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The Effects of 5-HTP and L-Theanine on Behavioral Stress Responses in *Drosophila melanogaster*

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ABSTRACT

Millions of people are impacted by anxiety disorders; however, there is still limited scientific evidence and research regarding numerous over-the-counter supplements claiming they are effective for anxiety. Fruit flies, *Drosophila melanogaster*, share more than 60 percent of their disease-related genes with humans. Therefore, fruit flies can be used as an effective animal model for evaluating the behavioral aspects of stress reactions.

A critical gap in the existing literature is the lack of controlled, dose-dependent comparative evaluations of multiple dietary supplements on stress-related behavioral responses within a single experimental framework. This study investigated how varying dosages (2, 2.5, 3 ppm) of 5-HTP (5-hydroxytryptophan) and L-theanine (an amino acid naturally found in tea leaves) affected locomotor activity, thigmotaxis, and light startle response in *Drosophila melanogaster*. Six treatment groups and one control group (only received *Drosophila* food and water) were used to test the dietary supplements, 5-HTP and L-theanine, at three different concentrations.

Results showed clear dose-dependent effects from both the 5-HTP and L-theanine. As concentration increased, locomotor activity and light-startle response both decreased whereas thigmotaxis increased for both dietary supplements. However, L-theanine produced stronger effects than 5-HTP. The results support the use of *Drosophila* as a model organism for investigating the behavioral effects of both dietary supplements and provide a foundation for future research on stress-related neuromodulation.

INTRODUCTION

Anxiety is one of the most common mental health conditions worldwide, affecting approximately 4-5% of the global population (Kopcalic et al., 2025). Despite the widespread use of over-the-counter supplements for anxiety, there is limited controlled research that exists regarding their safety and effectiveness. When treatment comes with risks like these, it raises an important question: are there safer alternatives? That question is what led to the research on 5-HTP and L-theanine.

A critical gap in the existing literature is the lack of controlled, dose-dependent comparative analyses of multiple dietary supplements on stress-related behavioral responses within a single experimental framework. Furthermore, physiological and behavioral indicators of stress in fruit flies are not consistently defined across studies.

The potential mechanism of action for each compound will be needed to better understand what causes the changes observed in the subjects' stress-related behavior. 5-HTP acts as a direct biochemical precursor to serotonin, a neurotransmitter that has been shown to have an association with mood regulation, emotional stability, and anxiolytic properties (Nakamura & Hasegawa, 2009). L-theanine exerts its effects through increasing GABA activity, which can promote relaxation. It has also been documented that increased levels of serotonin are associated with decreases in stress and improvements in behavioral responses. Although both supplements cause a calming effect, the differences in the pathways they use to achieve this effect demonstrate that they will affect behavior in different ways.

Prior research has produced inconsistent findings regarding the magnitude of stress-reducing effects associated with these compounds, particularly when comparing results across different study designs and models. This inconsistency is one of the primary limitations of the existing body of literature. Many studies evaluate the effectiveness of individual supplements but do not evaluate those supplements as part of a multi-supplement evaluation. As such, it remains ambiguous whether or not one supplement is more effective than another, nor was there substantial information available regarding when a particular supplement would be most helpful. Therefore, an area of research where a large gap currently exists was the controlled, dose-dependent evaluations of multiple dietary supplements for stress-related behaviors in a single experimental design. A goal of this study was to address the gap in research by directly comparing the anxiolytic effects of 5-HTP and L-theanine using similar methodologies and assessments of stress-related behaviors so that a more precise understanding can be obtained of their relative anxiolytic efficacies.

These differences are important to consider when evaluating the effects of behavioral outcomes because they show that reductions in stress related responses may not result from similar physiological pathways. However, the scientific literature regarding dietary supplements and anxiety is inconsistent. There is evidence that certain dietary supplements, including 5-HTP and L-theanine (which is naturally found in teas and some mushrooms), can effectively reduce symptoms of stress and anxiety; however, other studies found no significant effects of either supplement on reducing symptoms of stress and anxiety (Hidese et al., 2019). Studies examining the efficacy of dietary supplements are typically conducted using animal models. Because animal models do not experience the placebo effect and environmental factors that exist in human clinical trials, researchers feel that results obtained using animal models will be less variable.

However, few if any studies have examined the effects of multiple dietary supplements at the same time. As such, there is a need for controlled experimental designs to evaluate the effects of dietary supplements on anxiety. One type of organism commonly used as a model to examine anxiety-like behavior is the fruit fly. The advantages of using *Drosophila* include the ability to control the environment, manipulate dosages of a given substance, and produce highly consistent and reproducible results. Despite extensive

research on the genetic similarity between *Drosophila* and humans, there is still much to learn about how dietary supplements influence stress-like behavior in a controlled fashion. Specifically, very little research exists that examines more than one dietary supplement simultaneously and evaluates those supplements using multiple measures of stress-like behavior (i.e., locomotor activity, thigmotaxis, startle response). Finally, even though standard behavioral markers for measuring stress in *Drosophila* exist, there appears to be little consistency among studies regarding the application of these standards. Therefore, comparisons among studies that utilize *Drosophila* as a model cannot be made easily due to inconsistencies in methodology. This study attempted to address these issues by providing a comparative analysis of two widely used dietary supplements utilizing a controlled dose-response design and multiple behavioral measurements for assessing stress-like behavior.

Behavioral responses to dietary supplements in *Drosophila* provide insight into how neuromodulatory compounds influence stress-related behaviors in a controlled model system. 5-HTP and L-theanine were selected because they are commonly used OTC (Over-the-counter) supplements for stress, but have not been thoroughly tested in controlled, dose-dependent studies. As they work through different neurotransmitters, they provide a useful comparison for behavioral effects. Based on the literature review, this study hypothesized that increasing concentrations of 5-HTP or L-theanine would decrease locomotor activity and light startle response while increasing thigmotaxis in *Drosophila melanogaster*.

METHODS

The research question was investigated through a controlled experimental design using *Drosophila melanogaster* (fruit flies). There were 6 testing groups and 1 control group, which were tested for both supplements for anxiety (5-HTP and L-theanine) and 3 different dosages (2, 2.5, and 3 ppm). The control group received a cotton ball soaked solely in water and food. Three concentrations (2, 2.5, and 3 ppm) were tested for both 5-HTP and L-theanine.

Behavioral metrics in this research were selected based on their demonstrated utility in serving as indicators of stress/anxiety-like behaviors in animal models. Locomotion reflects total energy output and responsiveness, whereas decreased locomotion has been linked to sedative/calming effects. Thigmotaxis behavior is generally viewed as indicative of anxiety-related responses (Langen et al., 2011). The light-startle response assesses the level of neural responsiveness to outside stimuli and can be useful for assessing the extent to which subjects respond differently when exposed to differing stimulus conditions. The light startle response was scored on a scale from 1 to 5, where 1 indicated no response and a 5 indicated a maximum startle response. These three measures together provide a complete assessment of stress-related behaviors.

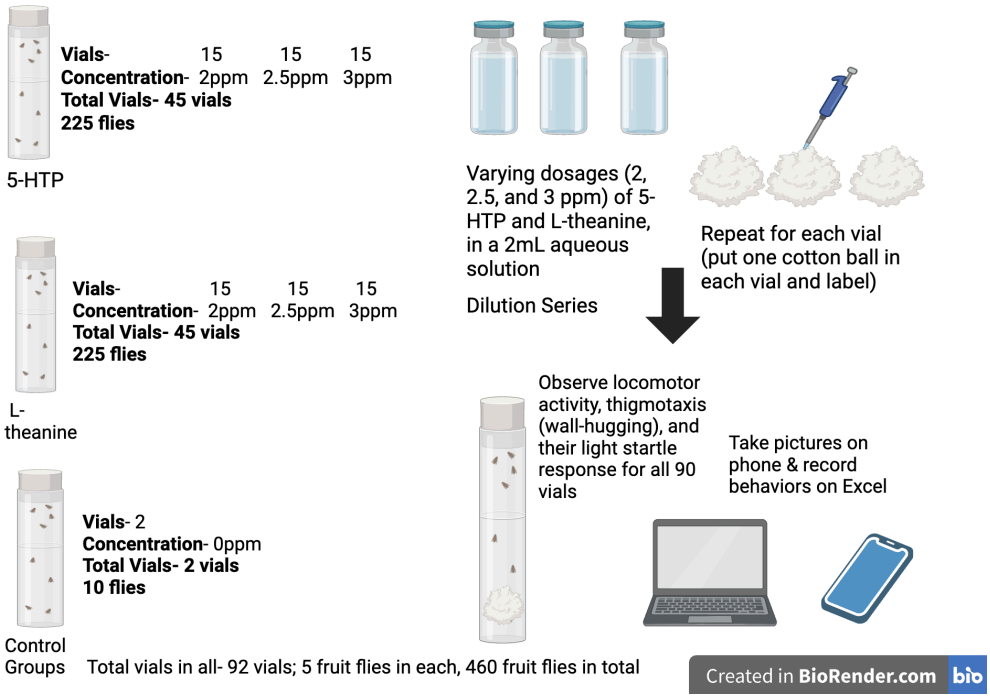
The 2 ppm, 2.5 ppm, and 3 ppm concentrations selected for this experiment range from low to moderate concentrations. Consequently, if any dose-dependent changes occur in behavioral responses within this range, they should be detectable. Moreover, the fact that all dosages were below known toxic ranges made it possible to observe trends in behavioral responses as dosage increased. Finally, using multiple vials per

treatment group and repeating trials would enhance the reliability of data collected by minimizing the impact of outlier values and individual variability.

The sample size included six tested groups representing two supplements tested at three concentrations, along with one control group. There were 5 fruit flies (Bronnec & Alexeyev, 2022) in each vial. Fifteen vials were used for each concentration, resulting in 92 vials and 460 total fruit flies. This sample size was selected to ensure sufficient statistical reliability and to minimize the influence of individual variability on observed outcomes.

Data were recorded for the flies' behaviors (locomotor activity, thigmotaxis, and their light startle response), recorded using a trail and video camera. Statistical analyses were performed using SPSS and graphs were created using Microsoft Excel. To conclude the experiment, the ethical and safe disposal of the *Drosophila melanogaster* was executed according to safe laboratory protocols. The *Drosophila melanogaster* were euthanized by freezing, and then they were put into the autoclave for sterilization. The sterilized contents were transferred to a waste container. All trials were done under the same environment, placed in a cabinet then examined after 2 days. The researcher was aware of treatment assignments during behavioral observations. Therefore, observer bias cannot be completely excluded.

Figure 1



Experimental Design. Data were collected by recording locomotor activity, thigmotaxis, and light startle response. All of the behavioral responses were analyzed to calculate mean and standard errors of each behavioral response at concentrations ranging from 2.0 to 3.0 ppm. Furthermore, tables and graphs were

made for the mean and standard errors of each behavioral response in Excel. Data trends were analyzed and interpreted.
(BioRender, 2025)

RESULTS

Figure 2
5-HTP and L-theanine effects on *Drosophila melanogaster*'s Locomotor Activity (Movement/Min)

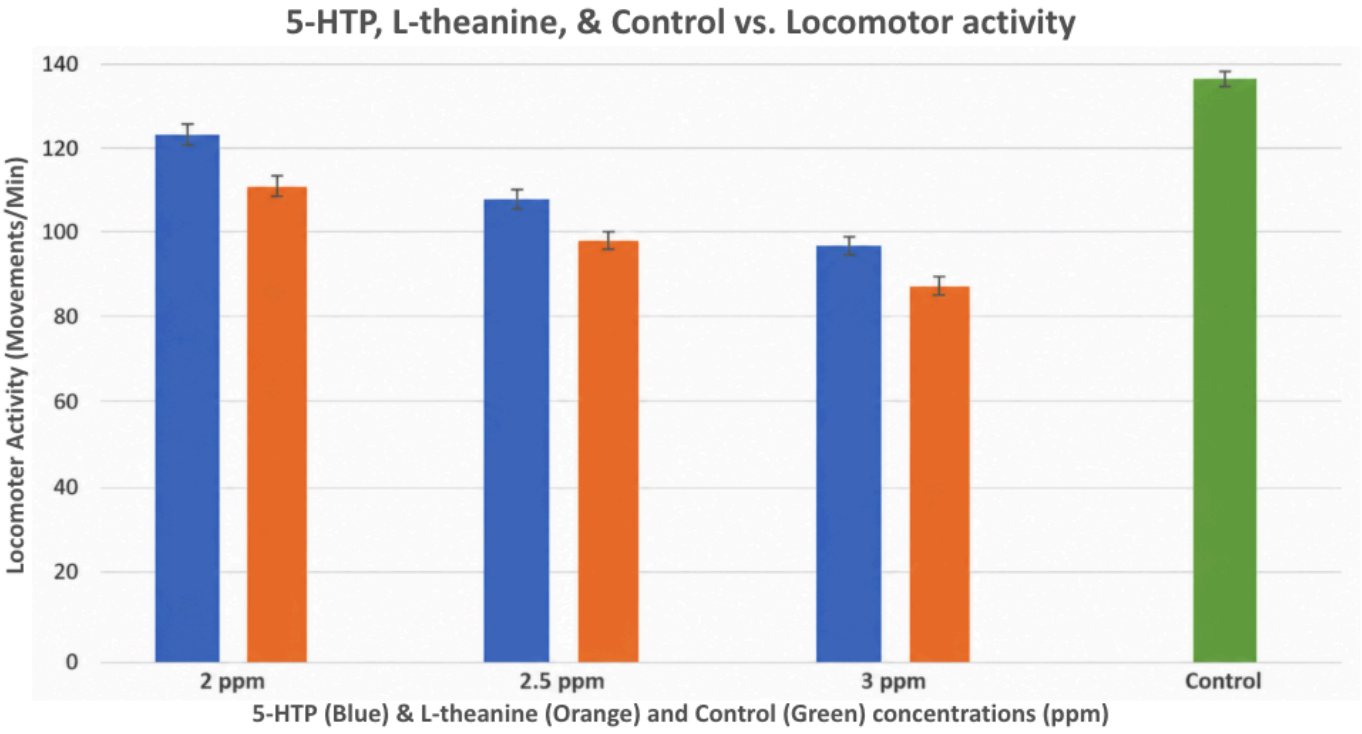


Figure 3
5-HTP and L-theanine effects on *Drosophila melanogaster*'s Wall Time (%)

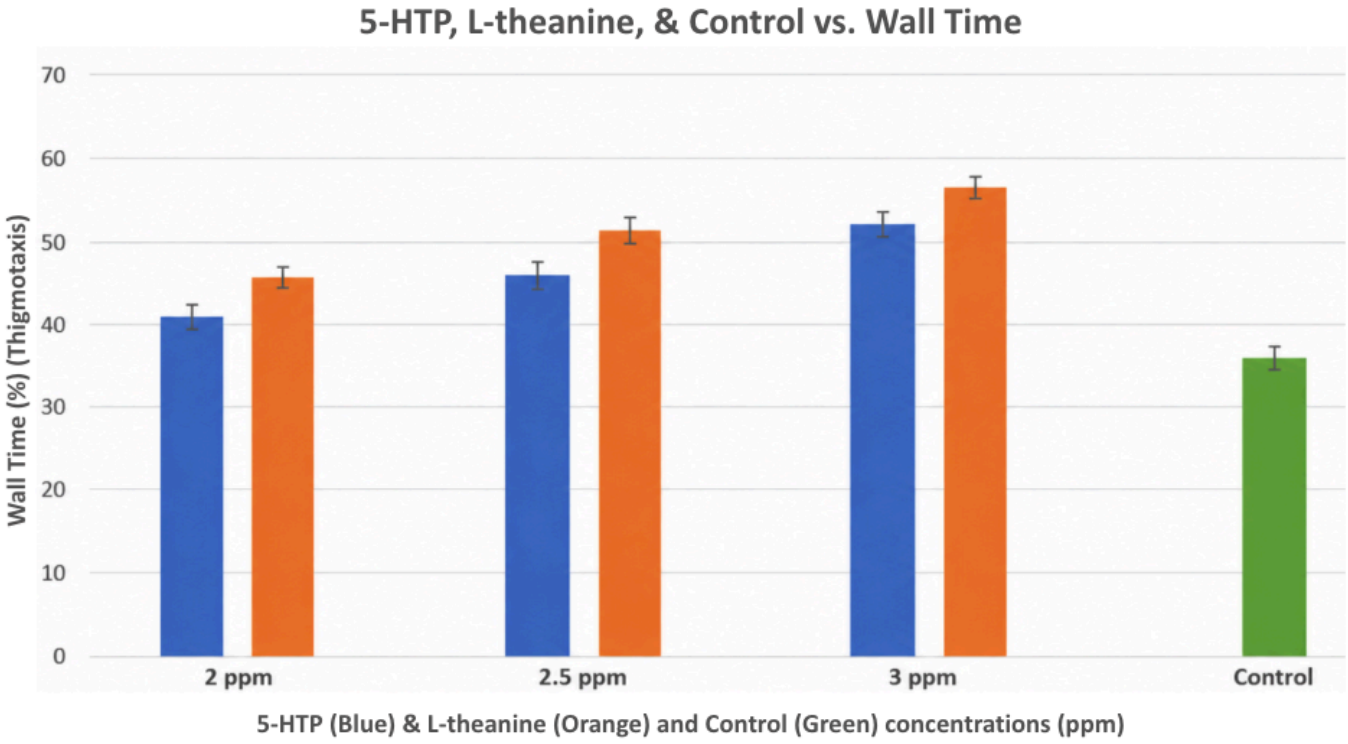


Figure 4
5-HTP and L-theanine effects on *Drosophila melanogaster*'s Light Startle Response (score out of 5)

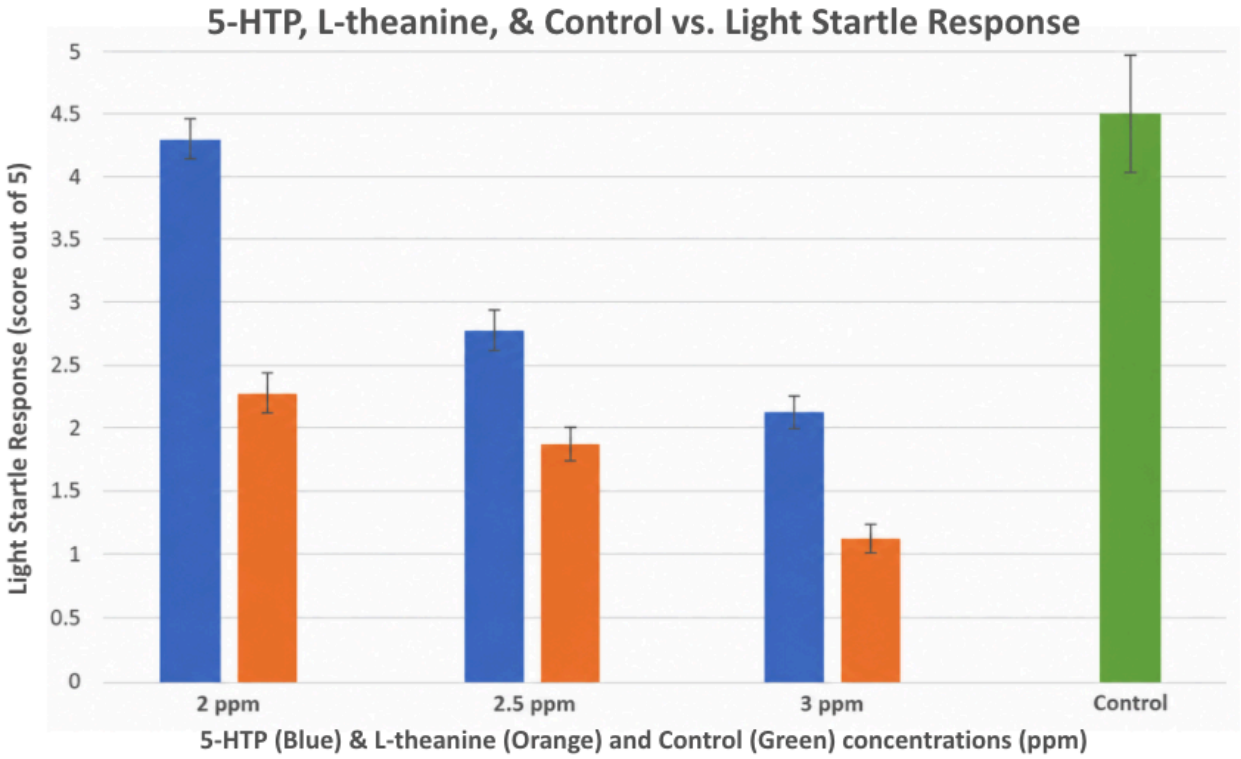


Table 1
Means and Standard Errors for Locomotor Activity, Wall Time, and Light Startle Response for 5-HTP and L-theanine from 2 ppm-3 ppm

Dietary Supplement	Concentration	Mean: Locomotor Activity	Standard Error: Locomotor Activity	Mean: Thigmotaxis	Standard Error: Thigmotaxis	Mean: Light Startle Response	Standard Error: Startle Response
Control	0	137	1	36.5	0.5	4.5	0.5
5-HTP	2	123.13	1.183	41.33	0.444	4.33	0.126
	2.5	108.6	0.716	46.33	0.909	2.8	0.107
	3	96.67	0.887	52.6	0.4	2.13	0.091
L-theanine	2	112.27	0.358	46.4	0.349	2.27	0.118
	2.5	98.67	0.843	51.4	0.388	1.87	0.091
	3	88.27	0.511	57.27	0.33	1.13	0.091

Note. Concentrations are in ppm (parts per million). Locomotor Activity is the movement of a fruit fly from one place to another (in Movements/Min), Thigmotaxis is "wall-hugging" behavior, staying at the edge or near the center of the arena (% Wall Time), and Light Startle Response is rapid, instinctual startle response to sudden decreases in light (score out of 5).

The results of this experiment indicated above show that there is a relationship in *Drosophila melanogaster* between concentration of L-theanine or 5-HTP administered, and behavioral response. Furthermore, L-theanine produced stronger effects than 5-HTP across all behavioral assessments at equivalent concentrations. For instance, locomotor activity decreased from 137 in the control group compared to 96.67 at 3 ppm in 5-HTP, while L-theanine decreased to 88.27 at 3 ppm. Similarly, light startle response decreased to 2.13 in 5-HTP at 3 ppm and 1.13 in L-theanine at 3 ppm.

Locomotor activity decreased as concentration increased for both 5-HTP and L-theanine. As shown in the above table 1, there was a dose-response effect for levels of locomotion as the negative slope in the graph indicated dose-dependent decrease. For 5-HTP, locomotor activity decreased from 123.13 at 2 ppm, to 108.6 at 2.5 ppm, and subsequently to 96.67 at 3 ppm. For L-theanine, activity decreased from 112.27 at 2 ppm to 98.67 at 2.5 ppm and then to 88.27 at 3 ppm. L-theanine produced lower locomotor activity (in movements per minute) than 5-HTP at all concentrations. Additionally, SPSS analysis (mean and standard error) confirmed a clear dose-dependent decrease in locomotor activity with low standard error values indicating consistent results across all trials.

Thigmotaxis (wall time) increased as concentration increased for both supplements (dose-dependent increase) as shown in table 1. For 5-HTP, wall time increased from 41.33% at 2 ppm to 46.33% at 2.5 ppm and then to 52.6% at 3 ppm. For L-theanine, wall time increased from 46.4% at 2 ppm to 51.4% at 2.5 ppm and then to 57.27% at 3 ppm. At all concentrations, L-theanine consistently produced higher wall time values than 5-HTP. SPSS analysis for mean and standard error confirmed that the results had a clear dose-dependent increase, along with low standard error values that indicated low statistical variability. The bar graph further supported this pattern, showing increasing wall time with higher concentrations (upward trend).

Light startle response decreased as concentration increased for both compounds (dose-dependent). For 5-HTP, response values decreased from 4.33 at 2 ppm to 2.8 at 2.5 ppm and then to 2.13 at 3 ppm. For L-theanine, response values decreased from 2.27 at 2 ppm to 1.87 at 2.5 ppm and then to 1.13 at 3 ppm. L-theanine consistently produced lower startle response values at all concentrations. SPSS analysis confirmed a dose-dependent decrease with low standard error values indicating reliable results. The graph supports this trend showing decreased responsiveness with increasing dosage.

Across all three behavioral measures, both supplements displayed consistent dose-dependent effects. Locomotor activity and light startle response decreased while thigmotaxis increased as concentration increased. Standard error values remained low across all trials indicating consistency in the data. L-theanine demonstrated greater overall changes than 5-HTP across all behavioral measures at all concentrations.

LIMITATIONS

The short lifespan of fruit flies limited the duration of observations. This meant that it was not possible to perform long-term studies with the fruit flies. Due to this, time constraints were in place and research had to be completed in a timely and reasonable manner. A significant limitation of this study was the number of control groups relative to the treatment groups. This may have reduced statistical accuracy when comparing treatment and control groups. These limitations may reduce the statistical power of the study and limit the generalizability of the findings, particularly when comparing differences between treatment groups.

A technical challenge was ensuring proper dosage. A technical limitation was the potential for minor variation in dosage preparation due to human error, despite efforts to standardize concentrations using serial dilution techniques. Procedures for Serial Dilution were followed by Zelterman et al. (2009).

Any variance in concentrations prepared could have had a profound effect on how subjects acted based on their behavior.

IMPLICATIONS

These findings showed a consistent, system-level behavioral response across multiple variables, strengthening the validity of the observed dose-dependent trends. These findings contribute to the understanding of dose-dependent neuromodulation by demonstrating that compounds acting through different neurotransmitter pathways can produce measurably distinct behavioral outcomes within a controlled model system. The findings from this study provide evidence that dietary supplements can produce measurable, dose-dependent modulation of stress-related behaviors in a controlled biological system. The reduced locomotion and light-startle behavior in animals for both 5-HTP or L-theanine indicate that the compounds may reduce stress-related responses. In contrast, the increasing thigmotaxis in animals resulted in higher levels for both compounds (Kopcalic et al., 2025). An increase in thigmotaxis was directly correlated to an anxiety-like response. However, in this study it may have also resulted from reduced locomotor activity. The flies remained near the edges more often as movement decreased. This created a conundrum between anxiety-like behavior and general movement suppression.

Finally, the differences in the effectiveness of the two supplements suggest that each operates through separate neural pathways (5-HTP through the synthesis of serotonin and L-theanine through increasing GABA activity). The stronger effect of L-theanine on light startle response can be explained by its influence on inhibitory signalling. L-theanine increases GABA activity, which reduces neuronal excitability more immediately, leading to a greater suppression of reflexive responses such as the light startle reaction. In contrast, 5-HTP acts through the synthesis of serotonin, which regulates behavior responses more gradually. As a result, the GABAergic pathway targeted by L-theanine likely produces more rapid and pronounced reductions in responsiveness compared to the serotonergic effects of 5-HTP. Therefore, the findings clearly reported here contribute to the overall body of knowledge relating to neurochemistry and behavioral responses through providing additional evidence for the significance of neurotransmitter regulation. In addition, the successful implementation of *Drosophila* as an experimental animal system for studying behavioral and pharmacologic responses is supported by these findings.

This study provides additional information on how neurotransmitter regulation affects behavior in Drosophila melanogaster. This study demonstrated that compounds that influence the serotonin and GABA systems will affect behavior in a model organism (such as Drosophila melanogaster) and therefore supports continued use of Drosophila melanogaster as a model organism for initial pharmacological studies. These findings support continued use of Drosophila melanogaster as a model organism for pharmacological research.

FURTHER RESEARCH

There is a need for more investigation into the neurobiological basis of the behavioral alterations seen in the flies treated with 5-HTP and L-theanine. To accomplish this, it is suggested to utilize neurochemical assays, fluorescence imaging or genetic expression analysis to investigate how each of these compounds affect neurotransmission, specifically concerning serotonergic and GABA (Gamma-aminobutyric acid)

transmission. These neurotransmitter systems have been demonstrated to play roles in regulating mood, stress, and behavioral reactions in both insects and humans. It is recommended conducting an even greater variety of doses than those used in the present study (such as, at least two times higher and lower) and establishing a more accurate dose-response relationship to ensure maximum benefit while avoiding adverse side effects like sedation and decreased responsiveness. Establishing an adequate dose-response relationship will enable us to better understand when an effect is due to pharmacologically active concentration of a compound vs. toxic concentration.

A second area of interest is to extend the study to include a broader array of behaviors, including feeding behavior, circadian rhythms, sleep cycles, reproduction, and social interactions. Our current study focused on stress related behaviors primarily measured using locomotor activity, thigmotaxis, and startle response. Although all three measures are valid indicators of stress-related behaviors, limiting the study to only three measures limits the ability to draw definitive conclusions regarding the impact of 5-HTP and L-theanine supplementation on total organismal physiology. Therefore, it would be beneficial to add more behavioral measures to gain a more comprehensive understanding of the overall effect of 5-HTP and L-theanine dietary supplements.

Long-term studies would be highly useful because they would determine if the benefits from 5-HTP and L-theanine supplementation last for extended periods of time. Furthermore, long-term studies would also address whether repeated exposure led to habituation, tolerance, and/or accumulation of effects. Understanding how 5-HTP and L-theanine works over longer durations of administration is important since many individuals use these supplements over long periods.

There are numerous ways this experimental design could be improved. One major improvement would be to greatly increase the sample size (especially for the control group) so that there is much less variation among individual animals. Another improvement would be to increase the number of control groups and implement a randomized controlled trial structure to minimize any biases inherent in comparing different treatments. With the arrival of new technology, many aspects of this experiment could be made more efficient and automatic. For example, computerized video tracking equipment could automatically track animal movements without requiring any human observation or intervention. Machine learning-based algorithms could analyze animal behavior based on video recordings taken during experiments. High resolution imaging systems could capture detailed images of the fly brains. These technologies would greatly aid to minimize human error and observer bias.

Further research should also involve comparative studies using additional dietary supplements and compounds that are recognized widely for their anti-anxiety and stress reducing properties, such as melatonin, magnesium, and ashwagandha. These types of studies would allow researchers to determine which compounds produce similar effects compared to 5-HTP or L-theanine, and evaluate possible interactions between compounds. These studies would deliver a comprehensive framework for evaluating the effectiveness of various dietary supplement combinations in treating anxiety and reducing stress.

Finally, it would be beneficial to conduct this type of research using more complex and different types of organisms such as rodents or other vertebrates. Studies conducted in vertebrates would provide an even more direct link between the previously described behavioral and neurological effects seen in *Drosophila* and potential applications in mammals (such as humans). Controlled human clinical trials would also eventually be required to confirm generalizations from these studies to humans. Human clinical trials could involve administering 5-HTP and L-theanine in a mixture with other compounds to subjects diagnosed with mild anxiety disorders. Measures included in human clinical trials could include both psychological assessments (questionnaires) and physiological measurement (heart rate variability and cortisol levels). Combining behavioral, neurological, and clinical approaches will ultimately provide a more complete understanding of how dietary supplements affect stress responses in both animals and humans.

CONCLUSION

The hypothesis was supported, as by increasing concentrations of both 5-HTP and L-theanine locomotor activity and light startle response decreased, whereas thigmotaxis increased. These outcomes were consistently observed across all trials, with L-theanine consistently producing stronger effects than 5-HTP at equivalent concentrations. L-theanine produced more pronounced effects than 5-HTP across all measured variables.

This experiment was conducted to investigate the effect of different amounts of 5-HTP and L-theanine on behavioral stress responses in *Drosophila melanogaster*, specifically focusing on locomotion, thigmotaxis, and the light-startled response. The results showed that each supplement produced measurable behavioral changes that were dose-dependent.

This study is significant as it fills an important gap in previous studies as it provides a comparative analysis of the effects of using two popular dietary supplements on a variety of behavioral measurements using a controlled experimental design. Additionally, the results show the significance of dosage when examining the efficacy or adverse effects of substances like those examined in this study.

Across all trials, locomotor activity and light startle response decreased, while thigmotaxis increased with increasing concentration, indicating a coordinated behavioral response to neuromodulatory input. L-theanine consistently produced stronger effects than 5-HTP for all behavioral responses. This highlights the significance of dosage and the differing behavioral outcomes produced by each compound. These findings directly support the original prediction that increasing dosage would produce measurable, dose-dependent changes in stress-related behavioral responses.

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