FLiACT PROTOCOL

CALCIUM IMAGING IN THE *DROSOPHILA* ANTENNAL LOBES

A - Materials

Imaging Set-up

- upright wide-field fluorescence microscope (BX51, Olympus) on an air table (Newport)
- 20x water immersion objective with a long working distance (NA 0.95)
- monochromator as a light source
- CCD camera
- Software for data acquisition and analysis (Till Vision)

In vivo preparation

- Orco-Gal4; UAS-GCaMP3 or GH146-Gal4; UAS-GCaMP3 flies
- custom-made Plexiglas mounting block with copper plate and built-in screws
- bee's wax
- two-component silicone
- plastic coverslips
- insect pins
- Ringer's solution
- soldering iron

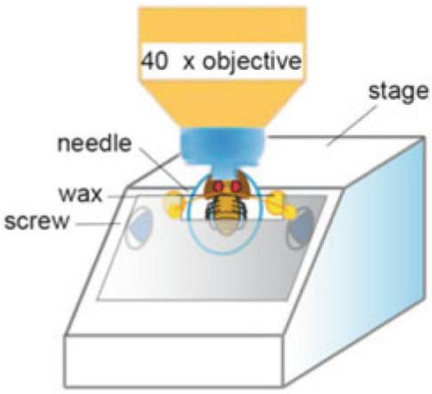
Odour stimulation set-up

- stimulus device: stimulus controller, flowmeters and glass pipettes

B - Procedures

Fly preparation for imaging

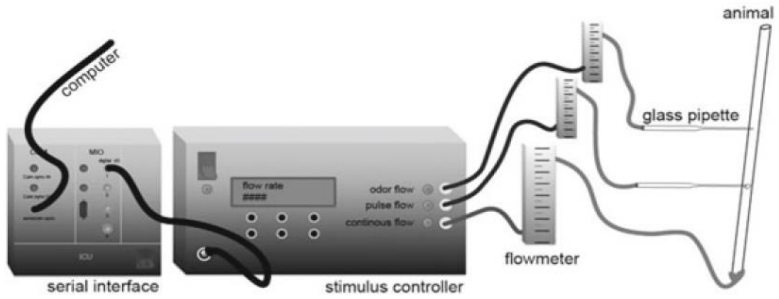
Flies are anesthetized on ice. One fly is fixed with the neck onto a mounting block. A small insect pin is placed in the front of the head; the thorax and abdomen are hanging. Pull antenna forward using a fine metal wire which is attached to a coverslip. Fix the coverslip to the front of the mounting block with wax. Push forward the coverslip with the metal wire by using the built in screws. This will give you access to the antennal lobes. Place a second plastic coverslip with a hole- seal it to the cuticule with silicone. Open a small brain window. Once the brain is exposed apply immediately Ringer's solution. With very fine forceps carefully remove all trachea to allow visualization of the ALs. Place the mounting block containing the fly under the microscope.



Odour stimulation

A glass pipe placed directly in front of the animal's antennae provides a constant airstream of 1L/min. This should not touch any part of the stage. Into this airstream, a stimulus airstream is added. The stimulus airstream is provided by two Pasteur pipettes, one of which is empty, providing a continuous stream of filtered air (*control stream*), and the other of which contains a piece of filter paper soaked in 6 µL odorant (*odour stream*) dissolved in a solvent (eg. mineral oil). The flow through each airstream is adjusted by a flowmeter, and humidified. The stimulus controller can be controlled by a computer, and regulates the airflow, so that into the constant airstream, a continuous stimulus airstream is added. At the moment when the odour stream is switched on, the control stream is switched off, and vice versa, so that odour stimulation is not accompanied by mechanical stimulation of the antennae.

To change the stimulus odorant, the stimulus pipette is exchanged. A mineral oil solvent should be used as a negative control, to ensure the apparatus is not contaminated with odours.



Data Acquisition

Bring the area of interest into the field of view, and find the grey value of the brightest point by hovering the cursor over this point. Adjust the exposure time so that this value is roughly 1000, so that upward and downward signal deflections can be detected. Define the area of interest (AOI – eg. the antennal lobes). Focus upward to the top of the AOI, and set this as (0,0,0). Make a z-stack of 100 steps (1µM each). From this stack, select the desired focal plane, and select so that signals are collected from only this plane.

Data Analysis

Movement correction – aligns the imaged structures to correct for XY movements.
Bleaching correction, to correct for bleaching of the fluorophore by excitation light.
Signal calculation – signal is presented as changes in fluorescence upon odour presentation: $\Delta F/F_0 = 100.(F_i - F_0)/F_0$, where F_i is the fluorescence signal at frame i and F_0 is the baseline fluorescence calculated from the average of several frames just before odour application.
Regions of interest – $\Delta F/F_0$ can be averaged over user-defined ROIs (eg. glomeruli, neurons).
Image representation – calcium responses are presented as false colour-coded images, by subtracting a frame just before odour application from one during application.
Peak responses – the highest value of $\Delta F/F_0$ during odour presentation, or the integral of the $\Delta F/F_0$ curve during presentation.
Signals can be mapped to specific glomeruli using the 3D antennal lobe atlas (Laissue et al., 1999).

Acknowledgement:
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