# Effects of Oral Supplement L-Theanine on Relaxation Indexed by Alpha Brainwave

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### **Abstract:**

**Background:** This study used electroencephalography (EEG) to measure brain wave activity to investigate the physiological impact of L-theanine, an amino acid found naturally in green tea, on a state of relaxation.

**Objective:** The purpose was to assess whether L-Theanine supplementation enhances relaxation by modulating brain waves in healthy middle-aged adults. This study focused on how L-Theanine affected alpha wave activities in different brain regions.

**Materials and Method:** Thirty healthy middle-aged participants were randomly assigned to receive either 200 mg of oral L-Theanine or a placebo. EEG recordings were obtained at baseline and 90 minutes after intake.

**Results:** Even though the results weren't statistically significant, there was a clear pattern of higher alpha wave power in the L-Theanine group, especially in the left frontal and parietal cortex 90 minutes after consumption, which suggests that they were more relaxed.

**Conclusion:** Despite the lack of statistical significance, the findings demonstrated that L-Theanine might modulate alpha wave activity. However, this finding may require further investigation with longer usage of L-theanine supplementation and extended observation periods.

**Keywords:** L-Theanine, Brain, Electroencephalogram, Brain wave activity, Alpha wave, Relaxation

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### Introduction

In our fast-paced modern society, preserving cognitive function is crucial for efficient learning and productivity, as distractions increase due to technology in daily life. The brain, as the principal organ of cognition, regulates essential functions for learning, such as attention, memory, perception, problem-solving, and selfcontrol. As individuals age, cognitive skills inherently decline, producing difficulties in memory retention and cognitive functioning. To address these issues, previous studies have focused on L-theanine, an amino acid that is naturally present in green tea and edible mushrooms.<sup>1,2</sup> L-Theanine is an amino acid that dissolves in water and is not a protein. Its backbone is glutamine, and it can also be found as an ethylamine derivative of glutamate. Its chemical formula is C7H14N2O3. L-Theanine can be obtained through various methods, including extraction from tea leaves, chemical synthesis, or biosynthesis.<sup>1</sup>

L-Theanine has attracted interest for its neuroprotective attributes, which foster neural connections and improve cognitive functions, including learning and memory. Studies indicate that L-Theanine can modulate brain waves, specifically the alpha wave linked to relaxation and focused attention. This alteration in cerebral activity emphasizes the potential of L-Theanine to enhance cognitive ability.<sup>3,4</sup> Previous studies have demonstrated that L-Theanine administration can enhance memory and cognitive function. It has been noted to improve memory and the functioning of the hippocampus, a brain region essential for long-term memory. L-Theanine has been shown to enhance mood, motivation, cognition, and memory by regulating neurotransmitters including dopamine, serotonin, 5-hydroxytryptamine (5HT), glycine, and GABA, while also reducing cortisol levels.5,6

Theanine, a prevalent non-protein amino acid, was first identified in green tea leaves in the 1940s by Sakato. The International Union of Pure and Applied Chemistry (IUPAC) refers to theanine chemically as 2-amino-4-(ethyl carbamoyl) butyric acid.<sup>7</sup> It usually appears in its L-(S) enantiomeric form. Theanine is distinctive in nature and is predominantly located in the Camellia genus, especially in tea-producing species such as C. sinensis var. sinensis and C. sinensis var. assamica. Theanine substantially influences the unique flavor of tea, with teas that possess elevated levels of theanine frequently considered superior in quality.8 An enzyme called theanine synthetase turns glutamic acid and ethylamine into theanine. This process mostly happens in the roots of tea plants before it is sent to the new shoots.9 Theanine concentrations in tea leaves may fluctuate based on factors including cultivation circumstances, tea grade, variety, and harvest timing. Certain investigations indicate that regulated exposure to sunlight can elevate theanine concentrations in tea. Throughout post-harvest processing, theanine concentrations remain stable across various tea types.10 Studies indicate that the use of theanine may enhance individual health and well-being, including stress reduction, enhanced cognitive function, and possible protective effects against specific diseases. Achieving doses linked to beneficial benefits exclusively through tea drinking may prove challenging due to the substantial volume required to attain them. The inclusion of caffeine in tea complicates the achievement of certain levels, potentially leading to side effects prior to realizing the desired benefits.11

L-Theanine demonstrates promising neuroprotective benefits and the capacity to boost cognitive function, especially memory, indicating its potential for regulating brain waves. The goal of this study is to learn more about how L-Theanine affects the

electroencephalogram in healthy middleaged people by looking at how taking supplements with L-Theanine affects their cognitive functions. This study aims to investigate the effects of L-Theanine supplementation on brain activity through EEG measurements taken at baseline and 90 minutes after intake. Previous investigations have primarily concentrated on the effects of L-Theanine in younger, healthy individuals or those with elevated health metrics; however, there is a lack of studies investigating its impact on the healthy middle-aged group. 1,3,4,12 This study seeks to address the issue by evaluating the activity of alpha brain waves after L-Theanine ingestion in healthy middle-aged persons, an age group that commonly utilizes supplements for relaxation and cognitive enhancement, hence yielding more significant insights into its effects. Thus, the aim of the present study was to determine the effect of L-Theanine in different regions of the brain and each hemisphere. When participants were administered L-Theanine, the brain waves' power was measured across various brain regions at different time points, including baseline and 90 minutes postingestion.

### **Materials and Method**

This study involved a randomized, double-blind, single-dose, placebo-controlled experiment. This study utilized a conceptual framework to investigate the impact of 200 mg L-Theanine oral supplementation on changes in alpha brain waves at various time intervals, namely at baseline and 90 minutes post-ingestion, in healthy middle-aged adults.

## **Participants**

Participants consisted of male and female volunteers aged 40 to 60 years, with a BMI ranging from 18.5 to 25 kg/m<sup>2</sup> and

no prior medical history. The trial was conducted at the MAS Neuroscience Center, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand. All participants were screened for the following inclusion and exclusion criteria. Inclusion criteria included (a) healthy male and female participants aged between 40 and 60 years old, (b) BMI 18.5-25 kg/m<sup>2</sup>, (c) healthy individuals with no underlying illness, and (d) non-shift workers with a regular sleep schedule, with bedtime being between 10 PM and 12 AM. (e) Not a regular alcohol drinker (an intake of > 14 U of alcohol per week for females and 21 U for males (a unit of alcohol in the United Kingdom is defined as 7.9 g or 10 ml of pure alcohol; an average beer has 10 ml of alcohol). A shot of whiskey comprises 18 ml of alcohol. (f) Participants who consented to refrain from all caffeine- and theaninecontaining items 24 hours before the test day; (g) Volunteers who gave written consent and agreed to follow the instructions. Exclusion Women who were pregnant or breastfeeding, people who had a history of psychiatric or emotional problems, people whose systolic blood pressure was higher than 140 mm Hg or their diastolic blood pressure was higher than 90 mm Hg and whose resting heart rate was lower than 40 beats per minute, people who had a history of substance abuse, people who smoked more than 5 cigarettes or the equivalent every day or drank more than 2 caffeinated drinks every day, people who had a family history of a disorder similar to schizophrenia, and people who took supplements a week before the test were not allowed to take part. The Mae Fah Luang University Ethics Committee on Human Research approved the study protocol (COA No. 088/2022, Protocol No.: EC 22033-20, authorized on May 19, 2022). All subjects gave informed consent, and the investigation followed their regulations.

#### **Materials**

Each participant received 200 mg of encapsulated purified L-Theanine for this investigation. The dosage was established based on the optimal effect noted in the previous study, 7 which demonstrated that 200 mg of L-Theanine exerted substantial effects on the human brain.

### Procedure

Participants were recruited based on specific inclusion and exclusion criteria and subsequently randomized into one of two groups: either the L-Theanine group or the placebo group. The lab assistant positioned the EEG electrode according to the 10-20 regulations. We performed the test twice: once at baseline and again 90 minutes after administering either L-Theanine or a placebo. Each session lasted for five minutes. We continuously recorded EEG during each five-minute test to monitor brain activity.

We recorded EEG activity at 90 minutes post-ingestion based on prior research and pharmacokinetic data. The brain detects levels of L-Theanine within approximately 30 minutes post-ingestion, indicating its relatively quick absorption.<sup>13</sup> Studies have observed alpha wave changes as early as 40 minutes after ingestion, with EEG monitoring conducted at various intervals, including 30-, 45-, and 60-minutes post-dose.13 But 90 minutes seemed like a good time to catch the full effect of a single 200 mg dose, since some research shows that the highest levels of L-Theanine in the brain happen one to two hours after consumption.<sup>14</sup>

This timeframe also helped minimize participant discomfort by reducing the need for repeated EEG recordings, which could lead to fatigue or fluctuations in alertness unrelated to the supplement itself. Previous research has also shown that L-Theanine effects last longer than this window.

For example, one study found that frontal alpha power increased significantly about three hours after taking it during a stress challenge. Given these considerations, 90 minutes provided an optimal balance between capturing L-Theanine's expected peak effects and maintaining participant comfort during the study session.

# Electroencephalographic Recording

The experiment employed an EEG apparatus based on the 10-20 system for the display, analysis, and recording of EEG activity. EEG signal frequency analysis was performed with a Fast Fourier Transform (FFT) technique over a 2-second interval. The frequency bands examined were delta (0.1-3.0 Hz), theta (4.0-7.0 Hz), alpha (8.0-12.0 Hz), and beta (13.0-30.0 Hz). For each subject, we initially measured the alpha wave power across a five-minute interval while they were seated in a relaxed state. The recorded power, quantified in microvolts, was subsequently averaged to yield one value denoting the group frequency band for each electrode. The electrodes were subsequently arranged to denote distinct regions of the brain, as illustrated below.

Group the electrodes for each brain region: Frontal area (F): FP1, FPZ, FP2, F7, F3, FZ, F4, F8; Temporal region (T): FC5, T7, CP5, FC6, T8, CP6; Parietal region (P): CP1, CP2, P3, PZ, P4, P7, P8; Central region (C): FC1, FC2, C3, CZ, C4; Occipital region (O): O1, OZ, O2.

Group the electrodes for the left and right hemispheres. Right: Fp2, F8, P8, O2; Left: Fp1, F7, P7, O1.

## Statistical analysis

We used SPSS version 23.0 to document the demographic data and outcome results in the current study. We averaged the power of the alpha waves across different conditions to measure the selective enhancement of

brain activity. To do so, a linear mixed-effects model predicted the power of frequency band (alpha), group (test, placebo), and time (baseline, 90 minutes). Baseline power was measured at 0 min and was used to control for initial differences in neural activity while accounting for variance between participants. The Mann-Whitney U test and linear mixed model were utilized for significance testing because of the data's non-normality. The comparison assessed the mean difference of each brain wave across several brain areas at baseline (before L-theanine administration) and 90 minutes post-administration among all subjects. A p-value less than 0.05, or 5%, qualifies as statistically significant.

### **Results**

Thirty participants were recruited and randomized to receive either L-Theanine (n=15) or a placebo (n=15). All participants successfully completed the experiment without difficulties. The sample size for identifying variations in mean changes in relative alpha power was established based on the findings of Evans, et al,15 who performed a comparable placebo-controlled crossover study examining the effects of a single 200 mg dose of AlphaWave® L-Theanine. We eliminated one outlier from each group during the analysis. The primary outcome measure of this study was the relative power of alpha brainwaves, recorded in microvolts, across various brain regions. The alpha power values presented in Table 1 represents the mean relative alpha activity recorded during each five-minute EEG session. These numbers are given as relative power ratios, which were found by comparing the activity in the alpha band to the overall EEG power spectrum. Higher relative alpha power indicates a greater predominance of alpha activity. Table 1 shows that the frontal region alpha power didn't change significantly in the placebo group (N = 14) at any time points after the drug was taken compared to the start (p=0.250). Similarly, no significant changes were observed in the temporal, parietal, central, occipital, left, or right regions at any of the post-ingestion time points. However, the frontal and temporal regions of the brain show a decreasing trend, whereas the parietal, central, occipital, left, and right regions show an initial increase in power and then return to baseline or slight decrease after 90 minutes. In the L-Theanine group (N = 14), however, the frontal region alpha power showed an increasing trend at 90 minutes compared to baseline (mean =  $0.33 \pm 0.09$ , p = 0.765). Although this trend was not statistically significant, it indicated a potential increase in alpha power overtime. Similar increasing trends were observed in the temporal (90 minutes: Mean =  $0.26 \pm 0.06$ , p = 0.662), parietal regions  $(90 \text{ minutes: Mean} = 0.25 \pm 0.09, p = 0.818),$ left hemispheres (90 minutes: Mean =  $0.22 \pm 0.04$ , p=0.161), and right hemispheres  $(90 \text{ minutes: Mean} = 0.25 \pm 0.06, p = 0.565),$ though these were also not statistically significant. Even though there were no changes that were statistically significant, the data show that the power of alpha waves increased in the frontal, temporal, and parietal areas, as well as in both the left and right hemispheres, after the L-Theanine group took it compared to the placebo group. These results suggest that the peak effect within the 90-minute test period occurs at the 90-minute mark and may potentially increase further if the experiment were extended.

Table 1 L-Theanine and placebo groups' alpha wave power in different brain areas at baseline and 90 minutes after consumption. The mean ± standard error of mean (SEM) values in μV is shown.

Alpha wave	Measurements				
	Baseline		90 minutes		
	Mean	SEM	Mean	SEM	<i>p</i> -value <sup>α</sup>
Placebo (N = 14)					
Frontal area	.31	.05	.23	.04	.25
Temporal area	.33	.09	.22	.05	.19
Parietal region	.17	.03	.14	.02	.50
Central area	.21	.04	.21	.06	.51
Occipital area	.15	.02	.15	.04	.42
Left hemisphere.	.29	.05	.22	.04	.16
Right hemisphere	.29	.06	.25	.06	.57
L-Theanine (N = 14)					
Frontal area	.22	.04	.33	.09	.77
Temporal area	.22	.05	.26	.06	.66
Parietal area	.18	.03	.25	.09	.82
Central area	.18	.03	.18	.04	.66
Occipital area	.17	.03	.18	.05	.84
Left hemisphere	.20	.04	.32	.09	.60
Right hemisphere	.23	.04	.29	.08	.89

<sup>&</sup>lt;sup>α</sup> *p*-value was calculated using Mann-Whitney test.

## **Discussion**

This study aimed to investigate the effects of L-Theanine on cognitive functions through changes in the activity of alpha brain waves measured using EEG. The study involved 30 healthy middle-aged participants, although one participant from each group was removed due to being an outlier, who were randomly assigned to either the L-Theanine group or the placebo group. The main results show that there were no statistically significant changes, but there was a trend toward higher alpha power after taking L-Theanine, especially in the left frontal and parietal regions.

After 90 minutes post-ingestion, alpha power increased from  $0.22 \pm 0.04$  at baseline to  $0.33 \pm 0.09$  in the frontal area and from  $0.18 \pm 0.03$  to  $0.25 \pm 0.09$  in the parietal area. These increases were more pronounced in the left hemisphere compared to the right one, where the alpha wave power rose from  $0.23 \pm 0.04$  to  $0.29 \pm 0.08$ . Even though these differences were not statistically significant (*p*-values of 0.765 for the frontal area, 0.818 for the parietal area, and 0.596 for the left hemisphere), the direction of change is consistent with what other research has shown, which is that L-theanine may increase alpha activity in these areas.

The increase of alpha power is generally associated with relaxation and focused attention.<sup>3,4</sup> In our results, the most significant change is in the frontal left region. The frontal regions, particularly on the left side, are associated with emotional regulation and attentional control.<sup>16</sup> Increased alpha activity in these regions has been interpreted as an indicator of a relaxed but alert mental state. 17 This pattern suggests that L-Theanine may promote a relaxed focus, potentially supporting cognitive processes that require sustained attention without inducing drowsiness. The parietal cortex, which is involved in the integration of sensory information and the allocation of attentional resources, also shows increased alpha power. Elevated alpha activity within this region has been consistently associated with internally directed attention and a reduction in the processing of external sensory stimuli.<sup>18</sup> Additionally, researchers believe that alpha oscillations in the parietal regions function as a gating mechanism, blocking irrelevant sensory input and promoting selective attention.<sup>19</sup> This study suggests that L-Theanine may help shift toward an internally focused cognitive state by inhibiting certain sensory inputs, potentially enhancing tasks that require mental imagery, working memory, or meditative awareness. Taken together, these findings suggest that L-Theanine may help shift the mind toward a focused and more relaxed cognitive state by reducing external sensory interference.

Compared to the placebo group, which typically showed reductions in alpha power from baseline to 90 minutes post-ingestion, the L-Theanine group showed a trend toward increased alpha activity. This contrast suggests that L-Theanine may affect the decline in alpha power typically observed during prolonged wakefulness or cognitive load. By maintaining or increasing alpha activity over time, L-Theanine may contribute to prolonged states of relaxation and

mental clarity, potentially counteracting the cognitive fatigue often experienced during extended periods of concentration. However, this hypothesis requires further investigation in future studies.

The earlier studies by Chu et al.<sup>20</sup> showed that taking 200 mg of L-Theanine dissolved in 100 ml of water caused alpha waves to appear mostly in the occipital and parietal regions of young female volunteers (aged 18-22), and the more recent randomized, triple-blind, placebo-controlled crossover study by Evans et al.15 found that 200 mg of AlphaWave® L-Theanine significantly increased frontal region and whole-scalp alpha power three hours after consumption in moderately stressed adults (19-60 years old). Our investigation targeted healthy middle-aged adults (aged 40 - 60), providing insights into the effects of L-Theanine across a broader age range; we also have a different observation period of 90 minutes. We observed that the increase in alpha power was most pronounced in the left frontal and parietal regions, which are areas associated with emotional regulation, attentional control, and sensory integration. According to these results, L-Theanine may change neural activity in different ways depending on age and brain region. This could affect how it is used to help middle-aged people feel calm and focused. Furthermore, it is possible that middle-aged adults require a longer latency period before significant changes appear, or that a single dose may be insufficient to provide significant effects in this population. Further research is warranted to explore these age- and brain region-specific effects, as well as their relevance to cognitive performance and emotional well-being.

To place our findings in a practical context, we considered how the observed effects of L-Theanine supplementation might translate to everyday tea consumption. On average, a standard cup of tea contains approximately 20 to 25 mg of L-Theanine,

though the amount can vary depending on the tea variety and brewing conditions.<sup>7,10</sup> Based on this estimate, the 200 mg dose used in our study would be equivalent to consuming about eight to ten cups of tea. It is unlikely that an individual would consume such a large quantity for relaxation purposes. Previous research has indicated that relaxation effects typically become more pronounced at higher doses of L-Theanine than what is commonly present in a single cup of green tea.<sup>13</sup> However, lower doses, like 50 mg from about two cups of tea, have been linked to increases in alpha wave activity within about 40 minutes, with effects lasting for at least 90 minutes.<sup>21</sup> This suggests that moderate consumption may elicit a mild state of relaxation, albeit less than the effects observed with a concentrated 200 mg dose of L-Theanine. Additionally, it is important to note that tea contains caffeine, which was not present in the pure L-Theanine capsules administered in our study. Caffeine and L-Theanine in tea are known to work together to produce a stronger effect, which is often described as a balanced state of relaxation with sustained focus. This is because L-Theanine can lessen some of the stimulating effects of caffeine.<sup>22,23</sup> From a practical standpoint, moderate tea consumption may contribute to stress reduction and relaxation, although the impact on alpha brain waves is likely to be more subtle compared to a higher dose L-Theanine supplement. For individuals seeking more pronounced relaxation effects without excessive caffeine intake or fluid consumption, options such as decaffeinated tea or L-Theanine supplements may offer a more effective alternative. This context helps highlight the real-world relevance of our findings and offers practical guidance for those interested in using L-Theanine to promote relaxation.

We acknowledge that 40 - 60 is a somewhat broad range, spanning two decades; we feel that it was appropriate for a pilot exploratory study, as it captures the population of middle-aged adults who might use supplements for relaxation or cognitive health. However, all participants met strict inclusion criteria to ensure relative health status. This range represents middle age, a life stage where subtle cognitive and neurophysiological changes begin. Research suggests that certain cognitive functions start to decline as early as the mid-40s.<sup>24</sup> We were interested in whether L-Theanine's relaxation and potential cognitive benefits would be evident in this age group, as many prior studies using EEG have examined younger adults.<sup>25,26</sup>

Both male and female participants were included to enhance the generalizability of our findings. We did not consider the sex differences in response to L-Theanine; rather, inclusion criteria were based on health and age parameters. It should be noted that we did not control for the menstrual cycle phase in female participants, as some studies indicated that alpha waves could be influenced by the menstrual cycle and progesterone level.27,28 However, we use a random assignment to treatment groups and ensure a balanced gender ratio, minimizing potential sex-related effects. We acknowledge this as a potential limitation in our design and recommend future studies account for menstrual phase to eliminate this variable.

### Conclusion

This study shows some early signs that L-Theanine may change brain activity, mainly by increasing the power of alpha waves in the left frontal and parietal regions. These are known to play important roles in controlling emotions, paying attention, and processing sensory information. While

the results didn't reach statistical significance, there is a consistent trend of increased alpha activity that suggests L-Theanine could help promote a relaxed yet alert mental state in healthy middle-aged adults. However, there are a few important limitations to acknowledge. The sample size was relatively small, and the study only explored the effects of a single dose. We also used a broad age range (40 - 60 years), and we didn't control for menstrual cycle phases in female participants, both of which could have introduced variability in the results. While we ensured a balanced gender ratio and randomized assignment, future studies should take these factors into account to better isolate L-Theanine's effects. Future research should explore different doses and timing to better understand how L-Theanine works over overtime and at varying levels. Longer-term studies could also determine whether regular use has long-term benefits. Including cognitive testing, like the CDR computerized battery, along with mood assessments such as visual analog scales, would give a clearer picture of how L-Theanine impacts both mental performance and emotional well-being. Adding biochemical measures, such as neurotransmitter levels and salivary cortisol, could also compare how L-Theanine affects other stress markers as well. And finally, excluding female participants during their menstrual cycles may help improve the results by reducing hormonal fluctuations that might influence brainwave activity.

### Contribution

Conceptualization: Nipapan Sangmanee and Phakkharawat Sittiprapaporn; Methodology: Nipapan Sangmanee and Phakkharawat Sittiprapaporn; Data Curation: Nipapan Sangmanee and Phakkharawat Sittiprapaporn; Resources: Phakkharawat Sittiprapaporn; Formal analysis: Chong Ie Yern and Phakkharawat

Sittiprapaporn; Validation: Phakkharawat Sittiprapaporn; Investigation: Phakkharawat Sittiprapaporn; Writing-original draft preparation: Chong Ie Yern and Phakkharawat Sittiprapaporn; Writing-review and editing, Phakkharawat Sittiprapaporn; Project administration: Phakkharawat Sittiprapaporn; Funding Acquisition: Nipapan Sangmanee and Phakkharawat Sittiprapaporn. All authors have read and agreed to the published version of the manuscript.

#### **Disclosure of interest**

The authors report no conflict of interest.

# **Data Availability Statement**

The data presented in this study are available within the article.

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