Olfactory Virtual Reality Simulations on *Drosophila* Larva Indicate that Attraction and Aversion are Not Opposite Behaviors

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Abstract

Drosophila melanogaster, commonly known as the “vinegar fly”, is a model organism for studying olfaction-induced behavioral activity. The behavior of positive chemotaxis or attraction from the activation of odorant receptors such as Or42a are well-characterized through extensive prior research. However, the behavior from the activation of aversive odorant receptors like Or49a are not well understood. To characterize aversion, and to test whether aversion and attraction have equal and opposite behaviors, I utilized the PiVR tracking system to simulate several odor conditions by applying light gradients on optogenetically modified third-instar larva. I have concluded that the characteristics of aversive behavior are not directly opposing the characteristics of attractive behavior through the analysis, and comparison of turn rate modulation, trajectories, and preference indexes between Or42a and Or49a light-stimulated larva.

Introduction

The detection of environmental cues is essential for an organism to survive. There are many ways to receive and respond to external stimuli and olfaction is one that senses external chemical signals. In *Drosophila*, the initial detection of chemicals is recognized through olfactory sensory neurons (OSNs) that express olfactory receptors (ORs). To distinguish between multiple chemicals, these ORs have specific binding sites for certain compounds. Following the recognition of a chemical, the organism can elicit a response that we can observe through their behavior. Chemotaxis can be characterized in two classes: positive chemotaxis or the movement towards a stimulus, and negative chemotaxis or the movement away from a stimulus. There are many olfactory receptors in *Drosophila* that induce positive chemotaxis or attraction, which are well-characterized through extensive behavioral studies on olfactory receptors such as Or42a. Positive chemotaxis is beneficial for organisms to: locate sources of odors, to find food, or to define the pheromones to find mates. There are also olfactory receptors that induce negative chemotaxis or aversion. Evolutionarily, third-instar *Drosophila* larva fall victim to predation of several organisms including a genus of parasitic wasps, *Leptopilina*. These wasps lay their eggs in the *Drosophila* larva, which later consumes the larva from the inside and kills it. To avoid these larval parasitoids, *Drosophila* larva have developed a predator-detecting olfactory circuit that is initiated with the activation of the olfactory receptor, Or49a, from the binding event with the wasp pheromone, iridomyremecin [3].

Attractive behavior in *Drosophila* is well understood through extensive studies on single-odor behavioral trials, where the modulation trends in running, stopping, head-casting, turn rate and accuracy, and weathervaning have been characterized [1,4]. Weathervaning is the mechanism in which larvae gradually curve toward higher concentration in a circular manner [5]. On the other hand, aversive behavior is not well understood. The Or49a OSN is a good candidate for classifying aversion since the neuronal circuitry has been already identified. With the limited time and resources in collecting the *Leptopilina* wasp pheromone, we will instead stimulate select neurons in the animal's "nose" via optogenetics to mimic exposure to a real odor.

Regarding their definitions, attraction and aversion may seem mutually exclusive, but this may not carry over in a behavioral analysis. Behavioral attraction has distinct modulations in run speed, turn rate, and turn accuracy in a larva's movement towards a stimuli, while behavioral aversion may not necessarily have those diametric modulations. In this experiment, I will be testing whether attraction and aversion display equal and opposite behaviors with a virtual reality simulation on optogenetically modified flies.

Methods

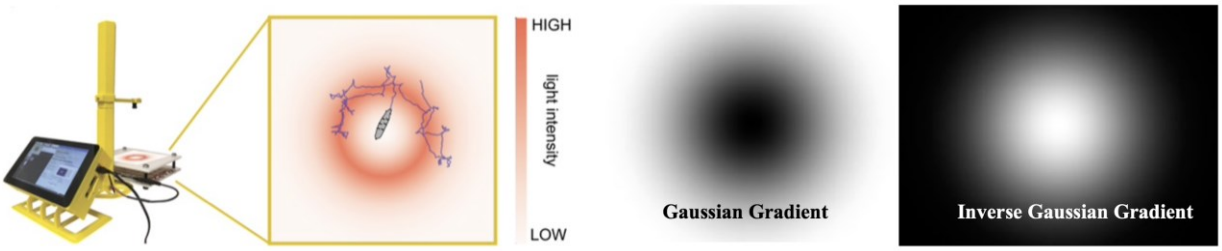
Genetic and Optogenetic Tools

ChrimsonR is a light gated ion channel that may induce neural activity when exposed to light. In flies, the specificity of ChrimsonR expression is based on the Gal4/UAS system. Here I selectively expressed ChrimsonR in two olfactory receptors governing attractive and aversive chemotaxis, Or42a and Or49a, respectively. I generated three fly crosses: a control group (W1118/UAS-ChrimsonR, attractive

chemotaxis group (Or42a-Gal4/UAS-ChrimsonR), and an aversive chemotaxis group (Or49a-Gal4/UAS-ChrimsonR).

Raspberry Pi Virtual Reality (PiVR) Tracking System and Virtual Reality Odor Gradients

To record the third-instar larva's activity, I used a behavioral tracker, PiVR, that is equipped with an LED light stage and a camera to record from above. The larva is carefully placed in the center of a 100mm petri-dish containing 1.5% agarose. The trials are 5 minutes long and the camera records the larva's head, tail, and centroid position at 30 frames per second. The 3 sensory environments seen in this experiment are no light stimulation, Gaussian gradient (since odor gradients follow a gaussian distribution, where the odor concentration is strongest in the center and gradually decreases further away) and inverse Gaussian gradient (to prevent the larva from touching the wall and compromising the trial). I will use a low-powered rig that emits light at roughly 1–2W/m^2 and a high-powered rig that emits light at roughly 40–50 W/m^2.



Results

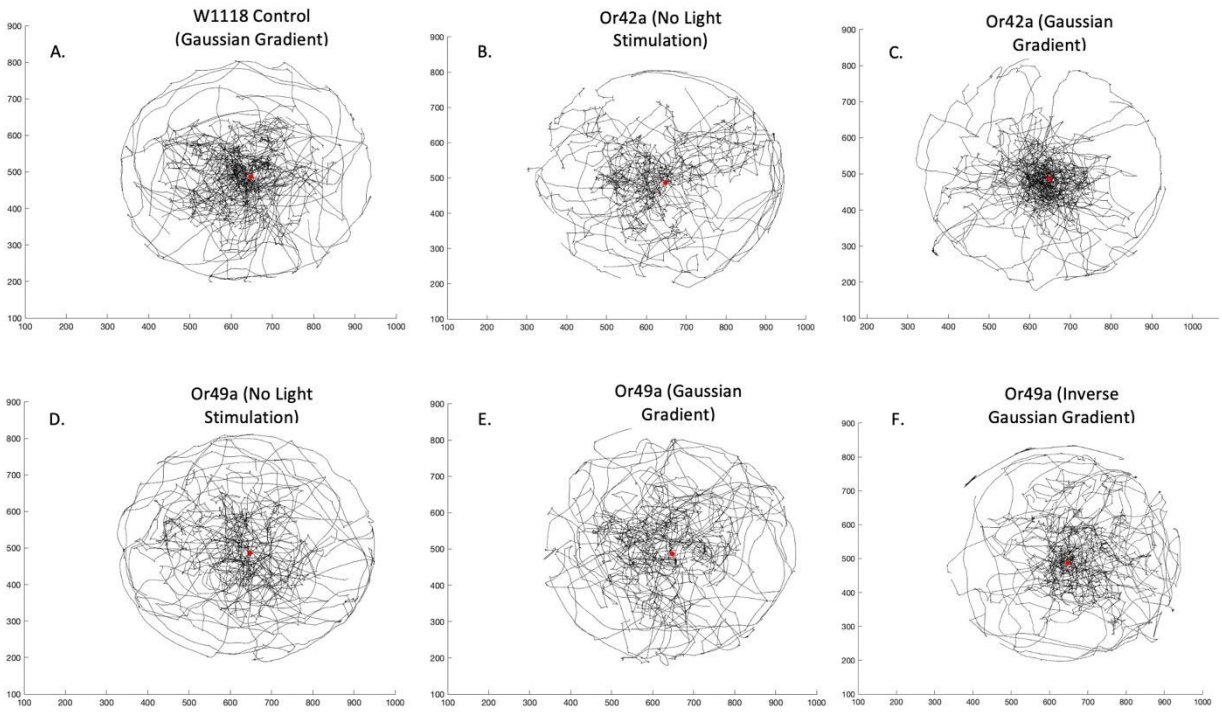


Figure 1. Each line represents a 5-minute trajectory of one larva and each graph represents the collection of trajectories of one larval group on the low-powered rig. The sample size of each larval group respectively is (n=31), (n=32), (n=33), (n=31), (n=32), and (n=34).

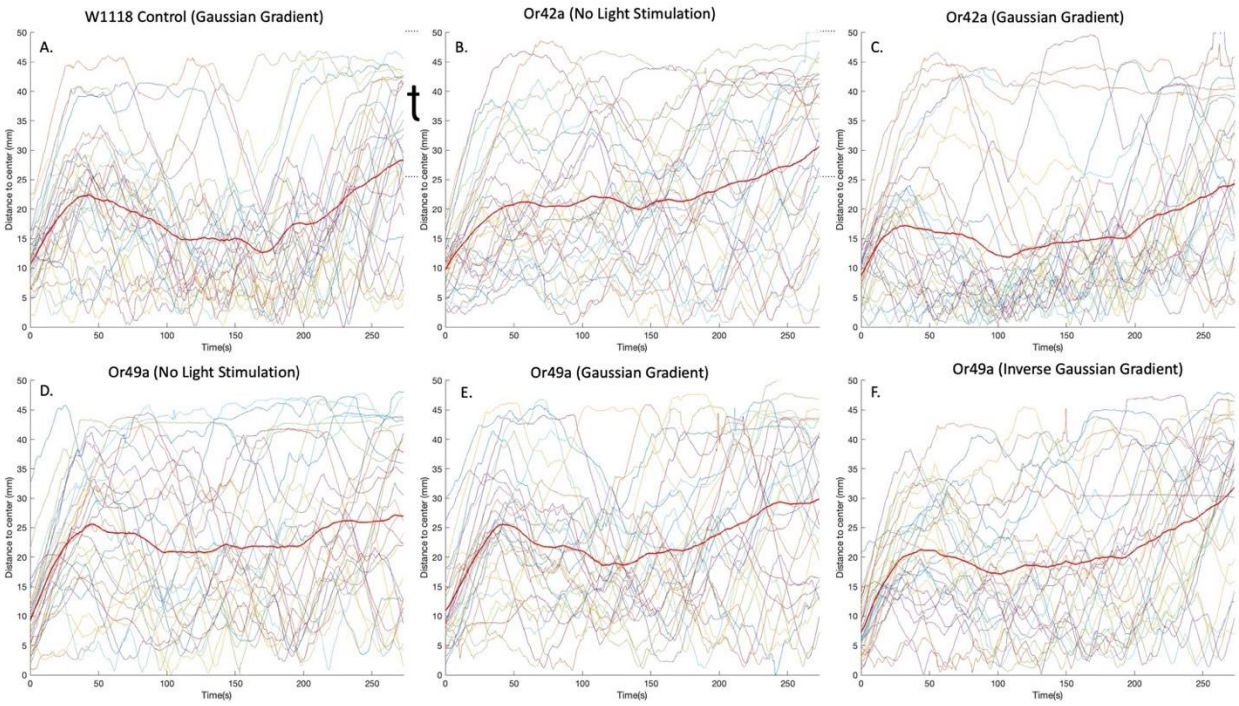
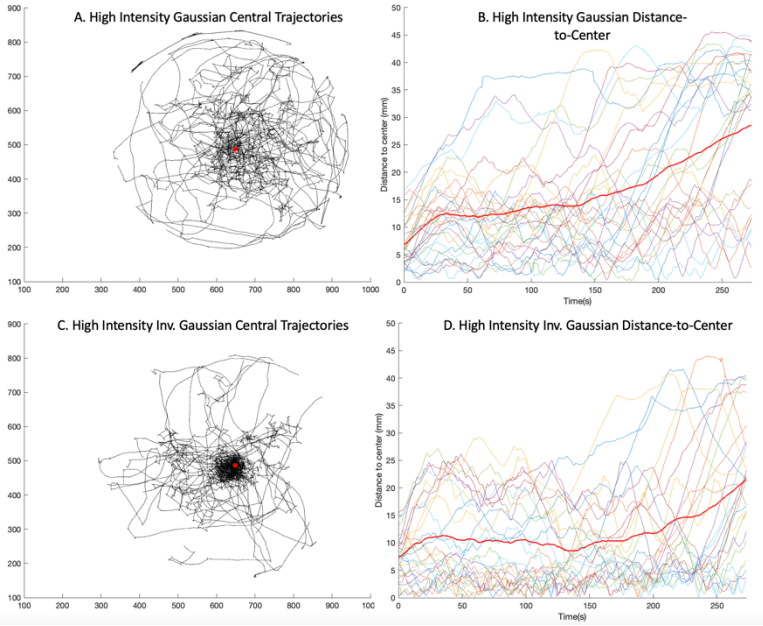


Figure 2. The larva's distance away from the center over the 5-minute trajectory for each group on the low-powered rig. The bold red line represents the mean distance away from the center.

Larval Groups in Different Light Conditions	Preference Index
W1118 (Gaussian Gradient)	0.6099
Or42a (No Light Stimulation)	0.501
Or42a (Gaussian Gradient)	0.711
Or49a (No Light Stimulation)	0.4755
Or49a (Gaussian Gradient)	0.4653
Or49a (Inverse Gradient)	0.5375

Table 1 and Figure 3. The Preference Index (PI) of each group in their respective sensory environments. The PI is the proportion of time spent inside the center circle over the total trial time. In this experiment, the area of the circle is 20% the area of the petri dish.



Or49a Larva in Different Light Conditions	Preference Index
Low Intensity Gaussian	0.46
High Intensity Gaussian	0.62
Low Intensity Inverse Gaussian	0.53
High Intensity Inverse Gaussian	0.69

Figure 4 and Table 2. Larval behavior on the high-powered rig seen through the central trajectories, distance-to-center, where-to-turn, the preference index data. The sample size of the two groups was n=30.

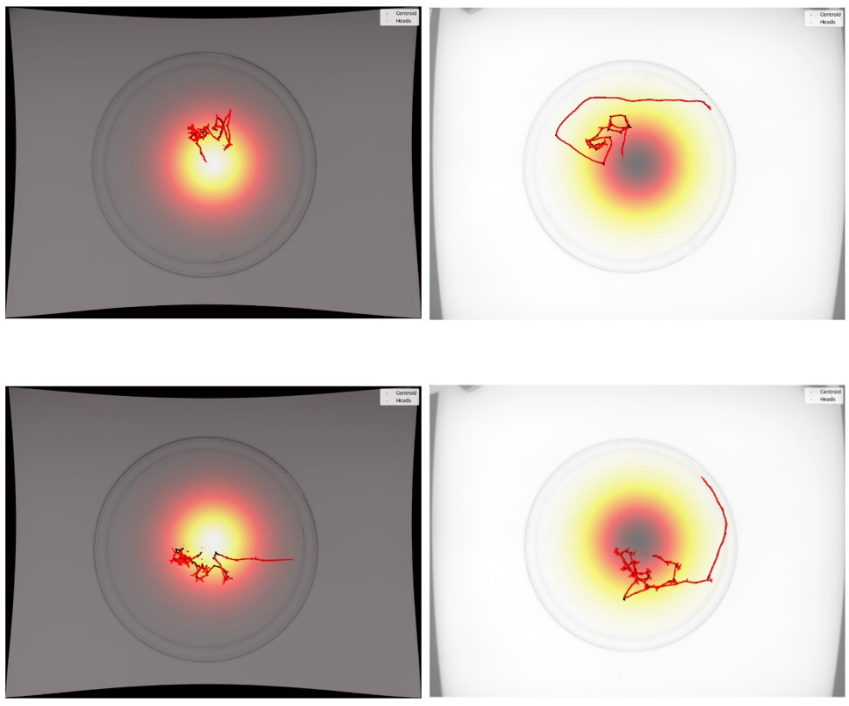


Figure 5. Low-powered (left) and high-powered (right) Gaussian gradient trajectories that display Or49a larval movement in red.

Discussion

Aversion, and attraction, are commonly defined as counterparts. Although odor receptors and neural pathways are structured similarly for opposing stimuli, an animal's behavioral response can vary greatly. For instance, in *Drosophila* larva, aversion may evoke high rates of reorientation to navigate away from the source, whereas attraction may evoke increased speed of crawling towards the source. We suspect that the mechanism of chemotaxis for innately aversive odors is a unique arrangement of behavioral decisions that may not be captured by the reciprocal decisions for attractive odors.

The compilation of trajectories in figure 1. allows us to infer upon the broad trends in the larva's movement over time. This helps us visually see where larva of a particular group tends to stay at, shown in the density of black lines in a given area. In the Or42a Gaussian group, we observe that the larva is attracted to the center of the petri-dish due to the high density of lines in the center. This is expected given that Or42a is known to elicit strong attraction [1]. In the Or49a Gaussian group, we observe an increase in larval trajectories along the side of the petri dish and a much lower density of larva in the center. Respective to the W1118 control group, the Or49a animals in the Gaussian gradient have an increased distribution in the petri dish. Although Or42a elicits attraction and Or49a elicits aversion, we can observe that the Or49a Gaussian group does not demonstrate opposite behaviors as the Or42a Gaussian group. For a more quantitative analysis of these trajectories, we can analyze and compare the PI of different groups. The Or42a Gaussian elicits a strong chemotaxis towards the center with a PI=0.711. Although Or49a inverse Gaussian should also elicit chemotaxis away from the sides due to a strong sensory stimulation, the PI=0.5375 shows that the larvae have no preference to be in the center at the lowest sensory stimulation. This indicates that the chemotaxis elicited by Or42a and Or49a are not opposites of each other but elicit their own distinct behaviors instead.

In figure 2. we observe that our optogenetic manipulations are properly inducing attractive and aversive behaviors. In the control group and no light stimulation groups, we observe that the average distance from the center is roughly around 25mm. Analyzing the Or42a Gaussian group, we observe strong attraction where the average distance from the center is decreased to roughly 15mm. Additionally, the Or49a inverse Gaussian group also shows aversion away from the sides and has an average distance of roughly 20mm. Although the attraction towards the center of the plate in Or42a shares similar trends to the aversion away from the sides of the plate in Or49a, the amount of modulation is dissimilar. Or42a seems to have a stronger influence on the larva's chemotaxis and promotes the animal to stay very close to the center, while Or49a seems to be repulsed from the outskirts yet indifferent about staying in the center. The behavior of Or49a inverse Gaussian seems to optimize their position where they prefer weak light stimulation—which is a behavior not found in attraction.

Although we were using the standard PiVR light intensity, we were not observing strong aversive chemotaxis. After the initial trials were performed on a standard PiVR that outputs light between 1–2 W/m^2, we conducted additional trials with Or49a light sensitive larva on a high-powered rig that outputs

light of roughly 40–50W/m^2. This increase in light intensity should allow us to see stronger aversive chemotaxis and display more substantial evidence for characterizing aversive behavior. In figure 4A and 4B, aversion in a gaussian gradient is observed as expected through the agitated movements in central trajectories. Although the distance-to-center and preference index results do not demonstrate clear aversion initially, both can be explained through the protocols of the assays. The distance-to-center graph and PI table display a higher concentration of larval activity near the center of the petri dish where the concentration of the simulated aversive odor is at its highest. Although this may show contradictory results, aversive behavior is still present. The protocol for this experiment requires the larva to be placed in the center of the petri dish before recording the trial. This placement of the larva in the center leads to an increase in larval activity near the center in the central trajectory plots of figure 4A and 4C. Regardless of larva position, the behavior of the larva seems to be very agitated with several tight turns and head casts between short bursts of runs seen in figure 5. Furthermore, placing the larva directly in the high intensity light may have resulted in a freezing behavior, which supports the lack of spatial exploration and explains the counter-initiative increase in PI. This indicates that the increase in light intensity does induce stronger and more prominent aversion.

Aversion across both light intensities displays a unique mechanism not seen in attraction. Chemo-attraction in larvae is characterized by the display of a modulation in run speed, stopping, head-casting, turn-rate and turn accuracy, and weathervaning [6]. Or49a which is known to elicit aversive behavior when stimulated demonstrates a distinct mechanism for exploration. Analyzing single trials of Or49a larva under both low-intensity and high-intensity Gaussian gradients, larval trajectories indicate that they tend to stay in regions away from high intensity light, but hover near the light gradient to experience minor stimulation. In figure 5, there are a few examples of this mechanism where larvae reside on the outskirts of the light gradient. This is surprising as Or49a is known to induce strong aversion with larvae staying clear of any light stimulation entirely [3]. This interesting behavior, not seen in attractive conditions, of hovering near aversive stimulation may indicate the larva's interest in exploration where they seem cautious yet curious about the aversive stimulus. More importantly, this indicates that the animal elicits aversive mechanisms independent and distinct from modulations of attraction.

Lastly, the Or49a high-intensity inverse gaussian gradient does not display identical modulation patterns to the Or42a gaussian gradient. In figure 3E and 3F, the Or49a larva in inverse gaussian conditions shows similar modulation patterns as Or42a larva in gaussian conditions but they are not identical. Figure 3D-E and table 2 demonstrates that the Or49a larvae generally stay 10mm from the center as opposed to the Or42a larvae staying only 15mm from the center in gaussian conditions. This positional shift closer to the center suggests that the behavior under the absence of aversive odors is not equal to the behavior under the presence of attractive odors. Thus, these trials suggest that the characteristics of aversive behavior are not directly opposing the characteristics of attractive behavior.

Moving forward, the characterization of aversive behavior with Or49a opens many avenues for further experiments. One avenue is to apply these findings with real wasp odors. Since there is evidence of behavioral modulations from light activated Or49a, the next step is to test the larva with the expected ligand of Or49a. Another avenue is to understand the integration of multiple signals, more specifically

the study of larva experiencing an aversive and attractive odor simultaneously and observing the decision-making process through its behavioral modulations. This allows the field of computational biology to better understand how larva modulate their behavior in realistic environments containing multiple odors.

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