Sensory Processing to Sequential Action Control

Durafshan Sakeena Syed
0000-0002-5729-0382

dsyed@ucsb.edu

1 Module 3 Instructor Guide — Sensory Processing to Sequential Action Control

1.1 Conceptual Framework

- Sensory-driven activation – mechanosensory input triggers grooming behavior
- Command-like neuron activation – specific neural populations bias or initiate motor programs
- Hierarchical suppression – competing actions are organized into structured sequences

Students investigate how these levels interact to produce ordered, flexible grooming behavior in *Drosophila melanogaster*.

1.2 Pedagogical Approach

- Observation – record natural grooming behavior
- Quantification – annotate and measure sequences
- Manipulation – test causal roles using optogenetics
- Interpretation – relate behavior to neural circuits

1.3 Learning Objectives

- Describe hierarchical organization of motor behavior
- Quantify behavioral sequences using time sampling
- Compare sensory-driven vs circuit-driven activation
- Interpret behavioral data in neural circuit context
- Evaluate selection and suppression of competing motor programs

1.4 Suggested Teaching Timeline (2–3 hours)

- 20 min: Introduction & Background
- 40 min: Data Collection (Dusting & Optogenetics)
- ~60 min: Data Analysis (Scoring & Graphing)
- 30 min: Discussion & Interpretation

1.5 Experimental Overview

1.5.1 Conditions

- Dust stimulation – natural mechanosensory activation
- Optogenetic activation – artificial activation of neurons

1.5.2 Key Experimental Question

- How do sensory input and specific neurons influence grooming sequence, timing, and selection?

1.5.3 Expected Outcomes

- Grooming follows an anterior ⟶ posterior progression
- Head grooming often occurs first
- Behavioral sequences are structured, not random

1.5.4 Optogenetic activation can:

- Induce specific actions in clean flies
- Override ongoing grooming sequences
- Alter timing and transition probabilities

Variability across flies is expected and can be used to discuss biological variability and experimental noise.

2 Data Analysis Options

- **Level 1 (Introductory):** manual sampling, % time, bar graphs
- **Level 2 (Intermediate):** frame scoring, ethograms, transition analysis
- **Level 3 (Advanced):** MATLAB/Python, transition matrices, automated classification

Optional statistics:

- Mann–Whitney U test (between groups)
- Paired t-test (within-fly comparisons)

3 Common Pitfalls & Troubleshooting

- Weak optogenetic activation → check retinal feeding and light conditions
- Scoring inconsistencies → enforce "dominant behavior per second" rule
- High variability → expected; use as discussion point

4 Key Conceptual Takeaways

- Grooming is hierarchical, not a simple reflex
- Sensory input biases but does not fully determine behavior
- Command neurons can initiate and dominate actions
- Sequences emerge from competition and suppression
- Motor control is dynamic and flexible

5 Preparation

Genetic crosses and fly husbandry (~15 days before the experiment)

5.1 Fly stocks:

- Obtain fly stocks from Bloomington Drosophila Stock Center.
- Keep different fly lines in separate vials/bottles.

For optogenetic (or thermogenic) experiments: Use flies carrying UAS-CsChrimson (for optogenetics) or UAS-TrpA1 (for thermogenetics).

These need to be crossed with GAL4 or Split-GAL4 driver lines.

5.2 Virgin Collection (for setting up crosses):

1. Identify virgins directly: Collect newly eclosed females (wings still folded, no sex combs on legs).
2. Alternative method (for beginners):
   - Collect pupae and place a single pupa per vial.
   - After eclosion, identify males and females.
   - All females eclosed in individual vials will be virgins (since no males were present).
   - These vials may also contain unfertilized eggs laid by virgin females.

> Tip
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> The largest number of virgins will eclose in the morning

Drosophila Workers Unite! is an excellent resource by Michele Markstein on how to do fly pushing/identifying gender differences/virgins and setting up the crosses.

5.3 Setting Up Crosses:

1. Collect males from the desired GAL4 or Split-GAL4 driver lines.
2. Place 6 virgin females (from UAS-CsChrimson or UAS-TrpA1) and 3–6 males (from a given driver line) together in one vial with fly food.
3. Maintain at room temperature (or recommended growth temperature for your experiment).

6 Transferring Parents

Transfer parent flies into new food vials every 5 days. This prevents the parental generation from mixing with the first generation of progeny.

> Tip
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> To separate flies safely, you can immobilize/anesthetize them by placing the vial on ice or in the refrigerator for ~1 minute.

6.1 Collecting Experimental Flies

Collect males from the first-generation progeny (correct genotype) for experiments. These flies will be ready for dusting or optogenetic/thermogenetic activation experiments.

6.2 Fly stocks:

Fly Genotype	Source	Cat#
Iav GAL4	Bloomington Stock Center (BDSC)	
R38B08 GAL4	BDSC	
DNg12	BDSC	
DNg11	BDSC	
MagoNote	BDSC/Simpson lab	
wPN	BDSC/Simpson lab	
UASCsChrimson	BDSC	
UAS TrpA1	BDSC	
AD-DBD-empty SPLIT	BDSC	
Canton S	BDSC	

6.3 Preparing flies for experiment (~3 days before the experiment)

Perform behavioral experiments on flies that are 3–6 days old after eclosion.

Control Flies (Wild-Type or Non-Optogenetic): These can be used directly for dusting experiments without any special preparation.

Experimental Flies (Optogenetic with CsChrimson):

Flies carrying UAS-CsChrimson must be fed on retinal-containing food for 3 days prior to the experiment. Retinal is required to make the Chrimson channel sensitive to light activation.