FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69217
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Project Page Link: https://review.jove.com/account/file-uploader?src=21111233

Title: FLEX: Flight Exercise Training Protocol for the Fruit Fly *Drosophila*

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Author Questionnaire

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

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes, Two rooms next to each other. 90 ft apart.**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 07
Number of Shots: 15

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

1.1. **Alexander Murashov:** Our research focuses on transgenerational susceptibility to neurological and metabolic disorders. We investigate molecular mechanisms, specifically bioenergetic changes that might transmit susceptibility across generations.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3.1*

What technologies are currently used to advance research in your field?

1.2. **Alexander Murashov:** Omics techniques like transcriptomics, proteomics, and metabolomics can help identify the mechanisms and causes of neurodegenerative disorders.

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

CONCLUSION:

What significant findings have you established in your field?

1.3. **Alexander Murashov:** We found that paternal Western diet reprograms offspring phenotype, including changes in activity, learning and memory, and feeding behavior.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3.2*

What research gap are you addressing with your protocol?

1.4. **Alexander Murashov:** Many protocols depend on forced climbing behavior. Flight exercise is more physiological and does not lead to injuries.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.6.3*

How will your findings advance research in your field?

1.5. **Alexander Murashov:** This protocol will utilize more physiological exercises that generate significant bioenergetic demands and minimize injuries.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.5*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. *Drosophila* Exercise Training and Post-Regimen Sample Preparation

Demonstrators: Giancarlo Lara and Steven Bradley

2.1. To begin, keep the *Drosophila* flies in an incubator or room maintained at 24 degrees Celsius with 60 percent humidity and a 12-hour light and dark cycle **[1]**.

2.1.1. WIDE: Talent placing fly vials inside an incubator with the temperature and humidity settings clearly displayed on screen. **NOTE: Interviews were shot by th**

2.2. Collect age-matched flies within 3 to 4 days after eclosion **[1]**. Randomly assign the flies to exercise and sedentary groups **[2-TXT]**.

2.2.1. Talent retrieving vials of newly eclosed flies and examining them closely.

2.2.2. Talent separating the flies into two groups labeled as "exercise" and "sedentary." **TXT: Use an equal number of flies in each group**

2.3. Provide both groups with 7 milliliters of formulation food **[1]** and use tape to secure the food vials inside the bowls **[2]**. If humidity cannot be controlled, provide water access by placing vials of wet cotton inside the bowls and taping them to the bowl walls **[3-TXT]**.

2.3.1. Talent pipetting 7 mL formulation food into empty vials and placing them in the bowls.

2.3.2. Talent taping each vial securely to the base of the bowl. **NOTE:** This shot was covered in 2.3.1

2.5.3 Talent placing wet cotton-filled vials into bowls and taping them securely to the inner walls. **TXT: Exercise flies for 5 consecutive days** **NOTE:** Show shot 2.5.3 after 2.3.2. VO is added here

2.4. Transfer flies into the bowls one day before the exercise regimen begins **[1-TXT]**. Cover the openings of the bowls with mesh and secure it using rubber bands **[2]**.

2.4.1. Talent transferring sedated flies into separate bowls. **TXT: Use brief and light CO_2 anesthesia**
AUTHORS: Please do not show the process of anesthesia here

2.4.2. Talent placing mesh covers over the bowl openings and fastening them with rubber bands.

2.5. Conduct the training in a room maintained at 24 degrees Celsius with 60 percent humidity **[1].** Secure the bowls to the exercise platform using bungee cords **[2]**. ~~If humidity cannot be controlled, provide water access by placing vials of wet cotton inside the bowls and taping them to the bowl walls **[3-TXT].**~~

2.5.2 Talent transferring the bowls into the training room. **NOTE:** Show 2.5.2 first and then 2.5.1. The VO has been inverted too

2.5.1 Talent wrapping bungee cords around each bowl to fix them onto the exercise platform.

~~2.5.3 Talent placing wet cotton-filled vials into bowls and taping them securely to the inner walls. **TXT: Exercise flies for 5 consecutive days**~~ **NOTE:** This shot has been moved after 2.3.2

2.6. Use two timers to control the motor. Set the first timer to start the machine at 8 AM and stop it at 3 PM daily **[1]**. Program the second timer to trigger a series of three motor revolutions every 5 minutes **[2]**. Each revolution should raise and drop the platform, inducing flight in the flies **[3]**. ~~Once the exercise session is complete, return the bowls to their incubator **[4].**~~

2.6.1. Shot of 2 timers then show the first timer being set to 8 AM and 3PM

2.6.2. Shot of the second timer being programmed to trigger revolutions every 5 minutes.

2.6.3. Shot of the platform's movement simulating flight in the flies.

2.6.4. ~~Talent placing the bowls back into the incubator at the end of the session~~. **NOTE:** Delete

2.7. At the end of the FLEX regimen, place the bowls in a cold room for 5 minutes to immobilize the flies **[1]**. Then, transfer the flies into new food vials for phenotypic analysis or into microcentrifuge tubes for molecular or biochemical assays **[2]**.

2.7.1. Talent placing bowls into a cold room. **NOTE:** This is slated as 2.6.4

2.7.2. Talent transferring flies into new food vials and microcentrifuge tubes, labeling them for downstream analysis.

Results

3. Results

3.1. The 5-day flight exercise regimen significantly reduced mortality in flies on a Western Diet **[1]**. Flight exercise also improved climbing ability in Western Diet-fed flies **[2]**.

3.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the shorter red bar labeled "WDE"*

3.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the higher red line labeled "WDE"*

3.2. Maximal oxygen consumption during complex I-supported respiration was reduced by 42% in flight muscles from Western Diet-fed flies compared to controls **[1]**, but was restored in flies from the Western Diet plus exercise group **[2]**.

3.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the tall blue bar labeled "CD" and the shorter orange bar labeled "WD" under the section "C1 (ProPyr/Mal/ADP)"*

3.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight the red bar labeled "WDE" under the section "C1 (ProPyr/Mal/ADP)" showing recovery in height relative to "WD"*

3.3. Offspring of Western Diet-fed fathers showed significantly more feeding licks than other groups **[1]**, while paternal exercise negated this increase **[2]**.

3.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the tall orange bar labeled "WFO"*

3.3.2. LAB MEDIA: Figure 5. *Video editor: Highlight the red bar labeled "WEFO"*

- **Drosophila**

Pronunciation link: https://www.merriam-webster.com/dictionary/drosophila merriam-webster.com
IPA: /droʊˈsɑːfəˌlə/
Phonetic Spelling: droh-SAH-fuh-luh

- **eclosion**

Pronunciation link: https://www.merriam-webster.com/dictionary/eclosion merriam-webster.com
IPA: /ɪˈkloʊʒən/
Phonetic Spelling: ih-KLOH-zhun

- **phenotypic**

Pronunciation link: https://www.merriam-webster.com/dictionary/phenotypic merriam-webster.com+2Cambridge Dictionary+2
IPA: /ˌfiː.noʊˈtɪp.ɪk/
Phonetic Spelling: fee-noh-TIP-ik

- **microcentrifuge**

Pronunciation link: https://www.howtopronounce.com/microcentrifuge howtopronounce.com+1
IPA: /ˌmaɪ.kroʊˈsɛn.trɪ.fjuʒ/
Phonetic Spelling: my-kroh-SEN-tri-fyo͞oz

- **eclosion**

Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/eclosion Cambridge Dictionary+1
IPA: /ɪˈkloʊ.ʒən/
Phonetic Spelling: ih-KLOH-zhun

- **regimen**

Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/regimen Cambridge Dictionary+1
IPA: /ˈrɛdʒ.ə.mən/
Phonetic Spelling: REJ-uh-muhn