

Submission ID #: 70697

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21562633>

Title: Semi-Automated Integration of Motor Healthspan and Lifespan Analysis Using FlyTrDT Pipeline in *Drosophila melanogaster*

Authors and Affiliations:

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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? **Yes**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? In the same building-ground floor and second floor

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. **Yes**

If **Yes**, please provide the **full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author willing to participate.

Dr Bipin G Nair, Dean, Amrita school of biotechnology, Amrita Vishwa Vidyapeetham 3

Current Protocol Length

Number of Steps: 31

Number of Shots: 50

Interviews

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

Authors: Answers are slightly edited in accordance with journal guidelines. Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

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

- 1.1. **Rajaguru Aradhya:** Our study investigates neurodegenerative mechanisms in *Drosophila* and identifies therapeutic strategies to preserve neuronal function and promote healthy aging.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Describe the research gap or limitation in existing methods and how this protocol addresses them.

- 1.2. **Rajaguru Aradhya:** Existing *Drosophila* aging studies measure lifespan and motor function separately; this protocol integrates both into one framework.
 - 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. **Parvathy Venugopal:** This protocol allows researchers to measure lifespan, locomotor healthspan, and the relationship between early motor function and long-term survival in *Drosophila*.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

NOTE: Testimonials were not recorded

Testimonial Questions (OPTIONAL):-

~~Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.~~

~~What value do you expect this video article to have for other researchers working in your field?~~

~~1.4. **Dr Bipin G Nair, Dean, Amrita school of biotechnology, Amrita Vishwa Vidyapeetham**
:- (authors will present their testimonial statements live)~~

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

~~How does the video format of your article help you to share your work with the broader-
scientific community?~~

~~1.5. **Dr Bipin G Nair, Dean, Amrita school of biotechnology, Amrita Vishwa-
Vidyapeetham:** (authors will present their testimonial statements live)~~

~~1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot,
looking slightly off-camera.~~

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Preparation of Standard Fly Food Medium

Demonstrator: Durga Nandan

- 2.1. To begin, gather the ingredients [1] to prepare the standard fly food medium [2]. Mix the contents thoroughly with a spatula until a uniform suspension forms [3].
 - 2.1.1. WIDE: Talent arranging the components and a container on the workbench.
Videographer: Please obtain CU shot as well for this shot.
 - 2.1.2. TEXT ON A PLAIN BACKGROUND:
Standard Fly Food Medium:
Cornflour – 6 g
Yeast – 5 g
D-glucose – 4 g
Sucrose – 3 g
Yeast extract – 1 g
Agar – 1 g
Malt extract – 0.25 g
Distilled water – 100 mL
 - 2.1.3. Talent mixing the contents in the container with a spatula until the suspension appears uniform.
- 2.2. Heat the mixture to approximately 85 to 100 degrees Celsius on an induction cooker for 12 to 15 minutes for volumes of 500 milliliters or greater, or for 5 to 10 minutes for volumes between 100 and 400 milliliters [1]. Stir continuously during heating [2].
 - 2.2.1. Talent heating the mixture on an induction cooker.
 - 2.2.2. Talent stirring the mixture continuously during heating.
- 2.3. Autoclave the heated mixture according to standard laboratory sterilization procedures [1]. After allowing the autoclaved medium to cool, monitor the temperature with a thermometer until it reaches 50 to 55 degrees Celsius [2].
 - 2.3.1. Talent placing the heated mixture in the autoclave.
 - 2.3.2. Talent checking the medium temperature with a thermometer.
- 2.4. Add 600 microliters of propionic acid and 100 milligrams of benzoic acid in 1 milliliter of 100 percent ethanol to the cooled medium [1] and mix thoroughly [2].

- 2.4.1. Talent adding propionic acid and benzoic acid in ethanol to the cooled medium.
- 2.4.2. Talent mixing the medium thoroughly.
- 2.5. Dispense 5 milliliters of food into each sterile vial measuring 10 centimeters in length and 20 millimeters in diameter [1].
 - 2.5.1. Talent dispensing food into the vial.
- 2.6. Leave the vials uncovered for at least 5 to 6 hours at room temperature [1]. Cover them with tissue paper to keep stray flies out [2].
 - 2.6.1. Shot of the vials kept uncovered at room temperature.
 - 2.6.2. Talent covering the vials with tissue paper.

3. Preparation of Drug-Containing and Control Food

Demonstrator: Nikhil Raj S

- 3.1. Prepare and cool a small batch of adult food as previously demonstrated [1].
 - 3.1.1. Shot of a small batch of adult food.
- 3.2. Prepare a stock solution of the test compound at the desired concentration using distilled water or dimethyl sulfoxide [1]. Dissolve the test compound in distilled water or dimethyl sulfoxide to generate a concentrated stock solution [2]. Add the stock solution to the cooled food to achieve the desired final concentration while maintaining identical vehicle concentrations across treatment and control groups [3].
 - 3.2.1. Shot of adding distilled water or dimethyl sulfoxide to the test compound.
 - 3.2.2. Shot of dissolving the test compound in the distilled water or dimethyl sulfoxide.
 - 3.2.3. Talent adding the stock solution to the cooled food.
- 3.3. After mixing thoroughly, dispense 2 milliliters of the drug-containing food into each experimental vial by layering it onto the surface of pre-solidified base food [1].
 - 3.3.1. Talent dispensing drug-containing food onto the surface of pre-solidified base food in an experimental vial.
- 3.4. Prepare control vials by adding an equivalent volume of vehicle alone to the food layered onto the base food [1].
 - 3.4.1. Talent adding the vehicle to the food layered onto the base food in a control vial.
- 3.5. Allow the layered food to solidify completely at room temperature before introducing flies [1].

3.5.1. Shot of the layered food placed at room temperature to solidify completely.

4. Sorting of Fly and Experimental Maintenance

Demonstrator: Nikhil Raj S

4.1. Cross the fly lines [1-TXT]. Obtain first filial generation or F1 (*f-one*) progeny expressing amyloid beta in neurons [2].

4.1.1. Talent transferring virgin elav-GAL4 females and UAS-APPE693G males into the same vial. **TXT: Fly lines: Virgin elav-GAL4 females × UAS-APPE693G males**

4.1.2. Shot of F1 progeny in the culture vial.

4.2. Maintain the flies on standard fly food at 25 degrees Celsius, approximately 60 percent relative humidity, and a 12-hour light and 12-hour dark cycle with carbon dioxide [1].

4.2.1. Shot of multiple fly culture vials containing adult flies on standard fly food arranged inside the incubator, with the incubator display showing 25 degrees Celsius, approximately 60 percent relative humidity, and a 12-hour light and 12-hour dark cycle.

4.3. Anesthetize the flies using carbon dioxide [1]. Collect newly eclosed adult male flies within a 2-to-3-day window [2] and transfer them into the appropriate food vials for each experimental group [3].

4.3.1. Talent anesthetizing the flies using carbon dioxide.

4.3.2. Talent collecting newly eclosed adult male flies.

4.3.3. Talent transferring male flies into drug-containing food vials and control food vials.

4.4. Replace the food every 3 days or earlier if food quality deteriorates [1].

4.4.1. Talent replacing the food in the fly vials.

4.5. Maintain the flies under these conditions until the designated ages for negative geotaxis assessment [1].

4.5.1. Shot of flies in the experimental vials.

4.6. Then, anesthetize small groups of flies briefly on an anesthesia pad, ensuring that total anesthesia exposure does not exceed 9 to 10 minutes [1].

4.6.1. Talent anesthetizing a small group of flies on an anesthesia pad.

4.7. Using a soft paintbrush, identify male flies while minimizing carbon dioxide exposure during sorting [1].

4.7.1. Talent identifying male flies with a soft paintbrush.

- 4.8. Place 10 male flies and 10 female flies in a 1-to-1 sex ratio into each fresh food vial according to the experimental design [1].
 - 4.8.1. Talent transferring 10 male flies and 10 female flies into a fresh food vial.
- 4.9. Repeat the sorting and aliquoting procedure until 4 to 6 replicate vials are established for each experimental condition [1].
 - 4.9.1. Shot of multiple replicate vials placed for each experimental condition.

5. Negative Geotaxis Assay

Demonstrator: Durga Nandan

- 5.1. In a transparent cylindrical climbing tube measuring 12 centimeters in height and 2 centimeters in diameter [1], mark a horizontal line 10 centimeters from the base of the tube [2].
 - 5.1.1. Shot of the transparent climbing tube.
 - 5.1.2. Shot of horizontal line being marked 10 centimeters from the base of the tube.
- 5.2. Position the tube vertically against a uniform white background to facilitate video tracking [1].
 - 5.2.1. Talent positioning the tube vertically against a uniform white background.
- 5.3. Mount a smartphone or digital camera on a stable stand at a fixed distance of approximately 15 to 20 centimeters from the climbing tube and maintain the same acquisition geometry across all recordings [1]. Ensure that the entire tube is within the frame [2] and record at approximately 30 frames per second and a minimum resolution of 1280 by 720 pixels [3].
 - 5.3.1. Talent mounting a smartphone or digital camera on a stable stand. **Author's NOTE: Instead of another stand, we used the stand available with phone stand itself**
 - 5.3.2. Show the entire climbing tube is within the camera frame.
 - 5.3.3. SCREEN: Screen-capture_1.mp4 00:02-00:15. **NOTE: Use the file uploaded on 04-07-2026**
- 5.4. Collect flies from experimental food vials without carbon dioxide anesthesia [1].
 - 5.4.1. Talent collecting flies from experimental food vials without carbon dioxide anesthesia.
- 5.5. Using a small funnel, transfer the flies gently into a clean climbing tube to prevent escape [1].

- 5.5.1. Talent transferring flies into a clean climbing tube using a small funnel.
- 5.6. Allow the flies to acclimatize in the testing chamber for 5 minutes and perform all climbing assays at the same time of day, such as in the afternoon, to minimize circadian variability [1].
 - 5.6.1. Shot of flies acclimatizing in the testing chamber.
- 5.7. Tap the tube firmly three times on a foam pad or mouse pad placed on a solid surface so that all flies fall to the bottom simultaneously [1]. Immediately start recording video for 10 to 12 seconds [2].
 - 5.7.1. Talent tapping the climbing tube three times on a foam pad.
 - 5.7.2. Talent starting video recording.
- 5.8. Allow the flies to climb freely for 10 seconds [1].
 - 5.8.1. Shot of flies climbing within the tube.
- 5.9. Record at least three independent trials per vial, allowing a 10-minute rest interval between trials [1].
 - 5.9.1. Shot of repeated climbing trials being performed for the same vial. **Author's NOTE: Two trials were taken separately for this. Hope that's okay.**
- 5.10. Save the recorded videos in MP4 format [1]. Transfer the recorded videos to a desktop computer for analysis [2].
 - 5.10.1. SCREEN: Screen-capture_2.mp4. **NOTE: Use the file uploaded on 04-07-2026**
 - 5.10.2. SCREEN: Screen-capture-3.mp4 00:15-00:25.
- 5.11. Perform the assay for each experimental condition, including control, drug-treated, and solvent control groups, and count the number of flies that reach or cross the 10-centimeter mark within 10 seconds [1]. Calculate the percent climbing activity [2-TXT]. Average the values across three trials and report the values as mean plus or minus standard deviation [3].
 - 5.11.1. Shot of flies crossing the 10-centimeter mark in the recorded video.
 - 5.11.2. SCREEN: Screen-capture-4.mp4 00:09-00:20. **TXT: % Climbing Activity: (E/T) × 100**
 - 5.11.3. SCREEN: Screen-capture-4.mp4 00:12-00:15.

Results

6. Results

- 6.1. Using conventional negative geotaxis scoring, at Day 7, climbing performance remained broadly comparable between amyloid-beta-expressing flies [1] and Canton S control flies [2], whereas by Day 28, amyloid-beta-expressing flies exhibited reduced climbing activity [3] relative to control flies [4].
 - 6.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight the box plot labelled 'elav-Gal4/UAS-Aβ42' under 'DAY 7'.*
 - 6.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the box plot labelled 'CS' under 'DAY 7'.*
 - 6.1.3. LAB MEDIA: Figure 3. *Video editor: Highlight the box plot labelled 'elav-Gal4/UAS-Aβ42' under 'DAY 28'.*
 - 6.1.4. LAB MEDIA: Figure 3. *Video editor: Highlight the box plot labelled 'CS' under 'DAY 28'.*
- 6.2. Control flies exhibited a mean climbing slope of 86.64 pixels per second at Day 7 [1], which approached zero by Day 28 at minus 1.50 pixels per second [2].
 - 6.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the red line segment labelled 'Most linear segment' and the text 'Slope = 86.64 px/sec'.*
 - 6.2.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the red line segment labelled 'Most linear segment' and the text 'Slope = -1.50 px/sec'.*
- 6.3. Amyloid-beta-expressing flies exhibited a climbing slope of 34.25 pixels per second at Day 7 [1], which declined to minus 72.25 pixels per second by Day 28 [2].
 - 6.3.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the red line segment labelled 'Most linear segment' and the text 'Slope = 34.25 px/sec'.*
 - 6.3.2. LAB MEDIA: Figure 4D. *Video editor: Highlight the red line segment labelled 'Most linear segment' and the text 'Slope = -72.25 px/sec'.*
- 6.4. Reduced locomotor performance in amyloid-beta-expressing flies was reflected by less sustained upward trajectories [1] and lower slope values [2].
 - 6.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the blue line labelled 'Mean vertical position' showing shorter upward movement.*
 - 6.4.2. LAB MEDIA: Figure 4D. *Video editor: Highlight the red line segment labelled 'Most linear segment' and the text 'Slope = -72.25 px/sec'.*

1. Cornflour
Pronunciation link: <https://www.merriam-webster.com/dictionary/cornflour>
IPA: /'kɔrn-, flau(-ə)r/
Phonetic Spelling: korn·flou·er
2. D-glucose
Pronunciation link: <https://www.merriam-webster.com/dictionary/glucose>
IPA: /'gluː koʊs/
Phonetic Spelling: gloo·kohs
3. Sucrose
Pronunciation link: <https://www.merriam-webster.com/dictionary/sucrose>
IPA: /'suː kroʊs/
Phonetic Spelling: soo·krohs
4. Agar
Pronunciation link: <https://www.merriam-webster.com/dictionary/agar>
IPA: /'eɪ, gar/, /'ɑː, gar/
Phonetic Spelling: ay·gar, ah·gar
5. Malt extract
Pronunciation link: <https://www.merriam-webster.com/dictionary/malt>
IPA: /'mɔlt/
Phonetic Spelling: mawlt
6. Distilled
Pronunciation link: <https://www.merriam-webster.com/dictionary/distilled>
IPA: /dɪ'stɪld/
Phonetic Spelling: dih·stild
7. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>
IPA: /sə'spen(t)-shən/
Phonetic Spelling: suh·spen·shuhn
8. Induction
Pronunciation link: <https://www.merriam-webster.com/dictionary/induction>
IPA: /ɪn'dɛk-shən/
Phonetic Spelling: in·duhk·shuhn
9. Autoclave
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔːtoʊ klɛv/
Phonetic Spelling: aw·toh·klayv
10. Sterilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilization>
IPA: /,ster-ə-lə-'zā-shən/
Phonetic Spelling: steh·ruh·luh·zay·shuhn
11. Thermometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermometer>
IPA: /θər'mɑːmɛtər/
Phonetic Spelling: thur·mah·muh·ter

12. Microliters
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
 IPA: /'maɪkroʊˌliːtər/
 Phonetic Spelling: my·kroh·lee·ter
13. Propionic acid
 Pronunciation link: <https://www.merriam-webster.com/dictionary/propionic%20acid>
 IPA: /ˌproʊpiˈɑːnɪk ˈæsɪd/
 Phonetic Spelling: proh·pee·on·ik a·sid
14. Benzoic acid
 Pronunciation link: <https://www.merriam-webster.com/dictionary/benzoic%20acid>
 IPA: /benˈzoʊɪk ˈæsɪd/
 Phonetic Spelling: ben·zoh·ik a·sid
15. Ethanol
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
 IPA: /'eθəˌnɔːl/
 Phonetic Spelling: eth·uh·nawl
16. Sterile
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>
 IPA: /'sterəl/, /'sterail/
 Phonetic Spelling: steh·rul, steh·ryle
17. Dimethyl sulfoxide
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dimethyl%20sulfoxide>
 IPA: /daɪˈmeθəl səlˈfɑːksaɪd/
 Phonetic Spelling: dye·meth·uhl sul·fok·side
18. Concentration
 Pronunciation link: <https://www.merriam-webster.com/dictionary/concentration>
 IPA: /ˌkɒn-sən-'trā-shən/
 Phonetic Spelling: kon·suhn·tray·shuhn
19. Vehicle (pharmaceutical)
 Pronunciation link: <https://www.merriam-webster.com/dictionary/vehicle>
 IPA: /'viːəkəl/, /'viːhɪkəl/
 Phonetic Spelling: vee·uh·kuhl, vee·hi·kuhl
20. Experimental
 Pronunciation link: <https://www.merriam-webster.com/dictionary/experimental>
 IPA: /ɪkˌsperə'men(t)əl/
 Phonetic Spelling: ik·sper·uh·men·tuhl
21. Solidify
 Pronunciation link: <https://www.merriam-webster.com/dictionary/solidify>
 IPA: /səˈlɪdəˌfaɪ/
 Phonetic Spelling: suh·lid·uh·fye
22. Filial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/filial>
 IPA: /'fɪliəl/
 Phonetic Spelling: fil·ee·uhl

23. Progeny
 Pronunciation link: <https://www.merriam-webster.com/dictionary/progeny>
 IPA: /'prɑːdʒəni/
 Phonetic Spelling: proj·uh·nee
24. Amyloid beta
 Pronunciation link: <https://www.merriam-webster.com/dictionary/amyloid>
 IPA: /'æməˌlɔɪd 'beɪtə/
 Phonetic Spelling: am·uh·loyd bay·tuh
25. Neurons
 Pronunciation link: <https://www.merriam-webster.com/dictionary/neuron>
 IPA: /'nɒr,ɑːn/, /'njʊr,ɑːn/
 Phonetic Spelling: nur·on, nyur·on
26. Virgin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/virgin>
 IPA: /'vɜrdʒən/
 Phonetic Spelling: vur·juhn
27. Elav-GAL4
 Pronunciation link: No confirmed link found
 IPA: /'iːləv gæl fɔːr/
 Phonetic Spelling: ee·lav gal·four
28. UAS-APPE693G
 Pronunciation link: No confirmed link found
 IPA: No verified IPA available
 Phonetic Spelling: yoo·ay·ess ay·pee·pee·ee six·nine·three gee
29. Eclosed
 Pronunciation link: <https://www.howtopronounce.com/eclosed>
 IPA: /ɪ'kloʊzd/
 Phonetic Spelling: ih·klohzd
30. Anesthetize
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
 IPA: /ə'nesθəˌtaɪz/
 Phonetic Spelling: uh·ness·thuh·tyze
31. Aliquoting
 Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
 IPA: /'æliˌkwɑːt/
 Phonetic Spelling: al·ih·kwaht
32. Replicate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/replicate>
 IPA: /'rep-lə-,kāt/ (verb), /'rep-lə-kət/ (adjective)
 Phonetic Spelling: rep·luh·kate
33. Geotaxis
 Pronunciation link: <https://www.howtopronounce.com/geotaxis>
 IPA: /ˌdʒiːoʊ'tæksɪs/
 Phonetic Spelling: jee·oh·tak·sis

34. Cylindrical
Pronunciation link: <https://www.merriam-webster.com/dictionary/cylindrical>
IPA: /səˈlɪndrɪkəl/
Phonetic Spelling: suh·lin·drih·kuhl
35. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌækwəˈzɪʃən/
Phonetic Spelling: ak·wuh·zish·uhn
36. Acclimatize
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimatize>
IPA: /əˈklaɪməˈtaɪz/
Phonetic Spelling: uh·kly·muh·tyze
37. Circadian
Pronunciation link: <https://www.merriam-webster.com/dictionary/circadian>
IPA: /sərˈkeɪdiən/
Phonetic Spelling: sur·kay·dee·uhn
38. Variability
Pronunciation link: <https://www.merriam-webster.com/dictionary/variability>
IPA: /ˌveriəˈbɪləti/
Phonetic Spelling: vair·ee·uh·bil·uh·tee
39. Conventional
Pronunciation link: <https://www.merriam-webster.com/dictionary/conventional>
IPA: /kənˈven(t)·shnəl/
Phonetic Spelling: kuhn·ven·shuh·nuhl
40. Locomotor
Pronunciation link: <https://www.merriam-webster.com/dictionary/locomotor>
IPA: /ˌloʊkəˈmoʊtər/
Phonetic Spelling: loh·kuh·moh·ter
41. Trajectories
Pronunciation link: <https://www.merriam-webster.com/dictionary/trajectory>
IPA: /trəˈdʒektəri/
Phonetic Spelling: truh·jek·tuh·ree
42. Pixels
Pronunciation link: <https://www.merriam-webster.com/dictionary/pixel>
IPA: /ˈpɪk_səl/
Phonetic Spelling: pik·sel