

L-THEANINE'S EFFECTS ON LEARNING AND MEMORY IN COLLEGE
STUDENTS

by

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ABSTRACT

Previous research has examined L-theanine as a possible supplement that can improve learning and memory and can decrease levels of anxiety, all while being side-effect free and safe to consume. These studies, however, have not used a double blind placebo controlled method. The purpose of the present study was to investigate L-theanine's effects on learning, memory, and anxiety in college students using a double blind, placebo controlled research design. Two hundred mg of L-theanine or a placebo were administered to 40 college students and performance on measures of learning, memory, and attention was assessed before and after drug administration. The results indicated that L-theanine did not have any significant effects on cognitive abilities or anxiety. Findings from the current research may have differed from previous research because of the sample of participants and the use of a more controlled research design.

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CHAPTER I

INTRODUCTION

Green tea can be traced back to ancient times and was used for a variety of medicinal purposes. Asian cultures believed tea could be used for headaches, fever, tooth decay, as an anti-inflammatory, for antihypertension, and as a sedative (Ross, 2007). The medicinal properties of tea may arise from L-theanine, an amino acid that is naturally found in green tea leaves and certain species of mushrooms. L-theanine is very similar to the structure of one of the primary excitatory neurotransmitters in the central nervous system, glutamic acid (Nathan, Lu, Gray, & Oliver, 2006). Yokogoshi et al. (1995) described L-theanine as a derivative of glutamic acid, which is referred to as glutamate in the brain when it is electrically charged or ionized. Glutamate is used by all neurons and glia cells in the body to help make new proteins, and it is the most abundant amino acid in the brain (Meyer & Quenzer, 2005). Glutamate is involved in many behavioral and physiological functions, such as synaptic plasticity, memory, learning, and general cellular metabolism (Meyer & Quenzer, 2005; White et al., 2016). Since L-theanine is so structurally similar to glutamate, it is important to consider what type of effect L-theanine might have on glutamate receptors.

Glutamate has three subtypes of ionotropic glutamate receptors: AMPA receptor, Kainate receptor, and NMDA receptor. These receptors depolarize the cell membrane of the postsynaptic cell and that leads to an excitatory response. Although glutamate is

involved in very important functions, it also has the potential to be very dangerous. Glutamic acid can be discharged into extracellular space and may lead to excitotoxicity (Sugiyama, Sadzuka, Tanaka, & Sonobe, 2001). Excitotoxicity can be caused by excessive exposure to glutamate and results in neuronal death or damage (Meyer & Quenzer, 2005). Neuronal cell death is most often caused by strong activation of NMDA receptors (Meyer & Quenzer, 2005). There is research that suggests L-theanine may possibly protect against neuronal death by binding to the glutamate receptors and inhibiting NMDA (Kakuda, Nozawa, Sugimoto & Niino, 2002; Sugiyama et al., 2001). Kakuda et al. (2002) found that L-theanine had a mechanism of binding to all three ionotropic glutamate receptors and this mechanism may offer neuroprotection by preventing neuronal death. Sugiyama et al. (2001) suggest L-theanine could also prevent glutamate from reaching toxic levels by acting on glutamate transporters, which inactivate release of glutamate at the synapse. Sugiyama et al. (2001) found the effects of L-theanine to be similar to glutamate transport inhibitors, which further suggests L-theanine's effect on glutamate transporters. Maruyama and Takeda (1994) also argue that L-theanine is a competitive antagonist on glutamate receptors and can inhibit synaptic release of glutamate. The role of the glutamatergic system has been extensively studied, particularly its role in learning and memory.

L-theanine has the ability to improve learning and memory because it is able to mimic and regulate glutamate, which is heavily involved in synaptic plasticity, learning, and memory (Meyer & Quenzer, 2005; White et al., 2016). Too much glutamate can

cause neuronal cell death and negatively affect learning and memory (Kakuda et al., 2002; Sugiyama et al., 2001). L-theanine can also improve learning and memory through its action on NMDA receptors (Meyer & Quenzer, 2005).

L-theanine Effects on Learning and Memory

Several researchers have concluded that NMDA receptors play a key role in learning and memory processes (Meyer & Quenzer, 2005; Riedel, Platt, & Micheau, 2003). Riedel et al. (2003) explained NMDA's role in encoding and how it can be seen throughout all animals. Because L-theanine indirectly acts on NMDA, it could increase learning and memory abilities in humans, and potentially prevent the development of neurodegenerative disorders (McEntee & Crook, 1993). Neurodegenerative disorders like Alzheimer's disease can often start in the form of mild cognitive impairment (Park et al., 2011). Park et al. (2011) investigated green tea extract and L-theanine's effect on cognitive functioning in participants who had a mild neurocognitive disorder. The Rey-Kim memory test was used to measure memory improvement and the Stroop test was used to measure attention (Park et al., 2011). Tea extract and L-theanine were found to increase delayed recognition in participants who had mild cognitive impairments. Park et al. (2011) suggested the combination of tea extract and L-theanine is able to improve memory by acting on memory retrieval. Additionally, word reading count on the Stroop increased after 16 weeks in participants of the tea extract and L-theanine group, suggesting an improvement in attention (Park et al., 2011). If L-theanine can improve memory retrieval and attention in participants with mild neurocognitive disorder, then it

is possible to see similar effects on healthy individuals. While there is not sufficient research on the effects of L-theanine in isolation on humans, there is plenty of research on how L-theanine affects learning and memory in the rat model.

Juneja, Chu, Okubo, Nagato, & Yokogoshi (1999) used the rat model to determine L-theanine's ability to enhance memory or learning. Operant conditioning was used to train rats to press a lever for food pellets. Learning ability was determined by measuring the length of time necessary for the rats to associate food reward with lever pressing. Learning ability was significantly improved in rats that were administered L-theanine when compared to the control group (Juneja et al., 1999). Rodent learning ability was also assessed through avoidance tasks. When given the opportunity, rats have been known to naturally navigate to dark areas. Researchers examined rats' avoidance behavior by pairing electric shock with movement to a dark area. Rats that were administered L-theanine showed greater cognitive ability by hesitating to move to the dark area, and they remained in the light area longer than rats that did not receive L-theanine (Juneja et al., 1999). This research shows that L-theanine helped improve rats' ability to associate shock stimuli with the dark areas of a room, and supports the conclusion that L-theanine has a positive effect on learning and memory during chronic stress. Additionally, Yokogoshi and Terashima (2000) used the rat model to examine L-theanine's effects on memory and learning. The Morris Water Maze was used as a tool to measure learning and memory abilities. Rats that were administered L-theanine were more consistent at reaching the platform than the control group, further suggesting L-

theanine's ability to improve memory (Yokogoshi & Terashima, 2000). Takeda et al. (2011) found that rats administered L-theanine increased their exploratory activities; the researchers associated this with enhanced object recognition memory. Because L-theanine was able to improve memory in the rat model, it is important to consider the research in humans.

Memory seemed to improve in humans when given L-theanine and caffeine in conjunction. Caffeine is known to improve reaction time and increase alertness and attention (Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008; Owen, Parnell, De Bruin, & Rycroft, 2008). L-theanine has been able to inhibit the stimulation or 'jitters' that are produced by caffeine and help to promote relaxation (Haskell et al., 2008). When taken in conjunction, L-theanine and caffeine seem to produce a synergistic effect (Owen et al., 2008). Caffeine at 50mg and L-theanine at 100mg increased the number of correctly identified words in word recognition tasks, and increased speed and accuracy in attention switching tasks. Haskell et al. (2008) researched the effects of caffeine, L-theanine, and a combination of caffeine and L-theanine on various cognitive tasks. The combination of L-theanine and caffeine were associated with significant increases in simple reaction time, working memory, delayed word recognition reaction time, and accuracy of sentence verification when compared to caffeine or L-theanine given separately (Haskell et al., 2008). L-theanine and caffeine may be a preferable combination when taken before completing a task that requires memory and concentration.

L-theanine may also improve learning and memory in healthy individuals because of its action on serotonin. Yokogoshi, Mochizuki, & Saitoh (1998) reported that L-theanine increased serotonin at the striatum, hippocampus, and hypothalamus in rats, but suppressed the general release of serotonin. The researchers did not go on to explain the implications of their findings, but an increase of serotonin at the hippocampus may contribute to the improvements in memory and learning that are observed with L-theanine.

Considering all research, it is apparent that L-theanine has a clear effect on learning and memory, in several different ways. First, L-theanine is able to mimic and regulate the most widely used neurotransmitter, glutamate, which is heavily involved in synaptic plasticity, memory, learning, and general cellular metabolism (Meyer & Quenzer, 2005; Nathan et al., 2006; Sugiyama et al., 2001; White et al., 2016). Second, L-theanine prevents neuronal cell death by binding to glutamate receptors and inhibiting NMDA (Kakuda et al., 2002; Sugiyama et al., 2001). Third, L-theanine can increase serotonin in certain areas of the brain, such as the hippocampus, which may also improve memory (Yokogoshi et al., 1998). The increase in serotonin that follows administration of L-theanine may indirectly improved learning and memory through its effect on stress and anxiety. L-theanine has been known to decrease stress and anxiety (Yoto, Motoki, Murao, & Yokogoshi, 2012), and it is also known that stress and anxiety hinder learning abilities and memory (Ivy et al., 2010; Popoli, Yan, McEwen, & Sanacora, 2012).

Furthermore, it is possible that L-theanine is able to improve learning and memory because it is decreasing stress and anxiety.

L-theanine Effects on Stress and Anxiety

Research suggests that the stress reducing properties of L-theanine could be directly translational to its effects on learning and memory (Popoli et al., 2012). Negative effects of stress and anxiety on learning and memory have been widely documented. Glucocorticoids are secreted during times of stress and have a direct effect on the release of glutamate in certain brain regions, such as the hippocampus (Popoli et al., 2012). High levels of glutamate at the hippocampus, a main structure involved in learning and memory (Meyer & Quenzer, 2005), can result in difficulties with working memory and overall learning ability (Popoli et al., 2012).

Among the many dangerous potential effects of stress, acute and chronic stress may contribute to the degradation of cognitive processing by increasing glutamate transmission (Popoli et al., 2012). Chronic stress has been found to impair learning and memory in humans because it can cause structural changes in the hippocampal neurons (Ivy et al., 2010). Chronic stress has also been known to cause the secretion of corticotropin, which has been found to act on hippocampal neurons and causes dendritic atrophy (Magariños & McEwen, 1995). Dendritic atrophy would result in impairments in learning and memory (Conrad, 2006). L-theanine may be effective in preventing the increase of glutamate transmission, changes to hippocampal neurons, and dendritic atrophy, by decreasing stress and anxiety.

L-theanine's effects on stress and anxiety have been well demonstrated in the rat model. In a study conducted by Tian et al. (2013), stress was induced in rats by using a chronic restraint mode, which involved polypropylene tubes that restrained rats for 8 hours a day for 21 consecutive days. Stress was measured through levels of corticotrophin, which is secreted in response to stress (Tian et al., 2013). The Morris Water Maze and a Step Through test were used to measure learning and memory ability. Tian et al. (2013) found that stressed rats performed worse on these tests of learning and memory. Additionally, the rats that received L-theanine took less time to complete the maze and they were more consistent at reaching the platform than the control group. The chronic restraint method impaired cognitive functioning (Tian et al., 2013). However, L-theanine was able to improve learning and memory by reversing the abnormal levels of corticosterone and catecholamine, thereby decreasing stress (Tian et al., 2013). The researchers also concluded that L-theanine was found to reverse the oxidative damage induced by stress (Tian et al., 2013). L-theanine was found to improve learning and memory in rats that had been under chronic stress, further suggesting that L-theanine improves learning and memory through its ability to decrease stress and anxiety levels.

Research suggests that L-theanine is able to decrease stress and anxiety by increasing alpha brain waves (Abdou et al., 2006; Yoto et al., 2012). Alpha brain waves are associated with a relaxed, but alert state, and an increase in mental concentration (Abdou et al., 2006; Yoto et al., 2012). Yoto et al. (2012) found that L-theanine started to produce alpha brain waves around 30 to 40 minutes after administration. The alpha wave

production was detected through an EEG at doses of L-theanine between 50 mg and 200 mg (Scheid et al., 2012). Researchers initially believed that L-theanine may only be useful in reducing stress for people who are classified as highly anxious, and its effects would be negligible for those already in a relaxed state. Contrastingly, Nobre, Rao, and Owen (2008) have found that alpha waves continued to increase even for participants who were already relaxed. Results indicated a greater increase in alpha brain waves in people who consumed 50mg of L-theanine in comparison in the control group (Nobre et al., 2008). Because L-theanine stimulates the production of alpha brain waves, which increases mental concentration, it should be able to improve mental performance in participants who are under a stressful task.

Kimura, Ozeki, Juneja, & Ohira (2007) conducted a study that examined the effects of L-theanine on stress-induced participants. A mental arithmetic task that took about 20 minutes to complete was used as a stressor, and stress was measured by examining heart rate, blood pressure, and salivary secretion of immunoglobulin. Immunoglobulin is used to measure immunity status and is found at low levels in people who have high anxiety (Kimura et al., 2007). Participants were also given a State Trait Anxiety Inventory to measure their perception of anxiety. Kimura et al. (2007) found that the group who received L-theanine had reduced heart rate, reduced blood pressure, and immunoglobulin levels were more normal when compared to the control group. The L-theanine group also subjectively perceived stress levels that were lower than the control

group. Kimura et al. (2007) found that L-theanine was able to influence physiological and psychological stress in humans.

Physiological effects of L-theanine are also noticed through decreases in blood pressure (Yoto et al., 2012; Yokogoshi et al., 1995). Yoto et al. (2012) measured L-theanine's effects on systolic and diastolic blood pressure in humans. An experimental group was given a stressful mental task, then 200mg of L-theanine, and then again asked to complete a stressful mental task. Blood pressure significantly decreased in individuals that ingested 200mg of L-theanine (Yoto et al., 2012), suggesting they had achieved a more relaxed state.

Stress and anxiety can also be decreased through GABA. GABA is widely known as an inhibitory neurotransmitter which blocks the release of dopamine and serotonin. The depletion of GABA tends to increase in relation to stress levels (Abdou et al., 2006). Research suggests that L-theanine may produce additional, secondary anxiolytic effects by acting on GABA (Mason, 2001). Additionally, this increase in GABA could be a potential factor leading to a suggested decrease in norepinephrine (Mason, 2001).

Summary and Purpose of Study

L-theanine is found in green tea and is structurally similar to glutamate, which is found throughout the brain and is involved in several important behavioral and physiological functions (Meyer & Quenzer, 2005). As the aforementioned research indicates, L-theanine potentially can affect learning and memory through a number of potential routes. L-theanine is able to mimic and regulate glutamate, which is one of the

ways it can affect learning and memory (Nathan et al., 2006; White et al., 2016). L-theanine also can affect learning and memory by preventing neuronal cell death and increasing serotonin in regions of the brain, such as the hippocampus (Kakuda et al., 2002; Sugiyama et al., 2001; Yokogoshi et al., 1998). L-theanine also potentially affects learning and memory by decreasing stress and anxiety levels (Yoto et al., 2012). Stress and anxiety have been shown to hinder learning and memory abilities (Ivy et al., 2010). L-theanine may decrease stress and anxiety by stimulating the production of alpha brain waves, decreasing corticosterone and catecholamine, decreasing blood pressure and heart rate, and increasing GABA (Abdou et al., 2006; Tian et al., 2013; Yokogoshi et al., 1995; Yoto et al., 2012).

Although there are several studies supporting L-theanine's effect on learning and memory in the rat model, there is not sufficient research conducted on humans. The L-theanine research that has been conducted on humans also involved caffeine. Caffeine, in conjunction with L-theanine, has a demonstrated effect on learning and memory in humans by increasing working memory and reaction time (Haskell et al., 2008; Owen et al., 2008). It is important to consider L-theanine's effects on learning and memory in humans without the presence of caffeine. However, there is a paucity of research that has examined the effects of L-theanine in isolation. Furthermore, there are no studies reported that have used a double-blind placebo controlled method to measure L-theanine's effects on learning and memory in humans. Also, there is no known research that explores L-theanine's effects on memory and anxiety in one investigation. Hence, the purposes of the

present study were to conduct a double-blind, placebo controlled investigation of the effects of L-theanine on memory and cognitive functioning and to assess the relationship this effect has with levels of anxiety. Therefore, based on previous research findings, this study assessed the following hypotheses:

Hypothesis 1: L-theanine was predicted to improve episodic memory in college students, as evidenced by an increase in immediate recall, delayed recall, and recognition.

Hypothesis 2: L-theanine was predicted to improve attentional functioning in college students, as evidenced by an improvement in scores on measures of attention and concentration.

Hypothesis 3: Because stress and anxiety have been found to impair learning and memory, the present study also predicted a negative correlation between memory and anxiety. Scores on an anxiety measure were predicted to decrease as scores on measures of memory increase. This relationship between anxiety and memory was predicted to be more evident and stronger in those who are taking L-theanine than the placebo group.

CHAPTER II

METHOD

Participants

The participants included a total of 40 (17 male and 23 female) undergraduate students from Middle Tennessee State University. The ages of the participants ranged from 18 to 23 ($M = 19.70$, $SD = 1.49$). Table 1 summarizes demographic information. Exclusion criteria included having any history of head injury, neurological illness, or current psychotropic medication use. Eligible participants were randomly assigned to either a placebo group or 200mg L-theanine group.

Materials

Beck Depression Inventory-II (BDI-II). The BDI-II is a self-report measure that screens for depression (Beck et al., 1996). Each participant was asked to read through 21 groups of items and circle one statement from each group that best described him or her. The items were endorsed by the participants on a scale of 0 to 3. Each group of items assesses a common symptom of depression. A total score was obtained by adding the points. Scores on the BDI-II fall between a range of 0 and 63, and the total score was the dependent variable of interest for this study.

According to psychometric research on the BDI-II, Beck (1996) reported a test-retest reliability of 0.93 to 0.96. Steer and Clark (1997) reported internal consistency of test items to be between 0.84 to 0.93. In regards to validity, studies have reported

concurrent validity ranging from 0.66 to 0.93 between the BDI-II and the earlier version of the BDI (Beck et al., 1996; Wong et al., 2000).

Hopkins Verbal Learning Test- Revised (HVLT-R). The HVLT-R (Brandt & Benedict, 2001) assesses verbal learning and memory with immediate recall, delayed recall, and delayed recognition scores being derived (Benedict, Schretlen, Groninger, & Brandt, 1998). The HVLT-R has a list of 12 words taken from three semantic categories, and is read to the participants over three successive learning trials (Benedict et al., 1998). Free recall scores were obtained for each learning trial. The HVLT-R also includes a 20 to 25 minute delay recall trial, which was also obtained from the participants (Benedict et al., 1998). After the delayed recall, a yes or no recognition task was presented and the participants were asked to respond 'yes' to all target words and 'no' to all non-target words (Benedict et al., 1998). There are a total of 6 different, but equivalent forms available, each using a different set of 12 words. The present study used Form 1 and Form 3. The dependent variables were the number of correct responses for the total recall (sum of all 3 learning trials), delayed recall, and recognition task.

Benedict et al. (1998) reported reliability coefficients falling within acceptable limits for the free recall trials (trial 1 = .55, trial 2 = .67, and trial 3 = .78). There is little evidence of practice effects (Benedict et al., 1998). A validity study reported that the HVLT-R has construct, convergent, concurrent, and discriminant validity (Woods et al., 2005).

Letter Number Sequencing (LNS). Letter Number Sequencing is a subtest from the Wechsler Memory Scale (WMS-III; Wechsler, 1997) and Wechsler Adult Intelligence Scale (WASI-IV) that measures divided attention, memory span, and short-term auditory memory (Pezzuti & Rossetti, 2017). The participants were read a sequence of numbers and letters and then were asked to recall the numbers in ascending order and the letters in alphabetical order (Pezzuti & Rossetti, 2017). The WMS-III Letter Number Sequencing subtest has 7 items with three trials each and the WASI-IV has 10 items, also with three trials each. The WMS-III LNS was used as Form A for this study and the WASI-IV LNS was used as Form B for this study and the dependent variable was the total raw score for each respective version of the test.

Regarding psychometric properties, Letter Number Sequencing on the WMS-III has a reported reliability coefficient of .82 between ages 16 and 89 (The Psychological Corporation, 1997). Letter Number Sequencing on the WMS-III has discriminant validity with measures that are not related to it as evidenced by low correlations (The Psychological Corporation, 1997). Both versions of the Letter Number Sequencing subtests are not significantly different, indicating that the two versions of the subtests are similar and can be used as alternate forms (Tulsky & Zhu, 2000).

Logical Memory (LM). Logical Memory is a test of episodic memory that involves the presentation of two short stories that the individual is asked to recall immediately after presentation, and then again after a delay of 20 to 30 minutes (Wechsler, 2009). The first story from the WMS-IV and the first story from the WMS-R

were used for the present study, with the WMS-IV story consisting of Form A and the WMS-R story consisting of Form B. The stories have similar length, similar complexity, but different topic content. Participants were asked to recall the information immediately after the stories were presented and then again after a 20 to 30 minute delay. The individual was scored based on the number of story details recalled correctly for both stories and all presentation trials. The dependent variable was the total number of correct immediately recalled details and the total number of correct delayed recall details.

According to psychometric research, the WMS-IV Logical Memory I has a reported reliability coefficient of 0.82 for ages 16 through 69 (Wechsler, 2009). The WMS-R has a reported interrater reliability of 0.97 (McGuire & Batchelor, 1998). In terms of validity, Wechsler (2009) reported a construct validity of 0.48 between Logical Memory I and the Verbal Paired Associates I. Construct validity is reported at 0.54 between Logical Memory II and Verbal Paired Associates II.

Mini Mental Status Exam (MMSE). The MMSE (Folstein et al., 1975) is a measure used to screen for cognitive impairment and general intellectual functioning (Spreen & Strauss, 1998). The examiner asked a series of questions to the participant, then recorded and scored the answers immediately. The questions on the MMSE examine whether the participant is oriented to person, place, time, and situation. Scores were based on the total number of correct answers up to 30 and the dependent variable was the total score.

Evidence of reliability is obtained from internal consistency studies, which report test-retest reliability for intervals of less than two months falling between 0.80 and 0.95 (Folstein, Folstein, & McHugh, 1975). The MMSE shows moderate to high correlations with other brief screening tests such as the Blessed Test and the Dementia Rating Scale (Foreman, 1987). The MMSE also shows moderate to high correlations with measures of intelligences, memory, attention, executive functioning, and concentration (Axelrod, Goldman, & Henry, 1992; Feher et al., 1992; Folstein et al., 1975).

State Trait Anxiety Inventory (STAI). The State-Trait Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) is a questionnaire that has two 20-item scales, one scale measures state anxiety and the other scale measures trait anxiety. The two subscales are balanced, meaning half of the items are positively worded and the other half are negatively worded (Conijn, Emons, van Assen, Pedersen, & Sijtsma, 2013). For the state anxiety scale, participants were asked to rate a 4-point scale from 1 (not at all) to 4 (very much) in terms of current anxious feelings. For the trait anxiety scale, participants were asked to rate another 4-point scale from 1 (almost never) to 4 (almost always). State anxiety occurs when a person perceives something as a threat, but the anxiety disappears once the threat is gone (Bados, Gomez-Benito, & Balaguer, 2010). State anxiety is a temporary response to a threat, whereas trait anxiety is more of a personality characteristic (Conijn et al., 2013). Trait anxiety can occur in the anticipation of a threat and presence of a threat, but it differs in intensity and duration (Bados et al.,

2010). Because the present study was only interested in current feelings of anxiety, the dependent variable was the total score on the state scale and not the trait scale.

According to psychometric research, alpha coefficients were .94 and .88 for the state anxiety scale and trait anxiety scale, indicating strong internal consistency (Stanley, Beck, & Zebb, 1996). Stanley et al. (1996) also found the state and trait subscales to correlate significantly. Positive correlations were reported between the STAI and other measures of anxiety such as the Worry Scale, demonstrating convergent validity (Stanley et al., 1996). Test retest reliability also was reported as strong for the STAI (Stanley et al., 1996).

Trail Making Test (TMT). The Trail Making Test (TMT) assesses speed for attention, sequencing, visual search, and motor function (Spreeen & Strauss, 1998). For Part A, the participant was instructed to draw a line connecting number 1 to number 2, number 2 to number 3, and so on until the number 25. For Part B, the participant was instructed to draw a line connecting number 1 to letter A, letter A to number 2, and number 2 to letter B and so on up to the number 13. The present study used two different forms of the TMT. Form A consisted of the traditional Parts A and B. To create a suitable alternate but equivalent form for the present study, Form B consisted of the upside down and mirror image of the traditional Parts A and B. The dependent variables were the time in seconds it took the participant to complete Part A and the time in seconds it took to complete Part B.

According to psychometric research, Fals-Stewart (1991) reported interrater reliability of 0.94 for part A and 0.90 for part B. Practice effects have been reported to exist after a short interval (Dye, 1979; Stuss, Stethem, & Poirier, 1987). Repeat testing caused continuing improvement for both part A and B (Durvasula et al., 1996). Alternate forms reliability for part A was reported as 0.89, and part B was reported to be 0.92 (Charter, Adkins, Alekoumbides, & Seacat, 1987). In terms of validity, part A and B were found to correlate 0.49 with each other, indicating they may measure different functioning (Heilbronner, Henry, Buck, Adams, & Fogle, 1991). Woodruff et al. (1995) explained the difference due to the location of the circles in part B. They are farther apart, therefore, requiring more perceptual processing ability than part A.

Procedure

After obtaining approval from the MTSU Institutional Review Board (See Appendix B), each participant was given a written informed consent that they read and signed prior to any administration. On test day, participants were given a questionnaire regarding demographics, medical history, and current medications. Participants were randomly assigned to one of two groups, one group received 200mg of L-theanine and the other group received a placebo. A 200mg dose of L-theanine was chosen because this is a dosage that was similar to those used in previous studies examining the effects of L-theanine on learning and memory. The placebo consisted of 140mg monocristaline cellulose that was encapsulated similarly to the L-theanine. Regarding the potential risks, L-theanine has no reported side effects in animals (Kakuda et al., 2000; Nathan et al.,

2006) or in humans (Bryan, 2008; Kobayashi et al., 1998), and has not been known to interact with other drugs. It is available over-the-counter in many pharmacies and vitamin shops. Given that only a single dose of L-theanine was administered, it was felt that this single dosage would minimize any potential adverse effects.

Participants first were administered the HVLt-R and the Logical Memory tests. These tests were administered first because of the delayed recall trial associated with each test. The order of administration of the HVLt-R and the Logical Memory tests were counterbalanced. The MMSE, BDI, State-Trait Anxiety Inventory, TMT, and LNS were then administered in randomized order. Each measure consisted of a Form A and Form B; forms for pre-test administration were randomly assigned. After completion of the pre-test measures, participants were administered 200mg of L-theanine or the placebo. A double-blind procedure was used to administer either L-theanine or the placebo, meaning the participant and the examiner did not know which pill the participant would be administered. The pills were numbered and placed in individual containers by someone other than the examiner. The examiner wrote down what number was written on the container so it could be later determined which pill the participant was administered. After administration of either L-theanine or a placebo, the participants were asked to read, "Crow Killer: The Saga of Liver-Eating Johnson" by Raymond W. Thorp and Robert Bunker (1983) for 35 minutes. Participants were retested after 35 minutes of L-theanine administration because research has indicated that L-theanine is able to cross the blood brain barrier as soon as 30 minutes after ingestion through the leucine preferred

transport system (Bryan, 2008; Nathan et al., 2006; Ota et al., 2014; Yokogoshi et al., 1998). Levels of L-theanine decrease in the brain after about five hours and are completely removed from the brain in twenty-four hours (Yokogoshi et al., 1998). After allowing L-theanine to take effect in the brain, post-test measures were administered. Alternate forms of the HVLT-R and Logical Memory tests were again given first because of recall trials. The tests again were presented in a randomized order. Alternate forms of the TMT and LNS were used. The participants were given a debriefing form and an opportunity to ask questions prior to leaving the assessment.

CHAPTER III

RESULTS

Initial Analysis

Initial analyses were conducted on age, education, weight, BDI, and MMSE to determine group equivalence. A series of one-way ANOVAs were conducted on each of these variables and the results indicated that there were no significant differences in any of the variables. Hence, it was determined that there was group equivalence for each of these variables (see Table 1).

Primary Analysis

To examine the primary hypothesis that L-theanine would improve episodic memory, a series of 2 (Time: Pre and Post) x 2 (Drug: Placebo and L-theanine) mixed factorial ANOVAs were conducted, with a repeated factor of Time and a between factor of Drug. The results indicated no significant interaction between Time and Drug for any of the dependent variables (see Table 2). There was no significant main effect for Drug for any of the dependent variables (see Table 3). However, a significant main effect for Time was found for LM-Immediate Recall $F(1,38) = 9.872, p = 0.003$, LM-Delayed Recall $F(1,38) = 5.170, p = 0.029$, HVLT-Delayed Recall $F(1,38) = 8.250, p = 0.004$, and HVLT-Retention $F(1,38) = 21.182, p = 0.000$ (see Table 4).

To examine the second hypothesis, that L-theanine will improve attention, a series of 2 (Time: Pre and Post) x 2 (Drug: Placebo and L-theanine) mixed factorial ANOVAs were conducted, with a repeated factor of Time and a between factor of Drug. The results

indicated no significant interaction between Time and Drug for any of the dependent variables (see Table 2). There was no significant main effect for Drug for any of the dependent variables (see Table 3). However, a significant main effect for Time was found for TMT A $F(1,38) = 21.839, p < 0.001$ (see Table 4).

To examine the third hypothesis, that differences between the placebo and L-theanine groups would emerge in the relationship between anxiety and memory, a series of Pearson correlations were conducted. Difference scores based on the STAI were obtained by subtracting the post STAI score from the pre STAI score. Correlations were then conducted between the STAI difference scores and the post treatment measures. These correlations were conducted separately for each group. There were no significant correlations found between the STAI difference score and any post memory treatment measures. See Table 5 for results.

Follow-Up Analysis

Examination of the raw data indicated that two of the participants scored within the severe range on the BDI-II. Given the potential deleterious effects of depression on memory and cognitive functioning it was decided to exclude these cases from the analyses. To ensure the data sample remained counter balanced, two more participants were randomly selected to be excluded from the analysis, leaving both groups equal and balanced. After these cases were excluded another series of one-way ANOVAS were conducted on age, education, weight, BDI, and MMSE to determine that the groups were still equal (see Table 6). Also, to ensure that the effects of depression were controlled, an

ANCOVA was conducted using BDI-II scores as a covariate. The results indicated a significant interaction between Time and Drug for the dependent variable HVLTR when controlling for depression, $F(1,34) = 4.638, p = 0.038$ (see Table 7). Results of within group comparisons indicated no significant difference between the Pre and Post measures for either the Placebo group ($F(1,16) = 1.058, p = .319$) or for the L-theanine group ($F(1,16) = .410, p = .531$). The results of between groups comparisons indicated no significant differences between the Placebo and L-theanine groups at either the pre-treatment ($F(1,33) = 0.027, p = 0.870$) or the post-treatment ($F(1,33) = 0.099, p = 0.755$).

CHAPTER IV

DISCUSSION

The initial analysis investigated the effect of L-theanine on learning and memory through a series of cognitive and memory tests before and after taking L-theanine or a placebo. However, the results indicated that no significant differences existed between the groups for any of the indices of memory and cognitive functioning. Review of the raw data indicated that two of the participants scored within the severe range on a measure of depression. Hence, a series of follow-up analyses were conducted to control for any potential effect from depression. The results of these follow-up analyses indicated a significant Group x Time interaction for the total recall score on the HVLTR. However, subsequent multiple comparisons indicated no significant differences either between or within the groups. Based on the results of the present study, L-theanine does not appear to have an effect on learning, memory, attention, or anxiety.

The findings from this present study are in contrast with previous studies that have found L-theanine to have significant effects on learning and memory. Park et al. (2011) indicated that L-theanine increased delayed recognition in participants who had mild cognitive impairments. Park et al. (2011) concluded that L-theanine was able to improve memory by acting on memory retrieval and by improving attention. Several studies that used a rat model found that L-theanine improved performance on tests that require object recognition memory (Takeda et al., 2011; Yokogoshi & Terashima, 2000). Another study that used humans found a dose of 100mg in conjunction with caffeine to

increase the number of correctly identified words in word recognition tasks and increased speed and accuracy in attention switching tasks (Haskell et al., 2008).

The present study's results are in contrast with previous studies that have found L-theanine to reduce anxiety. Results from Tian et al. (2013) found that stress induced rats performed better on learning and memory tasks when they were administered L-theanine. Researchers concluded that the rats performed better because their feelings of anxiousness decreased (Tian et al., 2013). L-theanine also reduced anxiety in participants who were undergoing a stressful mental task (Nobre et al., 2008). It has been well documented that L-theanine increases alpha brain waves, which are associated with an alert but relaxed state (Abdou et al., 2006; Yoto et al., 2012). While several researchers found L-theanine to decrease anxiety, the present research did not conclude the same finding.

The reason for the discrepancy between the previous studies examining the effects of L-theanine on memory and cognitive functioning and anxiety and the current findings may be the use of a more stringent double blind and placebo controlled investigation for the present study. A double blind placebo controlled study is a more precise way to study the effects of a drug or supplement. Hence, given the current findings, L-theanine may actually have no significant impact on memory and cognitive functioning and the findings of previous investigations may have been due to the presence of confounds rather than an actual effect of L-theanine.

Although the use of a more stringent research design may explain the aforementioned discrepancy, a more likely and somewhat surprising reason may be because of certain characteristics of the participant sample. Specifically, a major limitation of the current study was lack of reliable participants. When examining the participant scores more closely, it became apparent that approximately half of the sample performed at an impaired or borderline range on the memory measures when compared to a normative sample. This suggests that many participants were not putting forth their best effort. Because of the study's unreliable sample, it is hard to determine L-theanine's true effects on learning and memory. If the current study had more reliable participants and still did not find significance, then a higher dosage of L-theanine may have been necessary. Future studies may want to consider increasing this dosage, especially since L-theanine is safe at all dosage levels. Future studies also may consider increasing the 35 minute delay between pre and post administration. A longer delay might give L-theanine a better chance to enter the blood stream and affect brain functioning. The weight of participants may also be a factor that prevents L-theanine from being metabolized and distributed to the brain within the 35 minute delay. Another potential issue with this study was that participants did not have high levels of anxiety and anxiety did not differ significantly between pre and posttest administration. It is possible that L-theanine would have had a better effect on participants who had higher levels of anxiety. L-theanine may not have effects on learning and memory in individuals who aren't anxious.

In conclusion, the results of the present study are not an accurate representation of the possible effects of L-theanine. To prevent future research studies from gaining participants with low effort, other studies should incorporate an effort measure. If participants fail the effort measure, then they should be excluded from the study. Researchers may also want to consider using a more reliable population, such as graduate students or faculty members of a university. When using a reliable sample of participants, researchers may find that L-theanine does indeed improve learning and memory, but we cannot know this for certain unless researchers continue studying L-theanine. L-theanine offers the potential to improve cognitive abilities while being a safe supplement to consume, which makes L-theanine a supplement that should continue to be studied in the field of psychology.

REFERENCES

- Abdou, A. M., Higashiguchi, S., Horie, K., Kim, M., Hatta, H., & Yokogoshi, H. (2006). Relaxation and immunity enhancement effects of gamma-aminobutyric acid (GABA) administration in humans. *Biofactors (Oxford, England)*, *26*(3), 201-208.
- Axelrod, B.N., Goldman, R.S., & Henry, R.R (1992). Sensitivity of the Mini-Mental State Examination to frontal lobe dysfunction in normal aging. *Journal of Clinical Psychology*, *48*, 68-71. doi:10.1002/1097-4679(199201)48:1<68::AID-JCLP2270480110>3.0.CO;2-N
- Bados, A., Gómez-Benito, J., & Balaguer, G. (2010). The State-Trait Anxiety Inventory, Trait Version: Does it really measure anxiety? *Journal of Personality Assessment*, *92*(6), 560-567. doi:10.1080/00223891.2010.513295
- Beck, A.T. (1970). *Depression: Causes and treatment*. Philadelphia: University of Pennsylvania Press.
- Beck, A. T., Steer, R. A., & Brown, G. K. (1996). Beck depression inventory-II. *San Antonio*, *78*(2), 490-8.
- Benedict, R. H., Schretlen, D., Groninger, L., & Brandt, J. (1998). Hopkins Verbal Learning Test—Revised: Normative data and analysis of inter-form and test-retest reliability. *The Clinical Neuropsychologist*, *12*(1), 43-55. doi:10.1076/clin.12.1.43.1726
- Brandt, J., & Benedict, R. H. (2001). *Hopkins verbal learning test--revised: professional manual*. Psychological Assessment Resources.

- Bryan, J. (2008). Psychological effects of dietary components of tea: Caffeine and L-theanine. *Nutrition reviews*, 66(2), 82-90. doi: <http://dx.doi.org/10.1111/j.1753-4887.2007.00011.x>
- Charter, R.A., Adkins, T.G., Alekoumbides, A., & Seacat, G.F. (1987). Reliability of the WAIS, WMS, and Reitan Battery: Raw scores and standardized scores corrected for age and education. *International Journal of Clinical Neuropsychology*, 9, 28-32.
- Conijn, J. M., Emons, W. M., van Assen, M. M., Pedersen, S. S., & Sijtsma, K. (2013). Explanatory, multilevel person-fit analysis of response consistency on the Spielberg State-Trait Anxiety Inventory. *Multivariate Behavioral Research*, 48(5), 692-718. doi:10.1080/00273171.2013.815580
- Conrad, C. D. (2006). What is the functional significance of chronic stress-induced CA3 dendritic retraction within the hippocampus? *Behavioral and Cognitive Neuroscience Reviews*, 5(1), 41–60. <http://doi.org/10.1177/1534582306289043>
- Durvasula, R. S., Satz, P., Hinkin, C. H., Uchiyama, C., Morgenstern, H., Miller, E. N., ... Mitchell, M. (1996). Does practice make perfect?: Results of a six-year longitudinal study with semi-annual testing's. *Archives of Clinical Neuropsychology*, 5(11), 386.
- Dye, O.A. (1979). Effects of practice on Trail Making Test performance. *Perceptual and Motor Skills*, 48, 296. doi:10.2466/pms.1979.48.1.296
- Fals-Stewart, W. (1991). An interrater reliability study of the Trail Making Test (Part A and B). Unpublished manuscript.
- Feher, E.P., Mahurin, R.K., Doody, R.S., Cooke, N., Sims, J., & Pirozzolo, F.J. (1992). Establishing the limits of the Mini-Mental State. *Archives of Neurology*, 49, 87-92.

- Folstein, M. F., Folstein, S.E., & McHugh, P.R. (1975). 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, 12, 189-198. doi:10.1016/0022-3956(75)90026-6
- Foreman, M.D. (1987). Reliability and validity of mental status questionnaires in elderly hospitalized patients. *Nursing Research*, 36, 216-220. doi:10.1097/00006199-198707000-00004
- Haskell, C. F., Kennedy, D. O., Milne, A. L., Wesnes, K. A., & Scholey, A. B. (2008). The effects of L-theanine, caffeine and their combination on cognition and mood. *Biological psychology*, 77(2), 113-122. doi:10.1016/j.biopsycho.2007.09.008
- Heilbronner, R.L., Henry, G.K., Buck, P., Adams, R.L., & Fogle, T., (1991). Lateralized brain damage and performance on Trail Making A and B, Digit Span Forward and Backward, and TPT memory and location. *Archives of Clinical Neuropsychology*, 6, 251-258. doi:10.1016/0887-6177(91)90002-Q
- Ivy, A. S., Rex, C. S., Chen, Y., Dubé, C., Maras, P. M., Grigoriadis, D. E., & ... Baram, T. Z. (2010). Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. *The Journal Of Neuroscience*, 30(39), 13005-13015. doi:10.1523/JNEUROSCI.1784-10.2010
- Juneja L.R., Chu D., Okubo T., Nagato Y., & Yokogoshi H. (1999). L-theanine, a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Science & Technology* 10, 199-204.

- Kakuda, T., Nozawa, A., Sugimoto, A., & Niino, H. (2002). Inhibition by Theanine of binding of [3H]AMPA, [3H]Kainate, and [3H]MDL 105,519 to glutamate receptors, *Bioscience, Biotechnology, and Biochemistry*, 66, 2683-2686. doi: 10.1271/bbb.66.2683
- Kakuda, T., Yanase, H., Utsunomiya, K., Nozawa, A., Unno, T., & Kataoka, K. (2000). Protective effect of γ -glutamylethylamide (theanine) on ischemic delayed neuronal death in gerbils. *Neuroscience letters*, 289(3), 189-192.
- Kimura, K., Ozeki, M., Juneja, L. R., & Ohira, H. (2007). L-Theanine reduces psychological and physiological stress responses. *Biological psychology*, 74(1), 39-45.
doi:10.1016/j.biopsycho.2006.06.006
- Kobayashi, K., Nagato, Y., Aoi, N., Juneja, L. R., Kim, M., Yamamoto, T., & Sugimoto, S. (1998). Effects of L-theanine on the release of alpha-brain waves in human volunteers. *Nippon Nogeikagaku Kaishi*, 72(2), 153-157.
- Magariños, A. M., & McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. *Neuroscience*, 69(1), 83-88.
- Maruyama, M., & Takeda, K. (1994). Electrophysiologically potent non-competitive glutamate antagonists at crayfish neuromuscular junctions are also potent inhibitors of [3 H] MK801 binding to synaptic membranes from rat central nervous system. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 107(1), 105-110.
- Mason, R. (2001). 200 mg of Zen: L-theanine boosts alpha waves, promotes alert relaxation. *Alternative & Complementary Therapies*, 7(2), 91-95.

- McEntee, W. J., & Crook, T. H. (1993). Glutamate: Its role in learning, memory, and the aging brain. *Psychopharmacology*, *111*(4), 391-401. doi:10.1007/BF02253527
- McGuire, B. E., & Batchelor, J. (1998). Inter-rater Reliability of the WMS-R Logical Memory and Visual Reproduction Subtests in a Neurosurgical Sample. *Australian Psychologist*, *33*(3), 231-233. doi: 10.1080/00050069808257411
- Meyer, J., & Quenzer, L. (2005). *Psychopharmacology. Drugs, the brain, and behavior*. Sunderland, MA: Sinauer Associates, Inc.
- Nathan, P. J., Lu, K., Gray, M., & Oliver, C. (2006). The neuropharmacology of L-theanine (N-ethyl-L-glutamine) a possible neuroprotective and cognitive enhancing agent. *Journal of Herbal Pharmacotherapy*, *6*(2), 21-30. doi:10.1080/J157v06n02_02
- Nobre, A. C., Rao, A., & Owen, G. N. (2008). L-theanine, a natural constituent in tea, and its effect on mental state. *Asia Pacific Journal of Clinical Nutrition*, *17* (S1), 167-168.
- Ota, M., Wakabayashi, C., Matsuo, J., Kinoshita, Y., Hori, H., Hattori, K., ... Kunugi, H. (2014). Effect of L-theanine on sensorimotor gating in healthy human subjects. *Psychiatry and Clinical Neurosciences*, *68*(5), 337-343. doi:10.1111/pcn.12134
- Owen, G. N., Parnell, H., De Bruin, E. A., & Rycroft, J. A. (2008). The combined effects of L-theanine and caffeine on cognitive performance and mood. *Nutritional Neuroscience*, *11*(4), 193-198. doi:10.1016/j.biopsycho.2007.09.008
- Park, S. K., Jung, I. C., Lee, W. K., Lee, Y. S., Park, H. K., Go, H. J., ... Rho, S. S. (2011). A combination of green tea extract and l-theanine improves memory and attention in

- subjects with mild cognitive impairment: a double-blind placebo-controlled study.
Journal of Medicinal Food, 14(4), 334-343. doi:10.1007/s00213-015-3895-0
- Pezzuti, L., & Rossetti, S. (2017). Letter-Number Sequencing, Figure Weights, and Cancellation subtests of WAIS-IV administered to elders. *Personality and Individual Differences*, 104, 352-356. doi:10.1016/j.paid.2016.08.019
- Popoli, M., Yan, Z., McEwen, B. S., & Sanacora, G. (2012). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews Neuroscience*, 13(1), 22-37.
- Riedel, G., Platt, B., & Micheau, J. (2003). Glutamate receptor function in learning and memory. *Behavioural Brain Research*, 140(1-2), 1-47. doi:10.1016/S0166-4328(02)00272-3
- Ross, I. A. (2007). *Medicinal plants of the world, volume 3: Chemical constituents, traditional and modern medicinal uses*. Springer Science & Business Media. doi: 10.1007/978-1-59259-887-8
- Scheid, L., Ellinger, S., Altheld, B., Herholz, H., Ellinger, J., Henn, T., ... Stehle, P. (2012). Kinetics of L-theanine uptake and metabolism in healthy participants are comparable after ingestion of L-theanine via capsules and green tea. *The Journal of Nutrition*, 142(12), 2091-2096. doi: 10.3945/jn.112.166371
- Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.
- Spreen, O., & Strauss, E. (1998). *A compendium of neuropsychological tests*. New York, NY: Oxford University Press, Inc.

- Spreeen, O., & Strauss, E. (2006). *A compendium of neuropsychological tests. Third Edition*. New York, NY: Oxford University Press, Inc.
- Stanley, M. A., Beck, J. G., & Zebb, B. J. (1996). Psychometric properties of four anxiety measures in older adults. *Behaviour Research and Therapy*, *34*(10), 827-838.
doi:10.1016/0005-7967(96)00064-2
- Steer, R.A., & Clark, D.A (1997). Psychometric characteristics of the Beck Depression Inventory-II with college students. *Measurement and Evaluation in Counselling and Development*, *30*, 128-136.
- Stuss, D.T., Stethem, L.L., & Poirier, C.A. (1987). Comparison of three tests of attention and rapid information processing across six age groups. *The Clinical Neuropsychologist*, *1*, 139-152.
- Sugiyama, T., Sadzuka, Y., Tanaka, K., & Sonobe, T. (2001). Inhibition of glutamate transporter by theanine enhances the therapeutic efficacy of doxorubicin. *Toxicology Letters*, *121*(2), 89-96.
- Takeda, A., Sakamoto, K., Tamano, H., Fukura, K., Inui, N., Suh, S. W., ... Yokogoshi, H. (2011). Facilitated neurogenesis in the developing hippocampus after intake of theanine, an amino acid in tea leaves, and object recognition memory. *Cellular and Molecular Neurobiology*, *31*(7), 1079-1088.
- Tian, X., Sun, L., Gou, L., Ling, X., Feng, Y., Wang, L., ... Liu, Y. (2013). Protective effect of l-theanine on chronic restraint stress-induced cognitive impairments in mice. *Brain Research*, *1503*, 24-32. doi:10.1016/j.brainres.2013.01.048

- The Psychological Corporation (1997). *WAIS-III-WMS-III technical manual*. San Antonio: Author.
- Tulsky, D. S., & Zhu, J. (2000). Could test length or order affect scores on Letter Number Sequencing of the WAIS-III and WMS-III? Ruling out effects of fatigue. *The Clinical Neuropsychologist*, *14*(4), 474-478. doi:10.1076/clin.14.4.474.7205
- Wechsler, D. (2009). *WMS-IV administration and scoring manual*. San Antonio: The Psychological Corporation.
- White, D. J., de Klerk, S., Woods, W., Gondalia, S., Noonan, C., & Scholey, A. B. (2016). Anti-stress, behavioural and magnetoencephalography effects of an l-theanine-based nutrient drink: A randomised, double-blind, placebo-controlled, crossover trial. *Nutrients*, *8*(1), 53. doi:10.3390/nu8010053. doi:10.1016/j.acn.2005.06.007
- Wong, J. L., Wetterneck, C., & Klein, A. (2000). Effects of depressed mood on verbal memory performance versus self-reports of cognitive difficulties. *International Journal of Rehabilitation and Health*, *5*, 85-97.
- Woodruff, G.R., Mendoza, J.E., Dickson, A.L., Blanchard, E., & Christenberry, L.B. (1995). The effects of configural differences on the Trail Making Test. *Archives of Clinical Neuropsychology*, *10*, 408.
- Woods, S. P., Scott, J. C., Dawson, M. S., Morgan, E. E., Carey, C. L., Heaton, R. K., ... HIV Neurobehavioral Research Center (HNRC) Group. (2005). Construct validity of Hopkins Verbal Learning Test—Revised component process measures in an HIV-1 sample. *Archives of Clinical Neuropsychology*, *20*(8), 1061-1071.

- Yokogoshi, H., Kato, Y., Sagesaka, Y. M., Takihara-Matsuura, T., Kakuda, T., & Takeuchi, N. (1995). Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Bioscience, Biotechnology, and Biochemistry*, *59*(4), 615-618. doi: 10.1271/bbb.59.615
- Yokogoshi, H., Mochizuki, M., & Saitoh, K. (1998). Theanine-induced reduction of brain serotonin concentration in rats. *Bioscience, Biotechnology, and Biochemistry*, *62*(4), 816-817. doi: 10.1271/bbb.62.816
- Yokogoshi H., & Terashima T. (2000). Effect of theanine, r-glutamylethylamide, on brain monoamines, striatal dopamine release and some kinds of behavior in rats. *Nutrition* *16*, 776-777.
- Yoto, A., Motoki, M., Murao, S., & Yokogoshi, H. (2012). Effects of L-theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. *Journal of Physiological Anthropology*, *31*(1), 1. doi: 10.1186/1880-6805-31-28

APPENDICES

APPENDIX A

TABLES

Table 1
Descriptive Statistics for Group Differences

Variable	Placebo		L-theanine		Full Sample		Result
	M	SD	M	SD	M	SD	
Age	19.85	1.66	19.55	1.32	19.70	1.49	$F(1,38) = 0.40, p = 0.53$
Education	13.75	0.85	13.65	0.75	13.70	0.79	$F(1,38) = 0.156, p = 0.70$
Weight	150.55	26.96	172.60	43.23	161.56	37.27	$F(1,38) = 3.746, p = 0.06$
BDI	7.25	5.87	9.45	8.64	8.35	7.37	$F(1,38) = 0.888, p = 0.35$
MMSE	28.40	1.82	27.80	3.93	28.10	3.04	$F(1,38) = 0.384, p = 0.54$

Table 2
Descriptive Statistics and ANOVA Results for Time and Drug Interaction

Variable	Group	Time	M	SD	Result
LMI	Placebo	Pre	8.40	3.03	$F(1, 38) = 0.415, p = 0.523$
		Post	10.75	3.34	
	L-theanine	Pre	9.40	3.17	
		Post	9.75	5.12	
LMD	Placebo	Pre	7.95	3.58	$F(1,38) = 1.01, p = 0.321$
		Post	8.80	2.91	
	L-theanine	Pre	7.90	3.11	
		Post	10.10	5.00	
HVLT-TR	Placebo	Pre	23.25	5.15	$F(1,38) = 1.697, p = 0.201$
		Post	24.10	5.30	
	L-theanine	Pre	24.15	3.94	
		Post	23.35	4.50	
HVLT-DR	Placebo	Pre	8.40	2.39	$F(1,38) = 0.578, p = 0.452$
		Post	7.50	2.96	
	L-theanine	Pre	8.25	2.27	
		Post	6.75	3.04	
HVLT-Rec	Placebo	Pre	11.10	1.25	$F(1,38) = 0.608, p = 0.440$
		Post	10.90	1.33	
	L-theanine	Pre	11.25	1.25	
		Post	10.65	1.63	
HVLT-Ret	Placebo	Pre	92.50	23.44	$F(1,38) = 0.568, p = 0.456$
		Post	72.74	20.48	
	L-theanine	Pre	81.27	14.41	
		Post	67.07	22.93	
LNS	Placebo	Pre	15.00	4.77	$F(1,38) = 0.081, p = 0.778$
		Post	14.95	5.70	
	L-theanine	Pre	14.65	4.45	
		Post	15.40	5.26	
STAI	Placebo	Pre	37.30	8.44	$F(1,38) = 0.454, p = 0.505$
		Post	35.50	10.76	
	L-theanine	Pre	34.45	9.93	
		Post	34.10	10.59	

Table 2 Continued

Variable	Group	Time	M	SD	Result
TMT A	Placebo	Pre	29.10	8.74	$F(1,38) = 0.000, p = 1.000$
		Post	23.55	5.30	
	L-theanine	Pre	29.45	10.16	
		Post	23.90	6.54	
TMT B	Placebo	Pre	64.25	26.54	$F(1,38) = 0.012, p = 0.914$
		Post	59.65	25.21	
	L-theanine	Pre	63.45	17.33	
		Post	59.55	20.91	

Note: LM-I-Logical Memory Immediate Recall; LM-D- Logical Memory Delayed Recall; HVLT-TR- Hopkins Verbal Learning Total Recall; HVLT-DR- Hopkins Verbal Learning Test Delayed Recall; HVLT-Rec- Hopkins Verbal Learning Test Recognition; HVLT-Ret- Hopkins Verbal Learning Test Retention; LNS- Letter Number Sequencing; STAI- State Trait Anxiety Inventory; COWAT- Controlled Oral Word Association Test; TMT A- Trail Making Test Part A; TMT B- Trail Making Test Part B

Table 3
ANOVA Results for Drug

Variable	Result
LM.I	$F(1,38) = 0.349, p = 0.56$
LM.D	$F(1,38) = 0.411, p = 0.53$
HVLT.TR	$F(1,38) = 0.003, p = 0.96$
HVLT.D	$F(1,38) = 0.357, p = 0.55$
HVLT.RET	$F(1,38) = 2.469, p = 0.12$
HVLT.REC	$F(1,38) = 0.020, p = 0.89$
STAI	$F(1,38) = 0.514, p = 0.48$
LNS	$F(1,38) = 0.004, p = 0.95$
TMT.A	$F(1,38) = 0.025, p = 0.88$
TMT.B	$F(1,38) = 0.005, p = 0.95$

Table 4
ANOVA Results for Time

Variable	Result
LM.I	$F(1,38) = 9.872, p = 0.00^*$
LM.D	$F(1,38) = 5.170, p = 0.03^*$
HVLT.TR	$F(1,38) = 0.002, p = 0.97$
HVLT.D	$F(1,38) = 9.243, p = 0.00^*$
HVLT.RET	$F(1,38) = 21.182, p = 0.00^*$
HVLT.REC	$F(1,38) = 2.432, p = 0.13$
STAI	$F(1,38) = 0.998, p = 0.32$
LNS	$F(1,38) = 0.062, p = 0.81$
TMT.A	$F(1,38) = 21.839, p = 0.00^*$
TMT.B	$F(1,38) = 1.746, p = 0.19$

*Note: Statistical significance ($p < .05$) is indicated by **

Table 5
Correlation Between STAI and Post Treatment Measures

Variable	Result	
	<i>Placebo</i>	<i>L-theanine</i>
LM.I	$r = -0.111, p = 0.32$	$r = 0.156, p = 0.26$
LM.D	$r = -0.130, p = 0.29$	$r = 0.209, p = 0.19$
HVLT.TR	$r = -0.266, p = 0.13$	$r = 0.246, p = 0.15$
HVLT.D	$r = -0.239, p = 0.16$	$r = 0.150, p = 0.26$
HVLT.RET	$r = -0.204, p = 0.20$	$r = 0.154, p = 0.26$
HVLT.REC	$r = -0.030, p = 0.45$	$r = 0.335, p = 0.07$

Table 6
Descriptive Statistics and ANOVA Results for Group Differences when Controlling for Depression

Variable	M	SD	Result
Age	19.79	1.49	$F(1,35) = 0.048, p = 0.83$
Education	13.75	0.77	$F(1,35) = 0.046, p = 0.83$
Weight	164.08	37.73	$F(1,35) = 3.611, p = 0.07$
BDI	6.14	4.37	$F(1,35) = 0.115, p = 0.74$
MMSE	28.61	1.57	$F(1,35) = 0.397, p = 0.53$

Table 7
Descriptive Statistics and ANOVA Results for Time and Drug Interaction when Controlling for Depression

Variable	Group	Time	M	SD	Result
LMI	Placebo	Pre	8.89	3.25	$F(1,34) = 0.002, p = 0.97$
		Post	10.38	3.16	
	L-theanine	Pre	9.50	3.29	
		Post	10.94	5.01	
LMD	Placebo	Pre	8.44	3.20	$F(1,34) = 1.681, p = 0.20$
		Post	8.67	2.87	
	L-theanine	Pre	8.06	3.24	
		Post	10.00	4.99	
HVLT-TR	Placebo	Pre	22.50	5.03	$F(1,34) = 4.638, p = 0.04^*$
		Post	24.27	5.01	
	L-theanine	Pre	24.50	3.94	
		Post	23.50	4.72	
HVLT-DR	Placebo	Pre	8.27	2.34	$F(1,34) = 0.641, p = 0.43$
		Post	7.39	2.91	
	L-theanine	Pre	8.27	2.29	
		Post	6.72	3.21	
HVLT-Rec	Placebo	Pre	11.11	1.23	$F(1,34) = 0.161, p = 0.69$
		Post	10.83	1.38	
	L-theanine	Pre	11.22	1.23	
		Post	10.72	1.67	
HVLT-Ret	Placebo	Pre	94.94	23.42	$F(1,34) = 1.446, p = 0.24$
		Post	70.23	20.18	
	L-theanine	Pre	81.63	14.63	
		Post	65.92	23.96	
LNS	Placebo	Pre	14.89	4.14	$F(1,34) = 0.078, p = 0.78$
		Post	15.44	5.66	
	L-theanine	Pre	15.61	4.38	
		Post	14.88	5.29	
STAI	Placebo	Pre	37.00	8.46	$F(1,34) = 0.570, p = 0.46$
		Post	34.50	10.42	
	L-theanine	Pre	33.17	9.34	
		Post	32.39	9.68	

Table 7 Continued

Variable	Group	Time	M	SD	Result
TMT A	Placebo	Pre	30.11	7.93	$F(1,34) = 0.058, p = 0.81$
		Post	23.78	5.98	
	L-theanine	Pre	30.17	10.49	
		Post	24.44	6.68	
TMT B	Placebo	Pre	62.11	18.83	$F(1,34) = 0.045, p = 0.83$
		Post	60.56	24.98	
	L-theanine	Pre	62.06	17.70	
		Post	59.17	21.98	

*Note: Statistical significance ($p < .05$) is indicated by **

APPENDIX B

MTSU INSTITUTIONAL REVIEW BOARD APPROVAL FORM

IRB**INSTITUTIONAL REVIEW BOARD**

Office of Research Compliance, 010A Sam
Ingram Building, 2269 Middle Tennessee
Blvd Murfreesboro, TN 37129

**IRBN001 - EXPEDITED PROTOCOL APPROVAL NOTICE**

Friday, February 24, 2017

Principal Investigator **Melissa Nickel** (Student) Faculty
 Advisor Paul S. Foster

Co-Investigators NONE

Investigator Email(s) *mmn3a@mtmail.mtsu.edu; paul.foster@mtsu.edu*

Department Psychology

Protocol Title *L-theanine's effects on learning and memory in college students*

Protocol ID **17-2136**

Dear Investigator(s),

The above identified research proposal has been reviewed by the MTSU Institutional Review Board (IRB) through the **EXPEDITED** mechanism under 45 CFR 46.110 and 21 CFR 56.110 within the category (1) *Clinical studies of drugs and medical devices*. A summary of the IRB action and other particulars in regard to this protocol application is tabulated as shown below:

IRB Action	APPROVED for one year from the date of this notification
Date of expiration	2/28/2018
Participant Size	60 (SIXTY)

Participant Pool	Adult participants from the MTSU Psychology Research Pool with no history of head injury, no neurological illness and are not taking any psychotropic medications.	
Exceptions	1. Permitted to record participant names for administrative purposes only 2. If applicable, the participants may be compensated (extra credit)	
Restrictions	1. Mandatory informed consent 2. Destroy/delete identifiable information	
Comments	None	
Amendments	Date	Post-approval Amendments
		None at this time

This protocol can be continued for up to THREE years (2/29/2020) by obtaining a continuation approval prior to 2/28/2018. Refer to the following schedule to plan your annual project reports and be aware that you may not receive a separate reminder to complete your continuing reviews. Failure in obtaining an approval for continuation will automatically result in cancellation of this

Institutional Review Board

Office of Compliance

Middle Tennessee State University

protocol. Moreover, the completion of this study MUST be notified to the Office of Compliance by filing a final report in order to close-out the protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	IRB Comments
First year report	1/31/2018	TO BE COMPLETED
Second year report	1/31/2019	TO BE COMPLETED
Final report	1/31/2019	TO BE COMPLETED

The investigator(s) indicated in this notification should read and abide by all of the post-approval conditions imposed with this approval. [Refer to the post-approval guidelines posted in the MTSU IRB's website](#). Any unanticipated harms to participants or adverse events must be reported to the Office of Compliance at (615) 494-8918 within 48 hours of the incident. Amendments to this protocol must be approved by the IRB. Inclusion of new researchers must also be approved by the Office of Compliance before they begin to work on the project.

All of the research-related records, which include signed consent forms, investigator information and other documents related to the study, must be retained by the PI or the faculty advisor (if the PI is a student) at the secure location mentioned in the protocol application. The data storage must be maintained for at least three (3) years after study completion. Subsequently, the researcher may destroy the data in a manner that maintains confidentiality and anonymity. IRB reserves the right to modify, change or cancel the terms of this letter without prior notice. Be advised that IRB also reserves the right to inspect or audit your records if needed.

Sincerely,

Institutional Review Board
Middle Tennessee State University

Quick Links:

[Click here](#) for a detailed list of the post-approval responsibilities. More information on expedited procedures can be found [here](#).

APPENDIX C
CHECKLIST

Please read the following very carefully and indicate if you:

- Have had a significant traumatic brain injury
 - Have a neurological disorder
 - Are taking psychotropic medications, such as: Prozac (fluoxetine), Zoloft (sertraline), Klonopin (clonazepam), Abilify (aripiprazole), and others.
 - Are pregnant or nursing
-
- Eligible to participate.**
 - Not eligible to participate.**

Signature

Date

APPENDIX D

INFORMED CONSENT

Principal Investigator: Melissa Nickel

Study Title: L-theanine's Effects on Learning and Memory in College Students

Institution: Middle Tennessee State University

Name of participant: _____ Age: _____

The following information is provided to inform you about the research project and your participation in it. Please read this form carefully and feel free to ask any questions you may have about this study and the information given below. You will be given an opportunity to ask questions, and your questions will be answered. Also, you will be given a copy of this consent form.

Your participation in this research study is voluntary. You are also free to withdraw from this study at any time. In the event new information becomes available that may affect the risks or benefits associated with this research study or your willingness to participate in it, you will be notified so that you can make an informed decision whether or not to continue your participation in this study.

For additional information about giving consent or your rights as a participant in this study, please feel free to contact the MTSU Office of Compliance at (615) 494-8918.

1. Purpose of the study:

You are being asked to participate in a research study because we are interested in investigating how taking L-theanine affects memory and cognitive functioning.

2. Description of procedures to be followed and approximate duration of the study:

The study should take approximately 1.5 hours to complete. You will first be asked to complete a series of tests of memory and cognitive functioning, after which you will be given either 200mg of L-theanine or a placebo. After taking one of these, you will be asked to read a book for about 45 minutes. Following the 45 minutes, you will be asked to once again complete the tests of memory and cognitive functioning. You will also be asked to complete a questionnaire regarding your medical history.

3. Expected costs:

None.

4. Description of the discomforts, inconveniences, and/or risks that can be reasonably expected as a result of participation in this study:

Many studies have been conducted with L-theanine that have reported no adverse effects of this supplement, which is available over-the-counter in many vitamin shops.

The product label states that L-theanine is a dietary supplement that promotes relaxation and mood support. Consult your healthcare provider prior to use if you are pregnant, nursing, taking any medications or have any medical conditions.

The FDA has deemed L-theanine as, "generally recognized as safe". The FDA website says the following regarding the safety of L-theanine:

"A significant amount of information related to the safety of L-theanine is available for evaluation. In addition to a long history of safe consumption of L-theanine from its natural presence in tea, the safety of L-theanine has been the subject of a number of experimental studies in both animals and humans. The

evidence from historical safe consumption of L-theanine from tea and experimental studies together can be used to determine its safe use for human consumption.”

More information on L-theanine can be found on the FDA website:

<https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269524.pdf>

5. Compensation in case of study-related injury:

MTSU will not provide compensation in the case of study related injury.

6. Anticipated benefits from this study:

a) The potential benefits to science and humankind that may result from this study are that we may gain an increased understanding of the effects of taking L-theanine on memory and cognitive functioning.

b) The potential benefits to you from this study are that you will gain a better understanding of how research is conducted and you will earn credits for your course.

7. Alternative treatments available:

Not applicable.

8. Compensation for participation:

None.

9. Circumstances under which the Principal Investigator may withdraw you from study participation:

Noncompliance with the study procedures and failure to comply with instructions.

Also, you may be withdrawn if you have a history of any significant head injury, neurological illness, or are taking a psychotropic medication.

10. What happens if you choose to withdraw from study participation:

Participation in this study is strictly voluntary and there are no penalties for refusing to participate and there are no consequences from withdrawing from the study. The participants may choose to withdraw from the study at any point.

11. Contact Information.

If you should have any questions about this research study or possible injury, please feel free to contact **Melissa Nickel** at (586) 260-7153 or my Faculty Advisor, **Dr. Paul Foster** at 898-2007.

12. Confidentiality. All efforts, within reason, will be made to keep the personal information in your research record private but total privacy cannot be promised. Your information may be shared with MTSU or the government, such as the Middle Tennessee State University Institutional Review Board, Federal Government Office for Human Research Protections, if you or someone else is in danger or if we are required to do so by law.

13. STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS STUDY

I have read this informed consent document and the material contained in it has been explained to me verbally. I understand each part of the document, all my questions have been answered, and I freely and voluntarily choose to participate in this study.

Date

Signature of patient/volunteer

Consent obtained by:

Date

Signature

APPENDIX E
HISTORY QUESTIONNAIRE

Subject History and Demographics:

Subject Number:

Date of Birth:

Date of Study:

Sex:

Age:

Height:

Weight:

Handedness:

Education:

History of significant head injury (meaning loss of consciousness)? Y/N

If yes then explain. How long was the loss of consciousness?

History of neurological or psychological/psychiatric illness? Y/N

If yes then explain.

Currently taking psychotropic medications? Such as meds for depression or anxiety?

If yes then explain. What meds?