Sensory Processing to Sequential Action Control

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1 Background

Animals organize complex behaviors into structured sequences. In this module, you will test whether grooming behavior is organized as a hierarchical sequence and how sensory input and specific neurons can initiate or override this sequence.

When dust stimulates mechanosensory bristles, flies groom in a stereotyped anterior-to-posterior sequence: Head → Abdomen → Wing → Thorax These body sweeps are alternated with leg rubbing bouts. You will test how sensory stimulation and targeted neural activation influence this hierarchy.

1.1 Experimental Conditions

You will observe flies under two conditions:

1. Dust stimulation – natural sensory activation (dusting protocol)
2. Optogenetic activation – artificial activation of sensory or command neurons with light (1min light OFF → 3 min light ON)

1.2 Behavior Categories

H (Head), FL (Front Leg rubbing), BL (Back Leg rubbing), T (Thorax), A (Abdomen), W (Wing), WK (Walking), S (Standing)

2 Part 1: Behavioral Recording

Use a camera or cellphone to record behavior. Use slow motion (120fps or higher) if available to identify subtle grooming movements and transitions between actions. Record ~5 flies per condition. Duration: 3–5 min per fly Ensure the entire body is visible. Use consistent lighting and fixed magnification.

2.1 Scoring Technique:

Watch the video and pause every 1 second and record the behavior at that moment. If multiple behaviors occur, record the dominant behavior (the one occupying most of that second). Data Visualization for Students:

For the timeline Ethogram, use a simple grid where each box represents 1 second of "real" fly time. Here is an example

	Time (s)	1	2	3	4	5
Fly #1	Behavior	FL	H	FL	H	FL

3 Part 3: Behavioral Scoring

At 1-second interval, record the behavior of each fly.

Categories and action associated:

Category	Abbreviation	Example Action
Head grooming	H	Sweeps of head/ antennae
Front leg rubbing	FL	Rubbing front legs together
Abdomen grooming	A	Abdomen sweeps
Back leg rubbing	BL	Rubbing hind legs
Wing grooming	W	Wing sweeps
Thorax grooming	T	Thorax sweeps
Walking	WK	Locomotion
Standing	S	Immobile

Goal: Track order, duration, and repetition of each action.

4 Part 2: Quantitative Analysis

4.1 Step 1: Count Behavior Intervals

- Count number of intervals per behavior
- Calculate percent time: Percent Time = (Number of intervals for a behavior ÷ Total intervals) × 100

4.2 Step 2: Graphical Representation

- Bar graph: Percent time spent per behavior
- Timeline ethogram: Color-coded row per fly showing behavior over time Color suggestion for ethogram:
- Head: Purple | Abdomen: Dark Green | Wings: blue | Thorax: Pink | Front Leg: Orange | Back Leg: light green | Walking: Black | Standing: White

4.3 Step 3: Hierarchy Analysis

Measure: - % of flies starting with head grooming - Average duration of each action - Latency to first posterior grooming action - Number of sequence reversals - A:P Ratio (Anterior-to-Posterior) = (Head + Front Leg) ÷ (Abdomen + Wing + Thorax + Back Leg)

After scoring 60 seconds (or your full video), use this box to calculate your totals:

Condition	Expected A:P Ratio	Biological Meaning
Dust Only (Control)	High initially	Anterior dominance; sensory-driven sequence.
Command (e.g., Wing) + Dust	Low	Command neuron overrides the anterior hierarchy.

Total Anterior Intervals (H + FL): _____ Total Posterior Intervals (A + W + T + BL): _____ A:P Ratio (Line 1 ÷ Line 2): _____ Percent Time (Head): (Total H ÷ Total Intervals) × 100 = _____%

4.4 Step 4: Command Neuron Experiments

These neurons are known to bias or initiate specific grooming actions: Measure: - Probability of induced action - Latency to induced action - Whether natural sequence was interrupted Example: Does activating leg rubbing command neuron increase leg rubbing above baseline?

5 Part 4: Procedure Overview

5.1 A. Dust-Induced Grooming

1. Place fly in vial
2. Add small amount of dust (cornstarch or reactive yellow)
3. Gently shake/tap 10 times
4. Record grooming

> Tip
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> If it is difficult to transfer flies into the vial containing dust, anesthetize flies briefly on ice by placing vial in the ice box or refrigerator, allow flies to wake up before shaking and dusting

5.2 B. Mechanosensory Bristle Activation

1. Use flies expressing CsChrimson in bristle neurons
2. Keep in darkness until recording (keep vials in the cardboard box, for example)
3. Illuminate with red light (red laser pointer), (even white light will work)
4. Record grooming and analyze sequence

5.3 C. Command-Like Neuron Activation

5.3.1 Command neurons

- aDN / DNg12 ⟶ Head grooming
- DNg11 ⟶ Front leg grooming
- wPN ⟶ Wing grooming
- MagoNote ⟶ Thorax grooming
- LegPN ⟶ Leg rubbing (mostly back leg rubbing, also front leg rubbing)

5.4 Experiments

- Activate neurons in clean flies ⟶ observe induced behavior
- Activate neurons in dusted flies ⟶ test hierarchy override

6 Part 5: Interpretation Questions

- Does grooming follow an anterior-to-posterior pattern?
- Do command neurons initiate specific actions, and can they override the natural grooming sequence?
- Which actions dominate under each condition?
- What does this suggest about hierarchical suppression?
- How do command neurons interact with sensory input to control sequences?

7 Part 6: Data Recording Options

Option	Description	Tools
A	Manual time sampling	Excel / Google Sheets

Option	Description	Tools
B	Frame-based scoring	Excel with conditional formatting
C	Pre-coded dataset	CSV files, MATLAB, R, Python

 Tip

Color-coded ethograms and stacked bar graphs visually convey hierarchy and timing, even without coding.

8 Sample Data Recording Sheet: “The Snapshot Log”

Fly ID: _____
Condition: (Dust / Opto / Both)
Genotype: _____

Time (sec)	Behavior Code	Anterior (A)	Posterior (P)	Notes
1				
2				
3				
4				

Behavior Codes:
H (Head) · FL (Front Leg) · W (Wings) · T (Thorax) · A (Abdomen) · BL (Back Leg) · WK (Walking) · S (Standing)