Unraveling the Role of GABAergic Dysfunction in Catatonia: A GABAergic Investigation in Drosophila

Grant Proposal

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Abstract

Catatonia is a neuropsychiatric disorder characterized by a variety of behavioral, cognitive, and motor abnormalities. Despite it having a treatment, the neurobiological mechanisms underlying catatonia remain poorly understood where existing models fail to capture the full spectrum of symptoms. Recent studies link the development of catatonia to GABAergic dysfunction, especially disturbances in GABA-A receptor signaling. This work sought to fill important knowledge gaps by using *Drosophila melanogaster* as a model organism to examine the role GABAergic disruption plays in catatonia-like symptoms. This study used Drosophila with mutations in the rdl gene, which reduces GABAergic signaling. By comparing these mutants to wild-type Drosophila, this research assessed whether reduced GABA levels led to catatonia-like symptoms. These mutant flies were then induced with Ashwagandha powder, a natural compound that elevates GABA levels. The study looked at important behaviors such as locomotion and sensory responsiveness to see if ashwagandha supplementation improved GABAergic function and reduced catatonia-like symptoms.

The experimental groups were induced with various concentrations of ashwagandha powder through food supplementation. A chemosensory assay, which measures the flies' reaction to different chemicals, specifically attractants, was used to test sensory responsiveness. Additionally, a negative geotaxis assay was used to test motor function, which measured the flies' ability to climb after being placed at the bottom of a vial.

The data from these assays were statistically analyzed to compare the experimental and control groups, identifying behavioral differences that may be linked to GABAergic dysfunction. By examining locomotion and sensory responsiveness, this study aims to provide insight into the role of GABAergic signaling in catatonia and lay the groundwork for future research on its neurochemical basis.

Unraveling the Role of GABAergic Dysfunction in Catatonia: A GABAergic Investigation in Drosophila

Catatonia, a neuropsychiatric syndrome is characterized by a range of motor, behavioral, and cognitive symptoms, remains a mysterious condition with unclear underlying mechanisms (Kline et al., 2022). Patients with catatonia may exhibit symptoms such as mutism, immobility, echolalia (repeating others' speech), echopraxia (imitating others' actions), and waxy flexibility, where they maintain unusual body positions for extended periods (Rasmussen et al., 2016). The syndrome can be subdivided into different categories, with some forms being characterized by excessive movement or agitation, and others having decreased movement and responsiveness (Burrow et al., 2023). This diversity in the symptoms suggests that multiple underlying neurobiological pathways may be involved in the development of Catatonia.

Neurotransmitter systems associated with catatonia, particularly the GABAergic system play a significant role in regulating brain activity by inhibiting neural signals and maintaining a balance between excitation and inhibition in the brain, which is needed for normal cognitive and motor function. Dysregulation in the GABAergic system, which involves the neurotransmitter gamma-aminobutyric acid (GABA), has been observed in patients with catatonia and is believed to contribute to their motor and behavioral symptoms (Ariza-Salamanca et al., 2022). GABA is known to exert a calming effect on the brain by reducing excessive excitatory signals. Medications that enhance GABAergic activity, such as benzodiazepines, have been shown to reduce catatonic symptoms in many cases, further resulting in GABA dysfunction in this condition (Edinoff et al., 2021). However, benzodiazepines come with dependency risks, which increases interest in alternative treatments and animal models to study GABAergic pathways (Tan et al., 2011).

Previous Studies

Neurotransmitter dysregulation in catatonia have been studied using rodent models, where pharmacological alterations of the GABAergic and dopaminergic systems were shown to induce

catatonia-like behaviors. For instance, a prior study examined the role of GABAA receptors in neurodevelopment, particularly focusing on how deficiencies in the *gabrb3* gene in mice impacted social behaviors (Delorey et al., 2007). The researchers found that these *gabrb3*-deficient mice had abnormal social behaviors, an important deficit associated with autism spectrum disorder (ASD), suggesting that disruptions in GABAergic signaling can lead to behavioral abnormalities related to social interaction (Delorey et al., 2007). This link between GABAergic dysregulation and social deficits shows the potential for similar mechanisms to underlie catatonia-like symptoms, including social withdrawal and behavioral rigidity.

Knowledge Gaps

Despite these findings, many gaps remain in understanding catatonia's neurochemical and genetic bases. Current models have only been able to replicate certain aspects of the syndrome. Moreover, no studies have fully explored how interactions between the GABAergic and dopaminergic systems contribute to catatonia.

Introduction to Research Question and Hypothesis

This research aims to develop a *Drosophila melanogaster* model to study catatonia-like symptoms by investigating the effects of GABA-A receptor antagonism using Ashwagandha. Exposure to Ashwagandha is known to influence GABAergic neurotransmission, leading to noticeable changed in motor and sensory behaviors. Drosophila treated with Ashwagandha are expected to exhibit altered motor activity, such as increased locomotion, and changes in chemosensory responses. These changes provide a basis for understanding the neurochemical processes involved and would suggest that disturbances in GABAergic transmission play a role in the development of catatonia-like symptoms. Therefore, by using behavioral assays to measure locomotion and chemosensory responses, this research aims to develop a model for investigating the neurochemical foundations of catatonia and the role of GABA-A receptor dysfunction.

Introduce Methodology

This experiment will test the hypothesis by utilizing Drosophila melanogaster as a model organism for reduced GABAergic signaling to analyze behavioral changes. The wild-type flies are the control, with normal baseline behaviors. The experimental group consists of Drosophila with mutations in the rdl gene, which reduces GABA-A receptor function. By comparing these mutant flies to the wild-type group, the study will assess whether reduced GABA levels lead to catatonia-like symptoms. In testing for behavioral alterations, locomotion will be studied by assays involving geotaxis and chemosensory responsiveness, as well as social behaviors. Locomotion was studied by conducting a negative geotaxis test to measure the climbing ability. The chemosensory tests monitored the Drosophila's responses to attractants and repellents. After establishing baseline differences between wild-type and rdl mutant flies, the experimental group will be treated with Ashwagandha powder, a natural compound known to enhance GABAergic function. The same behavioral assays are then performed to see whether supplementation with Ashwagandha ameliorates catatonia-like symptoms. Finally, data from all groups was compared to find out if behavioral improvements were made, providing insights into the potential role of GABAergic signaling in catatonia.

Section II: Specific Aims

This proposal's objective is to develop a *Drosophila melanogaster* model to investigate the neurochemical mechanisms of catatonia, focusing on GABAergic neurotransmission and behavioral assays using bicuculline, a GABA-A receptor antagonist.

Our long-term goal is to define the neurochemical mechanisms causing catatonia and offer suggestions for possible treatment targets. The central hypothesis of this proposal is that bicuculline-induced GABAergic dysregulation causes noticeable behavioral abnormalities that resemble catatonia-like symptoms. The rationale for this project is the need to understand the fundamental mechanisms of the poorly studied neuropsychiatric disorder catatonia. While current research relies on complex

vertebrate models, the work proposed here is to create another animal model to gain a greater understanding of the cause of catatonia. Drosophila melanogaster serves as an alternative model especially due to its well-characterized genetic system and conserved GABAergic pathway which make it ideal for examining the neurochemical basis of catatonia (Baenas & Wagner, 2019).

Specific Aim 1: Use the negative geotaxis test to assess motor activity in Drosophila melanogaster with reduced GABA levels, induced with Ashwagandha, and compare their performance to wild-type flies to evaluate if increased GABA improves motor function.

Specific Aim 2: Evaluate the chemosensory response in flies with reduced GABA levels to investigate how GABA deficiency affects sensory perception and signaling.

Specific Aim 3: Assess locomotion and sensory responsiveness in Drosophila with lowered GABA levels and observe if Ashwagandha supplementation is able to eliminate these abnormalities and alleviate catatonia-like symptoms.

The expected outcome of this work is to gain a comprehensive profile on the behaviors of Drosophila Melanogaster in the presence of GABA-A receptor antagonistic conditions, emphasizing the idea that GABAergic neurotransmission plays a role in behaviors linked to symptoms of catatonia.

Section III: Project Goals and Methodology

Relevance/Significance

Catatonia is a neuropsychiatric disorder with an unclear cause and limited treatment options. Additionally, current animal models used to research this specific disorder are vertebrates which are not accessible to use all the time. Therefore, A scalable and reasonably priced model for examining the part GABA deficiency plays in catatonia is needed and is provided for by Drosophila Melanogaster. This research is significant as it will result in new findings regarding GABAergic signaling and its connection to catatonia-like symptoms.

Innovation

This research is novel as it investigates catatonia, a condition that is rarely modeled in invertebrates, using Drosophila melanogaster. By utilizing Drosophila with mutations in the rdl gene to reduce GABAergic signaling, this study provides a controlled model to examine the role of GABA dysfunction in catatonia. Additionally, by inducing these flies with Ashwagandha, a natural compound known to enhance GABA levels, the research explores its potential to reduce catatonia-like symptoms.

Methodology

Drosophila melanogaster will be used as the main model organism in this study to examine the role of GABAergic dysfunction in catatonia-like symptoms because of its well-mapped genetic pathways, quick life cycle, and ease of observation for behaviors like locomotion, chemosensory responses, and social interaction (Jeibmann & Paulus, 2009). The control group will be wild-type Drosophila, which will serve as a baseline for comparison, while the experimental groups will be Drosophila with mutations in the rdl gene, which reduces GABA signaling and simulates catatonic behaviors. These mutant flies will be treated with varying concentrations of Ashwagandha powder (0.3%, 0.6%, and 1.2%) to elevate GABA levels and potentially improve motor and sensory function. The Ashwagandha treatment will be administered through food supplementation to ensure consistent exposure among all flies, while the untreated control group will continue a standard diet.

There will be three assays completed once the experimental and control groups are prepared. The first assay that will be conducted will be a locomotion assay. This assay can be used to measure the impact of bicuculline on motor function. The flies will be placed in a controlled environment (an open-field area), and their movements will be recorded using tracking software. The behavioral parameters that will be analyzed to examine the motor activity include total distance traveled and the frequency of movement (Wiedemann, 2001).

In addition to the locomotion assay, a response to stimuli assay will be conducted. To do this, various chemical stimuli, such as attractants like sucrose and repellents like quinine, will be presented to Drosophila (Vang et al., 2012). The flies' preference or aversion to these chemicals will be measured using a two-choice paradigm to quantify their response.

A clustering assay will be used to investigate social behavior. For this assay, both the control and experimental drosophila groups will be placed in an observation chamber, and the flies' tendency to separate from one another or come together in social interaction will be tracked over time. Once these assays are completed, all the behavioral assays will be statistically examined to compare the experimental and control groups. Through this, it will be possible to define a relationship and see if GABA-A receptor antagonism caused by bicuculline exposure causes catatonia-like symptoms in Drosophila.

Specific Aim #1: **Evaluate motor activity in GABA-deficient Drosophila treated with Ashwagandha to assess improvements compared to wild-type flies.**

The aim will be to find how reduced GABA levels influence locomotion in Drosophila with the aid of negative geotaxis assays. The use of the mutant Drosophila strains for the gene, rdl, which affects the levels of the GABAergic signaling in wild-type flies and their various comparison; that includes induction by the natural extract, Ashwagandha at various concentrations in mutant flies. The rationale for this approach is that catatonia is a disorder of motor dysfunction, and since GABAergic dysfunction has been implicated in this disorder, studying how GABA restoration affects flies' motor activity can provide more information.

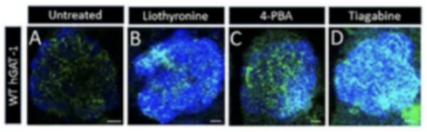
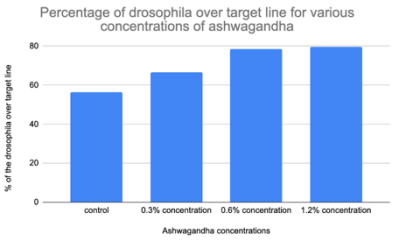


Figure 1: In Drosophila, pharmacological interventions alter the expression of the GABA transporter. In the antennal lobe, panels A-H show the astrocytic expression of both wild-type and mutant human GABA transporter 1 (hGAT-1) (Kasture et al., 2023).

Justification and Feasibility. GABAergic dysfunction is linked to significant motor and sensory deficits in animal models (Alharbi et al., 2024). However, the majority of research uses expensive and resource-intensive rodent models, which limits investigations. GABAergic impairments affect motor and sensory skills but there is no comparable work for Drosophila. *Drosophila melanogaster*, which provides a genetically feasible, affordable, and scalable platform to investigate GABAergic dysfunction, will be used in this project to bridge this gap. The GABAergic system of Drosophila is similar to that of mammals, which justifies its usage as a model (Martin & Krantz, 2014). As seen in figure 1, a figure from an article using drosophila as a model to explore GABA receptor function in another neuropsychiatric disorder, there is evidence that supports the idea involvement of GABAergic pathways in Drosophila. This supports the involvement of GABAergic dysfunction in neuropsychiatric conditions by demonstrating that disruptions in GABA signaling lead to observable behavioral changes. Since Drosophila share conserved GABAergic mechanisms with mammals, these findings justify their use as a model to explore how GABA deficiency contributes to catatonia-like motor problems and how restoring GABA levels may mitigate these symptoms. Additionally, this project hopes to offer new insights on the fundamental causes of catatonia that can guide future translational research by investigating motor and sensory abnormalities using well-established behavioral tests.

Summary of Preliminary Data. For my preliminary data, I conducted a single-factor ANOVA to compare the performance of wild-type control Drosophila, and three groups induced with different concentrations of Ashwagandha (0.3%, 0.6%, and 1.2%) in a negative geotaxis assay. Over the span of 10 seconds, I counted



Graph 1: This graph shows the relationship between the different Ashwagandha concentrations and the percentage of the drosophila that went past the target line. As the concentration increases, there was a greater number of drosophila that moved upward suggesting the idea that higher levels of GABA increases motor activity.

the number of flies that climbed to the top and averaged the results across three trials. The ANOVA resulted in a p-value of 0.02, indicating a statistically significant difference between the groups. This suggested that the Ashwagandha-induced concentrations influenced the climbing behavior of the flies, which could be attributed to alterations in GABAergic activity.

Expected Outcomes. The overall outcome of this aim is to establish a direct link between GABA-A receptor inhibition and motor dysfunction in Drosophila. This knowledge will be used to identify GABAergic contributions to motor impairments in catatonia and set the stage for additional treatments.

Potential Pitfalls and Alternative Strategies. Different concentrations of Ashwagandha may bring about different changes in the behavioral performance of Drosophila, especially when the flies adapt or develop tolerance to the treatment with time; this may be the time when concentrations can be changed to test for optimality. Other modulators of GABAergic transmission or natural products could be used to confirm and extend these observations, so the effects observed could be attributed exclusively to the given GABAergic modulation, uncomplicated by any other factor.

Specific Aim #2: Evaluate the impact of low GABA levels on sensory perception in Drosophila melanogaster.

The objective is to examine how reduced GABA levels affects the flies' ability to sense and respond to sensory stimuli using chemosensory assays. The percentage of flies that move toward or away from chemical attractants or repellents will be used to measure behavioral responses. The rationale for this approach is that GABAergic transmission is necessary for regulating sensory circuits and that neuropsychiatric disorders such as catatonia frequently affect sensory perception therefore, there may be a possible connection (Du et al., 2017).

Justification and Feasibility. GABAergic dysfunction is closely linked to significant motor and sensory deficits in both humans and animal models (Hasan et al., 2021). Figure 2 from a study investigates how GABA receptor manipulation affects locomotion in drosophila and found that it is feasible to use Drosophila to investigate the behavioral impacts of neurotransmitter dysregulation. The study demonstrated how altering GABAergic transmission changed motor behaviors; therefore, conduction chemosensory assays can allow for the measuring of specific behavioral changes linked to GABAergic dysfunction, such as modified responses to attractants and repellents. These assays are cost-effective as well as scalable. Moreover, the model organism offers a strong platform for connecting behavioral outcomes to neural mechanisms because drosophila consists of a genetically tractable system (Dionne et al., 2003).

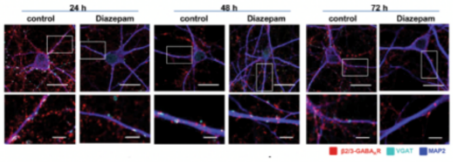
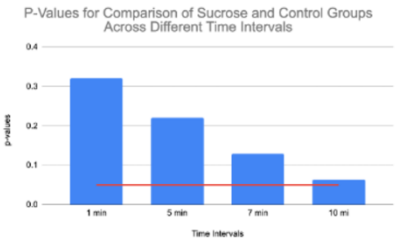


Figure 2: The figure illustrates how long-term diazepam use damages GABA-A receptor surface levels and GABAergic synapses in neurons, a process associated with sensory and motor impairments. By causing receptor internalization through a signaling cascade, this approach provides information on how GABAergic malfunction contributes to sensory problems (Nicholson et al., 2018).

Summary of Preliminary Data. In the preliminary data from the chemosensory assays, I have completed three trials with wild-type flies, testing their responses to sucrose versus water. As expected, more flies were attracted to the sucrose side; over the span of ten minutes. The average number of flies in each time interval across all three trials showed a consistent preference for sucrose. I performed a t-test for each time interval to see the statistical significance of these findings. The resulting p-values that came out were 1 min: 0.321, 5 min: 0.22, 7 min: 0.13, and 10 min: 0.064, indicating that the differences observed were not statistically significant, suggesting that while there is a trend, the chemosensory response of wild-type flies to sucrose was not strongly distinct from the control under these conditions.



Graph 2: This graph shows the p-values for each of the time intervals during the chemosensory assay. It is apparent that all the values for each of the time intervals is above 0.05 showing that the there is no statistical significance between sucrose and the control group amongst the wild-type Drosophila.

Potential Pitfalls and Alternative Strategies. One challenge in the experiment is the potential variability of the chemosensory response itself due to individual differences in flies or non-uniform assay conditions. The sucrose concentration may also not be at an ideal level to generate a behavioral response in all groups, leading to subtle or undetectable differences. These issues can be overcome by handling flies uniformly and performing each trial multiple times.

Section III: Resources/Equipment

The study will use basic Drosophila research tools such as fly vials, culture media, and incubators with regulated temperatures to maintain the experimental groups. Moreover, the study will use behavior assays that include a chemosensory response test and a negative geotaxis assay to assess sensory responsiveness and motor function. Then, ashwagandha powder will be used for the experimental group of Drosophila.

Section V: Ethical Considerations

This research complies with the ethical guidelines when using Drosophila melanogaster to observe the relationship between the GABAergic pathway and catatonia. In order to minimize damage and produce results that are scientifically credible, experimental techniques, including the administration of bicuculline, will stick to established recommendations. Overall, all data collection and analysis will comply with research integrity standards. Moreover, the study will not include vertebrate or human subjects.

Section VI: Timeline

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Section VII: Appendix

Section VIII: References

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