

The Effects of Dopamine Addiction on *Drosophila* Evolutionary Behavior

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Project Description

Dopamine (DA) is a neurotransmitter whose receptors play a critical role in numerous human neural disorders. DA receptors activate various effectors through G-proteins and signaling cascades.¹ The reduced numerical complexity of the *Drosophila Melanogaster* dopaminergic system facilitates the study of mechanisms of reward, memory, and addiction, while simultaneously allowing for targeted genetic manipulation.² Clusters of dopaminergic neurons project to the central complex, associated with motor activity, and the mushroom body (MB) neuropil - a fly brain structure critical for associative learning and memory.³ Changes in DA signaling in *Drosophila* have been associated with reward-seeking behaviors⁴ that mirror human behavior related to addiction and substance abuse.⁵ Increased dopaminergic signaling is often associated with positive emotions and strengthening positive associations in the brain.⁶ A decreased level of dopaminergic signaling can lead to a decrease in positive emotions and an increase in negative emotions, such as depression and anxiety.⁷ Additionally, decreasing dopamine levels after repeated dopaminergic firing results in characteristic addictive behavior,^{8,9} and *Drosophila* show addiction symptoms by ignoring “risk signals” to obtain dopamine release.¹⁰ Improving the understanding of dopamine's effects on addiction could provide insight into whether this condition can alter evolutionary behaviors; the innate, genetically encoded responses that have been shaped by natural selection. This study could contribute to previous research on evolutionary inherited mechanisms of repulsion and risk avoidance. However, further research is required to comprehend the full effect of dopamine's impact on evolutionary behavior. Therefore, analyzing dopamine addiction behaviors can help further research the neural flexibility of instinctive behavior. It is hypothesized that when *Drosophila* DA neurons are pan-neuronally activated during consumption of food containing inhibitory cues that are natural to male flies, it will be sufficient to override their natural evolutionary avoidance. By utilizing optogenetically modified fruit flies to target dopaminergic neurons, the results from this proposal should: first, construct genetically modified *Drosophila* flies using the Gal4/UAS system to chronically activate dopamine release paired with fly food containing cVA (an otherwise aversive cue to male flies); second, allow for the neuroanatomical identification and wire graphing of DA neurons, their projections, and innervation patterns in the CNS using fluorescent microscopy and 3D-reconstruction; and third, produce a model of addiction-like states in adult male *Drosophila melanogaster* to investigate the potential effects of dopamine addiction on evolutionary aversive behaviors. The dopaminergic system plays a central role in addiction by reinforcing drug-seeking behaviors through increased production of dopamine, which 'teaches' the brain to prioritize drugs over natural rewards.²⁶ From human inheritance, we know that substance use disorder is high, and early life trauma is a significant trigger for SUD development in humans. However, the epigenetic mechanism underlying this development is under-researched.

Health Relevance Statement

Drosophila melanogaster, commonly known as the fruit fly, is an ideal model organism to study neurogenetics due to their genetic similarity to humans, their fast breeding, and easy genetic manipulation.¹¹ This aligns with research showing that dopaminergic projections in the brain's reward circuit drive motivation and reinforce compulsive drug-seeking behaviors, a hallmark of substance use disorders.²⁶ The results of this study have the potential to contribute to the research on dopamine's role in addiction and interventions for substance use disorders in clinical populations using behavior readouts. These experiments could also evaluate how the manipulation of dopaminergic pathways can demonstrate how neural circuits mediate behavioral plasticity, providing insights into how disrupted dopaminergic signaling underlies conditions like Parkinson's or Alzheimer's. Likewise, the evolutionary angle offers a unique framework to study how neurochemical pathways evolve and are preserved, which could guide targeted therapies in humans.

Specific Aims

Dopamine levels in fruit flies modulate biological processes in *Drosophila* flies such as courtship, sleep, and arousal.² They have four G-protein coupled DA receptors: Dop1r1 (DUMB), Dop1r2 (DAMB), DOP2R (DD2R), and DopEcR.²³ **Of the roughly 100,000 neurons in the adult *Drosophila* brain, about 130 cells are dopaminergic.**¹² **The Gal4/UAS system in *Drosophila* has been utilized to study genetic expression and functions in previous studies.**¹³ **This study will use the Gal4 line 8848 to recognize dopaminergic neurons, and the UAS line 55135 to activate the dopaminergic neurons of interest. Once the crosses have been created, the dopamine receptors can be modulated via optogenetics.**¹⁴ This experimental cross has already been successfully achieved through the channelrhodopsin-dependent optogenetic techniques to study spatial and temporal control.¹⁵ Structural and functional studies involving channelrhodopsin and dopamine as the Gal4/UAS demonstrate that the channel opens rapidly upon blue light stimulation.¹⁶ **In this study, the utilization of chronic red-light activation will be conducted on dopaminergic cells in fruit flies to assess the strength of addiction, conversely to evolutionary adapted aversive mechanisms. *Drosophila melanogaster* serves as an effective and widely used model for studying addiction in the brain, offering valuable insights into the stimulating and sedating effects of drugs, as well as drug-related reward mechanisms.**¹⁷ **Addiction—particularly drug addiction—is considered a disorder,**²⁴ **marked in flies by excessive seeking behaviors that override natural evolutionary drives. Fruit flies present an ideal model for researchers to investigate specific aspects and systems of addiction in a controlled manner. In *Drosophila*, dopamine is expressed across multiple neuron clusters that connect with diverse brain regions.**¹⁸ **Dopaminergic neurons play a role in regulating a range of behaviors and functions, including vision, locomotion, arousal, and memory processes (both acquisition and consolidation). By focusing specifically on dopamine neurons in the context of addiction, researchers can better understand the**

disorder's impact on downstream dopaminergic pathways. This study will be utilizing evolved evolutionary traits in drosophila such as the natural repulsion to Cis-vaccenyl acetate (cVA). cVA is produced by drosophila melanogaster male and female flies primarily to encourage flies into mating. However, in the process, it also has an inherent repulsion impact on the males²². However, comparisons to other evolutionary developed behaviors with scents such as conditioned fear responses to predator scents have been limited in data. There is minimal research on scents that cause repulsed behaviors in drosophila which have been studied and are readily available for use. This study proposes the evaluation of addiction on evolutionary behavior. It is expected that male flies that are conditioned to associate food containing cVA scent with chronic dopamine release will override their instinctive behavior to avoid the naturally repulsive scent of cVA and demonstrate a preference for the modified food over the regular kind when artificial activation of DA release via channelrhodopsin is interrupted. To explore this hypothesis, three specific aims are proposed:

1. Constructing two different Drosophila lines to activate dopaminergic neurons using the Gal4/UAS system. To create both lines, we will cross genetically modified flies that have the Gal4 gene manipulated to be expressed in DA neurons only with a UAS line that allows for the visualization of targeted neurons with fluorescent microscopy and a UAS line that allows for optogenetic activation of the targeted neurons.
2. Identifying the neuroanatomical structure of the DA-ergic system and wire diagramming of dopamine neurons, their projections, and innervation patterns in the central nervous system of Drosophila using fluorescent microscopy and 3D reconstruction. For this, we will use the first line of flies, in which DA neurons express mCherry, a red fluorescent protein, under a specific wavelength of light. This allows for clear visualization of neuroanatomical structures associated with the dopaminergic system and serves as a control to confirm targeting accuracy within the correct brain region.
3. Studying the effects of addiction on evolutionary repulsive behaviors. For that, the second constructed fly line will be placed in a vial with fly food containing cVA, a naturally aversive substance for Drosophila males, while their DA neurons are chronically activated by channelrhodopsin. Then, they will be tested for their preference for cVA-containing food or regular food when the artificial release of dopamine is ceased.

Methods/Timeline

Comments on approach and procedure

A variety of research techniques will be employed in the experiments proposed throughout this application. Optogenetics, a technique that uses light to control ion channels in neural cells, will be used to activate dopaminergic neurons in Drosophila melanogaster. For that, the Gal4/UAS system will be used to create genetically modified flies that express light-sensitive proteins in

their dopaminergic neurons. Fluorescent microscopy and 3D reconstruction will be used to visualize and map the dopaminergic neurons, their projections, and innervation patterns in the fly brain. Behavioral assays will be utilized to assess the flies' behavior changes, such as their willingness to consume food containing aversive cues. As this experiment involves living subjects who are sensitive to their surroundings, minor setbacks are expected. In sum, completion of the proposed experiments in this application will provide data contributing to our better understanding of the neurobiology of addiction and reward, and importantly, the interplay between dopaminergic signaling and evolutionarily conserved behaviors.

Animal care/cell line generation

To create the experimental lines used in the study, flies of the Gal4 line 8848, which targets all dopaminergic neurons, were crossed with flies of the UAS line 35774 for visualization via MCherry, a red fluorescent protein (RFP) used to tag components in cells, and 55135 for optogenetic manipulation via channelrhodopsin. *Drosophila melanogaster* flies were raised in vials containing food (86% water, 0.574% agar, 6.30% cornmeal, 1.52% yeast, 4.65% dry molasses, 0.39% propionic acid, 0.15% methylparaben, 0.52% ethanol). To generate light-induced behaviors, 1:200 all-trans retinal was added to the food. Male and female pupae were isolated in vials containing food. Approximately 5 minutes before experimentation, adult flies were anesthetized using carbon dioxide.

Behavioral assay description

The assay utilized in this study will not follow a standard feeding assay; the fruit flies will be placed in a conditioned place preference assay (CPP). This will be used towards the goal of examining addiction traits within male *Drosophila melanogaster* in relationship to the evolutionary aversion of cVA. Three control fly groups will be used for this experiment. The first control, Group 1, consists of male wild-type flies that will be placed in cVA-containing food for one week. They will then be assessed for sensitization to cVA by being placed in DAM tubes with one side containing food with cVA and the other side containing food without cVA. The amount of time the flies spend on either side will be measured within 24 hours to determine preference. The second group, Group 2, will comprise a control group of 6 male flies not conditioned to associate food with cVA with dopamine release under chronic red light. The group will have flies crossed for 8848 x 55135 that will be each placed in the CPP assay using DAM tubes set up identical to the aforementioned ones for 24 hours. Group 3 will act as an experimental group containing 6 male fruit flies crossed with 8848 x 55135 in individual DAMs with the same setup. This group of male *drosophila* will be the group conditioned to associate cVA in food with dopamine release via chronic exposure to red light. This group of male fruit flies will also be measured for preference depending on the time spent on either side.

Experimental protocol

The flies used in this experiment were all male due to their natural aversion to cVA. Three groups of flies were used: wild-type flies and two genetically modified groups. Groups 1 and 3 were kept in a vial with food containing 5 μ l of cVA. Group 1 was kept in the vial for one week. Group 3 was kept in the vial under constant red light emission for 6 hours. Group 2 was also placed in a vial with cVA-containing food for 24 hours, but it did not undergo red light emission. The three groups were then individually placed in DAM tubes that contained cVA-containing food on one side and regular food on the opposite side. Group three was placed in the dams for 90 minutes to observe their behavior. The flies' behavior was then recorded over the next hour to assess each group's food preference.

Data analysis

The Drosophila Activity Monitor software (DAM) will record the data along with video footage for observations. DAM measures the locomotor activity of up to 32 individual flies, each in an 80mm-diameter tube. The 80mm length tubes are placed end-to-end in the centers of each tube pair, allowing a single fly to walk 2 tube lengths between the end cap which enables enhanced position-preference assays for odor, food, or light using a gap between the butted tube ends as a vent. Individual encounters will be clipped from full-length records and will be scored accordingly for behavioral patterns using Microsoft Excel spreadsheets, as well as for the analysis of the DAM data.

Research Strategy

Significance

Dopamine is crucial in modulating behaviors like learning, memory, and reward processing. In humans, dysfunctions in dopaminergic pathways are strongly correlated with addiction and substance use disorders (SUDs), with a significant interplay between dopamine signaling and behavioral regulation. The fruit fly dopaminergic system contains four g-coupled DA receptors: Dop1R1, Dop1R2, Dop2R, and DopEcR, which contribute to specific behavior outputs in flies.¹² Manipulation of dopamine in association with these receptors has provided unique insights into the interplay between neural circuitry, behavior, and environmental factors

Dating back to the early 2000s, parallels between dopaminergic signaling in fruit flies and mammals have been recognized.¹⁷ Thus, *Drosophila melanogaster* is an effective model for drug addiction due to its robust genetic tools and conserved neurochemical pathway. As a widely used model organism, dopaminergic signaling in fruit flies is integral to understanding reward and addiction-like behaviors in humans due to similar behavior responses. Addiction-like behaviors in fruit flies are typically characterized by persistent reward-seeking tendencies, often overriding innate aversive behaviors such as avoidance of harmful cues. For example, flies conditioned to associate a particular stimulus (e.g., light or food) with dopamine release will repeatedly seek that stimulus, even under conditions that would typically trigger avoidance, such as exposure to aversive pheromones like cVA (cis-vaccenyl acetate). Flies subjected to chronic dopamine

stimulation exhibit escalating reward-seeking tendencies, withdrawal symptoms upon dopamine termination, and risk-prone decisions. As a result, since these behaviors occur in quantifiable, recurrent patterns, it provides a stable framework for modeling addiction.

With the use of optogenetic techniques, activation of dopaminergic neurons can enhance the ability to examine dopaminergic dynamics in *Drosophila* as previous research has demonstrated precise control of dopamine and serotonin release in flies, linking these neurotransmitters to behavioral paradigms relevant to addiction.¹⁶ As such, this approach allows for a resolution in exploring the mechanisms of reward-seeking behavior, including overcoming natural aversive cues for dopaminergic rewards. In conjunction, the Gal4/UAS system provides a powerful method to target gene expression in precise neural populations, enabling the manipulation of DA signaling in behaviorally important circuits.

The role of DA in addiction-related behavior has been further investigated by studies demonstrating its involvement in risk-taking and reward persistence despite adverse conditions. Since dopaminergic systems can drive compulsive reward-seeking, a phenomenon that mirrors substance use disorders in humans, such findings underscore the translational potential of *Drosophila* research in understanding the neural foundation of addiction.¹⁵

Even with these insights, knowledge gaps remain in understanding the specific neuronal circuits and epigenetic mechanisms underlying dopaminergic regulation of behavior. Though prior research has linked dopamine with risk-related behaviors and withdrawal symptoms in *Drosophila*, the role of these neural circuits in evolutionary risk avoidance remains relatively underexplored. This study aims to address these gaps by utilizing renowned techniques including the Gal4/UAS system, optogenetics, and behavioral assays, to explore how dopaminergic activity influences risk-reward decision-making in *Drosophila*. These experiments will shed light on the connection between neural circuits, dopamine signaling, and behavior but can also provide translational insights relevant to human addiction and withdrawal mechanisms through generations. A more robust understanding of these mechanisms could lead to targeted therapeutic strategies for dopamine-related dysfunctions in humans.

Approach (preliminary data)

The use of the Gal4/UAS system is one of the most important tools in *Drosophila* genetics.¹⁹ In this system, the promoter of a target gene is inserted upstream of the yeast transcription factor gene Gal4, which will be expressed in all the cells containing the target gene.²⁵ The fly line containing the Gal4 is crossed with a line carrying a UAS transgene, which can be used to either visualize or manipulate the target cells.²⁵

For this proposal, flies with the Gal4 transcription factor of line 8848 (chromosome 3), which targets all dopaminergic neurons will be crossed with two different UAS lines. The first is the

UAS line 25774 (chromosome 2), which contains the MCherry, a red fluorescent protein (RFP) that is used to tag and visualize the target cells. The second is the UAS line 55135 (chromosome 2), which contains channelrhodopsin, which allows for activation of the DAergic neurons through optogenetics.

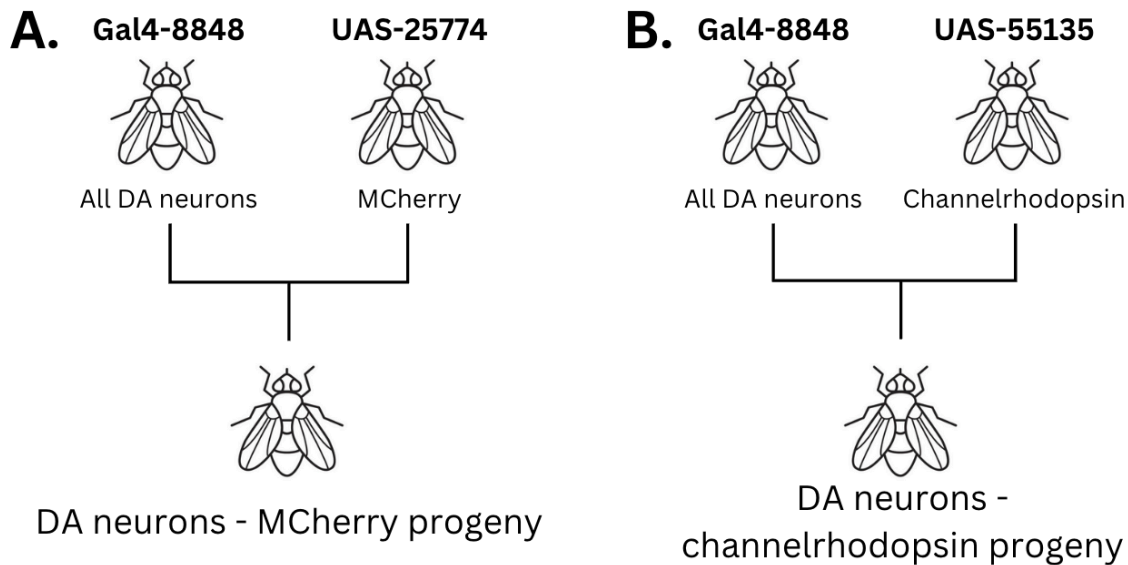


Figure 1. Visual representation of the Gal4-UAS crosses used in the experiment. This image shows the initial transgenic Gal4 and UAS lines used in the experiment, the crosses that were performed, and the resulting fly progenies. (A) shows the cross between the Gal4 line 8848 that targets all dopaminergic neurons with the UAS lines 25774, which expresses MCherry, allowing for the fluorescent visualization of DA neurons. (B) shows the cross between the Gal4 line 8848 that targets all dopaminergic neurons with the UAS lines 55135, which expresses channelrhodopsin, allowing for the optogenetic activation of DA neurons. The fly images used in this figure were drawn by Sherry Drawings.

The Gal4-8848/UAS-25774 transgenic flies were utilized to visualize the dopaminergic system in *Drosophila*, as all DA neurons were targeted with MCherry. The fly brain was dissected and mounted to be visualized under a fluorescent microscope. The results of this visualization can be seen in Figure 2.

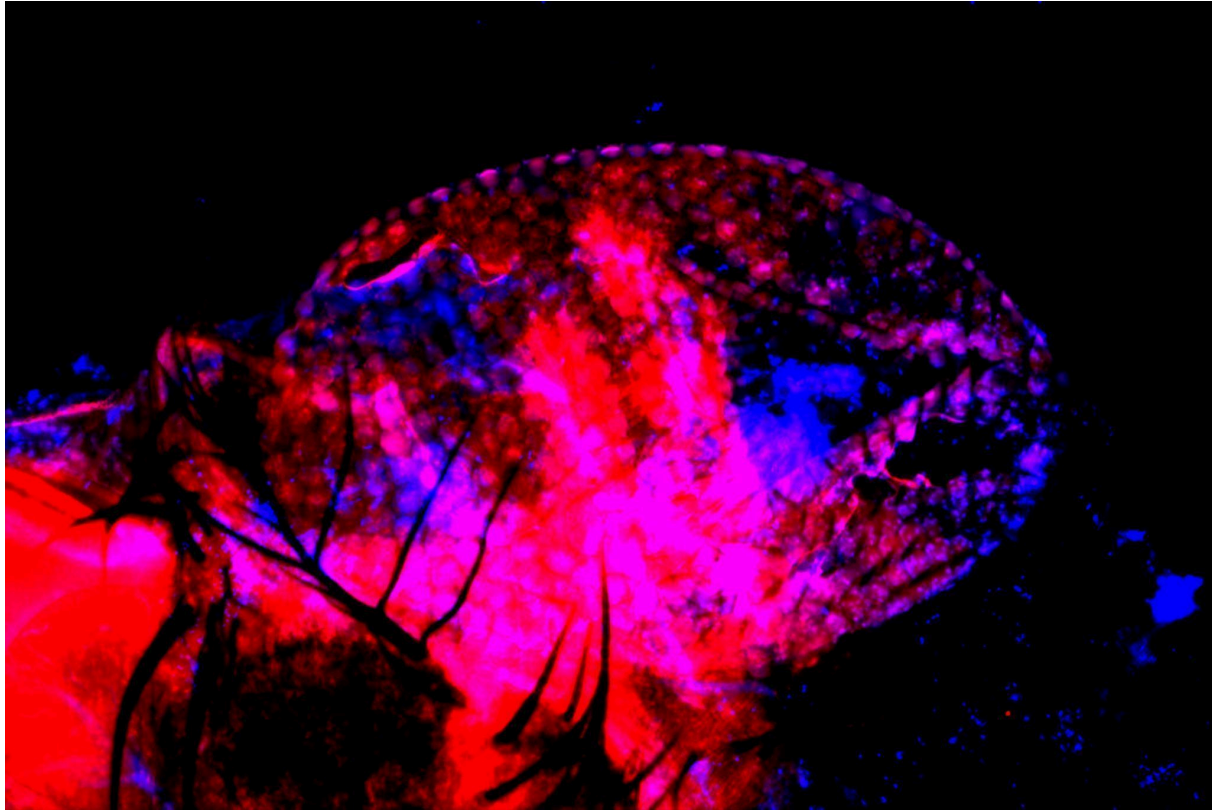


Figure 2. Drosophila dopaminergic system. This image shows a Drosophila dopaminergic system under a fluorescent microscope with 20x magnification. Flies from the Gal4/UAS cross 8848 x 25774 were used. The dopaminergic cells stained by MCherry are depicted in red and the cell nuclei stained by DAPI are shown in blue.

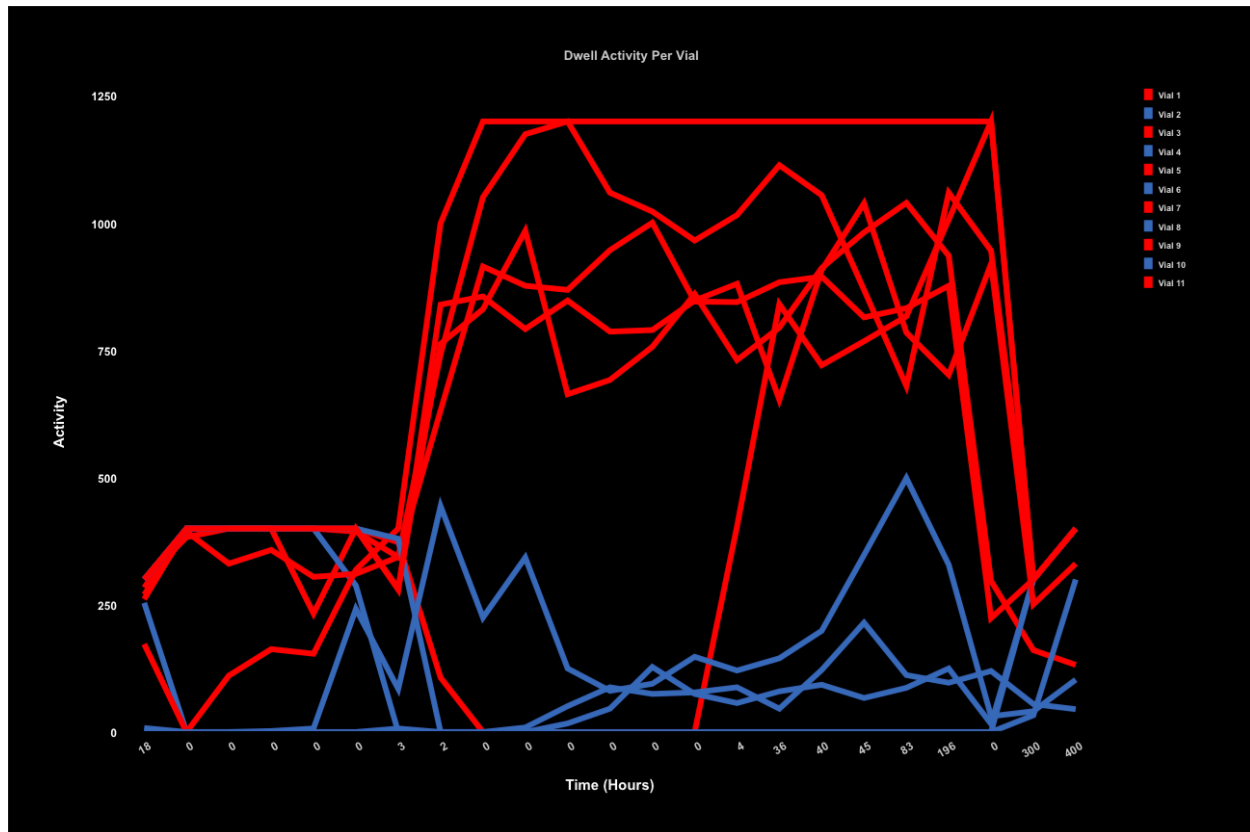


Figure 3. *Drosophila* were not sensitized to *cVA* scent. Wild-type *Drosophila* were placed in a vial with *cVA*-containing food for one week and then placed in DAM vials so their sensitization to the scent could be assessed. Red lines show vials with regular food and blue lines show vials with *cVA*-containing food. The flies clearly preferred regular food, indicating they were not sensitized to the scent of *cVA*.

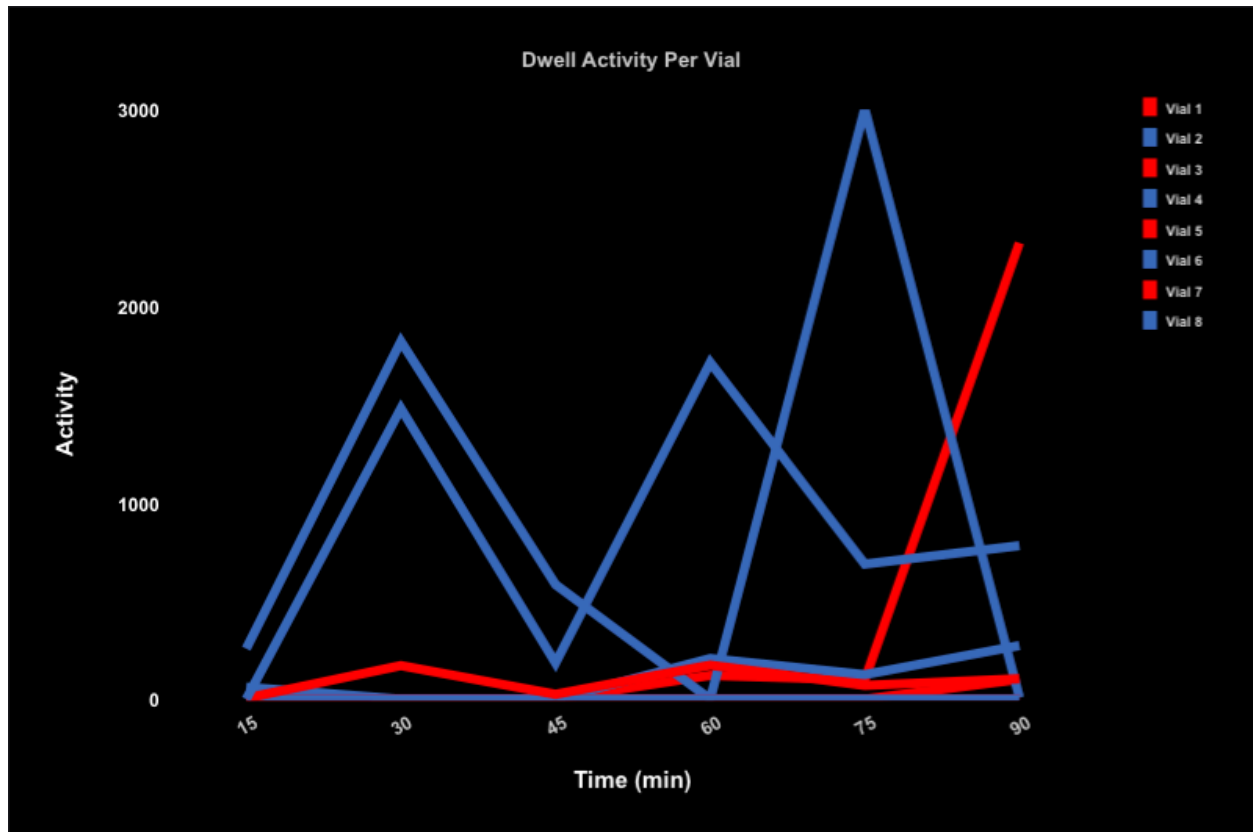


Figure 4. Six crossed (8848x55135) *Drosophila* were placed in cVA food for six hours while exposed to red light causing chronic dopaminergic activation. After being chronically stimulated the flies were placed in two combined DAM vials for 90 minutes. One vial contained cVA food, while the other contained normal food. Each fly was able to roam freely between the two vials. This graph represents the Dwell Count data (how long each fly spent in each area of the vial). The red lines represent the vials with normal food while the blue represents the vials with cVA food. Based on the graph it is clear that *drosophila* heavily favored the food with cVA.

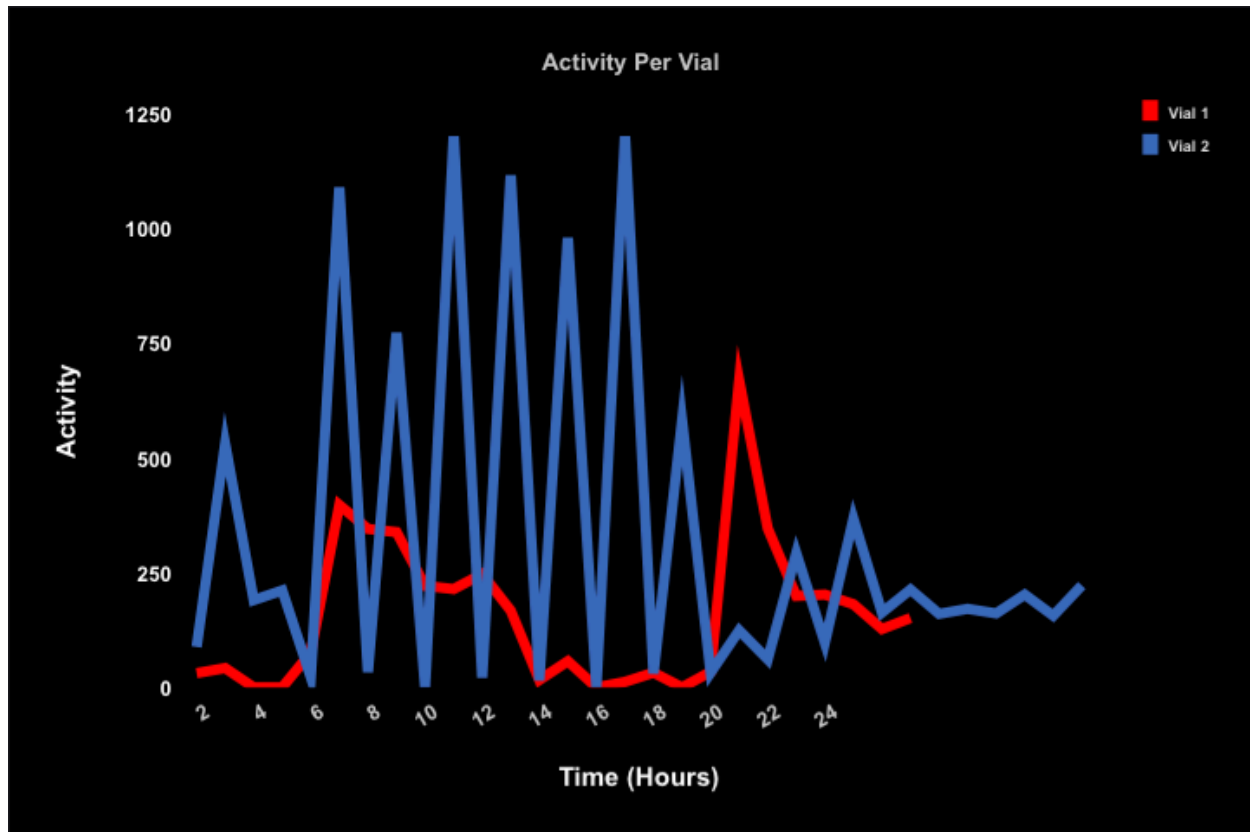


Figure 5. Six crossed *Drosophila* (8848x55135) were exposed to cVA food for 24 hours under red light, inducing chronic dopaminergic activation. Following this stimulation, only one fly survived. The surviving fly was then placed in a setup with two connected DAM vials for 24 hours—one containing cVA food and the other containing normal food. The fly had unrestricted access to move between the two vials. This graph illustrates the Dwell Count data for the fly over the 24-hour period. Red lines indicate vials with normal food, while blue lines represent those with cVA food. The graph clearly demonstrates a strong preference for cVA-containing food by the fly.

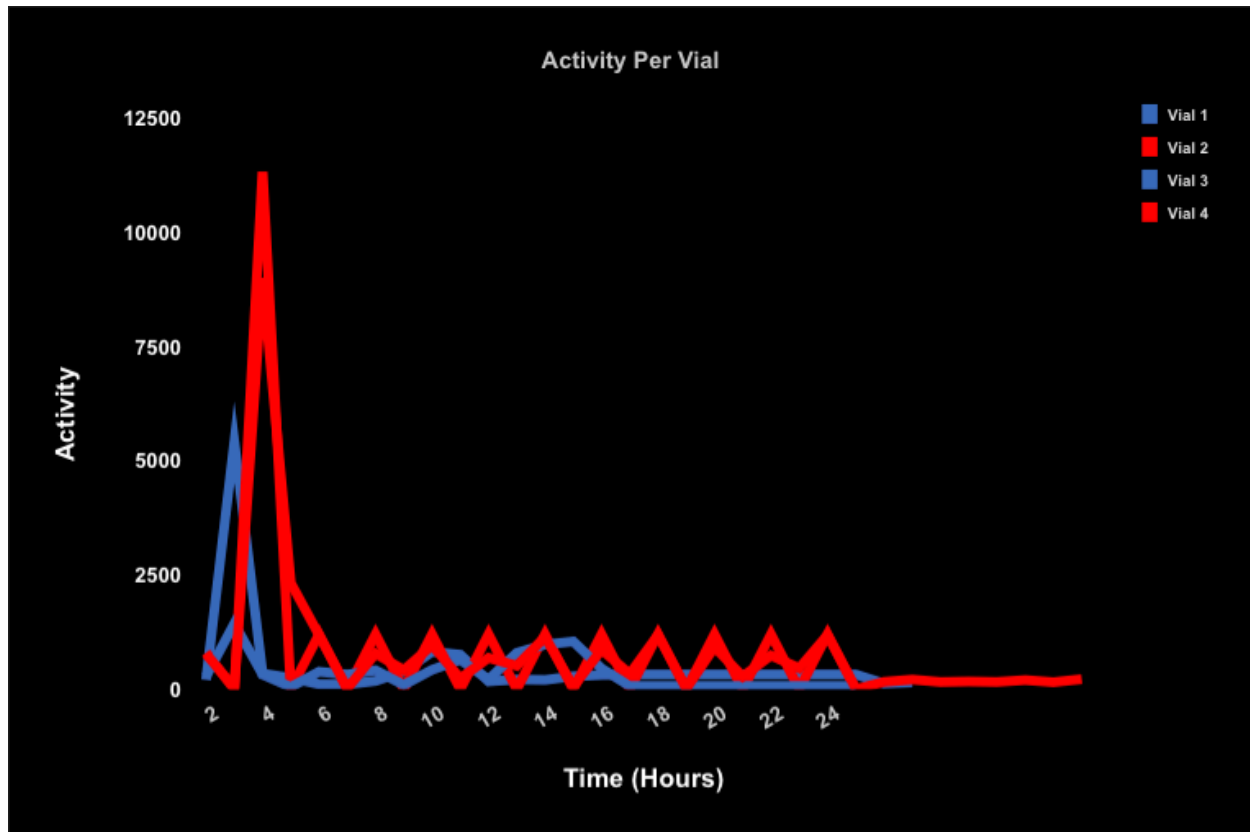


Figure 6. *Drosophila* used as a control were placed in identical initial conditions as previous red light-activated flies, however these flies did not receive chronic red light. After 24 hours these flies were placed in DAMs mirroring those of the red light-activated flies. Based on the graph the flies without chronic activation did not favor food with cVA.

Results

Two periods of observation produced findings that indicated a preference for the cVA. Using the DAM analysis, the chronically activated flies demonstrated a liking for the food containing cVA. This was consistent across the 90-minute interval and 24 hours period. The chronic red-light activation of dopaminergic neurons demonstrates addictive behavior through the flies' heightened activity in the cVa side of the tube. Though Group 2 flies did die, this will be related to insufficient resources to minimize error. As *Drosophila* are sensitive to fungi and possibly viruses, an infection could have spread, inexplicably killing the flies.

Approach (experimental design)

Specific aim 1: Constructing two different drosophila lines to activate dopaminergic neurons using the Gal4/UAS system

Experiment 1a: Using Gal4/UAS to create cross-genetically modified drosophila with light-sensitive channelrhodopsin activated dopaminergic neurons

Rationale: This experiment uses 2 Gal4/UAS crosses with dopamine, one for dopaminergic neuron visualization, and the other for light-sensitive activation of dopamine neurons. We will

use the first cross to map the DA neuronal complex in the fly brain (2a), and the second cross for experimenting with addiction (3a.)

Design: To produce both lines, we will cross genetically modified flies in which the Gal4 gene is engineered to be expressed only in DA neurons (drosophila line 8848), with one UAS line that enables visualization (drosophila line 25774 MCherry) of the targeted neurons via fluorescent microscopy and a UAS line (55135) that permits optogenetic activation of the targeted DA neurons via channelrhodopsin. The Gal4/UAS system is a powerful genetic tool we will use to study specific gene expression in fruit flies. In this system, the Gal4 gene is inserted into the fly genome under the control of an enhancer. When the enhancer is active, Gal4 is expressed; when inactive, Gal4 is not expressed. Different enhancers are active only in specific tissues, allowing for precise spatial control of gene expression. The Upstream Activation Sequence (UAS) is a DNA segment that, when bound by the Gal4 protein, promotes the transcription of a downstream gene of choice. We will begin with the components of the system separated into two fly lines. Our driver line contains the Gal4 gene under the control of a native tissue-specific enhancer, which ensures Gal4 expression occurs only in specific cells—in our case, dopaminergic neurons. Our second fly line, the reporter line, carries a reporter gene downstream of the UAS. When the two fly lines are crossed, both Gal4 and the reporter gene are expressed. In cells where Gal4 is expressed, Gal4 binds to the UAS, activating the expression of the reporter gene. In our experiment, we will use two reporter genes: MCherry, which encodes a red fluorescent protein for visualization, and a channelrhodopsin reporter, which allows for optogenetic control of neuron firing using light. This enables us to visualize with one line and manipulate the activity of dopaminergic neurons with the other. We will perform the cross by collecting 10 males from our 8848-driver line and 3-4 female pupae from each reporter line. Five males from the driver line will be placed in a vial with food and 3-4 female pupae from the MCherry line. The five remaining males will be placed in a vial with food and 3-4 female pupae from the Channelrhodopsin line. These flies will be labeled as F0 and is where the initial cross will be created. To ensure the cross is successful we will shine a red light on the MCherry cross. We expect to see the illumination of the fly's brain as the light is shone, this will serve as proof of a successful cross.

Specific aim 2: Identifying the neuroanatomical structures of the dopaminergic (DA) system

Experiment 2a: Visualizing the wire diagramming of dopamine neurons, their projections, and innervation patterns in the central nervous system of *Drosophila* with fluorescent microscopy and 3D reconstruction.

Rationale: Successfully visualizing and capturing images of the DA system in the drosophila brain using a fluorescent microscope

Design: To visualize the neuroanatomy of dopaminergic (DA) neurons labeled with MCherry, we will use the first line of flies mentioned. These flies express red fluorescence in their DA neurons when exposed to a specific wavelength of light, enabling clear visualization of their neuroanatomical structures. After confirming the success of the genetic cross in Experiment 1a, we will proceed with dissections to observe the DA neurons under a microscope. This process involves carefully removing the head of a fly from the MCherry-crossed line, delicately separating the eyes, and exposing the brain. Once a successful dissection is completed, we will use a microscope equipped with a light source optimized for the red fluorescence emitted by MCherry-labeled neurons. Using this setup, we will capture images of the illuminated neurons,

confirming the success of the cross and providing a detailed map of DA pathways within the *Drosophila* brain.

Specific aims 3: Studying the effects of addiction on evolutionary repulsive behaviors

Experiment 2a: Data collection through measurement of food preference in DAMs

Rationale: This experiment aims to begin the data collection on the effects of addiction on evolutionary repulsive behaviors through the measurement of male *drosophila* locomotor activity to observe food preference.²⁰ The food choices will consist of media containing cVA and non-manipulated media to confirm the results of chronic red-light activation of dopaminergic neurons which demonstrates addictive behavior.

Design: To determine that desensitization is not a factor towards the media preference, 6 male *drosophila* wildtype flies are placed in 2 interconnected DAMs each, containing cVA mixed media on one end and non-manipulated media on the other end. Both ends of the glass tube are plugged with cotton wool to allow airflow. The measurement of the locomotor activity for the wild flies will determine the sensitization influence of the behavior male *drosophila* exhibits with evolutionary repulsion for cVA.²¹ The control group of 6 male *drosophila melanogaster* crossed with Gal4 gene line 8848 and UAS line 55135 via channelrhodopsin will be placed in 2 interconnected DAMs each to observe the food preference behavior of the cVA mixed media and the non-manipulated media. This will serve as a comparison to the 6 male *Drosophila* flies containing the same cross as the control group, however, chronically exposed to red light activation of the DA neurons in the presence of cVA. These 6 male *Drosophila* flies will each be placed in DAMs, set up in an identical form to the previous groups of male flies, and observed for the measurement of their locomotion activity which will determine the food preference for the flies.

In conclusion, this study provides compelling evidence supporting the hypothesis that chronic activation of dopamine neurons in *Drosophila melanogaster* can override their natural evolutionary aversion to the cVA pheromone. The experimental results, as depicted in Figures 4 and 5, consistently showed that male fruit flies with chronically stimulated dopaminergic systems exhibited a significant preference for food containing cVA, a normally repulsive substance. This demonstrates a form of addiction-like behavior where the reward associated with dopamine release outweighs an innate avoidance mechanism. This finding reinforces the critical role of dopamine in reward-seeking behaviors and its capacity to modulate even evolutionarily conserved behaviors. The establishment of this *Drosophila* model of addiction-like states offers a valuable platform for future research into the neurobiological underpinnings of addiction and the potential for targeted therapeutic interventions in humans. This study's insights into the interplay between dopaminergic signaling and instinctive behaviors provide a unique evolutionary perspective on addiction, potentially guiding the development of novel strategies to address substance use disorders.

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