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The Role of Optogenetic Activation of Dopamine Neurons in Compulsive Grooming and Neural Changes in *Drosophila melanogaster*

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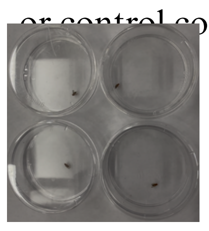
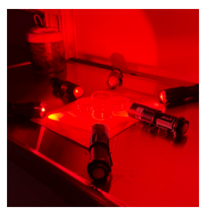
Introduction

- **Obsessive-Compulsive Disorder (OCD)** is characterized by uncontrollable and recurring thoughts (obsessions) and repetitive and excessive behaviors (compulsions) (National Institute of Mental Health et al., 2024)
- OCD affects approximately 2.3% of U.S. population (Ruscio et al., 2010)
- **Serotonin Hypothesis**
  - SSRIs (selective serotonin reuptake inhibitors) are used to target serotonin imbalance often found in OCD patients (Kellner et al., 2010)
  - Serotonin modulates the **cortico-striato-thalamo-cortical (CSTC) circuit** for inhibitory control (Desrochers et al., 2022)
  - Only 50% respond to SSRIs → suggests involvement of additional neurotransmitters
- **Dopamine's Emerging Role**
  - Dysregulated dopamine signaling in the CSTC circuit implicated in compulsive behavior (Gonzalez et al., 2025)
  - Neuroimaging studies have shown increased activity in orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and striatum of OCD patients (Melloni et al., 2012)
  - Rodent studies show increased dopamine in the dorsomedial striatum drives compulsive reward seeking (Seiler et al., 2022)
  - Dopamine is also linked to addiction and other compulsive disorders (Wise et al., 2020)
- **Compulsive Grooming** in *Drosophila melanogaster* mimics compulsions in OCD (Hatfield et al., 2025)
- **Central Complex and Mushroom Bodies** in *Drosophila* are analogous to human striatum and prefrontal cortex (Robinson et al., 2025)
- This study aims to determine if dopamine activation alone induces compulsive grooming in *Drosophila* and causes neural changes in the central complex or mushroom bodies
- It is hypothesized that optogenetic activation of dopamine neurons will cause increased grooming and observable neural changes

Methods

- **Subjects**
  - 28 *Drosophila melanogaster* were divided into 4 groups
    - 7 experimental males
    - 7 experimental females
    - 7 control males
    - 7 control females
  - All *Drosophila* were housed in standard molasses food (86% water, 0.574% agar, 6.3% cornmeal, 1.52% yeast, 4.65% dry molasses, 0.39% propionic acid, 0.15% methylparaben, and 0.52% ethanol)
- ***Drosophila* Lines**
  - Experimental *Drosophila*
    - GAL4 line #8848 x UAS line #55135 → red-light sensitive dopamine neuron activation
  - Control *Drosophila*
    - GAL4 line #8848 x UAS line #6795 → brain imaging
    - Wild *Drosophila* → behavioral assay
- **Experimental Setup**
  - *Drosophila* recorded in 7 sessions
  - Each session used 4 petri dishes (35mm x 10mm) in two rows on a white surface
  - Overhead camera ensured all *Drosophila* were in frame and clearly visible
- **Behavioral Protocol**
  - *Drosophila* lightly anesthetized using a standard freezer
  - One *Drosophila* per dish for individual observation
  - 30 minute acclimation period post anesthesia
  - Experimental group exposed to 10 minutes of red light
  - Control group underwent identical procedure without red light
  - 10 minute recording period immediately followed light or control condition





- **Behavioral Analysis**
  - Manual quantification of grooming behavior
  - Grooming bout: 2 seconds or more of uninterrupted grooming
  - Anterior grooming: front legs rubbing head or proboscis
  - Posterior grooming: mid/hind legs rubbing posterior
- **Neural Analysis**
  - 1 experimental and 1 control *Drosophila* were randomly selected for dissection
  - Analysis compared activity levels in central complex and mushroom bodies across groups.

Results

Figure 1: Total Grooming Bouts in Control and Experimental Groups

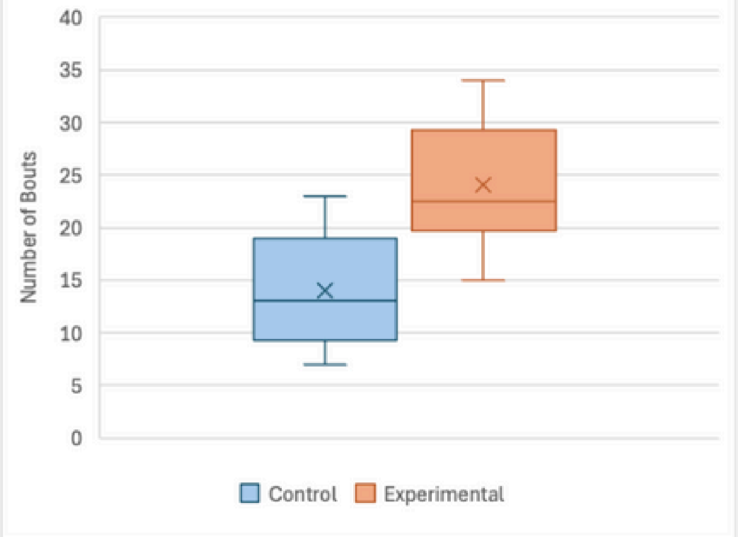


Figure 1: Total grooming bouts were significantly elevated in the experimental group (M = 24.071) compared to the control group (M = 14.000) ($p = 0.000929$).

Figure 2: Posterior Grooming Bouts in Control and Experimental Groups

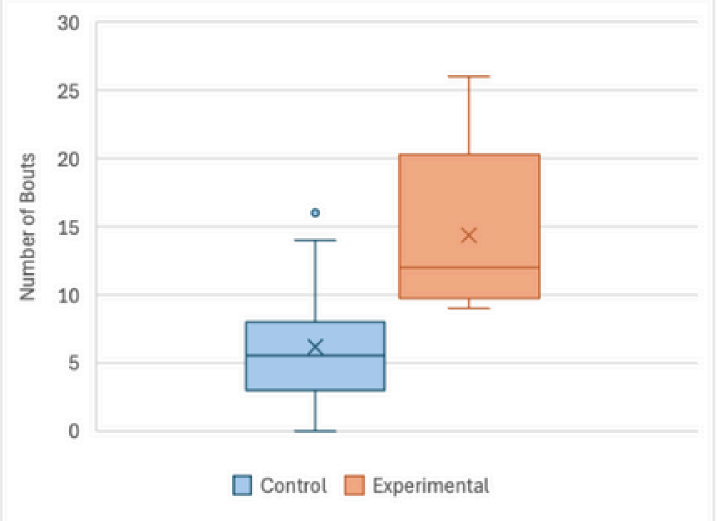


Figure 2: Experimental *Drosophila* engaged in significantly more posterior grooming (M = 14.357) compared to controls (M = 6.214). This difference was statistically significant ($p = 0.000228$).

Figure 3: Anterior Grooming Bouts in Control and Experimental Groups

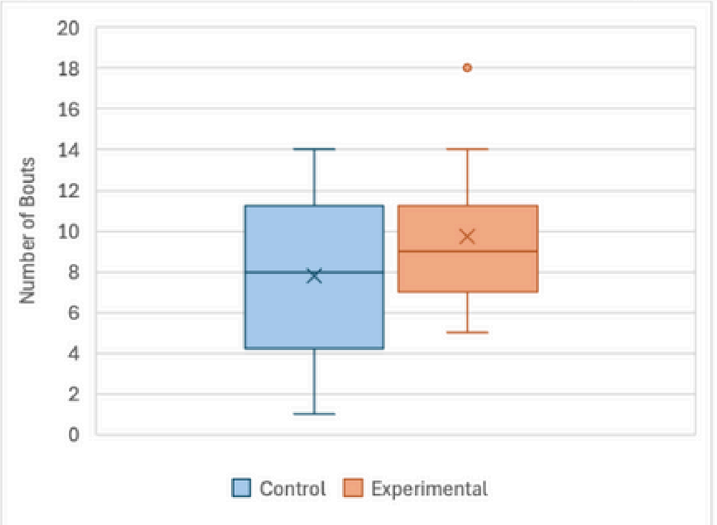


Figure 3: Experimental *Drosophila* showed a slightly higher mean number of anterior grooming bouts (M = 9.714) than controls (M = 7.786), but this difference was not statistically significant ($p = 0.219$).

Table 1: Summary Statistics and Significance Tests for Grooming Behavior in *Drosophila melanogaster*

Behavior Type	Group	Mean ± SD	P-value
Anterior Grooming	Control	7.786 ± 4.074	0.219
	Experimental	9.714 ± 3.347	
Posterior Grooming	Control	6.214 ± 4.411	**0.000228**
	Experimental	14.357 ± 5.639	
Total Grooming	Control	14.000 ± 5.251	**0.000929**
	Experimental	24.071 ± 5.700	

Table 1: Mean grooming bouts (± standard deviation) for control and experimental groups across anterior, posterior, and total grooming categories. p-values were calculated using two-tailed independent samples t-tests. Statistically significant differences are bolded. Results indicate that optogenetic dopamine activation significantly increased posterior and total grooming, but did not significantly affect anterior grooming.

Figure 4: Brain Imaging of Control *Drosophila melanogaster* without Dopamine Activation

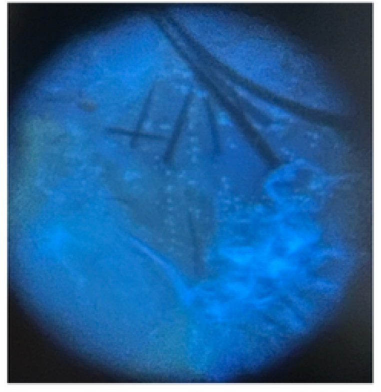


Figure 4: Image of a *Drosophila* brain expressing GFP without optogenetic dopamine stimulation. Baseline GFP expression is observed in key regions of interest. This serves as a control for neural activity.

Figure 5: Brain Imaging of Experimental *Drosophila melanogaster* with Dopamine Activation

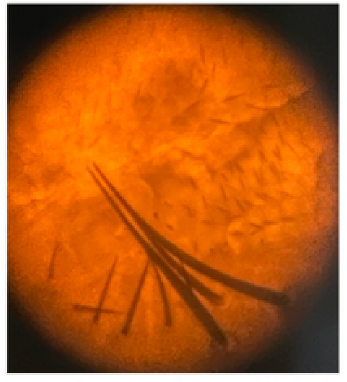


Figure 5: Image of *Drosophila* brain following optogenetic activation of dopamine neurons. Increased fluorescence is observed in key regions of interest driven by UAS line #55135, suggesting elevated neural activity associated with dopamine induced compulsive grooming behavior.

Figure 6: 3D Reconstruction of Dopaminergic Projections in the Central Complex and Mushroom Bodies

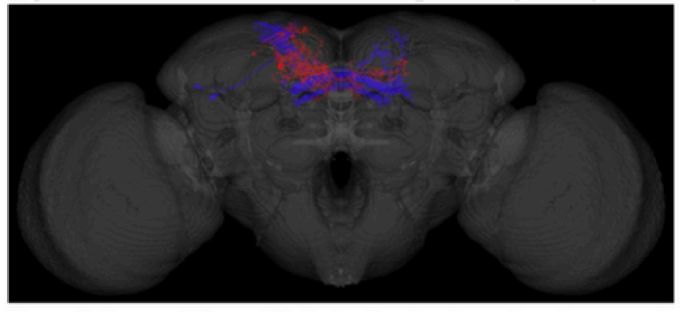


Figure 6: Image of *Drosophila* brain showing dopaminergic neuronal projections in the central complex (blue) and mushroom bodies (red). Red traces Kenyon cells and associated dopaminergic inputs within the mushroom body calyx and lobes, while blue traces dopaminergic inputs within the protocerebral bridge, fan-shaped body, and ellipsoid body (*Virtual Fly Brain*, n.d.).

Discussion

- **Key Findings**
  - Optogenetic stimulation of dopamine neurons in *Drosophila melanogaster* led to significant increase in total grooming bouts compared to controls
  - Experimental *Drosophila* averaged 24.071 grooming bouts compared to 14.000 in controls, demonstrating a statistically significant difference (p = 0.000929)
  - Increases were observed in both anterior and posterior grooming, suggesting a generalized effect on repetitive behavior
  - Brain imaging proved heightened activity in the central complex and mushroom bodies, which are functionally analogous to the human CSTC circuit implicated in OCD
  - These behavioral and imaging findings support the hypothesis that dopamine activation not only drives behavioral changes but also produces observable changes in brain activity within circuits homologous to the human CSTC circuit
- **Behavioral Insights**
  - Grooming increases were consistent across sexes and grooming regions, indicating the effect was not sex-specific or region-dependent
  - Some *Drosophila* proved modest behavioral changes, suggesting individual variability in dopamine sensitivity, similar to non-responders to OCD medication in clinical populations
- **Neural Implications**
  - Elevated dopamine signaling correlated with increased grooming and neural activation, confirming dopamine's role in driving compulsions
  - Fluorescence imaging proved increased activity in dopaminergic regions in experimental *Drosophila*
  - These results align with previous findings in rodents (Seiler et al., 2022) and human OCD patients (Melloni et al., 2012)
- **Relevance**
  - Confirms *Drosophila* as a viable, genetically tractable model for studying compulsive behavior and circuit-level dysfunction
  - Supports further exploration of dopamine targeting treatments, particularly for patients who do not respond to SSRIs
- **Limitations**
  - Grooming behavior may not capture the cognitive or emotional complexity of human OCD
  - Imaging was done after behavior, not in real time, limiting insight into neural activation timing
  - Human error (inconsistent handling, anesthesia exposure, etc.) and environmental factors (residual food, water, etc.) may have influenced grooming frequency
  - Small sample size and short duration of study (6 weeks)
- **Future Directions**
  - Incorporate real-time brain imaging to link neural firing with grooming behavior
  - Combine dopamine and serotonin manipulation to explore their interplay in compulsivity
  - Test additional genetic *Drosophila* lines to identify potential modifiers of compulsion
- **Conclusion**
  - Dopamine activation alone is sufficient to drive compulsive grooming behavior and neural changes in *Drosophila*
  - Confirms dopamine's direct role in compulsion and supports dopamine antagonists as a potential treatment path for SSRI-resistant OCD
  - Establishes a novel invertebrate model for future studies of circuit-level psychiatric disorders

References

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