

GCaMP fluorescent imaging of *Drosophila melanogaster* antennal lobe in response to odors

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1 Introduction

While electrophysiology is the most direct method to measure neuronal activity, it is invasive and is limited in the number of neurons that can be measured at a single time. One way to circumvent these problems is to measure calcium activity using GCaMP. GCaMP is a fluorescent calcium indicator that is activated when calcium binds to the calmodulin portion of GCaMP, which results in accessibility to the chromophore in the GFP portion of GCaMP. In order to evade the need to resort to invasive surgery, GCaMP can be expressed on surface neurons, such as the hairlike sensory neurons on the antennal lobe of the *Drosophila melanogaster* fly. Each sensory neuron expresses only one type of odor receptor protein, which results in differential activity of odor binding across the sensory neuron population. Our goal was to build an inverted scope that would be able to capture the neuronal activity of sensory neurons in response to different odor stimuli

2 Methods and Materials

2.1 Transgenic animals

Transgenic flies were prepared in the Jin Wang lab by Tuhin Chakraborty. The goal of the fly cross consisted of getting Or83b-Gal4 and UAS-GCaMP3 into the same flies. Or83b-Gal4 was used to target odorant receptor proteins on ciliated dendrites of specialized olfactory sensory neurons using the trapper gene, Gal4. In conjunction with UAS-GCaMP3, we were able to monitor calcium fluorescence in the cell bodies of most sensory neurons.

2.2 Inverted scope

The main microscope used was the Olympus IX50, an inverted scope with infinity objectives. The brightfield was built into the main microscope while the external light had to be retrofitted. The external light source was powered by a mercury lamp and was used to illuminate the subject through the dichroic mirrors. This enabled us to have a very specific frequency that hit the fly. An electronic shutter was placed between the mercury lamp and the objective to allow us to easily switch between the brightfield and the external lamp used to excite the GCaMP.

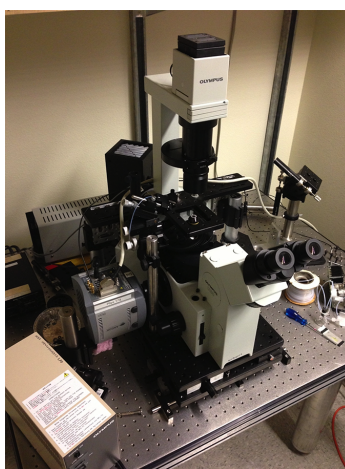


Figure 1: Full inverted scope setup.

In comparison to past replications of this project in the class, our project was novel in that we utilized an inverted scope setup instead of an upright scope. We used an Olympus IX50 inverted scope as the base of our setup.

2.2.1 Objectives

The Olympus IX50 inverted scope did not come equipped with working objectives. The correct objectives were ascertained qualitatively using a fly wing as the object to be resolved. The two objectives used were a 10X and a 40X. The first to position and set up the fly and the latter to get a higher resolution image to which record our experiment from.

2.2.2 Dichroic mirror

The inverted scope housed 4 built-in dichroic mirrors, the excitation and emission spectra of which were analyzed using a radiometric detector and SpectraSuite software. Excitation spectra were measured from the light emitted through the objective, and emission spectra were measured from the light emitted from the eyepiece. We narrowed down our selection of dichroic mirrors to those whose excitation and emission peaks closely resembled or overlapped with those of GCaMP (485 nm excitation, 530 nm emission). Final selection of the dichroic mirror relied on qualitative judgement of which dichroic mirror allowed for better visualization of fluorescence.

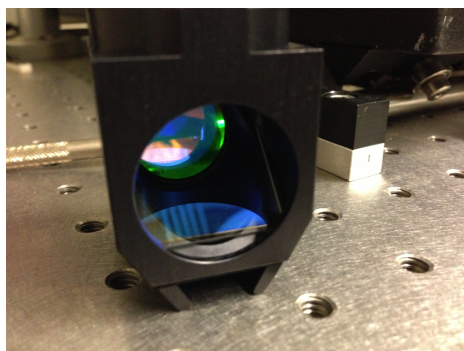


Figure 2: Example of dichroic mirror.

2.2.3 Light sources

Brightfield images were created by using an incandescent lamp to illuminate the sample from above. Epifluorescent images were created by using a broadband fluorescent lamp to illuminate, via the objective, the sample from beneath. Filters in the dichroic mirror enabled us to confine the fluorescent light illuminating the sample to a range surrounding the excitation peak of GCaMP (485 nm) and the fluorescent light reflected off the sample to a range surrounding the emission peak of GCaMP (530 nm).

2.3 Odor delivery

Odor stimuli were presented to the animals by transiently opening a solenoid valve for short bursts to let compressed air flow through the chamber (erlenmeyer flask) filled with scents. Constant air pressure of about 1-2 psi was maintained by switching to a second line of compressed air when the valve was closed. Four odors were used: isoamyl acetate (banana), ethyl hexanoate (apple), phenylethanol (rose), and vinegar.

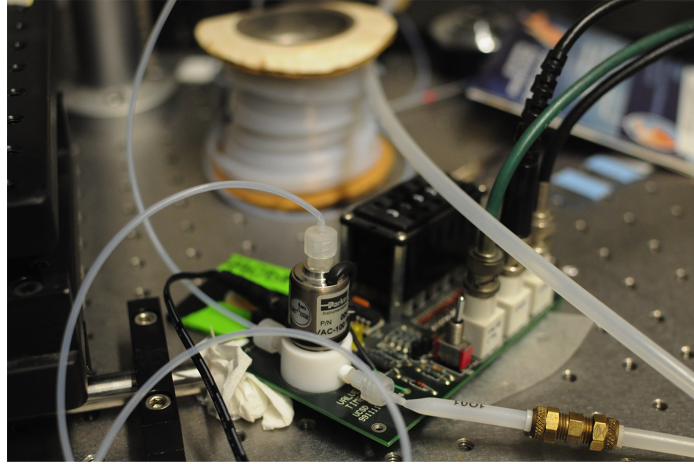


Figure 3: Odor delivery system.

2.4 Animal preparation

Because sensory neurons can be visualized on the surface of the antennal lobe, surgery was not required to prepare the flies for fluorescent imaging. However, the flies needed to be physically restrained so that the acquired images are not blurred by the movement of the antennae. A trap was constructed out of a 200 mL plastic pipette tip.

First, one centimeter pipette tip was sliced off with a razor blade and the rest was discarded. The flies were then temporarily immobilized by immersing a tube of flies into ice for about three minutes. Using forceps to hold a fly by its wings, we gently inserted the fly, head first, into the pipette tip. A tip of a pin wire was subsequently used to shove the fly deeper into the tip, taking care to only contact the tip of the pin with the inner surface of the pipette tip. Once the fly was stuck, the pointed part of the tip was sliced just in front of the fly head. The fly was gently pushed backwards through the newly cut hole in order to slice a little bit more off of the tip. Afterward, the fly was pushed back into the tip until its head just poked out with its antenna exposed. A small ball of clay was inserted behind the fly in order to prevent the fly from escaping.

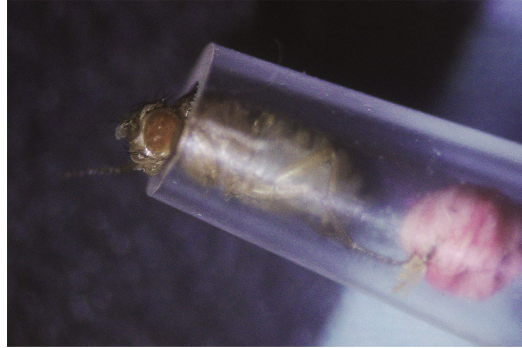


Figure 4: Fly head poking through a sliced pipette tip.

In order to view the entire antennal lobe in the field of vision, a glass hook was devised to hold up the antenna. Borosilicate glass capillary tubes was pulled into sharp electrodes. The hook was created by very quickly flicking the sharp tip of the sharp electrode over the tip of a flame.



Figure 5: A glass hook (bottom) holds up the antennal lobe.

2.5 Data acquisition

The camera used to capture images was an Andore IXON EMCCD. The image size was 1004 x 1002 pixels, within a 8 mm by 8 mm imaging area. Image acquisition was carried out by Andor Solis imaging software. Exposure time was set to 0.003 seconds, since the default value was too fast to produce a coherent image. Data acquisition was set to collect 50 frames and the subsequent series was exported as an .avi video format.

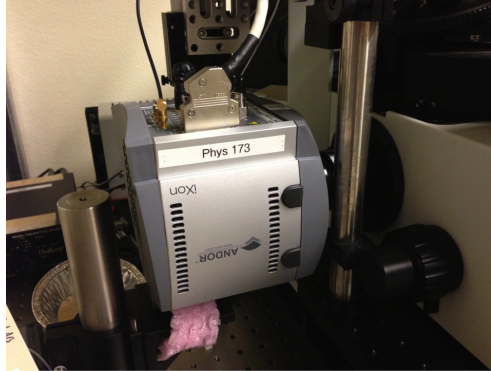


Figure 6: The EMCCD.

3 Results

3.1 Calibration of brightfield microscopy

The inverted scope was ascertained to require infinity objectives. With a 10x objective, we able to view the entire head of the fly in the image frame. Only with a 40x objective were we able to resolve the fine hairs of the sensory neurons. The antennal lobe filled the space of the image frame with a 40x objective. The 10x objective was used to maneuver the glass hook and center the antennal lobe. The 40x objective was used for collecting images. See Figure 5 for a 10x brightfield image.

3.2 Calibration of fluorescence microscopy

Two dichroic mirrors exhibited peak excitation and emission wavelengths that closely matched those of GCaMP (485 nm excitation, 530 nm emission). We ultimately chose to use the dichroic mirror with broader peaks (480-524 nm excitation, 530+ nm emission), because the other dichroic mirror's peaks were too narrow and precluded visualization of fluorescence. Qualitatively, both dichroic mirrors allowed us to visualize a fly wing dipped in fluorescence, but only the chosen dichroic mirror allowed us to visualize baseline fluorescence in the sensory neurons of the fly antennal lobe.

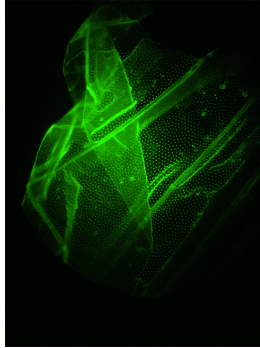


Figure 7: Fluoresceine dye in a fly wing.

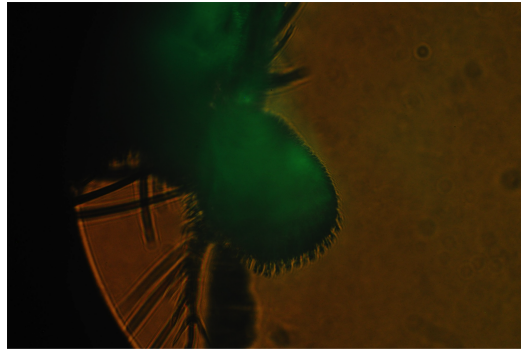


Figure 8: Baseline fluorescence of sensory neurons in the fly antennal lobe.

3.3 Odor experiments

We were only able to elicit responses to vinegar, and to no other odor. The fluorescent response was very bright and occurred very quickly when the odor was presented.

odor	fly 1	fly 2	fly 3	fly 4
vinegar	+	+	0	0
isoamyl acetate	-	-	-	-
ethyl hexanoate	0	0	0	-
phenylethanol	0	0	-	-

Table 1: Results of odor experiments. + indicates response, - indicates null response, and 0 indicates that the fly did not receive the odor.

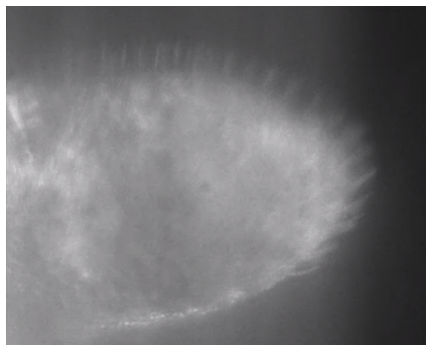


Figure 9: Fluorescent image of the antennal lobe before vinegar odor stimulation.

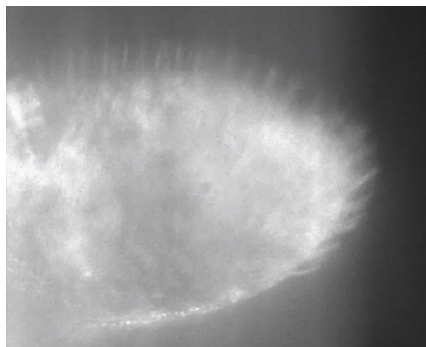


Figure 10: Fluorescent image of the antenna lobe shortly after vinegar odor stimulation.

The full video of the vinegar response can be viewed at <http://www.youtube.com/watch?v=fn7K5HjUcFo>.

4 Discussion

Our main quantitative measurement sought in this experiment was to see if we could get neural responses expressed through fluorescent proteins from different odors exposed to the *Drosophila melanogaster* fly. The experiment included setting up the proper materials and apparatuses in order to measure such experiments. The first step was to set up the inverted microscope and the odor delivery system and test to see that it worked accurately. The Inverted microscope used was not initially set up and had to be built up with minimal parts available. Through external resources and research an external light source and

an EMCCD camera were attached. The external light source and the EMCCD camera came off the original upright Zeiss microscope used in previous experiments. With the external light source we were able to get light of specific wavelength through the objective for our experiment. The odor delivery system was taken off the old microscope as well. Before running the experiments both the microscope and the odor delivery were tested and noted to be in working condition.

Before running our actual experiments Tuhin from Jin Wang’s lab gave us a short overview on setting up and running the experiment. Under his supervision we prepared a few flies and successfully ran the experiments in Jin Wang’s lab. The four flies we tested all gave positive results when exposed to certain odors. Each time the specific odor or hormone was puffed onto the fly, the antenna lobe reacted and fluoresced distinctly under the microscope. Once the new flies specific to our odors were genetically crossed we replicated the same experiments in our lab. Although the first flies prepared at Jin Wang’s lab were successfully run, once brought back to our lab we could not replicate it exactly. The first few flies that we got into the pipette tips did not stay alive. With a few practice flies we were able to mount the pipette holders on clay set on the glass slides and viewed under the microscope. The pipette worked in securing the body of the fly however many times the fly’s head was unstable and thus it was able to move its antenna lobe out of the plane of focus. This caused some difficulty in running the experiments initially and slowed down the measuring process as the preparation had to be done many times.

Once we were able to prepare the flies successfully we ran the results first with vinegar. The very first fly we used gave us a positive result when exposed to the odor. The video was recorded and saved using Andor Solis imaging while the experiment was reset for the next fly. After this first run however we did not obtain another positive result with any of the other flies, both different sexes and different batches, and with the other odors. Four different batches of flies were used and from each batch males and females were used. The odors were also delivered through new tubes and flasks to prevent contamination and a vacuum also set up behind the fly to provide circulation. These variables were tweaked after the first few runs however none of following flies gave us a positive result.

4.0.1 Technical Issues

With our limited resources we ran into a few technical issues that were mostly accounted for but still could not be completely resolved. This was the first time the experiment was run on an inverted scope and some adjustments had to be made. The fly would usually be on its side but on an upright it was rotated a little so the right and left antenna could be distinguishably in a different plane of focus through the microscope. When using the inverted scope the fly still stayed on its side, however it had to be turned the other way, exposing more of

its underside, in order for us to more accurately hold and measure its antenna lobe. It is not exactly known if this could alter the behavior of the fly but it did provide a variable that could have affected the results.

The biggest technical issue we could see in the experiment was the actual flies used. These were all prepared in advance for us by Jin Wang's lab, however it was unknown if each batch was screened beforehand to determine if it was the correct genetic cross we were looking for. To account for this we could have ran the same flies in Jin Wang's lab to ensure that they were positive GCaMP flies. The flies were also set up on different days which could have altered the ability of each batch to express the GCaMP.