Submission ID #: 68517
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20897733

Title: Behavioral Analysis of Locomotor Dysfunction in *Drosophila melanogaster* as a Readout for Neurotoxicity

Authors and Affiliations:

Zuzanna Tomkielska[1,2], Jorge Frias[1], Nelson Simões[1], Ana Casas[2], Duarte Toubarro[1]

[1]Center of Biotechnology of Azores (CBA), University of the Azores
[2]Mesosystem Investigação & Investimentos by Spinpark

Corresponding Authors:

Duarte Toubarro (duarte.nt.tiago@uac.pt)

Email Addresses for All Authors:

Zuzanna Tomkielska (2021117858@uac.pt)
Jorge Frias (jorge.mv.frias@uac.pt)
Nelson Simões (nelson.jo.simoes@uac.pt)
Ana Casas (ana@mesosystem.com)
Duarte Toubarro (duarte.nt.tiago@uac.pt)

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: 06/26/2025

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

.

Current Protocol Length

Number of Steps: 21
Number of Shots: 31

Introduction

REQUIRED:

1.1. **Duarte Toubarro:** This assay aims to develop and apply a high-throughput, cost-effective, and ethically favorable method for high-throughput screening of neurotoxic compounds using a fruit fly.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.15.1.*

What are the current experimental challenges?

1.2. **Duarte Toubarro:** Traditional assays offer limited, one-dimensional data, miss subtle impairments, and involve observer bias. They lack real-time, long-term monitoring, reducing sensitivity and reproducibility. Our protocol overcomes these through automation and tracking.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.2.*

What advantage does your protocol offer compared to other techniques?

1.3. **Zuzanna Tomkielska:** What makes our protocol advantageous is that it combines the classic climbing assay with real-time monitoring technology. This allows us to capture subtle motor impairments that might be missed by traditional methods, providing higher sensitivity and more precise behavioral data.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3.*

Protocol

2. Negative Geotaxis Assay

Demonstrator: Zuzanna Tomkielska

2.1. To begin, gently transfer the flies to an empty bottle **[1]**.

2.1.1. WIDE: Talent carefully tipping flies into an empty bottle.

2.2. Place the bottle containing the flies inside a box filled with ice, ensuring the entire bottle is covered **[1]**.

2.2.1. Talent placing the fly bottle into a larger box filled with crushed ice.

2.3. After 1 minute, gently tap the bottle to verify that all flies are anesthetized **[1-TXT]**.

2.3.1. Talent tapping the bottle lightly with a close-up shot of the flies in the bottle. **TXT: Place on ice for 1 more min to ensure full anesthesia**

2.4. Prepare a smaller container filled with ice **[1]**. Place a Petri dish or a flat, clean surface on top of the ice to create a chilled platform **[2]**.

2.4.1. Talent scooping ice into a smaller container.

2.4.2. Talent placing a Petri dish over the ice.

2.5. Now, invert the tube over the chilled Petri dish and gently tap to release the flies onto the cold surface **[1-TXT]**.

2.5.1. Talent inverting the tube over the chilled Petri dish and gently tapping the tube to release flies. **TXT: Confirm anesthesia by observing complete immobilization of the flies**

2.6. Carefully transfer the flies into empty vials **[1]**. Allow the flies to recover from anesthesia for 30 minutes in a controlled environment at 25 degrees Celsius, with a 12-hour light-dark cycle and 50 percent humidity **[2]**.

2.6.1. Talent placing the flies into vials.

2.6.2. Talent placing the vials in a controlled chamber or incubator.

2.7. Next, for microcapillary feed preparation, use a pipette to manually load 10 microliters of the test solution into each microcapillary tube, ensuring consistent volume across all samples **[1]**.

2.7.1. Talent pipetting test solution into microcapillary tubes.

2.8. Add 3 to 5 microliters of mineral oil to the open end of each microcapillary tube to

prevent evaporation and leakage during the experiment **[1]**.

2.8.1. Talent pipetting mineral oil into the open ends of microcapillary tubes.

2.9. Insert the pre-filled microcapillaries through holes in the cotton vial plugs, ensuring a secure fit **[1-TXT]**.

2.9.1. Talent inserting microcapillaries into vial plugs. **TXT: If needed, use previously cut tips to fit the cotton without blocking airflow**

2.10. Place the vials containing flies into an incubator set to 25 degrees Celsius with a 12-hour light-dark cycle and leave the vials in the incubator for the duration of the experiment **[1-TXT]**.

2.10.1. Talent placing vials into an incubator. **TXT: Manually replace microcapillaries with fresh food daily**

2.11. Every 12 hours, transfer the flies to a clean, empty vial to assess their locomotor activity **[1]**.

2.11.1. Talent transferring flies into a new vial.

2.12. Measure and mark a line 7 centimeters from the bottom of the vial using a permanent marker. This mark will serve as the climbing target **[1]**.

2.12.1. Talent drawing a line 7-centimeter from the bottom of the vial.

2.13. Position a camera or phone in a stable location to record climbing behavior **[1]**. Ensure proper lighting and a transparent background to enable clear visibility and analysis. Confirm that the experimental setup is compatible with the camera's field of view **[2]** and start recording **[3]**.

2.13.1. Talent mounting the camera in a fixed position in front of the vial.

2.13.2. WIDE: A shot of the whole setup with the transparent background and the talent looking through the camera to check the camera's field of view. Author's NOTE: 2.14.2 is included within the 2.13.2.

2.14.2. Talent pressing the record button on the camera. NOTE: This shot is moved here. Author's NOTE: 2.14.2 is included within the 2.13.2.

~~2.14. Tap the vial gently to bring the flies to the bottom **[1]**, then immediately start recording **[1]**.~~

~~2.14.1. Talent tapping the vial to bring flies to the bottom. **TXT: Avoid excessive force to prevent injury or disorientation**~~ NOTE: The authors requested to skip this shot.

2.14.2. ~~Talent pressing the record button on the camera.~~ NOTE: This shot is moved below 2.13.2.

2.15. Review the video recordings and measure the time taken by each fly to reach the 7-centimeter mark **[1]**.

2.15.1. SCREEN: The video recording being played and the time taken by the flies to reach the 7-cm mark being measured. **TXT: Assign the set maximum time to flies that fail to reach the line; Check for abnormal behaviors**

3. Real-Time Monitoring Assays

3.1. Prepare the feeding microcapillaries with the sample and mineral oil seal as shown previously **[1]**. Then, prepare individual locomotion chambers using clear plastic straws cut to approximately 6 centimeters in length **[1]**.

3.1.1. A shot of the prepared feeding microcapillaries with the sample and mineral oil seal.

3.1.2. A shot of the prepared locomotion chamber using clear plastic straws.

3.2. Seal one end of each straw with a transparent film to create an airtight closure **[1]**. Near the sealed end of the straw, create a small hole to insert the feeding microcapillary, ensuring it fits snugly and does not leak or move during the assay **[2]**.

3.2.1. Talent sealing one end of each straw with transparent film.

3.2.2. Talent puncturing a hole near the seal.

3.3. After anesthetizing and segregating the flies as shown earlier, gently place one anesthetized fly into each tube using a fine paintbrush **[1]**. Seal the open end of each tube with either transparent film or a cotton plug, allowing airflow while preventing escape **[2]**.

3.3.1. Talent placing an anesthetized fly into a straw using a paintbrush.

3.3.2. Talent sealing the open ends of the straws with transparent film or cotton plugs.

3.4. Now, insert a pre-filled microcapillary into each prepared chamber through the designated hole **[1]**. Place the assembled tubes into the assay arena, making sure the microcapillaries remain accessible for daily replacement **[2-TXT]**.

3.4.1. Talent inserting a microcapillary into the prepared hole in the tube.

3.4.2. Talent arranging tubes into the assay arena. **TXT: Allow flies to recover and acclimate for 30 min before proceeding**

3.5. Place the entire setup inside a chamber maintained at 25 degrees Celsius, with a 12-hour light-dark cycle and 50 percent humidity **[1]**. Connect the device to the local tracking system or network **[2]**.

3.5.1. Talent placing the setup into a climate-controlled chamber.

3.5.2. The device being connected to the local tracking system or network.

3.6. Access the software platform and locate the device assigned to the experiment. Ensure

the system is correctly tracking each fly based on visual markers **[1]**. Enter all experimental metadata and start recording the assay **[2]**.

3.6.1. SCREEN: Opening the platform and selecting the correct device. The tracking screen displaying flies with visible motion trails or ID tags. Author's NOTE: 3.6.2 is included within the 3.6.1

3.6.2. SCREEN: Filling in metadata fields and clicking Start Recording. **TXT: Replace food in the microcapillaries daily with fresh solution; Inspect flies daily for survival and normal behavior over 3 – 5 d** Author's NOTE: 3.6.2 is included within the 3.6.1

Results

4. Results

4.1. Climbing ability assessed using the negative geotaxis assay at 24 and 48 hours **[1]** shows similar performance between the control and treatment groups at 24 hours, indicating no immediate motor deficits **[2]**.

4.1.1. LAB MEDIA: Figure 1.

4.1.2. LAB MEDIA: Figure 1. *Video Editor: Highlight the two bars at 24 h.*

4.2. By 48 hours, treated flies take nearly twice as long to climb compared to controls, reflecting significant motor impairment **[1]**.

4.2.1. LAB MEDIA: Figure 1. *Video Editor: Highlight the two bars at 48 h.*

4.3. Continuous activity tracking reveals that **[1]** treated flies initially display heightened movement, but their activity sharply declines after 12 hours, suggesting early hyperexcitability followed by reduced motor function **[2]**.

4.3.1. LAB MEDIA: Figure 2A.

4.3.2. LAB MEDIA: Figure 2A. *Video Editor: Highlight the box plot at the top (Treatment).*

4.4. By 24 to 40 hours, treated flies show reduced movement compared to controls **[1]**.

4.4.1. LAB MEDIA: Figure 2B. *Video Editor: Highlight the orange and grey boxes in the 3rd row from the top.*

4.5. Overall, treated flies have approximately 20% reduced activity across 40 hours **[1]**.

4.5.1. LAB MEDIA: Figure 2C. *Video Editor: Emphasize the red plot.*

4.6. Circadian analysis shows that treated flies lose rhythmic movement by 48 hours **[1]**.

4.6.1. LAB MEDIA: Figure 3A. *Video Editor: Highlight the flatter red plot between 48 to 72*.

4.7. Treated flies prefer light zones more than controls **[1]**.

4.7.1. LAB MEDIA: Figure 4A, 4B. *Video Editor: Highlight the orange box in 4A and and the whole treatment part of the blue box plot in 4B.*