Analyzing probiotic impact in the gut microbiome of Drosophila to alleviate symptoms of Seasonal Affective Disorder

Grant Proposal

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Abstract

Seasonal affective disorder (SAD) affects an estimated 10 million Americans, while a greater amount experience milder forms of the depressive condition (Flick, 2019). Nothing can be done about changing seasons; summer will always transition into winter, and with it, people afflicted with seasonal depression will have a deterioration in their emotional state and productivity. However, this research aims to counter seasonal depression, not by manipulating outside factors, but by investigating how changes made within the organism result in improved responses to seasonality shifts. This objective will be accomplished through the investigation of the gut microbiome of the model organism *Drosophila melanogaster*. As a result of previous research linking the state of gut microbiota to mental health conditions, this study will implement probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* to the gut microbiome of seasonal depressive flies. Flies will be subjected to tests intended to record motivation and symptoms of Major Depressive Disorder. By conducting this study, the results will hopefully provide a scientifically proven method for decreasing the effects of SAD symptoms.

Keywords: seasonal depression, circadian misalignment, SAD, microbiome, probiotics

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Seasonal depression, also known as seasonal affective disorder (SAD), is a subtype of depression or bipolar disorder that begins and ends around the same time each year. This condition is due to the change in seasons, with most symptoms beginning in the fall and lasting throughout the winter months. Seasonal depression can still occur in the summer or spring, although this is less common. In a given year, 5% of the U.S population experiences seasonal depression (Cotterell, 2010). The degree of severity can vary, but those susceptible to it will be impacted both in a mental and emotional sense. SAD is classified as a form of Major Depressive Disorder (MDD) and its impacts can be devastating. People struggling with the disorder report social withdrawal, feelings of helplessness, low energy, weight gain, and oversleeping. Mental health professionals suggest that those afflicted with SAD seek out remedies such as talk therapy, antidepressants, and regulated exposure to UV light by a specialized lamp, along with some more natural courses of action such as exercising, spending time outdoors, and eating a healthy diet (Mayo Clinic, 2021).

When one consumes food, it passes through the digestive system. In various areas of the digestive system, most commonly the small intestine and large intestine, the gut microbiome can be found, an ecosystem holding trillions of bacteria along with viruses and fungi. All these organisms work symbiotically with the host and regulate a multitude of important functions necessary for the host's survival. Because of the connection between the Enteric Nervous System (ENS), located in the gastrointestinal system, and the Central Nervous System (CNS), as a result of the vagus nerve, there has been a large amount of research done establishing a link between the state of the gut microbiome and neurological conditions. According to Limbana et al. (2020), patients diagnosed with mental conditions, including depression, have demonstrated gut microbiome dysbiosis, an imbalance in bacterial composition such as the loss of beneficial bacteria, and diversity of microflora.

The fascinating nature of the gut-brain axis prompts the strong research involving the gut microbiota and neurodegenerative and psychiatric disorders. However, studies like these that examine depression typically focus on MDD or clinical depression, with not much investigation on the implications in response to seasonal depression. This study proposes to investigate an under-researched aspect of a widely researched field. While MDD and SAD are both clinically classified as depressive disorders, the way they are initiated differs. In the context of laboratory work, the model organism would have to undergo changes to circadian rhythm rather than be subjected to constant stressors to induce SAD symptoms, a notable difference from MDD experiments.

Because dysbiosis of the gut microbiota leads to negative mental health status, the reinvigoration of microflora will lead to an improvement in mental health. As corroborated by Zhang et al. (2015), a healthier gut microbiota will function more efficiently than a weakened one, with the ENS relaying signals to the CNS in accordance to the state, impacting mood. The most effective way to influence the gut microbiome is through the consumption of probiotics, live microorganisms with the purpose of improving or restoring the microbiome. Probiotics containing *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*, bacteria proven beneficial to microbial flora, will be administered to induced SAD *Drosophila* to measure impacts on the health of gut microbiome and record possible alleviation of depressive symptoms (Gao et al., 2022).

Section II: Specific Aims

This proposal's objective is to investigate whether the bacterial flora in the gut microbiome will impact a seasonal depressive organism's mental state and if SAD symptoms can be alleviated through the introduction of probiotics to a microbiome in dysbiosis.

The central hypothesis of this proposal is if probiotics containing *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* are administered to improve the gut microbiome of depressed *Drosophila*, then the *Drosophila* will show increased motivation to attempt to access a sucrose reward because the diversity and plentifulness of bacteria in the gut microbiome correlates with improved mental/emotional states.

Specific Aim 1: To regulate daylight exposure and cause the overproduction of melatonin to induce abrupt circadian rhythm disorientation in *Drosophila* in accordance with SAD symptoms.

Specific Aim 2: To analyze differences in behavior and the gut microbiota of control and depressive group.

Specific Aim 3: Administer *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* to improve health of microbiota.

The expected outcome of this study is to see a microbiome rehabilitation due to the addition of probiotics to correct dysbiosis, which will alleviate depressive symptoms induced by light and dark cycle shifts.

Section III: Project Goals and Methodology

Relevance/Significance

Polled by the American Psychiatric Association, out of 2,211 participants, one in five individuals reported moodiness and a loss of interest in usually enjoyed activities during the winter months (Faillace, 2021). Seasonal depression is common in places veering from the equator and impacts the lifestyle of a person, affecting them emotionally to varying degrees, ranging from miniscule to extreme. The more extreme side leads to a depressive disorder known as Seasonal Affective Disorder. Depressive disorders should always be taken seriously, no matter how they are initiated, as the extreme end of it can be fatal. 3.5% of deaths in the United States were attributed to anxiety/depression, legitimizing the severity of a depressive disorder as an illness that can take lives (Pratt et al., 2016).

Innovation

As previously mentioned in the abstract, gut microbiome and depression investigations are common in academic research, each one providing individualistic characteristics with either a new compound or serotonin receptor. This project, while investigating the same symptoms of depression, must be induced through the simulation of seasonal changes for the project to remain as accurate as possible. This means that the project will deviate from typical methods of inducing depression, such as achieving a state of "learned helplessness" through the subjection of constant stressors. To compare, Ries et al. (2017) had the drosophila group be subjected to repetitive 300 Hz vibrations for periods of time to induce a depressive state. This project's altered ways for SAD induction will explore the relationship between depressed drosophila and gut microbiome from the focus of seasonal changes.

Methodology

Drosophila must first be induced into a depressive state through circadian misalignment. The first step will be achieved with two methods. First, with reduced light exposure, flies will be exposed to varying periods of light and dark to simulate the reduced daylight during transitions to the winter season. The next goal is for the flies to undergo melatonin overproduction, an effect humans also sustain when experiencing seasonal depression (Mayo Clinic Staff, 2021). For context, melatonin is a chemical compound the body naturally produces to make one tired enough to doze off into sleep. It is inhibited by the properties of sunlight, causing melatonin levels to decrease and for the person to wake up. Having the flies exposed to long periods of darkness will raise melatonin levels, but for the project to maintain reliability to the disorder it emulates, additional actions for SAD induction will be taken. Excess melatonin in drosophila will be achieved through the feeding of a liquid melatonin supplement, diluted with water.

To assess for SAD, two tests will be performed, a motivational test and a test for anhedonia. For the motivational test, flies will have to complete sub-tests to measure climbing rate and walking activity. One of the sub-tests will be a test for climbing rate. Flies will have to climb over a 4.5 mm gap, an almost insurmountable distance for the size of the fly, in order to reach a sucrose reward at the other end. The number of attempts in comparison to the control will be measured.

A typical symptom of Seasonal Affective Disorder (SAD) is anhedonia, which is the loss of enjoyment, and it is characterized by, for example, a loss of appetite. For the anhedonia test, flies will be contained in a petri dish with a drop of a 30% sucrose solution in the center. For this test, the number of stops the flies make in their walk to taste the sucrose will be measured against the control group. Studies have observed that uncontrollable stress over several days can induce a depression-like state in flies that reduces voluntary, innate behaviors (Ries et al., 2017).

After assessing behavioral responses, the gut microbiome will be analyzed by observing isolated bacterial components in fecal matter. Comparisons will be drawn on the diversity and health of microflora between the depressive and control groups.

Probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* will be administered to improve the gut microbiome of depressed drosophila. The depressed group will be split up into smaller subsections which will receive a respective probiotic group so that one can draw comparisons to how well a probiotic performs on repairing the microbiome. Afterward, the same process for gut microbiome analysis will be performed.

Specific Aim #1: To regulate daylight exposure and increase melatonin levels to induce abrupt circadian rhythm misalignment in *Drosophila* in accordance with SAD symptoms.

Justification and Feasibility. Along with the shorter lengths of daylight, people who struggle with SAD report lower levels of vitamin D in their system and increased melatonin. As a result, these conditions will be incorporated as part of the project's first specific aim. Supplies will need to be obtained to construct and moderate the specific testing conditions. The supplies needed are accessible and can be easily acquired relatively inexpensively, decreasing any supply or cost barrier and improving feasibility. The lab where this project will be conducted has needed resources already available, streamlining the initial stages of experimentation. In addition, the length of time for the changes in light-dark cycles for the drosophila will not last longer than 7-8 days, as other circadian studies in the fruit fly don't tend to go past that duration of time. For example, one specific study altered the photoperiod (exposure an organism has to light) over a similar timeframe and recorded peaks in locomotive activity (Shafer et al., 2004). While the goals of the two projects are different, their methods for regulating daylight exposure are relevant for this study.

Table 1. Locomotor Rhythms Whose Times of Peak Activity Adjust for Photoperiod

Photoperiod[a]	Early Peak (h ± SEM)[b]	Late Peak (h ± SEM)[c]
LD 6:18[d]	20.4 ± 0.2 (22)	6.2 ± 0.1 (23)
LD 8:16[e]	22.1 ± 0.1 (23)	8.1 ± 0.06 (26)
LD 10:14[f]	23.6 ± 0.1 (62)	9.8 ± 0.1 (60)
LD 12:12[g]	0.2 ± 0.01 (57)	11.5 ± 0.03 (59)
LD 14:10[h]	0.3 ± 0.1 (51)	13.1 ± 0.05 (58)
LD 16:8[i]	0.7 ± 0.1 (52)	14.4 ± 0.1 (54)
LD 18:6[j]	0.5 ± 0.2 (26)	14.6 ± 0.1 (26)

Figure 2.1*. Recorded changes made to the light-dark cycles of Drosophila over a time (Shafer et al., 2004).*

Summary of Preliminary Data. Melatonin solutions had to be prepared to be implemented into the feed of the test group. In a one-to-one ratio of water and potato flakes, a melatonin concentration of 100ug/ml was administered in the water. The two groups were then placed into dark containers on a self-timer with a set light-dark cycle. The control group with about 80 flies was run on a 12/12 cycle. The test group with about the same number of flies began with a 12/12 cycle and began decreasing light hours by two every two days. .

Figure 3.1. *Both control (left) and test (right) environments on the first day of the light-deprivation period.*

Expected Outcomes. As the control group is in standard conditions with no modification to light reception and melatonin in feed, I expect it will remain a reliable marker for change in comparison to the test group. The test group, with these modified conditions, should begin to present reduced motivation and movement as their circadian rhythms are being abruptly changed and are being administered a chemical seen excessively in most SAD cases.

Potential Pitfalls and Alternative Strategies. Incorrect timed exposure to daylight and diet deviating from testing procedure may affect depressive levels afterwards in drosophila. To address this, every effort will be made to follow the specificity of the testing procedure.

Specific Aim #2: To analyze differences in behavior and the gut microbiota of control and depressive group.

Justification and Feasibility. As explained before, numerous studies suggest a strong connection between gut microbiota and mental health in humans. Investigating this correlation in Drosophila can provide insights into the basic mechanisms underlying such connections. In the past, Drosophila has been used as a model for gut microbiome analysis, due to its simplified and minimal numbers of bacterial strains. Drosophila is also a favored model for neurological and behavioral investigations because of their rapid reproduction and useful genetic factors.

Summary of Preliminary Data. Initially, to test the testing procedure and gain experience handling the model organism, the first behavioral assay was completed to determine the changes between flies exposed to various L/D cycles. With a minimal number of 5 flies in each cycle (12/12, 9/15, 7/17, 3/21), each individual fly was assessed on the gap-climbing paradigm for three times each. The control (12/12) displayed overall a 93% climbing rate. The 9/15 group displayed a 20% decrease in climbing attempts from the control. The 7/17 group displayed a 33% decrease from the control. The 3/21 group displayed a 60% in climbing attempts from the control.

For gut microbiome comparisons, vials holding upwards of 30 flies each from one control and one test group were set for 4 days. Fly feces were then collected to isolate DNA.

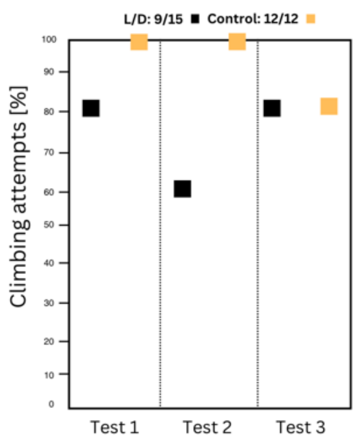

Figure 4.1 Graph showing comparison of climbing attempt percentage between the 9/15 group and the Control. (Top)

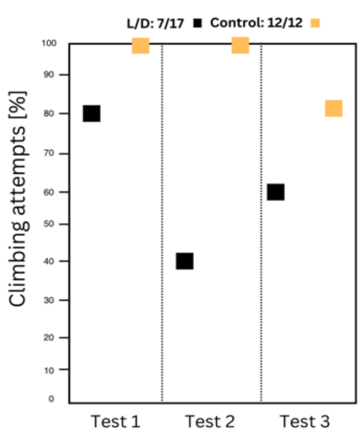

Figure 4.2 Graph showing comparison of climbing attempt percentage of 7/17 group and control. (Middle)

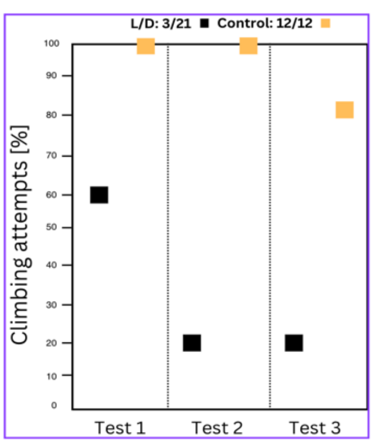

Figure 4.3 Graph showing comparison of climbing attempt percentage between 3/21 group and control. (Bottom)

Expected Outcomes. When re-doing this assay with a larger sample size, I expect the drosophila to exhibit similar trends in behavior like seen the first time. I also expect the abruptions in circadian rhythm the test group experiences to reflect dysbiosis when doing a microbiome comparison.

Potential Pitfalls and Alternative Strategies. Possible risks or safety concerns could be misinterpretation of data or incorrectly performed testing strategy. To address this, every effort is made by the researcher to be as precise and attentive to detail as possible.

Specific Aim 3**:** Administer *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* to improve health of microbiota.

Justification and Feasibility. According to Ghada Tafesh-Edwards, the *Drosophila* gut microbiota is dominated by bacteria, especially *Proteobacteria* (*Acetobacteraceae* and *Enterobacteriaceae*) and *Firmicutes* (*Lactobacillus* and *Enterococcus*) (Tafesh-Edwards G et al., 2023). For this study, the use of bacteria pertaining to *Lactobacillus* will be investigated, a lactic acid bacterial species with several benefits to human health. Besides the obvious benefits to gut health, a study from Kashan University of Medical Sciences, found that patients who received probiotic supplements, Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium bifidum, had significantly decreased Beck Depression Inventory total scores (Ghodarz Akkasheh et al., 2015).

Summary of Preliminary Data. To administer the probiotics to drosophila, the bacteria will be mixed with distilled water and poured into dry potato flakes. Then, there will be an interment period of 3 days before the next round of microbiome and behavioral analysis.

Expected Outcomes. Based on what previous data has shown, administering the probiotics will lead to improved performance on the behavioral assays. Because of the bidirectional relationship of the gut-brain axis, greater bacterial diversity in the microbiome has an impact on mood which should be reflected in the behavior of the drosophila.

Potential Pitfalls and Alternative Strategies. Possible risks or safety concerns could be misplacement of bacterial-holding agar plates resulting in contamination and misjudgment of microbiota. To address this, caution will be taken when transporting and observing samples so that results aren't impacted by unforeseen factors.

Section III: Resources/Equipment

Resources used by the researcher during lab experimentation are the following:

- Sharp/fine scissors and tongs to hold down the fly and clip their wings.
- FlyNap to anesthetize the flies.
- A microscope to see the flies in better clarity.
- Containers with foam lids to store the flies and fly food (potato flakes) mixed with distilled water along with yeast to keep them fed until the next day.
- Sharpie and tape to label each container.
- Gap-climbing paradigm and a pipette of a 30% sucrose solution.
- Small petri dishes with sucrose solution
- Fridge
- DNA isolation kit
- Centrifuge, vortex, and PCR equipment.
- Primordium Labs

Section V: Ethical Considerations

Because this project requires the live handling of Drosophila and will be utilizing live lactobacillus strains, every effort will be made to maintain a sanitary environment to ensure the safety of the researcher, others using the lab environment, and the flies. The flies involved with the experiment will be kept under humane conditions, food will not be deprived from them. When actively using the bacterial strains and introducing them to the flies, surfaces and equipment will be wiped down with a cleaning agent prior and after the lab period. The researcher will also wash their hands prior and after experimentation in the lab. For the disposal of the bacterial strains, a solution of bleach will be created and

Section VI: Timeline

1. Induce depressive state.
    a. Create areas for the drosophila to regulate L/D cycles (blue buckets w/ lamps) - 1/2/2023
    b. Place the flies in their respective pre-programed L/D cycle container for up 5 days 1/2 – 1/7
    c. Cut off the wings in preparation for gap-climbing paradigm - 1/9/24
2. Record behavioral changes
    a. Gap-climbing paradigm test and data analysis - 1/10/23
        i. Present prelim data on this assay - 1/19/23
    b. Incorporate other assays
        i. Stop-for-sweet paradigm (feeding assay) - 1/20/23
3. Gut microbiome analysis
    a. Begin fecal harvesting and ship to external lab - 1/25/23

4. Administer probiotics and record findings - 1/30/23

Section VII: Appendix

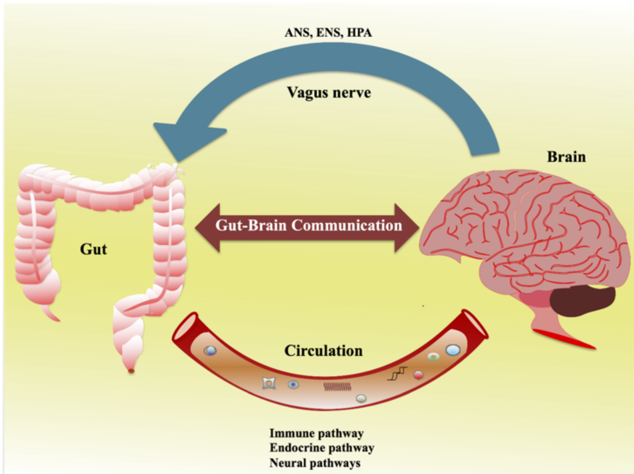

Figure 5.1 Schematic diagram showing the communication between the gut and brain, a bidirectional relationship that is strongly influenced multiple pathways, such as the ENS, ANS, Vagus nerve etc. (Suganya et al., 2020).

Section VIII: References

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