

Submission ID #: 69671

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21249703>

**Title: Validation of Hyperbaric Pressure System with Xenon Anesthesia
for *Drosophila melanogaster***

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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? **Yes, identification of the fly gender under stereoscope**

SCOPE: 2.1.1

Videographer: Please film all SCOPE shots with a SCOPE KIT

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

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 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: 29

Number of Shots: 49

Introduction

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

Authors: Please note that the questions will not appear on screen. Please answer in stand-alone statements with sufficient context.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Ekin Daplan:** My research aims to understand how general anesthesia works and there are several gases that are anaesthetics under hyperbaric conditions.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What research gap are you addressing with your protocol?~~

- 1.2. **Ekin Daplan:** This protocol lets us anesthetize fruit flies with xenon to study basic mechanisms of anesthesia.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.5. **Ekin Daplan:** Our protocol offers simplicity and speed, and it uses cheaper materials than traditional pressure chambers.
 - 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

- 1.6. **Ekin Daplan:** Maximum pressure is limited to approximately 4 atm, and imperfect sealing causes gradual pressure loss.
 - 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How will your findings advance research in your field?~~

- 1.7. **Ekin Daplan:** This setup enables reproducible measurement of anesthetic gas effects on behavior in small model organisms.

- 1.7.1. 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.

Videographer's NOTES:

: There is no sound of the clapperboard when I used my phone to do the board.

I only took headshots of Ekin. Luca was busy at the time so I gave Ekin the email address (author.liason@jove.com) to pass onto Luca to send over his headshots.

There was a noisy fridge near where I filmed the introduction. I was told this fridge could not be turned off and there was no other rooms available for me to relocate.

The rooms where I filmed the Introduction and Protocol were small & dark. I did my best to bring up the light levels in the room using the portable light I had with me.

It was my first time using the recommended NexYZ universal phone adapter. I'm not sure if I used it correctly and/or framed up the shots correctly. As I didn't have a photo or video to reference to.

Protocol

2. Xenon Anesthesia in *Drosophila melanogaster* Using a Hyperbaric Syringe Setup

Demonstrator: Ekin Daplan

2.1. To begin, collect 20 anesthetized female and male *Drosophila melanogaster* flies aged 1 to 3 days for each experiment [1-TXT].

2.1.1. Talent sorting 20 female and 20 male anesthetized flies. **TXT: Anesthesia: CO₂ inhalation; Let flies rest in vial with food for 24 h at 22°C** **Videographer's NOTE: Scope shots were also obtained for this step.** The stereo microscope clips file names are: 20260227_135027 & 20260227_135555. Located in the Step 2 folder within the Rushes folder. The first clip of the stereo microscope showed the female flies. The second clip of the stereo microscope showed the male flies

Video editor: Please add the wide shot first with the talent and then show the two scope clips side by side with split screen

2.2. Transfer the gases from their container gas cylinders into leakproof gas bags for experimental use [1]. Using silicone tubing, directly connect the gas cylinder outlet to the gas bag inlet to allow gas transfer [2].

2.2.1. Talent positioning the gas cylinder next to the gas bag. **Videographer's NOTE: Shots 2.2.1 & 2.2.2 were combined together. I wasn't aware that the Author combined the two shots together in the first take. So there is an error on the board. I decided to do another take with those shot numbers shown on the board.**

2.2.2. Talent connecting silicone tubing between the gas cylinder and the gas bag.

2.3. Attach a 25-millimeter-long silicone tubing to a polypropylene syringe [1]. Connect the polypropylene syringe to the gas bags and to the three-way tap [2].

2.3.1. Talent attaching silicone tubing to the polypropylene syringe. **Videographer's NOTE: Shots 2.3.1 & 2.3.2 were combined together. Take 3 is the best one to use as it had the best framing in my opinion**

2.3.2. Talent connecting the syringe tubing to the gas bag and three-way tap.

2.4. To assemble the hyperbaric setup, first position the LED light table in landscape

orientation on the table [1].

2.4.1. Talent placing the LED light table flat on the bench in landscape orientation.

2.5. Place the injection pump adjacent to one short side of the LED light table [1-TXT]. Then locate the camera above the setup to cover the region where the experimental syringe will be positioned [2].

2.5.1. Talent positioning the injection pump next to the LED light table. **TXT: Ensure pump orientation allows the experimental syringe to extend above the backlight**

2.5.2. Talent mounting and adjusting the camera above the experimental setup.

2.6. Now cut a 75-millimeter-long silicone tube [1]. Connect the three-way tap to the manometer using the silicone tubing from the central nozzle [2].

2.6.1. Talent cutting silicone tubing to length. **Videographer's NOTE: Shot 2.6.1 Take 2 should be used, as the tube was not cut in the prior take**

2.6.2. Talent connecting the tubing between the three-way tap and the manometer.

2.7. Use hose spring clamps on the nozzle at the manometer side and the three-way tap side to ensure the tubing is tightly secured [1].

2.7.1. Talent fastening hose spring clamps onto both tubing connections.

2.8. Then use the button marked with a double arrow on the injection pump control unit to set the speed [1] to 80 millimeters per minute and the duration to 38 seconds for automatic compression or decompression of the plunger [2].

2.8.1. Shot of the injection pump control panel. **Videographer's NOTE: Shots 2.8.1 & 2.8.2 were combined together. I asked the Author if she could increase the brightness of the control panel to make it more visible to my camera. The Author told me she couldn't increase the brightness.**

2.8.2. Shot of the speed and duration settings being adjusted.

2.9. Next, limit ambient light in the experiment room and turn on the LED light table [1]. Set the brightness level to 3 to increase video contrast [2].

2.9.1. Talent dimming room lights and switching on the LED light table. **Videographer's NOTE:**

2.9.2. Talent adjusting the LED brightness setting to level 3.

2.10. Remove the plunger from the polycarbonate syringe [1]. Insert a small amount of cotton into the syringe tip to prevent fly escape [2].

2.10.1. Talent removing the syringe plunger. **Videographer's NOTE: Shots 2.10.1 & 2.10.2 were combined together.**

2.10.2. Talent placing cotton into the syringe tip.

- 2.11. To transfer 40 flies into the polycarbonate syringe, remove the vial lid [1] and place the syringe barrel vertically on top of the vial so the barrel flange aligns with the vial opening [2].
- 2.11.1. Shot of vial lid being opened. **Videographer's NOTE:** Shots 2.11.1, 2.11.2, 2.12.1 & 2.12.2 were combined. These had to be combined due to time sensitivity. The Author told me the flies would fly away if we didn't combine the shots.
- 2.11.2. Talent positioning the syringe barrel onto the fly vial.
- 2.12. Rotate the assembly 180 degrees and tap to move the flies into the syringe barrel [1]. Then reinsert the plunger and place the syringe into the injection pump [2].
- 2.12.1. Talent rotating and tapping the vial and syringe to transfer flies.
- 2.12.2. Talent reinserting the plunger and loading the syringe into the injection pump.
- 2.13. Orient the syringe so the barrel markings face the camera for optimal visualization of the flies [1]. Secure the syringe by fastening both the barrel and plunger head to the injection pump using the adjustable screws [2].
- 2.13.1. Talent rotating the syringe to align barrel markings with the camera view.
- 2.13.2. Talent tightening the adjustable screws on the injection pump. **Videographer's NOTE:** Shot 2.13.2B is an extra shot. Showing a closer view of the panel & screws, as the Author and myself felt it could not be seen clearly in the prior shot.
- 2.14. Now remove the cotton from the syringe tip and connect the syringe to the three-way tap [1].
- 2.14.1. Talent removing cotton from the syringe tip and attaching the syringe to the three-way tap. **Videographer's NOTE:** Shot 2.14.1 Take 2 should be used. Take 1 had a slip up by the Author.
- 2.15. Start video recording [1]. Turn on the manometer then turn the stopcock to connect the manometer with the syringe and confirm zero kilopascal pressure [2].
- 2.15.1. SCREEN: Screen_2.15.1..mov 00:00-00:03 .
- 2.15.2. Talent switching on the manometer and turning the stopcock.
- 2.16. Now turn the three-way tap to the lateral position to allow air exchange with the syringe [1]. Using the manual control option of the injection pump, move the plunger from the 20-milliliter mark to the 2-milliliter mark [2].
- 2.16.1. Talent rotating the three-way tap to the lateral position.
- 2.16.2. SCREEN: Screen_2.16.2..mov 00:03-00:29 .
- 2.17. Aspirate 18 milliliters of anesthetic gas from the gas bag into the polypropylene syringe [1]. Connect the syringe to the lateral luer lock port of the three-way tap [2].

2.17.1. Talent drawing anesthetic gas into the syringe from the gas bag.

2.17.2. Talent connecting the syringe to the three-way tap.

2.18. Then use the manual option of the injection pump to move the plunger back to the 20 milliliter mark [1]. Turn the stopcock to connect the manometer with the syringe and observe the pressure reading [2].

2.18.1. SCREEN: Screen_2.18.1..mov 00:00-00:27 .

2.18.2. SCREEN: Screen_2.18.2..mov 00:00-end .

2.19. Start automatic plunger movement with the preset speed and duration settings [1]. Allow the plunger to reach the 5-milliliter mark over 38 seconds while monitoring pressure [2].

2.19.1. SCREEN: Screen_2.19.1..mov 00:20-00:39.

2.19.2. SCREEN: Screen_2.19.2..mov 00:00-end .

2.20. Leave the plunger at the 5-milliliter position for 20 seconds and note the peak pressure [1].

2.20.1. SCREEN: Screen_2.20.1..mov 00:00-00:20 .

2.21. Tap the syringe gently to dislodge flies adhering to the plunger and allow them to settle at the bottom [1-TXT].

2.21.1. Talent tapping the syringe lightly.

TXT: Start automatic decompression after 20 s Videographer's NOTE: Shot 2.21 may need the trimming, author wanted the first few seconds to be clipped out.

2.22. End the experiment once the plunger returns to the 20-milliliter mark and record the final pressure for leak control [1].

2.22.1. SCREEN: Screen_2.22.1..mov

3. Quantification of *Drosophila melanogaster* Emergence from Xenon Anesthesia Using Pixel-Based Analysis

3.1. Launch ScreenFlow to edit the videos [1]. Crop each recorded video to the region of interest corresponding to the first 5 milliliters of the syringe barrel [2].

3.1.1. SCREEN: Screen_3.1.1..mov 00:03-end .

3.1.2. SCREEN: Screen_3.1.2..mov 00:05-00:11, 00:25-00:30

3.2. Trim each video to the first 20 seconds following syringe decompression [1]. Export the videos as GIFs and duplicate them [2].

- 3.2.1. SCREEN: Screen_3.2.1..mov 00:22-00:40.
- 3.2.2. SCREEN: Screen_3.2.2..mov 00:10-00:34
- 3.3. Now, launch the Fiji software to remove the first and last frames from different duplicates [1]. Sequentially click on **Image, Video Editing, and Delete Frames [2]**.
 - 3.3.1. SCREEN: Screen_3.3.1..mov 00:04-00:19
 - 3.3.2. SCREEN: Screen_3.3.2..mov 00:13-end
- 3.4. Click on **Process, Binary, and Make Binary** to each GIF file to binary so flies appear white on a black background [1].
 - 3.4.1. SCREEN: Screen_3.4.1..mov 00:15-00:32
- 3.5. Open both binary files in Fiji [1]. Calculate the pixel difference between them by selecting **Process and Image Calculator** to generate a pixel difference file [2].
 - 3.5.1. SCREEN: Screen_3.5.1..mov 00:00-00:05, 00:16-end
 - 3.5.2. SCREEN: Screen_3.5.2..mov 00:02-00:18, 00:25-00:30
- 3.6. After saving the pixel difference file select **Analyze and Analyze Particles**, adjusting pixel size thresholds to improve signal-to-noise ratio [1].
 - 3.6.1. SCREEN: Screen_3.6.1..mov 00:11-end, AND
 - SCREEN: Screen_3.6.2..mov 00:02-00:10, 00:17-00:22
- 3.7. Save the generated CSV file containing pixel differences per frame [1]. Use the data to calculate cumulative movement across frames for statistical comparison [2].
 - 3.7.1. SCREEN: Screen_3.7.1..mov 00:11-00:19 .
 - 3.7.2. SCREEN: Screen_3.7.2..mov 00:00-End 3.

Results

4. Results

- 4.1. The experimental videos were processed by converting them into binary and difference images to prepare for pixel analysis [1].
 - 4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the Binary and Difference images sequentially*
- 4.2. Without thresholding, pixel differences accumulated over frames and elevated the baseline signal to approximately 20,000 [1].
 - 4.2.1. LAB MEDIA: Figure 3 (right panel). *Video editor: Highlight the curve showing the gradual increase in cumulative pixel difference from frame 0 to frame 600.*
- 4.3. Applying a pixel size threshold from 100 to 1000 reduced background signal and emphasized differences caused by fly movement [1]. Pixel difference values per frame remained close to zero before frame 200 [2].
 - 4.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight each curve from 0 to 1000*
 - 4.3.2. LAB MEDIA: Figure 5 (left panel). *Video editor: Highlight the flat region of the graph (right) from frame 0 to frame 200.*
- 4.4. The YOLOv11n model tracked flies with high per-frame accuracy, but frequent occlusions led to excessive fly ID assignments [1]. Due to tracking inconsistencies, fly movement was quantified by summing all ID movements per experiment and normalizing by a maximum of 200 IDs [2].
 - 4.4.1. LAB MEDIA: Figure 6.
 - 4.4.2. LAB MEDIA: Figure 7 *Video Editor: Please highlight C*
- 4.5. Flies exposed to 85% xenon recovered faster and moved more [1] than those exposed to 90% [2] or 95% xenon [3].
 - 4.5.1. LAB MEDIA: Figure 8. *Video editor: Highlight the red curve (85%)*
 - 4.5.2. LAB MEDIA: Figure 8. *Video editor: Highlight the green curve (90%)*
 - 4.5.3. LAB MEDIA: Figure 8. *Video editor: Highlight the blue curve (95%)*

1. Drosophila

Pronunciation link: <https://www.merriam-webster.com/dictionary/drosophila>

- IPA: /drə'sɑ:fələ/
Phonetic Spelling: ˈdrə·sɑ·fə·lə
2. melanogaster
Pronunciation link: <https://www.howtopronounce.com/melanogaster>
IPA: /ˌmɛlənoʊ'gæstər/
Phonetic Spelling: mɛ·lə·noh·gas·ter
3. anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /ə'nesθə'taɪzd/
Phonetic Spelling: ə·nes·thuh·tyzd
4. hyperbaric
Pronunciation link: <https://www.merriam-webster.com/dictionary/hyperbaric>
IPA: /ˌhaɪpər'bærɪk/
Phonetic Spelling: hy·per·bar·ɪk
5. polypropylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polypropylene>
IPA: /ˌpɑːli'proʊpəˌliːn/
Phonetic Spelling: pɑ·lee·proh·puh·leen
6. polycarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/polycarbonate>
IPA: /ˌpɑːli'kɑːrbəˌneɪt/
Phonetic Spelling: pɑ·lee·kar·buh·nayt
7. manometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/manometer>
IPA: /mə'nɑːmətər/
Phonetic Spelling: mʌ·nom·uh·ter
8. kilopascal
Pronunciation link: <https://www.merriam-webster.com/dictionary/kilopascal>
IPA: /'kɪləˌpæskəl/
Phonetic Spelling: kɪl·uh·pas·kuhl
9. decompression
Pronunciation link: <https://www.merriam-webster.com/dictionary/decompression>
IPA: /ˌdi:kəm'prɛʃən/
Phonetic Spelling: dee·kuhm·presh·uhn
10. aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æspəˌreɪt/
Phonetic Spelling: əs·puh·rayt
11. luer
Pronunciation link: <https://www.howtopronounce.com/luer>
IPA: /'luːər/
Phonetic Spelling: loo·er
12. quantification
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>

- IPA: /ˌkwɑːntəfəˈkeɪʃən/
Phonetic Spelling: kwon·tuh·fuh·kay·shuhn
13. xenon
Pronunciation link: <https://www.merriam-webster.com/dictionary/xenon>
IPA: /ˈziːnɑːn/
Phonetic Spelling: zee·non
14. pixel
Pronunciation link: <https://www.merriam-webster.com/dictionary/pixel>
IPA: /ˈpɪksəl/
Phonetic Spelling: pik·suhl
15. binary
Pronunciation link: <https://www.merriam-webster.com/dictionary/binary>
IPA: /ˈbɑːnəri/
Phonetic Spelling: bye·nuh·ree
16. threshold
Pronunciation link: <https://www.merriam-webster.com/dictionary/threshold>
IPA: /ˈθreʃˌhoʊld/
Phonetic Spelling: thresh·hohld
17. occlusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/occlusion>
IPA: /əˈkluːʒən/
Phonetic Spelling: uh·kloo·zhuhn
18. statistical
Pronunciation link: <https://www.merriam-webster.com/dictionary/statistical>
IPA: /stəˈtɪstɪkəl/
Phonetic Spelling: stuh·tis·tuh·kuhl
19. cumulative
Pronunciation link: <https://www.merriam-webster.com/dictionary/cumulative>
IPA: /ˈkjuːmjəˌleɪtɪv/
Phonetic Spelling: kyoo·myuh·lay·tiv
20. baseline
Pronunciation link: <https://www.merriam-webster.com/dictionary/baseline>
IPA: /ˈbersˌlaɪn/
Phonetic Spelling: bays·line
21. occlusions
Pronunciation link: <https://www.merriam-webster.com/dictionary/occlusion>
IPA: /əˈkluːʒənz/
Phonetic Spelling: uh·kloo·zhuhnz
22. normalization
Pronunciation link: <https://www.merriam-webster.com/dictionary/normalization>
IPA: /ˌnɔːrmələˈzeɪʃən/
Phonetic Spelling: nor·muh·luh·zay·shuhn
23. occluded
Pronunciation link: <https://www.merriam-webster.com/dictionary/occlude>

IPA: /ə'kluːdɪd/

Phonetic Spelling: uh·klood·id

24. fluorescence

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>

IPA: /flʊ'reɪsəns/

Phonetic Spelling: floo·res·uhns