-practice sAA ($P = 0.032$) and subjective stress measured by visual analogue scales ($P = 0.020$) [26].

3.2.2 Anxiety

The evaluations of anxiety are usually conducted by subjective questionnaires. In a study performed by Higashiyama et al. [27], participants were dived into two groups referred to as high and low anxiety propensity group according to the enquiry of manifest anxiety scale. Compared to the placebo group, the intake of *L*-theanine decreased heart rate ($P = 0.001\ 6$), enhanced visual attentional performance ($P = 0.000\ 1$), and improved reaction time response among healthy subjects in the high anxiety propensity group ($P = 0.001$) [27].

The anti-anxiety effects of *L*-theanine may vary depending on the intensity of anxiety. Lu et al. [28] compared the anxiolytic effects of *L*-theanine with alprazolam on anticipatory anxiety in humans. *L*-theanine treatment lowered the visual analogue mood scale (VAMS)-Tranquil score of participants compared to placebo and alprazolam significantly ($P < 0.05$), and the latter is a prescription drug used in curing anxiety clinically. However, no anxiolytic effects were observed between neither *L*-theanine nor alprazolam in an experimentally induced acute anticipatory anxiety model [28], potentially attributing to the intensity of anxiety used in this model was intensive. The absence of effectiveness of alprazolam was consistent with previous studies during experimentally induced anxiety. Alprazolam could perform anxiolytic effects preferring in objectively physiological measurements, such as skin conductance, startle response, and brain electrical activity [29]. The explanation of the different effect of alprazolam between subjective and objective measurements is the differential modulation of neurochemicals in neural circuits involved in the physiological measures and subjective self-reports [28].

A very recent study evaluated the anxiolytic effects of *L*-theanine (450 mg/day and 900 mg/day) on 46 participants with a clinical diagnosis of Generalised Anxiety Disorder (GAD). Although this study did not provide evidence of the beneficial effects of *L*-theanine in treating anxiety, participants with GAD who received *L*-theanine treatment reported improved sleeping satisfaction and improved insomnia symptoms [4].

3.2.3 Depression

Stressful life experiences have been related to severe depressive conditions as well as depressive symptoms. Depression and anxiety disorders are highly comorbid, with symptomatology representing affective, somatic and emotional dysregulation correlated with depression and anxiety [21]. About 20% to 25% of individuals enduring major stressful experiences would develop into depression [1].

An open-label study was conducted to evaluate the effects of theanine on depressive symptoms on twenty individuals with major depressive disorders [30]. *L*-theanine (250 mg/day) was added to participants' current medication for eight weeks. This chronic

administration of *L*-theanine decreased the Hamilton Depression Rating Scale (HAMD-21)-score ($P = 0.007$) and STAI ($P = 0.012$).

It is worth considering the dosage and duration of treatment of *L*-theanine in evaluating its relaxation effects, as these effects are dose-dependent and area-specific, as well as time-, drug-, and task-dependent. Several studies performed on animal samples have claimed that *L*-theanine reaches the therapeutic plateau at 400 mg/day, whereas this could not be generalised to more clinical studies on population. The highest dosage of human clinical trials until now is up to 900 mg/day [4]; other dosages of *L*-theanine used in clinical trials range 200–400 mg/day. The research conducted by Unno et al. [26] indicated 17-days of theanine intake (200 mg, twice a day, in total 400 mg/day) was able to provide the initial anti-stress effect assigned for potential long-term stress (11-week pharmacy practice). Similar duration of a 4-week clinical trial has also provided the evidence that stress-related Self-rating Depression Scale and STAI-trait were reduced by *L*-theanine significantly ($P = 0.019$ and $P = 0.006$, respectively), but with a lower dosage of 200 mg/day [8]. In addition,

Table 2
Relaxation effects of *L*-theanine in human clinical trials.

Type/task	Participants/Age (years)	Dosage	Outcomes	Study design	Reference
Acute stress task; mental arithmetic task for 20 min.	12 male participants, age (21.50 ± 1.38) years	Oral 200 mg dissolved in. 100 mL water; acute 1 day	*L*-theanine reduced heart rate ($P < 0.05$) HRV (LF/HF) ($P < 0.05$) and salivary immunoglobulin A ($P < 0.01$); STAI ($P < 0.01$); and VAS ($P < 0.01$) compared with placebo.	Placebo-controlled, double-blind	Kimura et al. [31]
Healthy; light-to moderate caffeine consumers; visual probe task (10 min) or facial expression discrimination task (15 min).	48 healthy participants, age (20.5 ± 2.0) years	200 mg in drinks; 250 mg caffeine; 1 day	*L*-theanine lowered reaction time in visual probe task ($P = 0.046$); and agonisted the caffein increased systolic BP and diastolic BP ($P = 0.017$ and $P = 0.008$).	Between subjects; double-blind	Rogers et al. [6]
Visual attention task; audio response test.	18 healthy participants, age (19 ± 1) years	200 mg/100 mL water; 1 day	*L*-theanine decreased heart rate ($P = 0.001\ 6$), elevated visual attentional performance ($P = 0.000\ 1$), and improved reaction time response among high anxiety propensity subjects compared to a placebo ($P = 0.001$), no effect on STAI.	Double-blind; placebo-controlled	Higashiyama et al. [27]
Anticipatory anxiety model of electrical stimuli; 3-full day repeated test.	16 healthy participants; 12 males (age (24.8 ± 5.4) years) and 4 females (age (29.0 ± 1.4) years)	200 mg; acute; repeated 3 measurements	*L*-theanine reduced subjective anxiety on the tranquil-troubled subscale of the VAMS than placebo and alprazolam ($P < 0.05$); no significant anxiolytic effects in the anxiety state.	Double-blind; placebo-controlled	Lu et al. [28]
Auditory oddball target detection task (5 min); arithmetic mental task (10 min); physical stress task, cold pressor test.	14 healthy volunteers, 8 men, 6 women; age (22.8 ± 2.1) years	200 mg; caffeine (100 mg); with 250 mL warm water; 1 day	Decreased systolic blood pressure in AMT (after mental task) 4, 5, 6, periods; and diastolic blood pressure at AMT 4 ($P = 0.006$) and AMT 6 ($P = 0.039$); decreased Tension-Anxiety score ($P = 0.004$).	Cross-over, randomized, placebo-controlled	Yoto et al. [25]
Stress, assigned to practice in a hospital or a drug store.	14 man, 6 women (theanin: age (22.5 ± 0.2) years; placebo: age (22.5 ± 0.1) years)	In total 400 mg; 200 mg, twice a day, 17 days	*L*-theanine decreased salivary alpha-amylase activity ($P = 0.032$); subjective stress ($P = 0.020$); VAS ($P = 0.020$).	Placebo-controlled; group comparison design	Unno et al. [26]
Healthy; Trail-Making Test, Stroop Test, Japanese version of the Brief Assessment of Cognition in Schizophrenia (BACS).	30 individuals with no major psychiatric illness (9 men and 21 women); age (48.3 ± 11.9) years	200 mg/day; 4 weeks	*L*-theanine decreased SDCS, STAI, and Pittsburgh sleep quality index (PSQI) ($P = 0.019$, 0.006 and 0.013); Stress; Self-rating depression scale (SDS), anxiety: STAI; sleep: PSQI; *L*-theanine improved verbal fluency and executive function scores ($P = 0.001$, and 0.031).	Randomized; placebo-controlled, crossover and double-blind trial	Hidese et al. [8]
Stroop test, and Brief Assessment of Cognition in Schizophrenia (BACS).	Major depressive disorder; 20 patients with MDD (4 men (41.0 ± 14.1) and 16 women (42.9 ± 12.0))	250 mg/day for 8 weeks	*L*-theanine decreased HAMD-21-score ($P = 0.007$), STAI ($P = 0.012$), PSQI ($P = 0.030$).	Open-label study	Hidese et al. [30]
Trail-Making Task; Stroop task.	46 participants with a DSM-5 diagnosis of Generalized Anxiety Disorder (18–75 years)	450 and 900 mg; 225 mg 1 capsule twice/day; 8 weeks	No effect in anxiety Hamilton Anxiety Rating Scale score ($P = 0.73$), insomnia severity index (ISI: $P = 0.35$), sleep satisfactory (ISI item 4, $P = 0.015$).	Double-blind, randomised; placebo-controlled	Sarris et al. [4]
Multi-tasking framework (mathematical processing, Stroop, memory search and psychomotor tracking).	34 healthy participants: 15 men (26.53 ± 5.04); 19 women (27.03 ± 5.61)	200 mg *L*-theanine in 430 mL drinks (alpha glycerylphosphorylcholine 25 mg; phosphatidylserine, 1 mg) and micronized chamomile (10 mg); acute 1 day	Subjective stress was decreased after 1 h ($P = 0.003$); salivary cortisol was reduced 3 h after *L*-theanine treatment ($P = 0.047$).	Double-blind, randomised; placebo-controlled, crossover	White et al. [44]
Carbohydrate response caused by sorbet consumption.	11 healthy males, age (27.7 ± 10.8) years	200 mg *L*-theanine in Mango sorbet (100 g); 1 day	No significant effect on blood pressure (systolic and diastolic), heart rate variability (over 90 min), heart rate ($P > 0.05$).	Randomize, double-blind, placebo-controlled, crossover	Williams et al. [46]
No task stressor; high consumption of caco-content increase blood pressure.	122 participants (age 18–25 years)	128 mg *L*-theanine in 40 g chocolate (3.2 mg/g of chocolate); 1 g of chocolate for each kg of body weight; 1 day	*L*-theanine decreased diastolic (3.65 mm Hg) and systolic (5.15 mm Hg) blood pressure (4–8 mm Hg) ($P < 0.000\ 1$ and $P < 0.05$).		Montopoli et al. [45]

the trials of acute single day intervention have been conducted; studies performed by Yoto et al. [25] and Kimura et al. [31] have shown that 200 mg/day of *L*-theanine manage to provide the anti-stress effect in the mental arithmetic task (Table 2).

3.2.4 Others: cognition and insomnia

Other functionalities of tea-theanine include improving memory by facilitating hippocampal synaptic efficiency. In the study mentioned above performed by Hidese et al. [30], cognitive function was improved after *L*-theanine treatment (250 mg/day) as shown by decreased response latency ($P = 0.001$) and error rate ($P = 0.036$) in the Stroop test, and increased verbal memory ($P = 0.005$) and executive function ($P = 0.016$) in the Brief Assessment of Cognition in Schizophrenia test [30]. A lower dose of *L*-theanine (200 mg/day) was applied to 30 healthy participants without clinical depression in a randomised, placebo-controlled, crossover, and double-blind trial [8]. The difference of the health status of individuals resulted in the inconsistency of the cognitive-enhancing effects; the score of Stroop test was not changed significantly, but the verbal fluency and executive function scores have been improved by *L*-theanine treatment significantly ($P = 0.001$ and $P = 0.031$) [8].

Oral treatment of *L*-theanine (200 mg) has been found to improve the quality of sleep in a manner defending anxiety and relaxation, as evidenced by reductions of intermittent awakening and the recovery from exhaustion and refreshed awakening. This was related to increased alpha brain wave that *L*-theanine simulated increased parasympathetic nerve system responses and decreased sympathetic nerve system responses [13], referring to a therapeutic effect not by sedation but through anxiolysis without inducing daytime drowsiness [13]. Similar improvement of sleep was observed in healthy participants' sleep disturbance shown as decreased scores of PSQI ($P = 0.030$) [8], as well as in participants diagnosed with GAD [4]. Although the study of Sarris et al. [4] has reported that *L*-theanine did not outperform placebo for insomnia severity on the Insomnia Severity Index ($P = 0.35$), the self-reported sleep satisfaction from participants was greater than placebo ($P = 0.015$), and *L*-theanine may improve sleep symptoms of sleep disturbance in cases of mild insomnia as their symptoms are relatively less entangled with severe symptoms of anxiety [4].

4. Mechanisms

4.1 Pre-synaptic effects: glutamine transporter

The analogous structure of *L*-theanine to glutamine makes it act as the antagonist of the binding sites with AMPA and KA, and as an agonist of NMDA site, inhibiting the glutamine transporters and blocking the reuptake of glutamine and glutamate [28]. Extracellular glutamine enters neurons via glutamine transporter, which is responsible for transporting glutamine into neurons from adjacent astrocytes, in which glutamine is hydrolysed by phosphate glutaminase into glutamate and stored in the vesicles. Yoneda et al. [32] reported that *L*-theanine has an inhibitory effect on glutamine rather than glutamate. Specifically, *L*-theanine suppresses the glutamine transporter, thereby inhibiting the conversion of glutamine to glutamate (Fig. 2). The decrease of intracellular glutamate leads to the insufficiency of the neurotransmitter pool for subsequent exocytotic release upon stimuli [33].

The glutamine transporter solute carrier 38a1 (Slc38a1) has a critical role in the penetration of glutamine into neurons. Slc38a1 is expressed in undifferentiated neural progenitor cells which are derived from the primary stem cells. *L*-theanine has the ability to selectively elevate the transcription of Slc38a1 in neural stem cells instead of neurons or astrocytes. Potential mTOR pathways have been suggested to be activated in response to *L*-theanine in undifferentiated P19 cells *in vitro* [34], whereas more studies are needed as results differ and there is an agreement that has not been reached on the activation of mTOR [32].

4.2 Post-synaptic effects: glutamate receptors

Another mechanism involved in the anti-stress action is that *L*-theanine competes for the receptors of glutamate, including NMDA, AMPA and KA [24]. Compared with the pre-synaptic effects, this action is weak as evidence by the low affinity of *L*-theanine to these three subtypes of ligands. The IC_{50} was reported 80 to

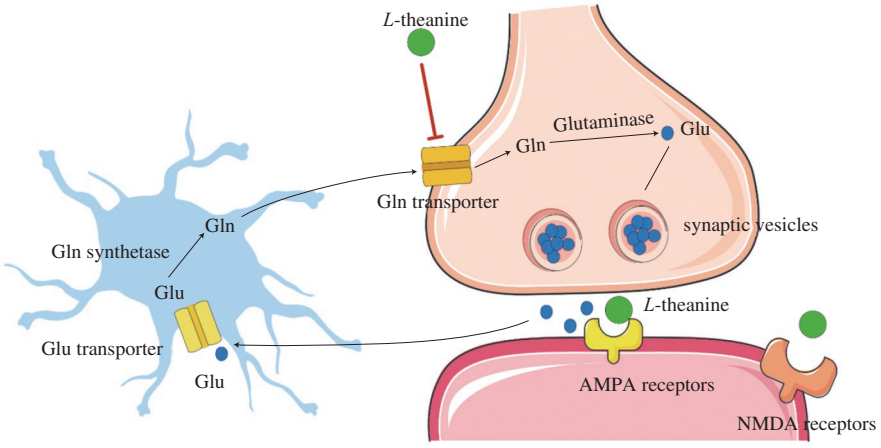

Fig. 2 Schematic representation of *L*-theanine pre- and post-synaptic effects. *L*-theanine suppresses glutamine (Gln) transporters to inhibit the incorporation of extracellular Gln into the neuron. *L*-theanine also competes for the receptors of glutamate (Glu), for instance, NMDA (*N*-methyl-*D*-aspartate) and AMPA (amino-3-hydroxy-5-methyl-4-isoxazole propionic acid).

30 000-fold less than that of glutamate. Nevertheless, this is still relevant in pharmacological activity; a study suggested that the *L*-theanine contributed to the prevention of neuronal cell death due to its antagonistic effect on AMPA receptors [33].

Yamada et al. [16] suggested that *L*-theanine might favour AMPA receptors instead of NMDA ones, as they found that the AMPA receptor antagonist inhibited the calming effects of *L*-theanine, and this result was in agreement with the inhibitory effect of *L*-theanine on AMPA receptors [35]. The increase of dopamine level by *L*-theanine injection was not changed by co-injection with 5,7-dichlorokynurenic acid, a potent antagonist at the glycine site of the NMDA receptor, whereas in another study, the NMDA receptor was proved to be targeted by *L*-theanine [36]. The increase of Ca^{2+} level by *L*-theanine was suppressed in the presence of non-competitive and competitive NMDA receptor antagonists (MK-801 and AP-5, respectively). In the behavioural test on mice, *L*-theanine improved MK-801 induced deficits in prepulse inhibition of acoustic startle, suggesting that *L*-theanine exerts its effects, at least in part, through agonistic action on NMDA receptors [36]. A recent study by Sebih et al. [37] found that *L*-theanine may partially act as a co-agonist of NMDA receptors by modulating GlunN2A and GluN2B gene expression based on the experiments performed on hippocampal neurons.

4.3 Neurogenesis

Exposure to stress is related to neurogenesis as stressful experiences decrease the number of new neurons in the dentate gyrus. *L*-theanine has been found to promote neurogenesis. Takarada et al. [34] evaluated the effects of *L*-theanine on dentate gyrus in the std-ddY mouse model. Mice subjected to psychosocial stress had a decrease in the number of cells labelled with BrdU, a marker of DNA synthesis, indicating a decline in neuronal proliferation in the brain. The ingestion of theanine to mice, before and after stress exposure (50–500 mg/kg for 5 days), was able to restore the number of clusters of BrdU-positive cells [34]. Together these results suggest that *L*-theanine has the functionality to facilitate neuroblasts to new mature neurons and thereby modulate the intact neuronal network functioning in the adult brain [38].

Chronic stress and depression decrease hippocampal volume and lead to cerebral atrophy [39]. In a study of the SAMP10 mice (senescence-accelerated mice), the decreased brain volume in response to psychosocial stress of confronting housing was suppressed by *L*-theanine [39]. The *L*-theanine treatment further increased two genes expression, transcription factor Npas 4 (neuronal PAS domain protein 4) and lipocalin 2. Npas4 regulates the formation and maintenance of inhibitory synapses by increasing the release of GABA and promoting the inhibition of excitatory neurons [40]. Lcn2 is secreted by astrocytes, regulating a variety of behavioural activities, including cognitive function, depression, and anxiety [41]. Lcn2 in cerebrospinal fluid is recognised as a marker to diagnose brain damage; and the inhibition of excessive lcn2 is a therapeutic target for chronic neuroinflammatory and neurodegenerative diseases in Alzheimer's disease, brain trauma and chronic stress [42]. The intake of *L*-theanine increased the release of GABA by increasing the expression of Npas4 and lcn2 to improve brain atrophy and stress vulnerability.

It has been shown that *L*-theanine may not only play an important role in protecting the brain from stress but other chronic nerve inflammation and neurodegenerative diseases [19,43]. Although the direct positive correlation between experimental promotion of adult neurogenesis and clinical treatment benefits has not been fully elucidated until now, *L*-theanine appears to alleviate symptoms related to the intact neuronal network coordination dysfunctions, ensuring the brain health and wellness.

5. *L*-theanine in food matrix

The anti-anxiolytic effect of *L*-theanine, together with associated mechanisms, concentration and models discussed above, are related to the purified *L*-theanine as the tablet or liquid form. It is essential to consider the delivery of *L*-theanine in the way of food matrix to meet the needs of daily consumption of health-promoting compounds. Till now there have been limited studies of intergradation of *L*-theanine into the food products. White et al. [44] found the nutrient-beverage containing 200 mg of *L*-theanine is able to reduce subjective stress response and salivary cortisol in healthy adults. However, the beverage used in the study contained other ingredients; for instance, phosphatidylserine (PS), alpha glycerylphosporylcholine, and chamomile. Another study based on food matrix is that *L*-theanine was added in cocoa chocolates (128 mg, 3.2 mg/g in chocolates), which decreased the diastolic and systolic blood pressure (4–8 mm Hg on average) due to *L*-theanine sympathomimetic inhibitory potential [45]. The similar anti-anxiolytic was not observed in another study using the mango sorbet as the food matrix. The study performed by Williams et al. [46] applied one-off ingestion of mango sorbet containing 200 mg of pure *L*-theanine among 18 health participants. Whereas no reductions were observed in heart rate, blood pressure, and no significant parasympathetic interactions were obtained by measuring HRV high-frequency band and low-frequency/high-frequency ratio as well [46]. The advantage of using mango sorbet as the food matrix is that it considered the stability of *L*-theanine, such as temperature and pH-environment (pH 5–6, less than 4 °C). This study, together with the study of Montopoli et al. [45], have featured no cognitive tasks or stimulus were performed on the participants; the only intervention to cause blood pressure fluctuation was attributed to the carbohydrate response caused by sorbet consumption, or by cacao. Further studies of *L*-theanine in the food matrix could be conducted by considering the external stressors on the blood pressure as potentially different mechanisms might be triggered.

It is necessary to assess the potential changes of *L*-theanine such biological compounds in the GI tract, including absorption, elimination, uptake, distribution and biotransformation. Studies of *L*-theanine as a mono supplement against other food compounds are required, as interactions and bindings of *L*-theanine with other food components would tangle its biological efficacy. One example is epigallocatechin gallate (EGCG), the AUC of EGCG ingestion without food was 2.7 and 3.9 times higher than that of EGCG intake with breakfast or embedded in the strawberry sorbet [47].

L-theanine needs to be readily available in bioavailable access concentrations to produce the desired physiological effects. Apart from the searching of the optimal dosage, the consideration of the weight of participants is recommended in the experimental design in future research. A consistent dose tailored for kilograms of body weight (kg/BW) is a possible way to optimise the effects

of *L*-theanine and evaluate the 'ideal' and customised dosage for individuals, as well as theoretically improving the bioavailability of *L*-theanine in the body.

6. Conclusion

This review summarises the anti-anxiolytic and relaxation effect of *L*-theanine from existing studies of animal models and human trials. Behavioural data collected concurrently might be limited as these effects are dose-dependent and area-specific, as well as time-, drug-, and task-dependent. More advanced methodologies and multidisciplinary knowledge, such as genomics, proteomics, metabolomics, and nutrigenomics, together with neuroimaging technology should be intergraded in the in-depth exploration of the mechanism of *L*-theanine prevention and treatment on brain diseases. It is equally necessary, though, to agree that more studies are needed that include *L*-theanine as a potential functional food ingredient compared alongside its pure encapsulated form to warrant any clinically relevant claims.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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