JOURNAL OF
EMERGING INVESTIGATORS

Article

Can Green Tea Alleviate the Effects of Stress Related to Learning and Long-Term Memory in the Great Pond Snail (Lymnaea stagnalis)?

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SUMMARY

Stress and anxiety have become more prevalent issues in recent years and teenagers are especially at high risk. Recent studies show that experiencing stress while learning can impair brain-cell communication, thus inhibiting learning. Green tea is believed to have the opposite effect, aiding in learning and memory retention. *Lymnaea stagnalis* is a pond snail with a simple nervous system and easily observable behaviors, making it an excellent model organism for human neurobiology. In this study, we used *L. stagnalis* to explore the relationship between green tea and a stressor that impairs memory formation to determine the effects of both green tea and stress on the snails' ability to learn, form, and retain memories. Memory was assessed using a conditioning procedure known as conditioned taste aversion (CTA), where snails are exposed to a sweet substance followed by a bitter taste with the number of biting responses being recorded. For the CTA groups, a total of 33 snails in 3 separate cohorts (Control (i.e. no stress), stressed, and stressed with green tea) was used in the trainings. Our results indicated that the best learning and memory occurred in the combination stressed with green tea group, specifically for long-term memory, although snails displayed good learning and memory during the short-term and intermediate trials. Stress was shown to be harmful to snail learning and memory for short-term, intermediate, and long-term memory. Thus, green tea not only alleviated the effects of stress, but also improved the snails' ability to learn and remember compared to their control counterparts (not stressed) group.

INTRODUCTION

In an ever more complex world, people are experiencing increased stress and anxiety. According to a 2017 survey conducted by The National Institute of Mental Health, over 40 million Americans were diagnosed with Generalized Anxiety Disorder (GAD), ranking GAD as 86% of all diagnosed mental health illnesses in the United States (1). The increase in stress and anxiety not only negatively affects adults but also the younger generation. In a 2014 American Psychological Association (APA) survey for stress, teens reported worse mental health and higher levels of anxiety and depression than all other age groups (2). In the annual survey, in 2013 teens first began to report higher levels of stress than adults – a trend that has since continued. In another survey, 61% of teens reported that they feel a lot of pressure to get good grades, 29% feel pressure to look good, 28% feel pressure to fit in socially, and 21% feel pressure to excel in sports (3). The pressures students face today have increased their anxiety levels and could potentially interfere with their ability to learn.

There are several natural remedies used to alleviate stress and anxiety. Green tea, made from the leaves of Camellia sinensis, has been consumed and applauded for its health benefits for thousands of years. It was explicitly used as a medicine during the Tang Dynasty (618-907 AD) in China (4). Recently, green tea was studied for its health benefits including prevention of cardiovascular disease, cancer, and cognitive dysfunction. It was shown that the consumption of green tea improved memory and attention in subjects with mild cognitive impairments (5). In a study at the University of Basel in Switzerland, researchers found that green tea extract enhances cognition in healthy subjects, specifically their working memory (6). Working memory, as defined by British cognitive psychologists Alan D. Baddeley and Graham J. Hitch, refers to "the short-term maintenance and manipulation of information necessary for performing complex cognitive tasks such as learning, reasoning, and comprehension".

Green tea contains flavonoids, a group of plant molecules believed to be responsible for the health benefits through cell signaling pathways and their antioxidant effects. It is made from leaves that have not undergone oxidation and therefore contains more antioxidants than other teas (7). The health benefits of green tea have been mainly attributed to a group of antioxidants called catechins. Catechins have previously been found to help improve memory and learning (8).

The Great Pond Snail, *Lymnaea stagnalis*, is a freshwater gastropod found in the Holarctic region (**Figure 1**). The snail possesses a N-methyl-D-aspartate (NMDA) receptor, which enhances the strength of synaptic connections in the nervous system and plays a vital role in memory and certain kinds of learning; this receptor is also found in the mammalian brain. Strengthening the synaptic connections due to activation of NMDA receptors is critical for learning in the pond snail as well as in humans (9). For this reason, the snail is widely used to research human learning and memory because of the similarity in nervous system structures and processes. Snails are also used because they are easy to train through conditioned learning, have easily observable behaviors linked to memory, and have large neurons. All these factors make the snail an excellent model organism to study neurobiology.

Audesirk et al. concluded that the best way to train *L. stagnalis* was by using Conditioned Taste Aversion (CTA) (10). CTA is an operant conditioning procedure achieved by exposing an organism to a sweet taste (conditioned stimulus), which evokes a feeding response, followed by a bitter taste (unconditional stimulus), which evokes a withdrawal response. The goal of CTA is to have the organism associate the two tastes, so that when presented with the sweet taste in the future, the snail will avoid it, remembering its training. Retention of a training behavior is considered memory. Short-term memory only lasts minutes, while intermediate memory lasts 1–2 hours, and long-term memory lasts longer than 18 hours.

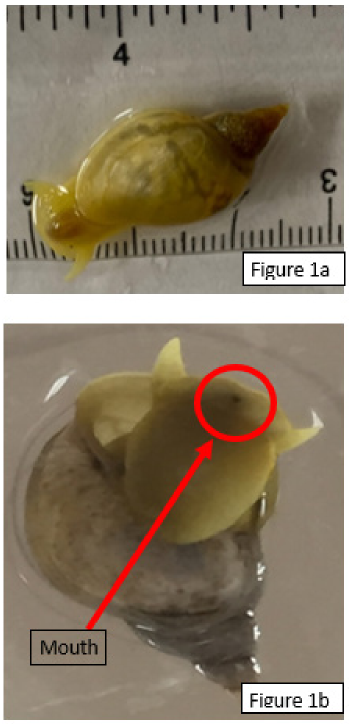

Figure 1: (a) Lymnaea stagnalis is approximately 2 cm in length with a very simple nervous system. (b) The underside of the snail observed by a mirror. The mouth is labeled to indicate how biting is observed to assess learning and memory.

Scientists have tested the effects of several memory-improving flavonoids and other substances to determine which chemical compounds could improve snail memory. A flavonoid called epicatechin, which is found in green tea, cocoa, red wine, and blueberries, was found to help improve memory and learning in *L. stagnalis* (11). The reason this specific flavonoid was studied was because medical researchers had discovered the many health benefits of green tea in humans, such as memory improvement. The beneficial impact on memory that green tea causes is most likely due to the flavonoids and phytochemicals improving the cognitive functions of the nervous system (12). A 2012 study tested the effect of chocolate, known to contain memory-improving flavonoids, on the memory of the snails (13). The results showed that the snails exposed to chocolate demonstrated better learning and memory than those not exposed.

Recent studies have also used pond snails to study stress and anxiety. Understanding how stress and anxiety affects snails may help researchers to better understand green tea's effect on humans. There are several factors that can cause enough stress to impact memory and learning in snails. In one study, scientists exposed snails to the predatory scent of crayfish. After the exposure, snails were unable to learn and form memories (14). A different study found that when pond water was depleted of calcium- essential for shell strength and rebuilding- stressed snails were unable to learn and form memories (15). A quick and simple way to stress snails is by overcrowding 15 to 20 individuals in 100 mL of pond water for 1 hour prior to training. Under these conditions, snails also lose their ability to learn and remember (16, 17). By training snails and observing their behaviors following exposure to stressful situations, it is evident that the stressful event resulted in some impairment of memory or prevented any memories from forming.

The significance behind training these snails and observing their learning and memory patterns is that the results from such studies can be related to human neurology. The purpose of this study was to determine if green tea could improve the memory of the snail, if stress interferes with learning and memory of the snail, and if green tea could alleviate the effects of stress on learning and memory in the snail. We hypothesized that green tea would improve learning and memory, stress would inhibit learning and memory, and green tea would be able to reverse the stress-induced learning and memory deficits in the snails.

RESULTS

In this study, memory was assessed using a conditioning procedure known as conditioned taste aversion (CTA), where snails are exposed to a sweet substance followed by a bitter taste with the number of biting responses being recorded. For the CTA groups, a total of 33 snails in 3 separate cohorts (Control (i.e. no stress), stressed, and stressed with green tea) was used in the trainings. Our results indicated that the best learning and memory occurred in the combination stressed with green tea group, specifically for long-term memory, although snails displayed good learning and memory during the short-term and intermediate trials. Stress was shown to be harmful to snail learning and memory for short-term, intermediate, and long-term memory. Thus, green tea not only alleviated the effects of stress, but also improved the snails' ability to learn and remember compared to their control counterparts (not stressed) group, suggesting green tea is effective in alleviating the effects of stress.

Effect of Green Tea on Learning and Memory

First, we studied the effect of green tea on short-term, intermediate-term, and long-term memory formation in *L. stagnalis*. We trained and compared two groups of 20 snails: one group trained only in pond water and the other group trained in a 1:4 ratio of green tea to pond water. One-way ANOVA analysis indicated for the pond water group that there was no significant difference among the groups ($F_{3,76}$ =

2.27, p = 0.0877). The ANOVA results for the green tea group indicated that a post-hoc Tukey Test should be conducted ($F_{3,76}$ = 76.87, p < 0.0001). The results from the post-hoc Tukey Test indicated that there was significant difference between the pre-test and 10-minute post-test (p = 0.001), the pre-test and 1 hour post-test (p = 0.001), and the pretest and 24 hour post-test (p = 0.001). We defined good learning and memory as the snails taking less bites during the post-test than the pre-test. We defined poor learning and memory as snails who experienced no change or an increase in number of bites. We found that 80% of the snails trained in pond water displayed good short-term (ST) memory, 55% displayed good intermediate (IM) memory, and only 45% displayed good long-term (LT) memory. We found that 100% of the snails trained in green tea displayed good ST memory, IM memory, LT memory **(Figure 2)**.

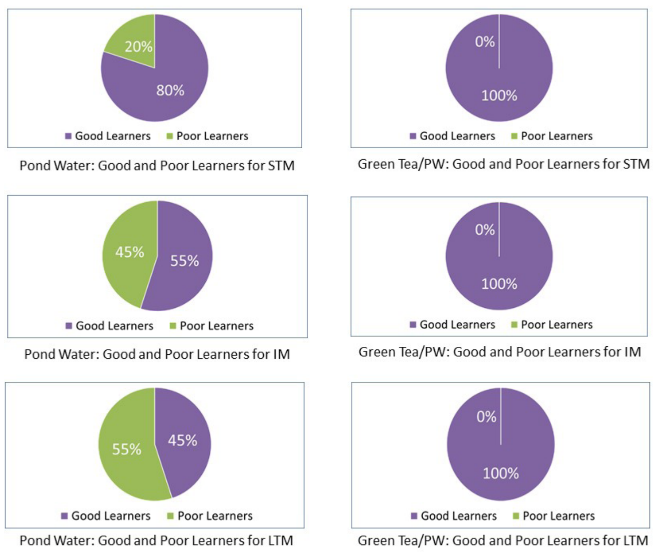

Figure 2: Effect of Green Tea on Learning and Memory. Percent of Good vs. Poor Learners of snail CTA training in pond water and pond water/green tea. The number of bites post CTA training for each snail group (n=15) was used to determine percent of good learners (snails that retained memory/showed a decrease in number of bites) and percent of poor learners (snails that didn't retain memory/showed an increase or no change in number of bites). All snails trained in pond water/green tea learned and retained the CTA training memory.

Effect of Stress and Stress with Green Tea on Learning and Memory

The mean number of bites for each test group was measured as a readout for stress **(Figure 3).** When the snails were first trained in pond water, most of them displayed good ST memory, but not LT **(Figure 3)**, which was expected since *L. stagnalis* does not naturally have good LT memory (15). However, after being stressed, most snails were not able to remember the training at all and took many bites during post-tests. The stressed snails exposed to green tea showed improved ST, IM, and LT memory, taking very few bites of sucrose in one minute.

Green Tea and Stress without CTA training

We conducted a one-way ANOVA on the number of bites in each trial for both the green tea group without CTA training and stress group without CTA training data. The ANOVA results for the green tea group indicate that there was no significant difference among the groups ($F_{3,56}$ = 0.717, p = 0.546). The ANOVA results for the stress group indicated that there was no significant difference among the groups ($F_{3,56}$ = 0.698, p = 0.557). The results from the green tea test and stress test indicate that neither green tea nor stress had a significant effect on the feeding response of the snails.

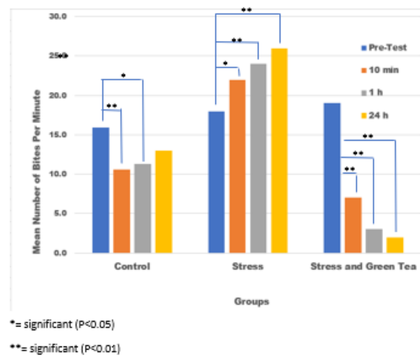

Figure 3: Effect of stress and stress with green tea on learning and memory. Mean number of bites for each group. Each cluster of bars represent a treatment group (n=33), while the smaller bars represent the individual trial [blue: pre-test, orange: 10 min (ST memory), grey: 1 h (IM memory), yellow: 24 h (LT memory)]. Asterisks highlight where the differences lied among the groups according to the Tukey Tests. These results show that stress negatively effected snail memory

Pond Water CTA results

We conducted a one-way ANOVA on the number of bites in the four trials in the pond water group **(Table 1).** The ANOVA results for the group indicated that there was no significant difference among the groups ($F_{3,128}$ = 4.57, p = 0.0045). In the pond water trials, there was a significant decrease in the number of bites for the 10-minute post-test as compared to the pre-test (p < 0.01, **Table 2**), indicating that these snails displayed excellent short-term memory. They retained that memory for the 1-hour post-test (p < 0.05, **Table 2)**, but displayed an increase in the number of bites during the 24-hour post-test, indicating a gradual loss of memory **(Figure 3)**. Seventy-six percent of the snails displayed good ST memory, sixty-nine percent displayed good IM memory, and only fifty-five percent displayed good LT memory.

Group	p-value	F-value
Pond Water	0.0045	F(3,128) = 4.57
Stress	9.50E-07	F(3,128) = 11.55
Stress and Tea	1.11E-16	F(3,128) = 122.24

Table1: ANOVA *p*-values and *f*-values. One-way ANOVA test results comparing the pond water, stressed, and combination stress with green tea water groups. The results indicate that a significant difference exists within each of the three groups.

Stress CTA results

We conducted a one-way ANOVA on the CTA training with stress group **(Table 1)**. The ANOVA results for the data indicated that there was a significant difference among the groups and that a post-hoc Tukey Test should be conducted ($F_{3,128}$ = 11.55, p < 0.0001). The results from the post-hoc Tukey Test **(Table 2)** indicated that there was significant difference between the pre-test and 10-minute post-test (p = 0.05), between the pre-test and 1-hour post-test (p = 0.001), and between the pre-test and 24-hour post-test (p = 0.001). Eighteen percent of the snails displayed good ST memory, eighteen percent of the snails displayed good IM memory, and only three percent of the snails displayed good LT memory. Stress was detrimental to the snails' learning and memory, as the mean number of bites post-training showed a statistically significant increase **(Figure 3)**.

Comparison	Pond Water	Stress	Green Tea
Pre-Test vs 10 min	0.0051**	0.0143*	0.0010**
Pre-Test vs 1 h	0.0190*	0.0010**	0.0010**
Pre-Test vs 24 h	0.2556 (ns)	0.0010**	0.0010**
10 min vs 1 h	0.0899 (ns)	0.6938 (ns)	0.0034 (ns)
10 vs 24 h	0.4196 (ns)	0.0456*	0.0010**
1 h vs 24 h	0.6637 (ns)	0.3929 (ns)	0.6688 (ns)

Table 2: Tukey Pairwise Comparisons. Tukey Test results comparing the pond water, stressed, and combination stress with green tea water groups. We used Post-hoc Tukey Test results to generate pairwise comparisons to determine the significant differences in the trials. *= p<0.05; **= p<0.01; ns= nonsignificant.

Green Tea/Stress CTA results

We conducted a one-way ANOVA on the number of bites in green tea post stress group. The ANOVA results for the data indicated that there was a significant difference among the groups and that a post-hoc Tukey Test should be conducted ($F_{3,128}$ = 122.24, p < 0.0001). The results from the post-hoc Tukey Test indicated that there was significant difference between the number of bites taken pre-test and 10-minute post-test (p = 0.001), the pre-test and 1-hour post-test (p = 0.001), and the pre-test and 24-hour post-test (p = 0.001). Ninety-seven percent of the snails displayed good ST memory, ninety-seven percent of the snails displayed good IM memory, and one-hundred percent of the snails displayed good LT memory. The mean number of bites post training had a statistically significant decrease indicating that the training was successful **(Figure 3)**.

DISCUSSION

The recent increase in stress and anxiety levels and our attempts to alleviate and remedy with the use of ancient holistic herbs and medicines, such as green tea, led us to investigate whether green tea can alleviate the negative impact stress has on learning and memory formation. The simple nervous system of *L. stagnalis*, combined with the ease of training and observing learned behaviors, make *L. stagnalis* an excellent model organism to study neurobiology. The purpose of this study was to determine if green tea could improve the memory of the snail, if stress interferes with learning and memory of the snail, and if green tea could alleviate the effects of stress on learning and memory in the snail.

From the results of our first experiment examining the effect of green tea on memory and learning, we concluded that green tea was effective in enhancing learning and memory formation in *L. stagnalis*. Once that was determined, our second experiment examined if green tea would be able to reverse the effects of stress on learning and memory. Before any CTA training, the effects stress and green tea would have on the snails' appetite needed to be determined. The stress and green tea controls were conducted to ensure two factors — that green tea did not act as an appetite suppressant and that exposure to stress did not cause the snails to 'stress-eat'. The stress and green tea controls indicated that there was no measurable effect of green tea or stress on snail appetite. The results here indicate that green tea significantly improved the snails' ability to retain training. Therefore, the main goal was to determine how stress would impact learning and memory and if green tea would be able to alleviate the effects of stress. The pond water group in our second experiment confirmed that the one-trial training was effective, and the snails could be conditioned. The goal of the stress CTA trials was to confirm that stress would affect the snails' ability to learn and remember the CTA. Since the snails displayed an increase in number of bites post-training, stress appeared to have interfered with the snails' ability to learn and remember. The results of the stressed snails exposed to green tea indicate that green tea was able to alleviate the effects of stress and reverse the stress-imposed learning/memory deficit in the snails. All the snails were good learners in the stress with green tea trials with the greatest improvement in their long-term memory. These results indicate that green tea can reverse the effects of stress on learning and memory. To counter the effects of stress, consumption of green tea could reverse stress-induced learning or memory deficits.

The data supports the hypothesis that not only does green tea improve learning and memory, but it can also alleviate the detrimental effects of stress on learning and memory in *L. stagnalis*. The greatest improvement, however, was seen in the long-term memory, suggesting that green tea has the strongest effects on long-term memory. Since the neurobehavioral processes in *L. stagnalis* have been shown to be like those in humans (10), the conclusions from our research can be applied to human neurology. Green tea may have the greatest effects on human long-term memory instead of short-term. In this study, stress was shown to negatively impact learning and memory in *L. stagnalis*, while green tea seemed to reverse that effect and aid in learning and memory. Since *L. stagnalis* is widely used in neuroscience research, we believe that they can be especially helpful in research on learning and memory formation.

Besides the green tea, stress also played a major factor in the findings of this study. Stress caused the snails to take a dramatic increase in the number of bites and a high percentage of stressed snails were poor learners, as they showed an increase in number of bite post-training. Stress was detrimental to the snails' learning and memory. This supports the theories that stress is very harmful to human learning and memory. High stress levels in adolescents have a direct correlation with lower grades and poor learning and memory, so much so that it can lead to memory and learning deficits if stress levels are kept high (3). To decrease stress levels and reverse the learning/memory deficit, green tea was extremely effective. Green tea is a natural way to reduce stress levels and prevent deficits. A natural way to improve memory has been something scientists have been searching for and based on our results green tea shows the ability to increase learning and memory in snails under stress.

MATERIALS AND METHODS

Care and Maintenance of *L. stagnalis*

L. stagnalis snails (obtained from Patsy Dickinson and Stephan Hauptman, Bowdoin College) were kept in a 10-gallon tank; with 50% of the water being Poland Springs water and 50% pond water (1.8 g of Instant Ocean/1 gallon of deionized water). The water was replaced every week and the waste was taken out of the tank every other day. Snails were fed organic romaine lettuce three times a week. The tank temperature was maintained between 18° and 22°C. To maintain calcium levels, oyster shells were crushed and added to the water. The average size of the snails was 2 cm. The age of the snails cannot be determined due to varying lab conditions affecting the rate of growth.

Conditioned Taste Aversion (CTA)

Snails were deprived of food for 24 hours prior to training. This was important because during training, the number of bites the snail took was being measured, so food deprivation ensured that the snails were hungry and would take bites of the solution. As it is known that *L. stagnalis* feeding behaviors undergo both appetitive and aversive classical conditioning (10), the number of bites taken per minute was chosen as an indicator of memory formation.

The first step of CTA was the pre-test. During the pre-test, the snail was placed in a petri dish elevated above a mirror (used to observe the snail bites). Next, the snail was exposed to 5 mL of a 0.5% sucrose solution. A timer was set for one minute, and the number of bites of sucrose the snail took was recorded. Counting the number of bites is an easily observable technique used in previous studies (16). The contents of the dish were then emptied, and the pond water was replenished **(Figure 4)**.

Ten minutes after the pre-test, the snail was exposed to the sucrose solution again for only 15 seconds. After the 15 seconds, the contents of the dish were emptied, and the pond water was replenished. Five seconds later, the snail was exposed to 5 mL of a 0.5 % bitter KCl solution. The KCl was left in the petri dish for 15 seconds, then the contents of the dish were emptied, and the pond water was replenished.

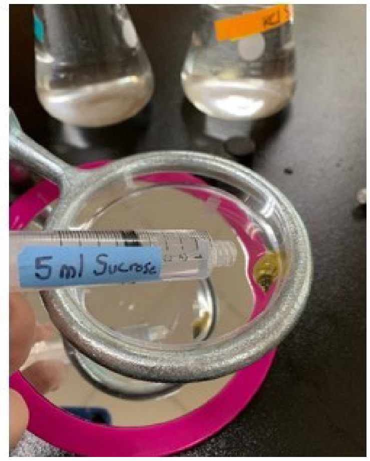

Figure 4: CTA used for all experiments. The conditioned taste aversion procedure (first step). The snail was given 5 mL of a 0.5% sucrose solution (conditional stimulus; shown) followed by 5 mL of a 0.5% KCl solution (unconditional stimulus; not shown). If snails demonstrated good learning, they would avoid biting the sucrose. However, if the snails were poor learners, they would not show any aversion to the sucrose.

Ten minutes later, the first post-test was conducted. The procedure of the post-test was identical to that of the pre-test. For the 10-minute post-test, the snail was exposed to the 5 mL of sucrose solution again. A timer was set for one minute and the number of bites was observed and recorded using a mirror. Two more post-tests were conducted to reach a total of three post-tests per snail. The three post-tests were conducted 10 minutes, 1 hour, and 24 hours after conditioning.

Green Tea Control and Green Tea CTA

In order to ensure that the green tea had no effect on the snails' appetite, 15 snails were deprived of food for 24 hours and then exposed to 5 mL of 0.5% sucrose solution. A timer was set for one minute, and the number of bites of sucrose the snail took was recorded. Then, the contents of the dish were emptied, and the pond water was replenished.

The snails were then placed in a 1:4 dilution of green tea to pond water and once again exposed to 5 mL sucrose solution with the number of bites taken recorded for 1 minute. This was followed by the 10-minute, 1-hour, and 24-hour post-tests. This specific concentration of green tea was previously used and found to be effective (14).

Thirty-three snails underwent CTA training in green tea to determine the effect of green tea on memory and learning. The CTA in green tea training followed the same procedure as above, apart from the snails being placed in a 1:4 dilution of green tea to pond water during the training. Lipton decaffeinated green tea was used to ensure that the caffeine itself did not affect the snails' appetite. There is no evidence that the other components of green tea affect appetite.

Stress Control and Stress CTA Procedure

To determine if stress influenced the snails' appetite, 15 snails were exposed to 5 mL of 0.5% sucrose solution. A timer was set for one minute and the number of bites of sucrose the snail took was recorded, then the contents of the dish were emptied, and the pond water was replenished. The snails were then stressed by crowding 15–20 snails in 100 mL of pond water for 1 hour **(Figure 5)** and then once again exposed to 5 mL of 0.5% sucrose solution with the number of bites taken recorded for 1 minute.

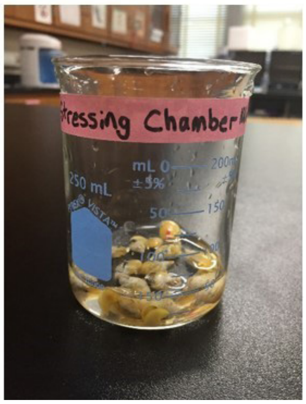

Figure 5: Stressing Chamber used for all experiments. Snails were stressed prior to training by overcrowding 20 snails in a beaker with only 100 mL of pond water for 1 hour.

Prior to any further tests, in order to ensure that the snails used in the trials did not retain any memory of a previous CTA training, the snail's memory was assessed in a pre-test in addition to allowing 3–5 days to pass before any additional trials. If a snail had a lower number of bites/minute as compared to previous pre-test results, then they would not be used that day. After the pre-test, the snails were stressed. To stress the snails, 15– 20 of them were crowded in 100 mL of pond water in a beaker for 1 hour **(Figure 5).** After stressing for one hour, the snails were trained using the CTA procedure, omitting the pre-test, since it was conducted prior to stressing the snails. Once again, post-tests were conducted 10 minutes, 1 hour, and 24 hours after conditioning.

Stress and Green Tea Procedure

After the snails were no longer stressed and forgot their conditioning once more (3–5 days later), they were given another pre-test. This pre-test was crucial since it once again evaluated if the snails had forgotten their training or not. Snails that had not forgotten previous training were placed back into their tank and another snail was chosen. Ten minutes after the pre-test, the snails were stressed using the stressing procedure above. After being stressed, the snails were trained using the CTA procedure, but instead of being trained in pond water, they were trained in a 1:4 dilution of green tea to pond water. After being trained in the green tea, the normal post-tests followed. Between 15–20 snails had to be in the stressing chamber to induce stress by crowding. Since 20 snails could not be trained all at once, some snails were used as placeholders.

Statistical Analysis

Data are expressed as the mean number of bites per minute. Statistical significance ($p < 0.05$) was determined using a one-way analysis of variance test (ANOVA) to determine if there was a significant difference the two treatment groups and the control group. Once it was determined that there was a significant difference, a Tukey Pairwise Comparison was used to determine where the differences lie among the groups.

ACKNOWLEDGEMENTS

We would like to thank Mary Simons, Richard Kurtz, Dr. Ken Lukowiak, and the JEI editors for their mentorship and guidance through the duration of this process. Their contributions were greatly appreciated.

Received: August 21, 2020
Accepted: November 5, 2020
Published: January 30, 2021

REFERENCES

1. "Mental Illness." *The National Institute of Mental Health*, 2017. nimh.nih.gov/health/statistics/mental-illness.shtml
2. Bethune, S. "Teen Stress Rivals That of Adults." *American Psychological Association,* vol. 4, no. 4, 2014. doi: 10.1037/e508692014-007.
3. Graf, N. and Horowitz, J. "Most U.S. teens see anxiety and depression as a major problem among their peers." *Pew Research Center*, 2019. www.pewsocialtrends.org/2019/02/20/most-u-s-teens-see-anxiety-and-depression-as-a-major-problem-among-their-peers/
4. Yang, Chung S., et al. "Recent Scientific Studies of a Traditional Chinese Medicine, Tea, on Prevention of Chronic Diseases." *Journal of Traditional and Complementary Medicine*, vol. 4, no. 1, 2014, pp. 17–23., doi:10.4103/2225-4110.124326.
5. Deka, A. and Vita, J. "Tea and cardiovascular disease." *Pharmacological Research*, 2011. doi: 10.1016/j.phrs.2011.03.009
6. Schmidt, A., et al. "Green tea extract enhances parieto-frontal connectivity during working memory processing." *Psychopharmacology*, 2014. doi: 10.1007/s00213-014-3526-1
7. "What are the health benefits of green tea?" Medical News Today, 2017.
8. Gottumukkala, R., Nadimpalli, N., Sukala, K., Subbaraju, G. "Determination of catechin and epicatechin content in chocolates by high-performance liquid chromatography." *International Scholarly Research Notices*, 2014. doi: 10.1155/2014/628196
9. Mayford, M., Siegelbaum, S., Kandel, E. "Synapses and

memory storage." *Cold Spring Harbor Perspectives in Biology,* 2012. doi: 10.1101/cshperspect.a005751

10. Audesirk, G., Audesirk, T., Alexander, J. "One-trial reward learning in the snail *Lymnaea stagnalis*." *The Journal of Neurobiology*, vol. 15, 1984, pp. 67-72. doi: 10.1002/neu.480150107

11. Knezevic, B., Kotamatsuzaki, Y., Freitas, E., Lukowiak, K. "A flavonoid component of chocolate quickly reverses an imposed memory deficit." *The Company of Biologists*, vol. 219, 2016, pp. 816-823. doi: 10.1242/jeb.130765

12. Whiteman, H. "Green tea may boost working memory." *Medical News Today*, 2017

13. Fruson, L., Dalesman, S., Lukowiak, K. "A flavanol present in cocoa [(-)epicatechin] enhances snail memory." *The Journal of Experimental Biology*, vol 215, 2012, pp. 3566-3576. doi: 10.12421/jeb.070300

14. Hughes, E., Shymansky, T., Swinton, E., Lukowiak, K., Swinton, C., Sunada, H., et al. "Strain-specific differences of the effects of stress on memory in *Lymnaea*." *The Company of Biologists*, vol. 220, 2017, pp. 891-899. doi: 10.1242/jeb.149161

15. Lukowiak, K., Sunada, H., Teskey, M., Dalesman, S. "Environmentally relevant stressors alter memory formation in the pond snail *Lymnaea*." *The Journal of Experimental Biology,* vol. 217, 2014, pp. 76-83. doi: 10.1242/jeb.089441

16. Dalesman, S. and Lukowiak, K. "How stress alters memory in 'smart' snails" PLoS ONE, 2012. doi:10.1371/journal.pone.0032334

17. Dalesman, S. and Sunada, H. "Combining stressors that individually impede long-term memory." *PLoS ONE*, vol. 8, 2013. doi: 10.1371/journal.pone.0079561