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EDUCATION SCIENCE INNOVATION

Methods and protocols

Device for optogenetic activation of Neurons in Drosophila melanogaster

Research Project

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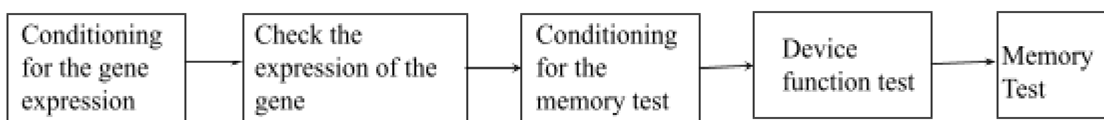
1. Introduction:

In this document we will talk about the materials and methods that are carried out during the project entitled "Device for optogenetic activation of Neurons in Drosophila melanogaster", however it should be noted that this project was carried out in the laboratory of brain plasticity, so many materials are from the laboratory and different protocols are performed by laboratory personnel, however they are part of the results that support the project.

2. All parts of the process:



The conditioning for the memory test and testing memory stage is carried out by the laboratory staff, due to the duration of the project, the student was unable to perform these processes.

2.1. Conditioning for the gene expression

To prepare tubes you need to stir 15 ml of fly medium (provided by the laboratory kitchen) in a beaker together with 68 µl of retinal with a concentration of 1mM. To prepare bottles, multiply all amounts by 8. Bottles and tubes should be placed in the black box and then placed in the incubator overnight to allow the retinal to adhere to the fly media. Flies are introduced into the tubes and bottles for 3 days in the black box and inside the incubator at 18°C.

2.2. Check the expression pattern of the Cschrimson.mVenus

To verify whether we have the Drosophila line (VT050660), the student does immunolabeling without antibody. The following steps are required for this process.

a. Fixation: Fixation overnight @ 4°C in PFA 16% diluted in PBS-T 0.6%.
b. Dissection: it must be performed in PBS 1X on ice (round tray and pillbox, forceps, remove as much trachea as possible).
c. Fixation: Fix for 1h under agitation on the shaker plate in 500µl of PFA 4% (PFA 16% diluted in PBST 1%).
d. Washing: Wash 3x with PBS for 20 min under agitation on the shaker plate.

e. Mounting : On a slide, stick an eyelet, put a drop of Prolong medium (4,5µl) in the center of the eyelet and then with the help of forceps recover the brains and put them in the drop of Prolong medium. Cover with a coverslip.
f. Conservation of the slides : In the refrigerator at 4°C and in the dark, until the day they will be used.

After performing this series of steps, the sample is ready to be put under the confocal microscope.

Solutions:
- PFA 4% : 10 ml of PFA 16% + 30ml of PBST (conservation 1 week) -> In the fridge at 4°C (PFA 16% : in a cupboard under a hood)
- PBS : Dilute one PBS tablet in 200ml of MiliQ water (shelf life 1 month) -> In the fridge at 4°C
- PBST : PBS 1X (tablet) + Triton X100 at 0.6%.

Product references :
- PFA 16 % : 15710 Electron Microscopy Science supplier
- PBS pellet: P4417-100 TAB supplier Sigma
- Triton x100 : X100-500ML supplier Sigma

2.3. Conditioning of the memory test

During this part, the flies are conditioned to test the formation of long-term memory. **REMINDER: This session is conducted by laboratory personnel.

a. Before the memory conditioning experiment:

For the preparation of the odor bottles, they have been filled with paraffin oil between 100-200 ml. Under the hood, add the proper volumes of each odor in the right bottle: 58µL of Octanol in RED bottles and 52µL of 4-Methylcyclohexanol in BLUE bottles using the proper pipettes.

Then, close the bottles with the proper caps (labeled O and M, with large pipes). After that, put the odor bottles, the bottles with the flies, and the collector device into the behavioral room for 30 min (the behavioral room is at 25°C and has a humidity of 80).

b. Turning-on and check-up of the setup:

When the time is up, connect the odor bottles, plug the barrels to the current and the airflow (2l/min). On the software, go to the odor test and click odor 1 (Octanol). Check that bottles are bubbling properly then let bubble for about 30 sec. Then stop the odor 1 and start air 1. Repeat for odor 2 and air 2.

c. Loading and launch of protocol:

There are 2 groups of flies, the first one will have the electric discharge related to the octanol odor (odor 1) and the second one will have the electric discharge related to the Methylcyclohexanol odor (odor 2).
With the help of a funnel, put the first group of flies into the holes of the barrels. Connect the barrels to the electric current and the air supply tubings. Turn off the light, exit the room and start protocol.
-Protocol= 5 cycles are performed, each lasting 5 minutes. In each cycle, odor 1 is applied, followed by air + shock, then odor 2 is applied, followed by air.
Collect the flies with the collector device and put them in new bottles (only with fly media without retinal).

2.4. Device function test

To test the performance of the device, the flies that are subjected to the device must show the "moonwalk" phenotype, just after performing memory conditioning. Gene (CsChrimson.mVenus) activation is performed by means of red light (660 nm) at 500 Hz intensity for 5 minutes, and back to the behavioral incubator (at 18°C for 24 hours).
(Repeat the previous steps with the second group of flies but in the protocol exchanges odors, described in 2.3 and 2.4).

2.5. Memory Test

Introduce each group of flies into the test machine and leave them for 30 seconds without odor. After this time, expose the flies to the two odors with an air flow of 8 l/min for 1 minute, so that they can choose between odors. After this time, the flies that chose each odor are counted.
**REMINDER: This session is conducted by laboratory personnel.