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Title: Multimodal Behavioral Phenotyping of Stress-Induced Depression-like States in *Drosophila melanogaster*

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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

☐ Incorrect

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? **No**

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: **06/15/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 15

Number of Shots: 32

Interviews

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Samira Jelizaveta Garajeva:** Our research aims to establish a multimodal behavioral framework for quantifying stress-induced, depression-like states in *Drosophila melanogaster*.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Samira Jelizaveta Garajeva:** This protocol applies to screening antidepressant compounds, investigating gene-environment interactions, and exploring neurobiological mechanisms behind behavioral variability in *Drosophila*.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Samira Jelizaveta Garajeva:** This protocol measures motor output, motivation for passive coping, sensory-guided decision-making, circadian structure, and inter-individual behavioral variability.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Samira Jelizaveta Garajeva:** Controlling environmental factors like temperature, humidity, and handling is critical to minimize carryover effects and reduce confounding locomotor suppression.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Samira Jelizaveta Garajeva**: Future studies can investigate sex-specific and age-related stress responses and integrate pharmacological manipulations to clarify underlying neurochemical mechanisms.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Vibration Stress Induction and Forced Swim Assays in *Drosophila melanogaster*

Demonstrator: Samira Jelizaveta Garajeva

- 2.1. To begin, using a spatula, collect male *Drosophila melanogaster* within 24 hours of eclosion under light carbon dioxide anesthesia [1]. Place flies in groups of 10 into polypropylene or acrylic vials containing standard food [2] and seal the vials with cotton plugs [3].
 - 2.1.1. Talent collecting male *Drosophila melanogaster* after eclosion under light carbon dioxide anesthesia.
 - 2.1.2. Talent placing 10 flies into an empty polypropylene or acrylic vial. Notes: 2.1.2 and 2.1.3 were combined
 - 2.1.3. Talent sealing the vial with a cotton plug.
- 2.2. Place the tubes on a vibration platform to expose them to mechanical vibration at 300 hertz [1]. Apply repeated cycles consisting of 45 minutes of vibration followed by 15 minutes of recovery for a total of 6 hours per day over 3 consecutive days.[2]. Maintain control flies under identical housing conditions without exposure to vibration [3].
 - 2.2.1. Talent placing the fly vials on the vibration platform.
 - 2.2.2. Talent setting up the vibration and recovery cycle.
 - 2.2.3. Talent placing control fly vials away from the vibration platform.
- 2.3. For forced swim test, collect adult flies within 24 hours of eclosion [1] and maintain them under constant light and controlled environmental conditions [2].
 - 2.3.1. Talent collecting adult flies after eclosion.
 - 2.3.2. Talent placing the collected flies under constant light and controlled environmental conditions.
- 2.4. Prepare multi-well chamber slides and the overhead camera to record behavior for 2 minutes and calculate the latency to first immobility [1]. Fill each well with approximately 2 milliliters of 0.08 percent sodium dodecyl sulfate solution at room temperature [2]. Note: The VO has been added per the revised shot.
 - 2.4.1. Talent arranging the multi-well chamber slides on the work surface and positioning the overhead camera above the chamber slide. NOTE: 2.5.2 was combined with this step as per the author's request.
 - 2.4.2. Talent filling each well with sodium dodecyl sulfate solution.
- 2.5. Without anesthesia, transfer a single fly into each well immediately before recording [1]. Use an overhead camera to record behavior for 2 minutes and quantify the latency

~~to first immobility [2].~~

2.5.1. Talent transferring one fly into each well.

~~2.5.2. Talent positioning the overhead camera above the chamber slide.~~ NOTE: The author requested to combine this with 2.4.1

3. Long-Term Activity Monitoring and Open-Field Locomotor Assays

3.1. Briefly anesthetize fruit flies for 5 seconds using carbon dioxide for sorting [1]. Place individual flies into glass Drosophila Activity Monitor or DAM (D-A-M) tubes containing food [2]. Seal with cotton plugs [3].

3.1.1. Talent anesthetizing fruit flies with carbon dioxide.

3.1.2. Talent placing one fly into each glass DAM tube containing food.

3.1.3. Talent sealing the DAM tube with cotton plugs. Notes: 3.1.2 and 3.1.3 were combined.

3.2. Insert the tubes into the Drosophila Activity Monitor system and record locomotor activity continuously for 48 hours under controlled temperature and light-dark conditions [1]. NOTE: VO is edited as per the revised shot.

~~3.2.1. Talent inserting the tubes into the Drosophila Activity Monitor system. Talent placing the assembled DAM system in the incubator and connecting it to the computer.~~

~~3.2.2. SCREEN: Show continuous locomotor activity recording under controlled temperature and light-dark conditions.~~ NOTE: Not filmed.

3.3. For the open-field locomotor assay, briefly anesthetize fruit flies for 5 seconds using carbon dioxide [1]. Transfer individual flies into rectangular 25-by-75-millimeter arenas, with 1 fly per arena [2].

3.3.1. Talent anesthetizing fruit flies with carbon dioxide.

3.3.2. Talent transferring one fly into each rectangular arena.

3.4. Allow the flies to acclimate to the constant, low-intensity diffuse white light arena environment for 20 minutes before recording [1]. Record behavior under constant illumination using an automated video-tracking system [2].

3.4.1. Talent leaving flies in the arenas under low-intensity diffuse white light.

~~3.4.2. SCREEN: Show the automated video-tracking system recording fly behavior under constant illumination.~~ Author's NOTE: We replaced the scenes shot directly from the screen with footage of the talent working with the software.

4. Y-Maze Turning Behaviour and Phototactic Activity Assays

- 4.1. Briefly anesthetize fruit flies for 5 seconds using carbon dioxide [1]. Place individual flies into Y-maze chambers cut into 1.6-millimeter-thick black acrylic with a laser engraver [2].
 - 4.1.1. Talent anesthetizing fruit flies with carbon dioxide.
 - 4.1.2. Talent placing one fly into each Y-maze chamber.
- 4.2. Allow the flies to explore freely for an extended recording period of 20 minutes [1]. Record left or right turning decisions during exploratory locomotion for 1 hour [2].
 - 4.2.1. Talent allowing flies to move freely in the Y-maze chambers.
 - 4.2.2. ~~SCREEN:~~ Show recorded left and right turning decisions during exploratory locomotion. Author's NOTE: We replaced the scenes shot directly from the screen with footage of the talent working with the software.
- 4.3. For phototactic assay, load individual flies into FlyVac chambers without using carbon dioxide anesthesia and allow them to acclimate for 10 minutes before initiating trials [1].
 - 4.3.1. Talent loading individual flies into FlyVac chambers.
- 4.4. Present repeated binary light-dark choices to each fly [1]. Record choices across 40 trials using automated detection [2].
 - 4.4.1. Talent initiating binary light-dark choice trials.
 - 4.4.2. ~~SCREEN:~~ Show automated detection recording choices across 40 trials. Author's NOTE: We replaced the scenes shot directly from the screen with footage of the talent working with the software.
- ~~4.5. Calculate the light choice probability per fly [1] and the average time between light choices as a measure of activity [2]. Quantify behavioral variability across individuals using median absolute deviation [3].~~
 - ~~4.5.1. ~~SCREEN:~~ Show calculation of light choice probability for each fly.~~
 - ~~4.5.2. ~~SCREEN:~~ Show calculation of the average time between light choices.~~
 - ~~4.5.3. ~~SCREEN:~~ Show quantification of behavioral variability using median absolute deviation. Author's NOTE: We show a talent working on statistics, as proposed in the next scene. It seems these sections are just redundant~~
- 4.6. For data analysis, process the behavioral data from stressed and control groups across all assays and use permutation-based tests to evaluate group differences [1].
 - 4.6.1. Representative shot of talent analyzing the processed data.

Results

5. Results

- 5.1. In the forced swim assay, stressed flies adopted immobility more rapidly [1] and showed prolonged passive episodes compared to control flies [2].
 - 5.1.1. LAB MEDIA: Figure 2. *Video editor: Please highlight the height of the plot corresponding to X-axis title "Depressed"*
 - 5.1.2. LAB MEDIA: Figure 2. *Video editor: Please highlight the height of the plot corresponding to X-axis title "Control"*
- 5.2. In terms of long-term locomotor activity, continuous DAM recordings revealed that control flies were significantly more active than stressed flies [1].
 - 5.2.1. LAB MEDIA: Figure 3. *Video editor: Please emphasize the higher values of the control group corresponding to the depressed group in the graph.*
- 5.3. In the open-field locomotor assay, control flies displayed sustained locomotion, frequent directional changes, and traveled significantly greater distances in 1 hour [1] compared to stressed flies that showed reduced displacement and slower movement [2]. In contrast, the stressed flies showed significantly higher maximum acceleration than control flies [3].
 - 5.3.1. LAB MEDIA: Figure 4A. *Video editor: Please highlight the thick line in the middle of the purple-colored violin plot and the corresponding Y-axis height.*
 - 5.3.2. LAB MEDIA: Figure 4A. *Video editor: Please highlight the thick line in the middle of the blue color violin plot and the corresponding Y-axis height.*
 - 5.3.3. LAB MEDIA: Figure 4B. *Video Editor: Please highlight the width and the height of the central thick line in blue color violin plot, followed by the thick line and width of the purple color plot.*
- 5.4. In the Y-maze assay, control flies covered significantly longer distances in 1 hour than stressed flies [1]. Contrastingly, the stressed flies reached significantly higher maximum acceleration than control flies [2]. In terms of turning bias, the group difference between control and depressed flies were non-significant [3].
 - 5.4.1. LAB MEDIA: Figure 5A. *Video editor: Please highlight the difference in the heights of the central thick lines within the purple and blue plots sequentially.*
 - 5.4.2. LAB MEDIA: Figure 5B. *Video editor: Please highlight the difference in the heights*

of the central thick lines within the purple and blue plots sequentially.

5.4.3. LAB MEDIA: Figure 5C. *Video editor: Please emphasize the purple and blue box plots and highlight the term “ns” on top of the graph.*

5.5. In the FlyVac phototactic test, the groups did not differ in the number of completed trials [1]. The stressed flies showed a lower light-choice probability than control flies, indicating a shift toward the dark arm [2]. Further, the depression induction did not measurably alter between-fly variability in phototaxis index [3].

5.5.1. LAB MEDIA: Figure 6A. *Video editor: Please highlight the similar distributions of purple and blue dots within their corresponding color boxes in the graph.*

5.5.2. LAB MEDIA: Figure 6B. *Video editor: Please highlight that the blue box plot is lower than the purple box plot in the light-choice probability graph.*

5.5.3. LAB MEDIA: Figure 6C. *Video editor: Please emphasize the purple and blue box plots and highlight the term “ns” on top of the graph.*

Pronunciation guides:

1. *Drosophila melanogaster*
Pronunciation link: <https://www.merriam-webster.com/dictionary/Drosophila>
IPA: /drə'sɑ:fələ ,melənəʊ'gæstər/
Phonetic Spelling: dru·sah·fuh·luh meh·luh·noh·gas·ter
2. *Eclosion*
Pronunciation link: <https://www.merriam-webster.com/dictionary/eclosion>
IPA: /ɪ'kloʊʒən/
Phonetic Spelling: ih·KLOH·zhuhn
3. *Anesthesia*
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>
IPA: /,æn.əs'θi:.zə/, /,æn.əs'θi:.zjə/
Phonetic Spelling: an·uhs·THEE·zhuh; an·uhs·THEE·zyuh
4. *Carbon dioxide*
Pronunciation link: <https://www.merriam-webster.com/dictionary/carbon%20dioxide>
IPA: /,kɑ:r.bən daɪ'ɑ:k.səɪd/
Phonetic Spelling: KAR·buhn dye·OK·syde
5. *Polypropylene*
Pronunciation link: <https://www.merriam-webster.com/dictionary/polypropylene>
IPA: /,pɑ:.li,prəʊ.pə'pi:.li:n/
Phonetic Spelling: pol·ee·PROH·puh·peen
6. *Acrylic*
Pronunciation link: <https://www.merriam-webster.com/dictionary/acrylic>
IPA: /ə'krɪlɪk/
Phonetic Spelling: uh·KRIL·ik

7. Vibration
Pronunciation link: <https://www.merriam-webster.com/dictionary/vibration>
IPA: /vaɪˈbreɪʃən/
Phonetic Spelling: vy-BRAY-shuhn
8. Hertz
Pronunciation link: <https://www.merriam-webster.com/dictionary/hertz>
IPA: /hɜːts/
Phonetic Spelling: hurts
9. Locomotor
Pronunciation link: <https://www.merriam-webster.com/dictionary/locomotor>
IPA: /ˌloʊ.kəˈmoʊ.tər/
Phonetic Spelling: loh·kuh·MOH·ter
10. Multi-well
Pronunciation link: <https://www.merriam-webster.com/dictionary/well>
IPA: /ˈmʌl.ti ˈwel/
Phonetic Spelling: MUL·tee wel
11. Sodium dodecyl sulfate
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium>
IPA: /ˈsoʊ.di.əm doʊˈdes.əl ˈsʌl.fet/
Phonetic Spelling: SOH·dee·uhm doh·DESS·uhl SUL·fayt
12. Immobility
Pronunciation link: <https://www.merriam-webster.com/dictionary/immobility>
IPA: /ˌɪm.moʊˈbɪl.ə.ti/
Phonetic Spelling: im·moh·BIL·uh·tee
13. Drosophila Activity Monitor
Pronunciation link: <https://www.merriam-webster.com/dictionary/Drosophila>
IPA: /drəˈsɑːfələ ækˈtɪvəti ˈmɑːnətər/
Phonetic Spelling: druh·SAH·fuh·luh ak·TIV·uh·tee MON·uh·ter
14. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˈɪŋ.kjə.beɪ.tər/
Phonetic Spelling: ING·kyuh·bay·ter
15. Acclimate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimate>
IPA: /əˈklaɪ.mert/
Phonetic Spelling: uh·KLY·mayt
16. Diffuse
Pronunciation link: <https://www.merriam-webster.com/dictionary/diffuse>
IPA: /dɪˈfjuːs/
Phonetic Spelling: dih·FYOOS
17. Automated
Pronunciation link: <https://www.merriam-webster.com/dictionary/automated>
IPA: /ˈɔː.tə.meɪ.tɪd/
Phonetic Spelling: AW·tuh·may·tid
18. Y-maze
Pronunciation link: <https://www.merriam-webster.com/dictionary/maze>

- IPA: /waɪ meɪz/
Phonetic Spelling: why mayz
19. Laser engraver
Pronunciation link: <https://www.merriam-webster.com/dictionary/laser>
IPA: /ˈleɪ.zə ɪnˈɡreɪ.vər/
Phonetic Spelling: LAY·zer en·GRAY·ver
20. Exploratory
Pronunciation link: <https://www.merriam-webster.com/dictionary/exploratory>
IPA: /ɪkˈsplɔːr.ə.tɔːr.i/
Phonetic Spelling: ik·SPLO·uh·tor·ee
21. FlyVac
Pronunciation link: No confirmed link found
IPA: /ˈflaɪ.væk/
Phonetic Spelling: fly·vak
22. Phototactic
Pronunciation link: <https://www.merriam-webster.com/dictionary/phototaxis>
IPA: /ˌfoʊ.toʊˈtæk.tɪk/
Phonetic Spelling: foe·toh·TAK·tik
23. Binary
Pronunciation link: <https://www.merriam-webster.com/dictionary/binary>
IPA: /ˈbaɪ.nə.ri/
Phonetic Spelling: BYE·nuh·ree
24. Permutation
Pronunciation link: <https://www.merriam-webster.com/dictionary/permutation>
IPA: /ˌpɜː.mjəˈteɪ.ʃən/
Phonetic Spelling: per·myuh·TAY·shuhn
25. Behavioral
Pronunciation link: <https://www.merriam-webster.com/dictionary/behavioral>
IPA: /bɪˈheɪv.jə.əl/
Phonetic Spelling: bih·HAYV·yer·uhl
26. Acceleration
Pronunciation link: <https://www.merriam-webster.com/dictionary/acceleration>
IPA: /əkˌsel.əˈreɪ.ʃən/
Phonetic Spelling: ak·sel·uh·RAY·shuhn
27. Phototaxis
Pronunciation link: <https://www.merriam-webster.com/dictionary/phototaxis>
IPA: /ˌfoʊ.toʊˈtæk.sɪs/
Phonetic Spelling: foe·toh·TAK·sis
28. Median absolute deviation
Pronunciation link: <https://www.merriam-webster.com/dictionary/median>
IPA: /ˈmiː.di.ən ˈæb.səˌluːt ˌdiː.viˈeɪ.ʃən/
Phonetic Spelling: MEE·dee·uhn AB·suh·loot dee·vee·AY·shuhn