EFFECTS OF ORAL SUPPLEMENT L-THEANINE ON COGNITIVE FUNCTIONS AND RELAXATION: ELECTROENCEPHALOGRAPHIC STUDY

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MASTER OF SCIENCE
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THIS THESIS IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANTI-AGING AND REGENERATIVE MEDICINE

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Ie Yern Chong

Thesis Title	Effects of Oral Supplement L-Theanine on Cognitive Functions and Relaxation: An Electroencephalographic Study
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ABSTRACT

This study investigates the effects of a single 200 mg dose of L-Theanine, an amino acid in green tea, on brain wave activity using electroencephalogram (EEG). The aim was to evaluate if L-Theanine enhances cognitive performance and relaxation by modulating alpha, beta, and theta waves and whether these effects are uniform across the brain. Fifteen healthy middle-aged participants received L-Theanine, with EEGs taken at baseline, 30-, 60-, and 90-minutes post-ingestion.

The results showed non statistically significant but observable trend of increased alpha and beta wave power, particularly in frontal, temporal, and parietal regions at 90 minutes, while theta wave activity generally decreased. Comparison of fast brain waves revealed a relatively greater increase in alpha over beta power while comparing Z-scores, indicating a shift toward a balanced alpha-beta state and suggesting relaxed alertness.

Lateral analysis showed no significant differences between whole brain, left and right hemispheres, implying a uniform effect across the brain. This balanced reduction in beta dominance supports a calm yet alert state.

These trends, although not significant, highlight L-Theanine's potential in modulating brain wave patterns, enhancing cognitive function and relaxation. Further studies with longer observation periods are suggested to confirm these effects and clarify their significance.

Keywords: L-Theanine, EEG, Brain Wave Activity, Alpha Waves, Beta Waves, Theta Waves

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ABBREVIATIONS AND SYMBOLS

5-HT	5-Hydroxytrptamine, Serotonin
ATP	Adenosine triphosphate
BMI	Body mass index
EEG	Electroencephalogram
FDA	Food and Drug Administration
GABA	Gamma-Aminobutyric Acid
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IUPAC	International Union of Pure and Applied Chemistry
MCI	Mild cognitive impairment
µV	Microvolts

CHAPTER 1

INTRODUCTION

1.1 Background

Maintaining cognitive function is essential for daily life and productivity. The brain, as the central organ responsible for cognition, governs various important functions for learning, including attention, memory, perception, problem-solving, and self-control. However, as individuals age, cognitive functions naturally decline, posing challenges to memory retention and cognitive performance.

To address these challenges, many have turned to supplement to aid with this, one of such is L-Theanine. L-Theanine is a naturally occurring compound found in green tea and edible mushrooms (Casimir et al., 1960; Sakato et al., 1956). L-Theanine is a water-soluble, non-protein amino acid with a glutamine backbone and is also present as an ethylamide 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 (Casimir et al., 1960).

L-Theanine has garnered attention for its neuroprotective properties, which promotes neuronal connectivity and enhances cognitive functions such as learning and memory. Studies have shown that L-Theanine is capable of modulating brain waves, particularly the alpha wave which is associated with relaxation and focused attention. This modulation of brain activity highlights the potential of L-Theanine in optimizing cognitive performance (Park et al., 2011; Yoneda et al., 2019).

Research has shown that L-Theanine supplementation can improve memory and brain function. It has been observed to enhance memory and the functioning of the hippocampus, a brain region crucial for long-term memory. Additionally, L-Theanine has been found to positively influence mood, motivation, cognition, and memory by modulating neurotransmitters such as dopamine, serotonin, 5-hydroxytrptamine (5HT), glycine, and GABA, while decreasing cortisol levels (Tamano et al., 2013; Vuong et al., 2011).

Given its promising neuroprotective effects and its potential to enhance cognitive function, particularly memory, L-Theanine shows its potential to modulate brain waves. By investigating the effects of L-Theanine supplementation on cognitive functions, in healthy middle-aged individuals, this study aims to further dive deeper into the role of L-Theanine effect on the Electroencephalogram in different wave lengths.

1.2 Importance of Research

The significance of this study is to dive deeper into the impact of L-theanine supplementation on brain wave using the EEG at various time intervals post-consumption while volunteers relax.

While prior research has predominantly focused on the influence of L-theanine on alpha brain waves, there remains a lack of studies examining its effects on other wavelengths. This research aims to fill this gap by assessing the broader spectrum of brain wave activity following L-theanine consumption, thus providing more valuable insights on its effects.

1.3 Research Objective

The objective of this study is to examine the impact of single dose oral L-theanine 200mg supplementation on cognitive function and relaxation by measuring brainwave changes at various time intervals in different brain regions. Additionally, the study aims to determine whether L-theanine's effects are distributed across the whole brain equally or show lateralization.

1.4 Research Assumptions

The research assumes that taking L-theanine supplements can potentially modulate brain waves which can then be detected by the electroencephalogram. These changes could possibly suggest an increase in cognitive function or relaxation.

1.5 Conceptual Framework

The study is based on a conceptual framework that explores the effects of 200mg L-theanine supplementation on brain wave changes at different time points mainly at baseline, 30, 60, 90 mins post ingestion.

1.6 Hypothesis

1.6.1 A single dose oral 200mg L-theanine improves cognitive function and relaxation based on EEG reading within 90-mins.

H1: Alpha, beta, and theta wave power will show a significant increase from T1 (baseline) to subsequent measurements at T2, T3, and T4 following L-theanine consumption.

1.6.2 L-theanine effect on fast brain waves, specifically alpha and beta, is comparable.

H1: Alpha and beta wave power will increase at a similar rate post-L-theanine intake.

1.6.3 L-theanine's effect is distributed across the whole brain.

H1: There will be no significant difference in wave power changes between the left and right hemispheres

1.7 The Scope of Research

This paper aims to investigate the effects of L-theanine supplementation on cognitive function, particularly focusing on changes in brain wave changes post-consumption while relaxed. While previous studies have primarily examined L-theanine's impact on alpha brain waves, this research will also explore its effects on other wavelengths, addressing a gap in existing literature. It also aims to determine whether L-theanine’s effects are distributed across the whole brain equally.

By using electroencephalography (EEG) the study aims to provide insights into L-theanine's potential as a cognitive enhancer and relaxant and its relevance for optimizing this function.

1.8 Term Definition

1.8.1 L-Theanine

L-Theanine, a non-protein amino acid primarily found in green tea leaves, has been extensively studied for its potential to promote relaxation and reduce stress, some papers even state its potential effect on cognitive functions (Li et al., 2022; Park et al., 2011; Williams et al., 2020). It is known to modulate neurotransmitters like dopamine and serotonin, promoting relaxation and improving mood (Tamano et al., 2013).

1.8.2 Electroencephalogram, EEG

Electroencephalography (EEG) is a non-invasive method used to measure brain wave activity, providing insights into different cognitive states. It measures electrical activity through electrodes placed on the scalp, capturing variations in brain wave patterns such as Delta (0.5-4 Hz), Theta (4-7 Hz), Alpha (8-12 Hz) and Beta (13-30 Hz) waves (Henry, 2006).

1.8.3 Cognitive Function

Cognitive functions encompass a range of mental processes involved in acquiring, processing, storing, and applying knowledge. These include abilities such as attention, memory, learning, reasoning, problem-solving, and decision-making (Park et

al., 2011). Cognitive functions are essential for daily activities and overall mental performance, enabling individuals to interact effectively with their environment.

1.8.4 Relaxation

Relaxation refers to a state of reduced physical and mental tension, characterized by a sense of calm and reduced stress levels. This state is typically associated with an increase in alpha brain waves, which reflect a calm and alert mind (Evans et al., 2021). Relaxation is crucial for mental recovery and maintaining overall well-being

CHAPTER 2

LITERATURE REVIEW

2.1 Theanine

Theanine, a common non-protein amino acid, was originally discovered in green tea leaves in the 1940s by Sakato (Sakato, 1949). Known chemically as 2-amino-4-ethylcarbamoyl butyric acid by the International Union of Pure and Applied Chemistry (IUPAC) (Li et al., 2022). It naturally occurs predominantly as the L-(S) enantiomer. Theanine is unique in nature, and it's primarily found in the *Camellia genus*, particularly in tea-producing plants such as *Camellia sinensis var. sinensis* and *Camellia sinensis var. assamica*. Theanine contributes significantly to the distinctive taste of tea, with teas containing higher levels of theanine often regarded as of higher quality (Chu, 1997).

Biosynthesis of theanine occurs in tea plants from glutamic acid and ethylamine via the enzyme theanine synthetase, it occurs mainly in the roots and subsequently transferred to the developing shoots (Deng et al., 2008). Theanine levels in tea leaves can vary due to factors such as growing conditions, tea grade, variety, and time of harvest. As shown in some trials, controlled exposure to sunlight can increase theanine levels in tea. And despite post-harvest processing, theanine levels remain consistent across different types of tea (Hara, 2012).

Studies suggest that theanine consumption can positively impact health and well-being of individual taking them, this includes stress reduction, improved learning ability, and potential preventive effects against certain diseases. However, reaching doses associated with positive effects solely through tea consumption may present a challenge due to the significant quantity one would need to consume to reach them. Additionally, the presence of caffeine in tea further complicates achieving these doses, potentially causing side effects before the desired benefits are experienced (Janet et al., 2015).

Synthesis and Biosynthesis of L-Theanine

1. Chemical Synthesis of L-Theanine:

L-Theanine was initially chemically synthesized in 1942 by Lichtenstein, yielding 90g/kg$_{-1}$ by treating pyrrolidone-5-carboxylic acid with aqueous ethylamine for 20 days at 37◦C (Lichtenstein, 1942). Various synthetic approaches have since been developed, including large-scale production methods involving the reaction of γ-benzyl glutamate in the presence of trityl chloride and ethylamine, as well as a two-step approach involving the dehydration of L-glutamic acid to L-pyrrolidone carboxylic acid followed by ring opening in the presence of ethylamine (Kawagishi & Sugiyama, 1992). More recently, L-theanine has been synthesized in four steps starting from commercially available N-phthaloyl-L-glutamic acid, resulting in an overall yield of 700 g kg$_{-1}$ (Gu et al., 2004). Chemical synthesis offers a simple, convenient, and cost-effective alternative to direct extraction from natural sources or biosynthetic methods. However, certain limitations exist, including consumer resistance to synthetic additives in food supplements and the need for protection and deblocking procedures for reactive groups, which can increase synthesis time and cost (Li et al., 2022). Additionally, synthetic theanine is typically produced as a racemic mixture of L- and D-forms, whereas theanine occurs naturally predominantly as the L-(S) enantiomer (Zhang et al., 2010).

2. Biosynthesis of L-Theanine:

In the tea plant, L-theanine is biosynthesized from glutamic acid and ethylamine by the enzyme theanine synthetase (Zhang et al., 2010). However, this enzyme is too labile for commercial-scale production (Li et al., 2022). Alternative methods for enzymatic synthesis have been developed using bacterial enzymes such as glutaminase, glutamine synthetase, and γ-glutamyltranspeptidase. Glutaminase from *Pseudomonas nitroreducens* has been utilized to simultaneously hydrolyze glutamine to ammonia and glutamic acid and catalyze the reaction of glutamine with ethylamine to form theanine. Glutamine synthetase and related enzymes from various bacterial sources have also been employed for the synthesis of theanine from glutamic acid and ethylamine (Vuong et al., 2011). Despite some limitations, such as the requirement for continuous ATP supply or high concentrations of ethylamine, enzymatic biosynthesis of theanine shows promise for industrial-scale production. Enzymes offer the advantage

of producing the naturally occurring L-(S) enantiomer of theanine, overcoming the racemic mixture produced by synthetic methods (Li et al., 2022).

2.2 L-Theanine Action on the Brain

2.2 1 Reduces Anxiety and Stress Level

Several studies discuss in a meta-analysis conducted by Williams et al. (2020) suggest that L-theanine supplementation at doses ranging from 200 to 400 mg per day exhibits promising anti-stress and anxiety-reducing properties. These effects are observed through various mechanisms, including the modulation of physiological stress markers such as salivary alpha-amylase and the inhibition of glutamatergic neurotransmission (Kakuda et al., 2002; Unno et al., 2013). However, it's important to note that doses exceeding 400 mg may not confer additional benefits, indicating a potential ceiling effect in its efficacy (Williams et al., 2020).

While L-theanine appears to be particularly effective in individuals with elevated anxiety levels, its impact on cognitive performance remains variable. Some studies report improvements in attentional performance and reaction time, especially in those with high anxiety (Higashiyama et al., 2011). Additionally, higher doses (450–900 mg) may not be beneficial, particularly in those with generalized anxiety disorder (Sarris et al., 2019).

The presence of L-theanine in green tea complicates the assessment of its specific physiological and psychological effects when consumed alongside other polyphenolic compounds. Green tea drinkers often exhibit lower depressive symptoms, suggesting a potential role for L-theanine; however, the presence of catechins and other constituents in green tea makes it challenging to isolate L-theanine's effects (Hintikka et al., 2005). Further research is needed to uncover its mechanisms of action and optimize its therapeutic use, especially concerning dosage and interactions with other compounds.

2.2.2 Increases Alpha Waves

Alpha waves, important in indicating states of relaxation and calmness, are a crucial component of human brain activity, reflecting reduced stress and anxiety levels. Studies have consistently demonstrated the ability of theanine administration to significantly increase alpha wave activity, thereby promoting a relaxed mental state and enhancing overall feelings of tranquility and well-being (Evans et al., 2021). For instance, Chu et al. (1999) found that ingestion of a 200 mg theanine solution led to a notable increase in alpha brain waves in the occipital and parietal regions of volunteers' brains after just 40 minutes, underscoring its relaxation-inducing effects. This dose-dependent rise in alpha wave intensity aligns with previous findings suggesting rapid absorption of theanine into brain tissue within 30 minutes of ingestion.

Moreover, recent research by Evans et al. (2021) demonstrated that participants who received a single dose of AlphaWave™ L-theanine exhibited significantly heightened alpha power in the frontal region compared to those receiving a placebo, particularly during eyes-open segments, indicating continuous relaxation effects. The increase in whole-scalp alpha power in this paper further substantiates these findings, emphasizing the potential of oral L-theanine to modulate brain activity towards a more relaxed state compared to placebo administration.

2.3 Electroencephalogram

Recording brain activity through Electroencephalogram (EEG) plays a crucial role in unraveling the mysteries of brain function through electrical measurements. This technique uses an EEG headset, offering flexibility in positioning electrode sensors across 25 different nodes. These nodes are established according to the International 10/20 system (Henry, 2006). The numbers '10' and '20' stand for the specific distances between each sensor position, with variations of either 10% or 20% along the head's length and width. The electrode placements are specified by letters, with 'F' for frontal lobe, T' for temporal lobe, 'C' for central, 'P' for parietal lobe, and 'O' for occipital lobe. Additionally, each letter is followed by either 'Z' or numerical values. 'Z' signifies the midline separating the left and right hemispheres, while even numbers

indicate positions on the right hemisphere and odd numbers on the left (Henry, 2006; Lim et al., 2020).

In this experiment we plan to determine the effect of L-theanine effect of regions of the brain and differences between the left and right hemisphere.

Different regions of the brain are separated into these electrodes as they are closely correlated to each other and have been similarly shown by (Bakhtiari et al., 2023; Kothare, 2014).

Frontal region (F): FP1, FPZ, FP2, F7, F3, FZ, F4, F8

Temporal region (T): FC5, T7, CP5, FC6, T8, CP6

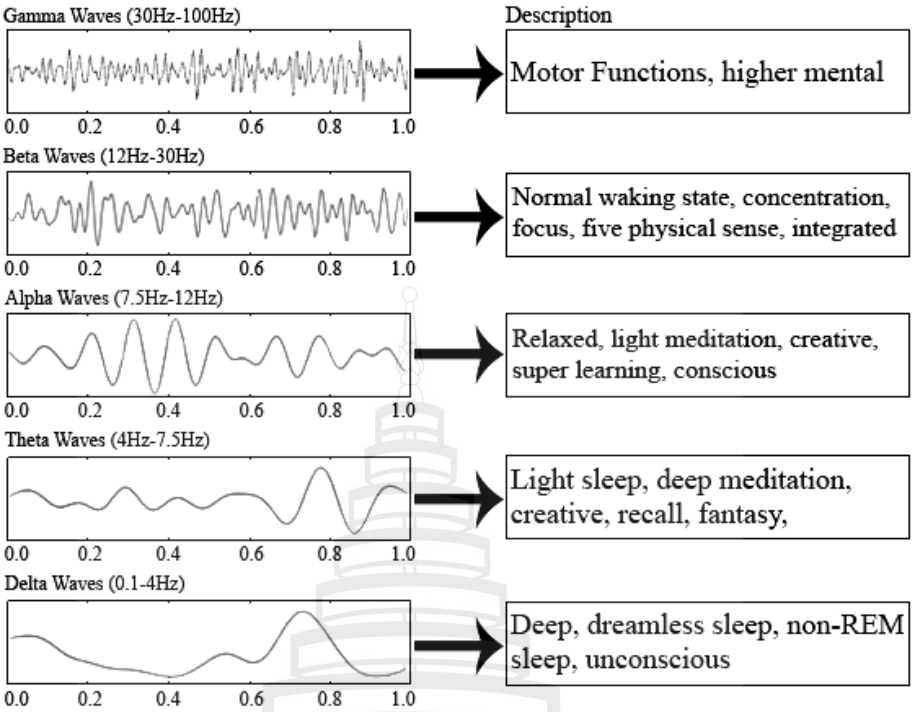
Parietal region (P): CP1, CP2, P3, PZ, P4, P7, P8

Central region (C): FC1, FC2, C3, CZ, C4

Occipital region (O): O1, OZ, O2

To ensure the balance of brain signals between its left and right hemispheres, it's important to maintain symmetry in the number and placement of electrodes. For the frontal lobe, it plays a role in concentration and problem-solving, which is in electrode sensors at Fp1 and Fp2 positions. Similarly, the parietal lobe, for working memory, for electrodes at F7 and F8 positions. The temporal lobe's role in auditory processing and long-term memory, electrodes are positioned at P7 and P8. Lastly, electrodes at positions O1 and O2 for the occipital lobe, important for visual processing and sight functions (Lim et al., 2020).

EEG band power reflects the neural circuitry and offers valuable insights into brain activity across different mental states (Elston, 2003). The brain waves are divided into delta, theta, alpha, beta, and gamma bands. The EEG graphs with descriptions of each brain wave are shown in Figure 2.1. EEG power has been associated with various cognitive traits and psychopathological conditions (Tran et al., 2006). Resting EEG power provides a direct reflection of brain activity and is stable over time, making it an ideal candidate for studying brain function. Multivariate analyses of EEG data help unravel the complex interplay of factors shaping brain activity, offering a deeper understanding of brain function and dysfunction. EEG recordings, obtained using scalp electrodes and analyzed through spectral analysis techniques, provide researchers with a comprehensive view of brain activity and its underlying mechanisms (Zietsch et al., 2007).



Source Srimaharaj et al. (2018)

Figure 2.1 Brainwave Frequencies and Descriptions

Type of Brain Waves

1. Delta Waves: Delta waves, spanning from 0.5 to 4Hz, are associated with deep sleep and restorative rest, indicating a state of deep relaxation and rejuvenation. Prominently observed in frontocentral head regions during deep sleep, delta waves play a crucial role in facilitating restorative sleep cycles. However, their presence in awake states may signal generalized encephalopathy or focal cerebral dysfunction, serving as valuable markers for diagnosing neurological conditions and assessing sleep quality (Nayak & Anilkumar, 2020; Sucholeiki, 2008).

2. Theta Waves: Theta waves, ranging from 4 to 7Hz, signify transitional states between wakefulness and sleep, observed during drowsiness, early sleep stages, and heightened emotional states. Most prominent in fronto-central head regions, theta activity provides insights into emotional arousal levels and potential neurological abnormalities. While theta waves are strongly detectable during dreaming and deep meditation, their presence during automatic tasks or daydreaming suggests reduced consciousness. Research also associates theta waves with memory consolidation,

creativity, and psychological well-being, highlighting their importance in cognitive functioning and emotional regulation (Nayak & Anilkumar, 2020; Sucholeiki, 2008).

3. Alpha Waves: Alpha waves, ranging from 8 to 12Hz, are indicative of a relaxed mental state and are typically observed when the eyes are closed and during periods of mental relaxation. Characteristically present in normal awake EEG recordings, especially in the occipital head region, alpha waves signify a state of calmness and relaxation. Variations in alpha rhythm, such as slowing, asymmetry, or non-reactivity, can suggest underlying neurological dysfunction, aiding in the assessment of brain health and cognitive functioning (Nayak & Anilkumar, 2020; Sucholeiki, 2008).

4. Beta Waves: With a frequency range of 13 to 30Hz, beta waves reflect active thinking and alertness, often increasing during periods of cognitive engagement and decreasing during deeper sleep stages. Predominantly observed in frontal and central head regions, beta waves serve as markers for heightened cognitive activity and alert mental states. Abnormalities in beta activity, such as focal attenuation or regional differences, may indicate cortical injuries, dysfunction, or the effects of sedative medications, offering insights into brain activity levels and cortical integrity (Nayak & Anilkumar, 2020; Sucholeiki, 2008).

5. Gamma Waves: Gamma waves, operating at frequencies between 32 to 100Hz, represent a state of heightened perception and cognitive processing, often associated with learning and problem-solving tasks. These fast and high-frequency brainwaves reflect simultaneous information processing from different brain areas, indicating peak mental states and enhanced cognitive functions. Notably observed in individuals engaged in long-term meditation practices, gamma waves demonstrate strong and regular patterns, suggesting heightened perceptual abilities and cognitive flexibility. Research on gamma rhythms underscores their importance in sensory perception, integrating neural networks, and facilitating various cognitive functions, emphasizing their role as key biomarkers of cognitive processing and brain function (Nayak & Anilkumar, 2020; Sucholeiki, 2008).

2.4 Safety and Toxicity

Based on available research and regulatory guidelines, L-Theanine appears to be safe for consumption within certain limits. The Food and Drug Agency (FDA) suggests that daily intake should not exceed 1200 mg, as higher doses could potentially pose health risks (Vuong et al., 2011; FDA, 2006). However, typical dietary intake levels are much lower, with an estimated daily intake of 628 milligrams per person and a 90th percentile value of 1284 mg/day per person (Türközü & Şanlier, 2017). Acute and sub-acute toxicity tests conducted by the Japan Food Additives Association have deemed L-Theanine, as safe for consumption (Juneja et al., 1999). Studies on rodents have shown that high doses of L-Theanine, up to 4000 mg per body weight for 90 days, did not result in toxic effects (Borzelleca et al., 2006). Pathological studies also indicate that any observed effects were not dose related. Additionally, long-term studies on rats consuming L-Theanine at concentrations of up to 5% of their diet did not show any chronic toxicological or tumorigenic effects. Overall, current evidence suggests that L-Theanine is safe for human consumption within recommended limits and does not exhibit significant toxicity even at high doses (Türközü & Şanlier, 2017).

In addition to the safety information above, a meta-analysis of studies on L-theanine supplementation reveals its potential anti-stress and anxiety-reducing effects at doses ranging from 200 to 400 mg per day (Williams et al., 2020b). These studies, most of which were designed as randomized, double-blind, placebo-controlled trials, demonstrate the efficacy of L-theanine in improving various psychological parameters. For instance, one study observed no side effects with a dosage of 400 mg per day over an eight-week period, while another study with a higher intake of 900 mg over 10 weeks reported minimal adverse events (Sarris et al., 2019). Notably, even at high doses, L-theanine did not exhibit significant toxicity in animal models or human subjects. Despite the potential benefits of L-theanine, further research is needed to understand its long-term effects and its interactions with other compounds, particularly in the context of green tea consumption where multiple bioactive compounds may influence physiological and psychological outcomes. Overall, L-theanine appears to be

safe and well-tolerated, with potential therapeutic benefits for stress and anxiety management.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

This is a single-arm trial, where participants are given a single dose of oral L-theanine 200 mg and changes on the EEG are measured over time.

3.2 Study Population

Middle aged Male and Female volunteers, from ages 40 to 60 years with a BMI between 18.5 – 25 kg/m² and no previous history of mental illness or brain injury.

3.3 Study Location

MAS Neuroscience Center, School of Anti-Aging and Regenerative Medicine Mae Fah Luang University, Bangkok, Thailand.

3.4 Sample Size Determination

Different change from baseline of Alpha power (Evans et al., 2021), μ= 296.2

σ_{before} = $S_{1=}$ 29.45 σ_{after} =S_2=28.068

Where

$$S_p^2 = \frac{[S_1^2(n_1 - 1) + S_2^2(n_2 - 1)]}{[n_1 + n_2 - 2]}$$

$$S_p^2 = \frac{(28 - 1)29.45^2 + (28 - 1)\ 28.068^2\ ]}{[28 + 28 - 2]}$$

= 826.56

To calculate the sample size by two mean dependences, using the formula

$$n = \frac{(z_{\alpha/2} + z_{\beta})^2 \sigma^2}{\mu_d^2}$$

Set

α = 0.05 (two tailed) $Z_{0.025}$ = 1.96

β = 0.10 (one tailed) $Z_{0.100}$ = 1.28

$$n = \frac{(z_{\alpha/2} + z_{\beta})^2 \sigma^2}{\mu_d^2}$$

$$= \frac{(1.645 + 0.84)^2 826.56}{(296.2 - 316)^2}$$

= 13.03

≈

Where

n = sample size

S = σ = Standard deviation

$S^2_p = \sigma^2$ =Pooled variance

The expected dropout rate is 10%.

n ≈15

Total sample size 15

3.5 Inclusion Criteria

3.5.1 Healthy male and female participants aged between 40-60 years old.

3.5.2 BMI 18.5-25 kg/m².

3.5.3 Healthy individual with no underlying mental illness.

3.5.4 Non-shift worker, A regular sleep pattern, bedtime between 10 pm -12 am.

3.5.5 Participants who agree to abstain from all caffeinated and theanine containing products 24hours prior to test day.

3.5.6 Volunteers who give written consent and agree to follow the instructions.

3.6 Exclusion Criteria

3.6.1 Pregnancy and breastfeeding women.

3.6.2 Participants who personal history of psychiatric or emotional problems.

3.6.3 Participants with blood pressure exceeding 140 mmHg systolic or 90 mmHg diastolic and a resting heart rate <40 per minute.

3.6.4 Participants with a history of any drug abuse.

3.6.5 Participants who Family history of a schizophrenia-like illness.

3.7 Discontinuation Criteria

3.7.1 Participants who cannot comply with the instructions and fail to arrive to the study.

3.7.2 Participants who develop allergic or hypersensitive reactions from L-theanine.

3.7.3 Participants who want to drop out from the trial for any reason.

3.7.4 Participants who suffered illness from other medical conditions during the trial.

3.7.5 Participants who had less than 6 hours of sleep before the night before the experiment

3.8 Variables of the Study

3.8.1 Independent variables: 200mg L-theanine oral supplement.

3.8.2 Dependent variables: EEG changes, in each brain region, left and right hemisphere and type of brain wave.

3.9 Research Materials

3.9.1 Electroencephalogram

The experiment utilized an EEG device using the 10-20 system, for displaying, analyzing, and recording EEG activity. Frequency analysis of EEG signals was conducted using a Fast Fourier Transform algorithm with a 3-second interval. Frequency bands considered included 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).

3.9.2 L-Theanine Capsule

For this study, 200 mg of purified L-theanine was encapsulated for each participant. This dosage was determined based on the optimal effect observed in a previous study (Vuong et al., 2011), which indicated that 200 mg of L-theanine had significant effects on the human brain.

3.10 Research Procedure

3.10.1 Volunteers between 40-60 years of age are recruited in accordance with the inclusion and exclusion criteria.

3.10.2 Volunteers are thoroughly explained about the research purpose, detailed procedure, and anticipated risks and benefits of the study.

3.10.3 Participants are requested to fill out the form and sign the informed consent.

3.10.4 Subsequently, participants were scheduled for a follow-up session to undergo the EEG test later.

3.10.5 On the day of the test, participants received detailed instructions on how to experiment is done and to relax during the whole experiment.

3.10.6 To ensure accuracy and minimize errors, participants were given 15 minutes to rest before the EGG is put on.

3.10.7 The EEG electrode was then placed on by the lab assistant based on the 10-20 system. Once the EGG was set in place, the participant was given 200mg of L-

theanine. The recording was done four times: initially at baseline (T1), then 30 minutes after ingesting L-theanine or placebo (T2), followed by sessions at 60 minutes (T3), and 90 minutes (T4). Each recording lasted for three minutes.

3.10.8 Once completed, participants can return home. If participants experience any discomfort, they were instructed to notify the researcher.

3.11 Data Collection

Data of each electrode was recorded in the EEG software and then processed into single value representing group frequency band for each individual electrode as explained below, and stored into an excel file in the university laptop.

For each participant, we first recorded the power of theta (4-8 Hz), alpha (8-13 Hz), and beta (13-32 Hz) waves over a 3-minute period. The recorded power, measured in microvolts (μV), was then averaged to obtain a single value representing group frequency band for each individual electrode. The electrodes are then grouped to represent different regions of the brain as shown.

For each brain region, it is represented by the sum average these electrodes

Frontal region (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 (Bakhtiari et al., 2023)

For the whole brain, left and right hemisphere, sum these electrodes

Whole brain: Fp1, Fp2, F7, F8, P7, P8, O1, O2

Right: Fp2, F8, P8, O2

Left: Fp1, F7, P7, O1 (Lim et al., 2020)

The study protocols received approval from the Mae Fah Luang University Ethical Committee in Chiang Rai, Thailand, under approval number COA 254/2021. Prior to participation, all individuals involved in the study provided their informed consent.

3.12 Statistical Analysis

Documentation of the medical records and the outcome results in this study were recorded by Microsoft Excel 2019 and all the analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 23.0. We compared the average power of theta, alpha, and beta waves at each time point. The Kolmogorov-Smirnov test was used to check for normality. Due to the non-normality of the data the Friedmann test was used to analysis if there were any changes between all time frames, then a post-hoc analysis would be done. The Wilcoxon pair-match tests was used to analyze changes from baseline and other timeframes. The comparison evaluated the mean difference of each brain wave in different regions of the brain at baseline (T1, before taking L-theanine) with 30 minutes (T2), 60 minutes (T3) and 90 minutes (T4) after taking L-theanine among all participants. A p-value below 0.05, or 5%, is considered statistically significant.

The Mann-Whitney U test was used to compare changes in alpha and beta wave power, assessing mean differences across brain regions at the same time points to determine if both waves changed similarly. Additionally, comparisons were made across the whole brain, as well as the left and right hemispheres, to determine whether L-theanine's effects were distributed equally throughout the whole brain. Z-values were included to indicate the direction and strength of observed changes, A p-value below 0.05, or 5%, is considered statistically significant.

3.13 Ethical Consideration

This study follows the Good Clinical Practice (GCP) guidelines, an internationally recognized ethical and scientific quality standard for the design, conduct, recording, and reporting of trials involving human participants, as outlined by the International Conference on Harmonization (ICH).

The GCP guidelines ensure the protection of human rights for clinical trial participants, guarantee the safety and efficacy of new compounds, provide standards

for conducting clinical trials, and define the roles and responsibilities of clinical trial sponsors, investigators, and monitors. The ethical considerations included:

3.13.1 Participants were fully informed about the study's objectives, methodologies, and potential adverse effects.

3.13.2 Participants gave their informed consent willingly before the study began and had the freedom to withdraw at any time without any penalty.

3.13.3 Participation in this study was completely free, with no financial or personal conflicts of interest between the researchers and participants.

3.13.4 200mg L-theanine was previously used with no adverse effect and is FDA approved.

3.13.5 In any case, if the problem occurs, the researcher will help and take responsibility for the subjects as much as possible.

3.13.6 All volunteers' information is highly confidential.

CHAPTER 4

RESULTS

Fifteen volunteers were enrolled for the experiment (n=15). All participants completed the experiment without complications. During the analysis, one outlier was removed from each group for alpha and beta waves, and two outliers were removed from the Theta group.

From the results, we observe no statistically significant changes in brain wave activity across all brain regions over the four-time frames as shown in Tables 4.1-4.3. However, there are trends that suggest potential effects of L-theanine on cognitive functions, shown in Figure 4.1-4.3. These trends indicate that L-theanine may still influence brain wave patterns, potentially enhancing cognitive performance and promoting relaxation.

1. Analysis 1: Changes in Brain Wave Power Across Different Regions Over Four Time Points

This analysis examines the variations in brain wave power over time, providing insights into the effects of L-theanine as they progress. Each brain wave is analyzed separately to understand the distinct changes occurring within each region, as detailed below.

1) Alpha Power Analysis:

Alpha waves are associated with a relaxed state (Evans et al., 2021; Nayak & Anilkumar, 2020).The analysis of alpha power revealed no statistically significant differences across all brain regions over the observed time frames. The Friedman test was employed to evaluate changes in alpha power in the Frontal, Temporal, Parietal, Central, and Occipital regions, yielding the following results: ($\chi^2 = 1.48$, df = 3, p = 0.69; $\chi^2 = 1.41$, df = 3, p = 0.70; $\chi^2 = 0.86$, df = 3, p = 0.83; $\chi^2 = 0.56$, df = 3, p = 0.91; $\chi^2 = 2.92$, df = 3, p = 0.40), respectively. Similarly, comparisons of alpha wave power between the left and right hemispheres also showed no significant changes over time (Left: $\chi^2 = 1.11$, df = 3, p = 0.77; Right: $\chi^2 = 1.19$, df = 3, p = 0.76).

Given that all p-values exceed 0.05, no significant changes in alpha power were observed. Nonetheless, post-hoc Wilcoxon tests were conducted, comparing baseline (T1) with subsequent time points, to explore potential trends since changes from this time frame appeared most prominent, as seen in Table 4.1. The largest change was observed between T1 and T4, with Z-scores for the Frontal, Temporal, Parietal, Central, and Occipital regions as follows: (Z = 0.49, p = 0.62; Z = -0.24, p = 0.81; Z = -0.28, p = 0.78; Z = -0.39, p = 0.70; Z = -0.16, p = 0.88). The comparison between hemispheres produced (Z = 0.73, p = 0.46) for the left and (Z = 0.25, p = 0.81) for the right.

These results suggest that although statistically significant differences were not detected, variations in Z-scores indicate differences in the direction and magnitude of changes. Figure 4.1 illustrates an upward trend in alpha power within the frontal, temporal, and central regions, as well as in both hemispheres, with a more pronounced effect in the left hemisphere, suggesting a potential effect of L-theanine in increasing alpha wave power. This suggests that L-theanine has the potential to promote calmness and relaxation.

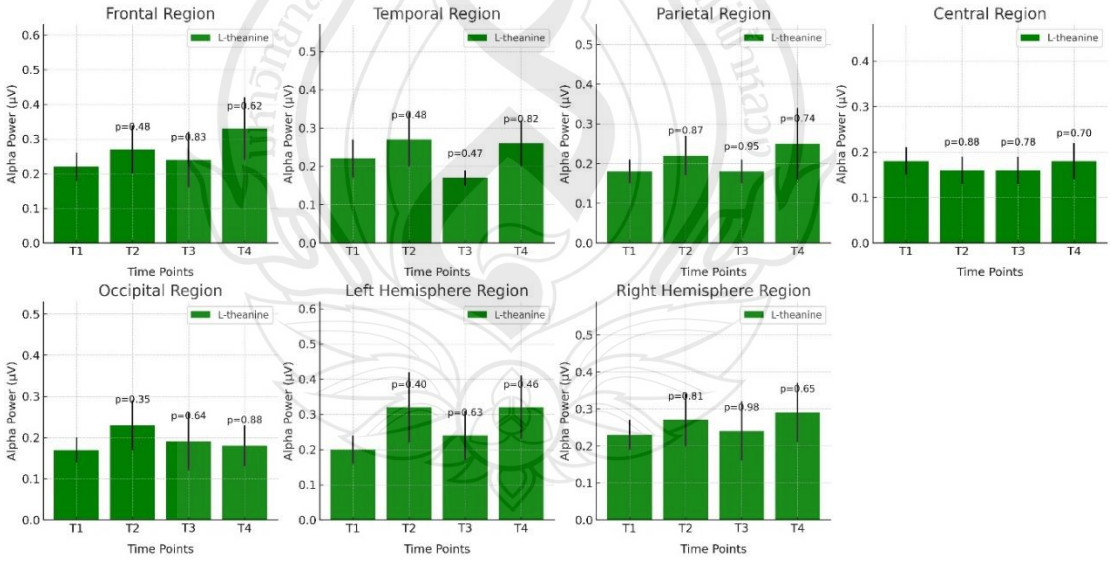


Figure 4.1 Alpha Wave Power in Different Regions of the Brain at Different Time Frames After Consumption of L-Theanine

2) Beta Power Analysis:

Beta waves are associated with increased cognitive function (Nayak & Anilkumar, 2020). The analysis of beta power across time frames also revealed no significant changes in all brain regions. The Friedman test results for changes in the Frontal, Temporal, Parietal, Central, and Occipital regions were: ($\chi^2 = 6.31$, df = 3, p = 0.10; $\chi^2 = 5.71$, df = 3, p = 0.13; $\chi^2 = 2.34$, df = 3, p = 0.51; $\chi^2 = 1.97$, df = 3, p = 0.58; $\chi^2 = 3.74$, df = 3, p = 0.29). Comparisons between hemispheres showed no significant changes over time (Left: $\chi^2 = 2.01$, df = 3, p = 0.57; Right: $\chi^2 = 2.89$, df = 3, p = 0.41).

As p-values were consistently above 0.05, no significant differences in beta power were observed. However, post-hoc Wilcoxon tests were conducted to further explore possible trends, focusing on differences between T1 and other time points (see Table 4.2). The most notable change occurred between T1 and T4. The results for Frontal, Temporal, Parietal, Central, and Occipital regions are as follows: (Z = 1.51, p = 0.13; Z = 1.61, p = 0.11; Z = 0.63, p = 0.53; Z = 1.31, p = 0.19; Z = 1.07, p = 0.28) respectively. Comparisons between hemispheres resulted in (Z = 1.10, p = 0.27) for the left and (Z = 1.19, p = 0.23) for the right.

While the overall differences were not statistically significant, the Z-score variations suggest some degree of change in beta power direction and magnitude, the positive score indicates there is an increase in beta power. Additionally, Figure 4.2 displays an increasing trend in beta power across all regions, particularly around the 90-minute time point, suggesting a potential effect of L-theanine in increasing beta wave power. This increase in beta power is associated with improved cognitive functions such as attention, memory, and problem-solving skills. Therefore, L-theanine may contribute to a general enhancement of cognitive performance through its effect on beta wave activity in the brain.

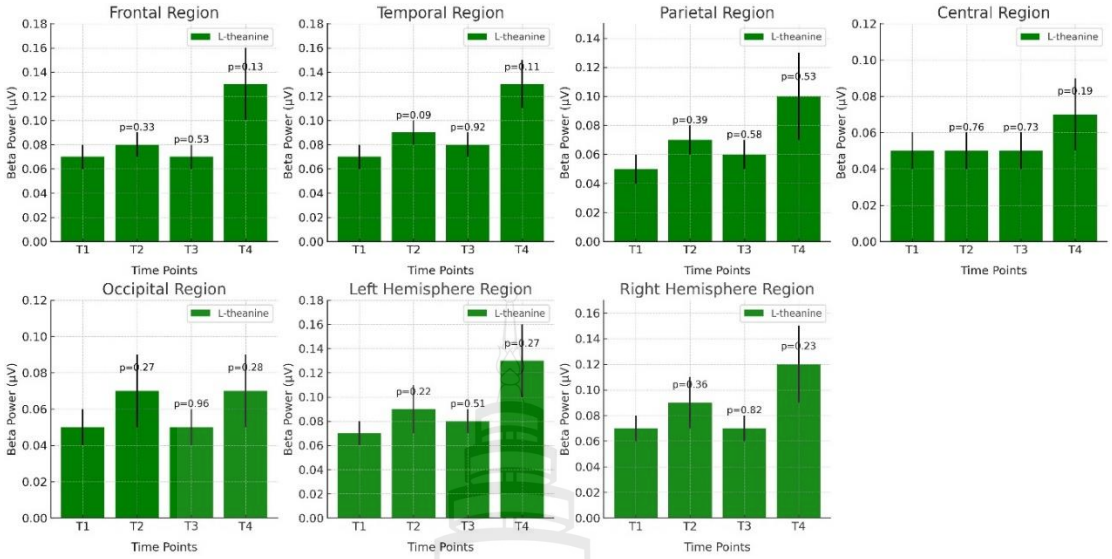


Figure 4.2 Beta Wave Power in Different Regions of the Brain at Different Time Frames After Consumption of L-Theanine

3) Theta Power Analysis:

Theta waves are associated with heighten emotional state, it is also the state seen in deep meditation or a transitional state between wake and sleep(Nayak & Anilkumar, 2020). Analysis of theta power also indicated no significant differences across the examined brain regions over time. The Friedman test results for the Frontal, Temporal, Parietal, Central, and Occipital regions were as follows: ($\chi^2 = 5.73$, df = 3, p = 0.13; $\chi^2 = 4.56$, df = 3, p = 0.21; $\chi^2 = 4.92$, df = 3, p = 0.18; $\chi^2 = 5.64$, df = 3, p = 0.13; $\chi^2 = 3.36$, df = 3, p = 0.34). Similarly, comparisons between the left and right hemispheres showed no significant changes over time (Left: $\chi^2 = 3.24$, df = 3, p = 0.36; Right: $\chi^2 = 3.72$, df = 3, p = 0.29).

Despite the lack of statistical significance, post-hoc Wilcoxon tests were carried out to assess potential trends, focusing on differences between baseline (T1) and subsequent time points (see Table 4.3). The largest shifts were noted between T1 and T4. The results for Frontal, Temporal, Parietal, Central, and Occipital regions are as follows: (Z = -1.36, p = 0.17; Z = -1.26, p = 0.21; Z = -2.35, p = 0.02; Z = -1.73, p = 0.08; Z = -1.92, p = 0.06). The hemispheric comparisons yielded (Z = -1.26, p = 0.21) for the left and (Z = -1.80, p = 0.07) for the right.

These findings suggest that while statistical significance was not reached, the negative score in Z-scores indicates subtle decrease in theta power. This is particularly notable in the parietal region, where the results suggest a time-dependent trend in theta power modulation, warranting further investigation. Additionally, Figure 4.3 shows a decreasing trend in Theta power across all regions, suggesting that L-theanine may have effects lower Theta power across all brain regions. This could potentially improve alertness as an increase in theta wave power is associated with mental fatigue, reduced cognitive capacity, and a decreased ability to maintain attention and vigilance during monotonous tasks (Craig et al., 2012).

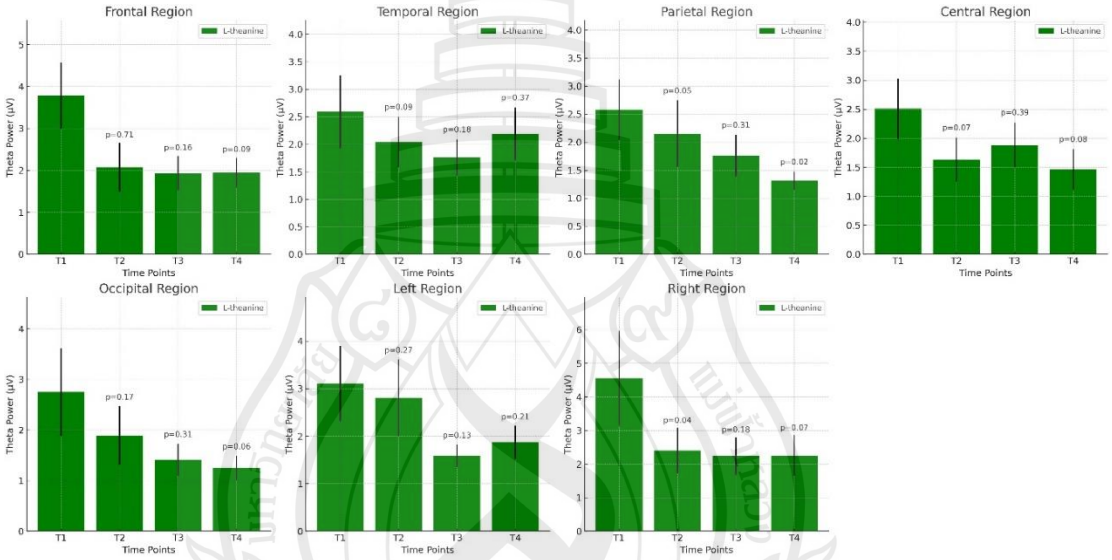


Figure 4.3 Theta Wave Power in Different Regions of the Brain at Different Time Frames After Consumption of L-Theanine

Table 4.1 Alpha Brain Wave Power in Brain Regions for L-Theanine Group at Time T1(Baseline), T2 (30 Mins-post Ingestion), T3 (60 Mins-post Ingestion) and T4 (90 Mins-post Ingestion). Values Presented Are Mean ± Standard Error of Mean (SEM)

EEG Power in each region (μV)		Alpha Wave										
		T1		T2			T3			T4		
L-Theanine (n=14)		Mean	SEM	Mean	SEM	p-value [α]	Mean	SEM	p-value [α]	Mean	SEM	p-value [α]
FP1, FPZ, FP2, F7, F3, FZ, F4, F8	Frontal	0.22	0.04	0.27	0.07	0.48	0.24	0.08	0.83	0.33	0.09	0.62
FC5, T7, CP5, FC6, T8, CP6	Temporal	0.22	0.05	0.27	0.07	0.48	0.17	0.02	0.47	0.26	0.06	0.82
CP1, CP2, P3, PZ, P4, P7, P8	Parietal	0.18	0.03	0.22	0.05	0.87	0.18	0.03	0.95	0.25	0.09	0.74
FC1, FC2, C3, CZ, C4	Central	0.18	0.03	0.16	0.03	0.88	0.16	0.03	0.78	0.18	0.04	0.70
O1, OZ, O2	Occipital	0.17	0.03	0.23	0.06	0.35	0.19	0.07	0.64	0.18	0.05	0.88
Fp1, Fp2,F7, F8, P7, P8, O1, O2	Whole	0.22	0.04	0.28	0.08	0.70	0.23	0.07	0.93	0.31	0.09	0.83
Fp2, F8, P8, O2	Left	0.20	0.04	0.32	0.10	0.40	0.24	0.07	0.63	0.32	0.09	0.46
Fp1, F7, P7, O1	Right	0.23	0.04	0.27	0.07	0.81	0.24	0.08	0.98	0.29	0.08	0.65

Note [α] p-value was calculated using Wilcoxon Match-Pair test, this analysis was used to compare changes from baseline (T1, 0min).

Table 4.2 Beta Brain Wave Power in Brain Regions for L-Theanine Group at Time T1(Baseline), T2 (30 Mins-post Ingestion), T3 (60 Mins-post Ingestion) and T4 (90 Mins-post Ingestion). Values Presented Are Mean ± Standard Error of Mean (SEM)

EEG Power in each region (μV)		Beta wave										
		T1		T2			T1			T4		
L-Theanine (n=14)		Mean	SEM	Mean	SEM	p-value [α]	Mean	SEM	p-value [α]	Mean	SEM	p-value [α]
FP1, FPZ, FP2, F7, F3, FZ, F4, F8	Frontal	0.07	0.01	0.08	0.01	0.33	0.07	0.01	0.53	0.13	0.03	0.13
FC5, T7, CP5, FC6, T8, CP6	Temporal	0.07	0.01	0.09	0.01	0.09	0.08	0.01	0.92	0.13	0.02	0.11
CP1, CP2, P3, PZ, P4, P7, P8	Parietal	0.05	0.01	0.07	0.01	0.39	0.06	0.01	0.58	0.10	0.03	0.53
FC1, FC2, C3, CZ, C4	Central	0.05	0.01	0.05	0.01	0.76	0.05	0.01	0.73	0.07	0.02	0.19
O1, OZ, O2	Occipital	0.05	0.01	0.07	0.02	0.27	0.05	0.01	0.96	0.07	0.02	0.28
Fp1, Fp2 , F7, F8, P7, F8, O1, O2	Whole	0.07	0.01	0.09	0.02	0.29	0.09	0.02	0.34	0.12	0.03	0.21
Fp2, F8, P8, O2	Left	0.07	0.01	0.09	0.02	0.22	0.08	0.01	0.51	0.13	0.03	0.27
Fp1, F7, P7, O1	Right	0.07	0.01	0.09	0.02	0.36	0.07	0.01	0.82	0.12	0.03	0.23

Note [α] p-value was calculated using Wilcoxon Match-Pair test, this analysis was used to compare changes from baseline (T1, 0min).

Table 4.3 Theta Brain Wave Power in Brain Regions for L-Theanine Group at Time T1(Baseline), T2 (30 Mins-post Ingestion), T3 (60 Mins-post Ingestion) and T4 (90 Mins-post Ingestion). Values Presented Are Mean ± Standard Error of Mean (SEM)

EEG Power in each region (μV)		Theta Wave										
		T1		T2			T1			T4		
L-Theanine (n=13)		Mean	SEM	Mean	SEM	p-value [α]	Mean	SEM	p-value [α]	Mean	SEM	p-value [α]
FP1, FPZ, FP2, F7, F3, FZ, F4, F8	Frontal	3.78	0.79	2.07	0.58	0.71	1.93	0.41	0.16	1.94	0.36	0.09
FC5, T7, CP5, FC6, T8, CP6	Temporal	2.59	0.66	2.04	0.46	0.09	1.76	0.33	0.18	2.19	0.48	0.37
CP1, CP2, P3, PZ, P4, P7, P8	Parietal	2.58	0.54	2.15	0.59	0.05	1.76	0.37	0.31	1.31	0.16	0.02
FC1, FC2, C3, CZ, C4	Central	2.51	0.52	1.63	0.38	0.07	1.88	0.39	0.39	1.46	0.35	0.08
O1, OZ, O2	Occipital	2.75	0.87	1.89	0.58	0.17	1.41	0.32	0.31	1.25	0.24	0.06
Fp2, F8, P8, O2	Left	3.11	0.79	2.81	0.81	0.27	1.59	0.24	0.13	1.87	0.35	0.21
Fp1, F7, P7, O1	Right	4.55	1.41	2.40	0.68	0.04	2.24	0.56	0.18	2.25	0.61	0.07
FP1, FPZ, FP2, F7, F3, FZ, F4, F8	Frontal	3.78	0.79	2.07	0.58	0.71	1.93	0.41	0.16	1.94	0.36	0.09

Note [α] p-value was calculated using Wilcoxon Match-Pair test, this analysis was used to compare changes from baseline (T1, 0min)

2. Analysis 2: Comparative Assessment of Alpha and Beta Power in Each Region at Individual Time Points

The analysis aimed to compare alpha and beta brain wave activities across various brain regions (Frontal, Temporal, Central, Parietal, Occipital, Left, and Right) over four time points (T1 to T4). A Mann-Whitney U test was used due to the non-normally distributed nature of the data, focusing on the ranks of observations rather than their means. The resulting Z-scores from this test provide insights into the relative dominance of beta wave activity over alpha waves, while p-values assess the statistical significance of these differences. A p-value of 0.05 or below was considered indicative of statistical significance. The results reveal that all tested groups have p-values < 0.05, suggesting significant differences between alpha and beta power. Therefore, the focus of the analysis shifts to interpreting the Z-scores to better understand these differences.

1) Temporal and Regional Patterns in Z-Scores

The analysis of Z-scores reveals both temporal shifts and regional differences in the dominance of beta and alpha wave activity across the brain as shown in Figure 4.4. Initially, at T1 and T2, there is a strong dominance of beta waves across most brain regions, as indicated by more negative Z-scores. For example, in the Frontal region at T1, a Z-score of -3.64 with a p-value of <0.01 highlights a significant dominance of beta wave ranks over alpha wave ranks, suggesting a more alert or cognitively engaged state. Similarly, the Occipital region, associated with visual processing, shows peak beta activity at T3, with a Z-score of -4.13 and a p-value of <0.01, indicating heightened attentional or visual processing.

By T4, the Z-scores across various regions, including the Frontal, Temporal, Central, Parietal, and Occipital regions, become less negative, indicating a shift towards a more balanced distribution between alpha and beta waves. For instance, the Frontal region with its Z-score increasing from -3.64 at T1 to -2.42 at T4, reflecting a significant reduction in beta wave dominance and suggesting a shift towards a more relaxed or resting state as alpha waves gain relative prominence.

Overall, the analysis highlights a trend of early beta wave dominance, particularly during the initial time points, that gradually diminishes over time, as alpha waves become more prominent. This shift suggests a transition from a highly active,

alert state to a more relaxed state, reflecting the effects of time and possibly the influence of L-theanine across different brain regions.

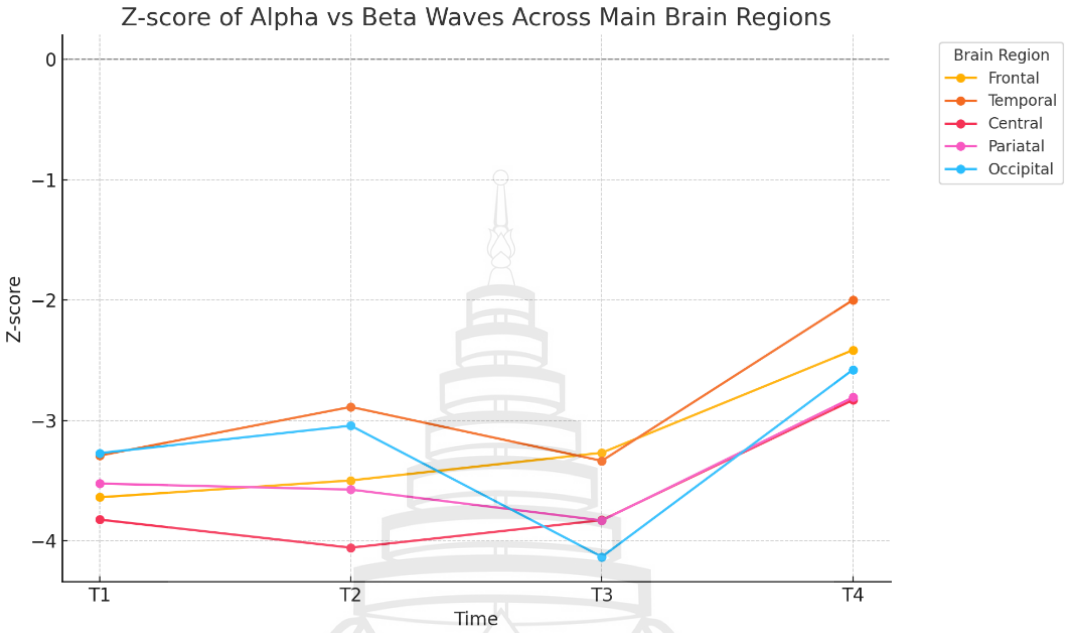


Figure 4.4 Z-score of Alpha vs Beta Waves Across Main Brain Regions

2) Analysis of Lateralized Brain Activity

A time-series analysis of the left and right hemispheres was conducted separately to explore potential differences in rank-based brain activity between the two sides. Both hemispheres exhibited similar trends in Z-scores over time as shown in Figure 4.5, showing pronounced beta dominance at T3 (Z-scores around -4.13 with p-values of <0.01), indicating that beta wave activity was significantly higher than alpha wave activity during this period, likely reflecting peak increase of cognitive engagement or attention. By T4, the Z-scores for both hemispheres increased closer to zero (Z-score of -2.58 with a p-value of 0.01), suggesting a substantial reduction in beta wave dominance and a shift towards a more balanced state between alpha and beta waves. The similarity in these trends indicates that L-theanine's influence on brain wave activity was relatively symmetrical, without significant lateralization. This suggests that its effects on alpha and beta wave activity were consistent across both hemispheres, with each showing a significant reduction in beta dominance by the end of the observation period (T4).

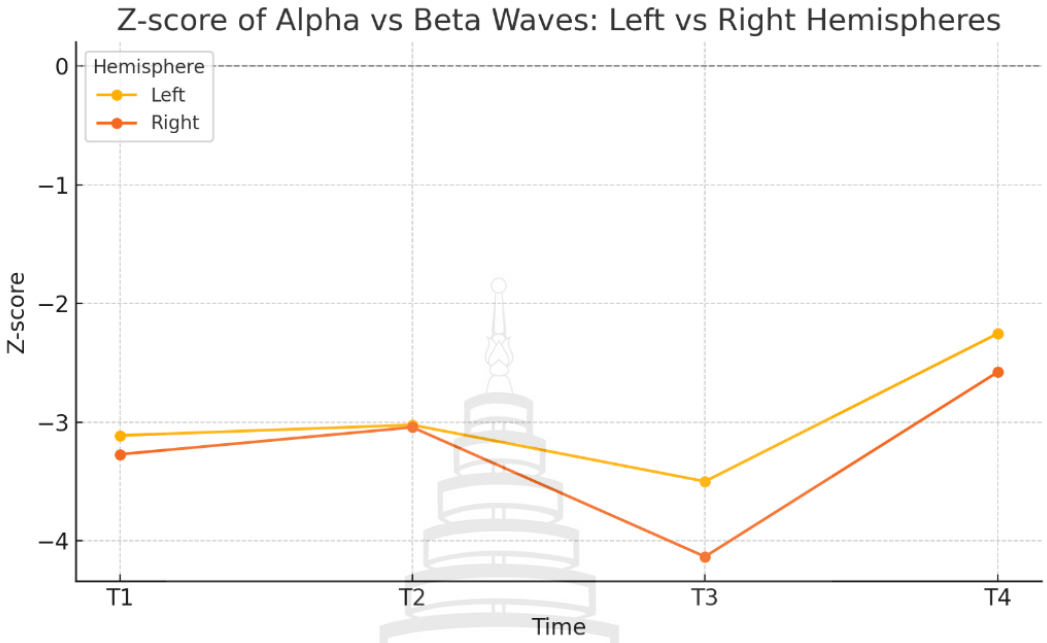


Figure 4.5 Z-score of Alpha vs Beta Waves: Left vs Right Hemispheres

3) Summary of Findings

The analysis revealed a consistent pattern of beta wave dominance across most brain regions during early time points, with significant differences between alpha and beta wave activities. This dominance peaked at T3 and then diminished by T4, indicating a shift towards a more balanced state between alpha and beta waves. The transition suggests a shift from heightened cognitive engagement to a more relaxed state as the session progressed, potentially influenced by L-theanine.

Additionally, the similarities in Z-score trends between the left and right hemispheres indicate that the effects of L-theanine on brain wave activity were largely symmetrical, with no significant lateralization. This uniform response across hemispheres supports the idea that L-theanine's impact on brain wave modulation is consistent across both sides of the brain, leading to a gradual reduction in beta dominance by the end of the observation period (T4).

3. Analysis comparing difference between Whole brain, Left and Right Hemisphere

This analysis examines Z-scores derived from the Mann-Whitney U test, comparing the ranks of alpha and beta wave activities across different brain regions

(Left Hemisphere vs. Whole brain, Right Hemisphere vs. Whole Brain, and Left Hemisphere vs. Right Hemisphere) over four time points (T1 to T4). Across time points, the Z-scores for both alpha and beta waves remain close to zero, indicating minimal differences in rank-based dominance between the whole brain and its hemispheres, a graph the Z-score were plotted to compare as seen in Figure 4.6. Alpha wave Z-scores are closer to zero across all time points, indicating relatively similar ranks between regions. Beta waves, on the other hand, show slightly more variation, particularly in the Whole vs. Right comparison at T3 with a Z-score of -0.547, although this difference is not statistically significant ($p = 0.58$). For both wave types, Z-scores become less negative by T4, indicating a reduction in rank differences between the left and right hemispheres, suggesting a trend toward a more balanced wave distribution over time. However, all p-values exceed the 0.05 threshold ranging from ($p = 0.58$ - 0.95), indicating that none of the differences are statistically significant. This suggests that while there are slight variations in wave dominance, they are not sufficiently pronounced to be considered significant across the studied regions and time points. Overall, the findings suggest that L-theanine's effects on brain wave activity are distributed evenly across the examined regions, without strong lateralization or significant shifts over time.

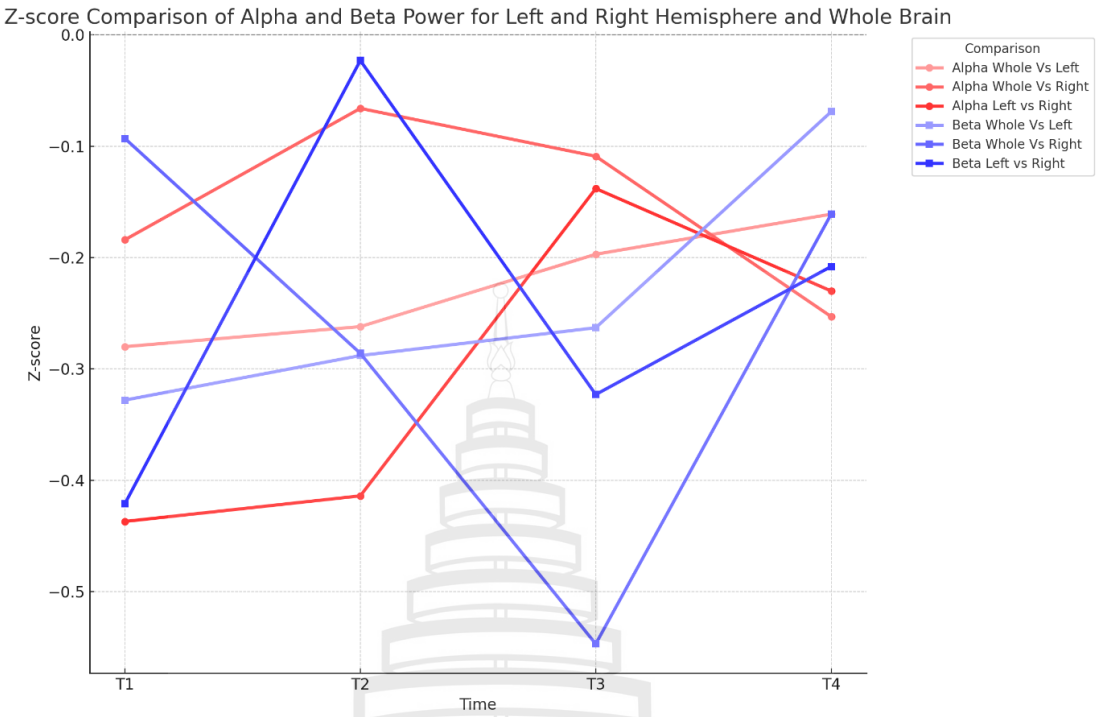


Figure 4.6 Z-score Comparison of Alpha vs Beta Power for Left and Right Hemisphere and Whole Brain

CHAPTER 5

DISCUSSION

5.1 Discussion

The aim of the current study was to find out whether there was an influence of a single dose of 200 mg L-theanine on cognitive function by analysing brainwaves during different time points. In the current study, the influence of L-theanine brainwaves modulation was tested through an experimental design.

It has been shown that L-theanine may contribute to improving attention, thus enhancing working memory and executive functions (Baba et al., 2021). This study specifically used EEG to measure brain wave activity to relate these changes to cognitive function.

The study indicated that although not statistically significant, there show an increasing trend in Alpha and Beta wave power and a decrease in Theta wave power after taking L-theanine. The most noticeable increases started at the 90th minute suggesting that further measurement may have greater increase in power causing results to be significant. As previously observe in another similar study by (Evans et al., 2021) state that a 200 mg dose of L-theanine significantly increased alpha wave power, peaking in efficacy at 3 hours post-ingestion.

The increasing trend of Alpha wave power following the consumption of L-theanine is likely due to its structural similarity to the excitatory neurotransmitter glutamate in the brain. L-theanine can potentially block glutamate receptors in the central nervous system, which may result in increased Alpha wave power (Kakuda, 2011). Additionally, L-theanine exerts a relaxant effect by enhancing GABA levels, thereby increasing the expression of dopamine and serotonin in the brain, further contributing to the rise in Alpha wave power (Kim et al., 2019).

In our findings, we noted the largest changes in brainwave power is in Beta wave power, although these changes were not statistically significant. Beta waves are low amplitude, fast waves present during aroused and actively engaged mental

activities (Nayak & Anilkumar, 2020). Our results showed that the changes predominantly occurred in the frontal, temporal, and parietal regions. The frontal region is associated with higher cognitive functions such as problem-solving, planning, and decision-making. The temporal region is involved in processing auditory information and is crucial for memory and language comprehension. The parietal region plays a significant role in processing sensory information, spatial orientation, and coordinating movements (Kumar & Bhuvaneswari, 2012; Teplan, 2002).These observed trends in increased Beta power suggest that L-theanine may positively influence these critical brain functions, potentially enhancing overall cognitive performance.

Our finding also suggests that there is a decrease in Theta wave power, normally Theta wave is associated with relaxation as well and is the state between wakefulness and sleep, it also helps with memory consolidation (Nayak & Anilkumar, 2020). However some research has shown that while awake, theta wave activity significantly increases during mental fatigue, particularly when individuals engage in tasks that are monotonous, they are also associated with states of reduced alertness or drowsiness, becoming more prominent as the brain's ability to maintain cognitive control and vigilance diminishes (Craig et al., 2012). Hence a decrease in Theta wave may suggest that L-theanine could potentially increase alertness and overall mental cognition.

It is important to note that delta wave activity was also monitored during this experiment; however, it remained at zero throughout. This is expected, as delta waves are typically observed during sleep. Their presence during wakefulness could indicate potential underlying pathological conditions (Assenza & Di Lazzaro, 2015).

We also compare the changes of fast brain waves namely Alpha and Beta wave to see if there differences in its changes. Results show a decrease in Z-scores of Alpha versus Beta waves across all regions, suggesting a reduction in beta wave dominance over time. Initially, at earlier time points (such as T1 and T2), beta wave dominance was more pronounced, indicating a heightened state of alertness. However, by T4, the Z-scores become less negative, indicating a shift towards a more balanced distribution between alpha and beta wave activity. This change suggests a transition from a state of high cognitive activity towards a more relaxed or resting state (Höller et al., 2012; Hurless et al., 2013), potentially influenced by L-theanine’s calming effects.

This trend of decreasing Z-scores contrasts with the findings from when comparing brain wave power across different regions over four time points, where the absolute increase in beta power was observed to be larger than that of alpha power across all time points as seen in Figure 4.1 and 4.2. This difference highlights an important distinction between changes in relative dominance Z-scores and absolute power levels. While beta power might increase more significantly in terms of absolute values, the Z-scores indicate that the relative rank-based dominance of beta waves decreases over time compared to alpha waves.

This could imply that although beta wave activity remains active, but the relative alpha waves power increases more as the session progresses. Essentially, the absolute beta power may still rise, reflecting ongoing improvement in cognitive engagement, but the proportional presence of alpha waves grows, contributing to a shift towards a more balanced state. This balance suggests that L-theanine might support a state of relaxed alertness, maintaining cognitive function while reducing the relative dominance of beta waves that is associated with heightened stress or mental strain (Höller et al., 2012; Hurless et al., 2013).

Another aim of the experiment is to determine whether if L-theanines effect affects the whole brain equally or lateralizes to one hemisphere. The analysis of lateralized brain activity revealed no significant differences across the whole brain, left and right hemispheres in terms of alpha and beta power. The similarity in Z-score trends across both hemispheres indicates that L-theanine's influence on brain wave activity is symmetrical, without any lateralization. When further compared with whole-brain results, it also supports this balanced pattern of influence. Analysis of whole-brain Z-scores over time shows similar reductions in beta dominance, consistent with the observations across individual brain regions and hemispheres. Both these comparisons also show that the p-value is >0.05, further stating that there was no statistical significance between them. The absence of lateralization implies that L-theanine's effects on brain activity are evenly distributed. A previous study examined the effects of L-theanine and found no significant changes in alpha wave activity across all brain regions, though a non-significant trend towards greater alpha activity was noted in the posterior region (White et al., 2016). In another similar study by Gomez-Ramirez et al., (2009)found that L-theanine selectively reduces alpha power particularly in posterior

and right-lateralized areas, however the participants were engagement in a demanding task, contrasting with our study where our alpha activity was observed during a more relaxed or passive state, hence no lateralization happens. These findings could potentially mean that L-theanine effect affects the whole brain equally while in a relaxed state.

These findings provide valuable insights into the time-dependent effects of L-theanine on brain wave changes and the distribution of effect on the brain. The shift from beta wave dominance to a more balanced alpha-beta state suggests that L-theanine may help facilitate a transition from a cognitively active state to one that supports relaxation. Moreover, the symmetrical effects across both hemispheres further underscore the broad and uniform nature of L-theanine's influence on brain wave patterns, enhancing its potential as a supplement for achieving a calm yet focused mental state.

5.2 Limitation of Study

This experiment utilizes electroencephalogram (EEG) to investigate brain wave activity, which may help predict changes related to cognitive function and relaxation. However, to provide more definitive conclusions, additional tasks should be conducted alongside EEG. Cognitive assessments such as the Mini-Mental State Examination (MMSE), the Rey Auditory Verbal Learning Test, or the Go/NoGo task would offer complementary insights into cognitive functions (Park et al., 2011).Similarly, measurements of biochemical markers like cortisol and alpha-amylase, along with psychological tests such as the Perceived Stress Scale (PSS) and Beck Anxiety Inventory (BAI), could be used to correlate EEG findings with relaxation and stress levels (Higashiyama et al., 2011)

Another limitation of this study is the time constraints and the relatively small sample size. Due to the limited time frame available for recruitment and data collection the study was restricted in the number of participants and the duration of experimental sessions. A larger sample size and a longer study period would enhance the statistical power of the results and allow for a more thorough investigation of the effects of L-

theanine on brain wave activity. The usage of a single dose 200mg L-Theanine might be too little to see significant changes in healthy individuals, this dose however has shown improvement in those with high anxiety scores (Higashiyama et al., 2011).

5.3 Suggestions for Future Research

Based on the analysis and results from the current study, several directions for future research are recommended to further investigate the effects of L-theanine on cognitive functions and brain wave activity. Firstly, extending the observation period longer than the current 90 minutes could provide more definitive insights into changes in brain waves of L-theanine's effects, as the current short observation periods may contribute to the lack of statistically significant results.

Comparative studies with other known cognitive enhancers, such as caffeine, magnesium or vitamin B, could help position L-theanine in the wider context of cognitive enhancement and better understand its unique effects (Boyle et al., 2022). Implementing long-term studies to assess the chronic effects of L-theanine supplementation on cognitive function and brain activity. These studies could involve daily supplementation over weeks or months, tracking changes over time.

Given that different tasks may influence brain wave patterns differently, future research should involve task-specific EEG analysis to isolate the effects of L-theanine on cognitive functions.

5.4 Conclusion

In conclusion, a single 200 mg dose of oral L-theanine did not result in statistically significant enhancements in cognitive function or relaxation, as measured by EEG, within the initial 90 minutes post-administration. However, observed trends indicate a potential for L-theanine to influence brain wave activity over extended time frames or with adjusted dosages. Specifically, an increase in alpha and beta wave power and a decrease in theta power were noted, suggesting that L-theanine could contribute to enhanced relaxation and cognitive engagement under optimized conditions.

Additionally, the analysis revealed that L-theanine’s effects appear to influence the whole brain equally, with no observed lateralization between the left and right hemispheres, reinforcing its potential as a broadly acting supplement. These findings, although preliminary, highlight L-theanine's possible role in modulating cognitive function and relaxation, warranting further investigation with larger sample sizes, extended recording periods, and additional cognitive task as a comparative. Addressing the limitations of the present study, future research should aim to explore the broader applicability of L-theanine in enhancing cognitive performance and its effects on brain wave distribution across different contexts and conditions.

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