Submission ID #: 61549
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18772493

Title: Planarian Scrunching as a Quantitative Behavioral Readout for Noxious Stimuli Sensing

Authors and Affiliations:

Ziad Sabry[1], Christina Rabeler[1], Danielle Ireland[1], Kevin Bayingana[1], Eva-Maria S. Collins[1,2]

[1]Biology Department, Swarthmore College, Swarthmore, PA, USA
[2]Physics Department, UC San Diego, La Jolla, CA, USA

Corresponding Authors:

Eva-Maria S. Collins (ecollin3@swarthmore.edu)

Email Addresses for All Authors:

zsabry1@swarthmore.edu
kbaying1@swarthmore.edu
dhagstr1@swarthmore.edu
crabele1@swarthmore.edu
ecollin3@swarthmore.edu

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

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

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

Current Protocol Length

Number of Steps: 21
Number of Shots: 33

Videographer: All SCREEN shots have been provided by the authors, no need to film them.

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ziad Sabry:** This protocol allows researchers to quantify planarian behavior and dissect the mechanisms underlying this behavior by combining quantitative behavioral analysis with molecular and chemical perturbations.

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

1.2. **Ziad Sabry:** This technique is easily accessible to all skill levels as it does not require advanced instruments or specialized software to get quantitative behavioral readouts of free-moving planarians.

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

OPTIONAL:

1.3. **Kevin Bayingana:** "Scrunching" can be used to study nociception in planarians and serves as a sensitive endpoint to assay disrupted nervous system function caused by xenobiotics and disease.

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

Introduction of Demonstrator on Camera

1.4. **Ziad Sabry:** Demonstrating the procedure will be Christina Rabeler, the lab manager from my laboratory.

1.1.1. INTERVIEW: Author saying the above.

1.1.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Quantitative Planarian Behavior Assays

2.1. Begin by placing a dimmable LED panel upon a flat surface, which will provide a uniform white background and can be used as an adjustable light source to obtain appropriate contrast **[1]**. Place a 100-millimeter Petri dish arena on the LED panel **[2]**.

2.1.1. WIDE: Establishing shot of talent positioning the LED panel.

2.1.2. Talent placing the Petri dish on the panel. Videographer NOTE: take 1 - we realized several shots after the take that the petri dish had water in it, so we reshot this at the very end using a petri dish without water in it. (The shots with the empty petri dish are Files 6H0A4280 and 6H0A4281)

2.2. Mount a camera on a ring stand above the arena **[1]** and adjust its position, height, and focus as necessary so that the entire arena is centered within the field of view and is in focus **[2]**. *Videographer: This step is important!*

2.2.1. Talent mounting the camera.

2.2.2. Talent adjusting the camera position and focus. *Videographer: If possible, show the field of view of the camera.*

2.3. Fill the arena with approximately 25 milliliters of the appropriate exposure media to half-maximum volume **[1]**. Turn on the LED panel and turn off any other light sources that may negatively affect recording quality **[2]**.

2.3.1. Talent filling the arena.

2.3.2. Talent turning on the LED panel and turning off other light sources.

2.4. Drop a planarian at the center of the arena using a transfer pipette. When testing a chemical solution, transfer the planarian with as little of planarian water as possible so that the concentration of the chemical is not significantly changed **[1]**. Begin recording data as image sequences in a native Fiji format **[2-TXT]**. *Videographer: This step is important!*

2.4.1. Talent dropping the planarian.

2.4.2. Talent starting recording. **TEXT: TIFF, GIF, JPEG, PNG, DICOM, BMP, PGM, or FITS**

2.5. For gliding experiments, record 1 to 2 minutes of gliding behavior. For scrunching or peristalsis experiments, record long enough to capture at least 3 consecutive oscillations occurring in a straight line. Once the experiment is completed, terminate the recording **[1]**. *Videographer: This step is important!*

2.5.1. Talent recording planarian, then terminating the recording at the appropriate time point.

2.6. If the planarian reaches the boundary of the arena without satisfying the termination criterion, pipette the planarian back to the center of the arena **[1]**. *Videographer: This step is important!*

2.6.1. Planarian reaching the boundary of the arena and talent pipetting it back to the center.

3. Data Analysis

3.1. Open the raw image sequence for an experiment in Fiji. Convert it to 8-bit and use the arrow tool or slider at the bottom of the image stack to pan through the image sequence **[1]**.

3.1.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 0:00 – 2:06. *Video Editor: Skip or speed through the import.*

3.2. To extract a time period and region of interest, draw a region of interest encompassing the full path of a planarian using the rectangle tool. Right click on the image stack and select **Duplicate**, check the box for **Duplicate stack**, enter the first and last frames of the sequence of interest, and click **OK [1]**.

3.2.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 2:07 – 2:40. *Video Editor: Speed up as necessary.*

3.3. For gliding experiments, extract a period of gliding where the planarian moves at least twice its body length. For scrunching or peristalsis experiments, extract an instance when the planarian undergoes a minimum of three consecutive and complete body oscillations in a straight line **[1]**.

3.3.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 2:40 – 3:13.

3.4. Apply a threshold to the duplicated image stack to binarize the image and extract the planarian from the background. Adjust the sliding bars so that the entire planarian is highlighted in red. The exact values are dependent on imaging quality **[1]**.

3.4.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 3:14 – 3:25.

3.5. Leave the boxes for **Dark background**, **Stack histogram**, and **Don't reset range** unchecked. Scroll through the image stack to ensure a good threshold range, and then click **Apply [1]**.

3.5.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 3:25 – 3:34. *Video Editor: Emphasize the boxes in the Threshold window.*

3.6. In the **Convert Stack to Binary** window, set the Method to **Default** and the Background to **Light**. Uncheck all boxes in this window and then click **OK**. A binarized image showing a black planarian on a white background will appear. Make sure that the entire planarian is visible in all frames of the image sequence **[1]**.

3.6.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 3:35 – 3:44.

3.7. Set measurements by clicking **Analyze** and **Set Measurements**. Check the boxes for **Area**, **Center of mass**, **Stack position**, and **Fit ellipse**, then click **OK [1]**. Select the open image stack and select **Analyze** and **Analyze Particles [2]**.

3.7.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 3:45 – 3:59.

3.7.2. SCREEN: 61549_12.2-1.2.10_t1.mp4. 4:00 – 4:05.

3.8. In the **Analyze Particles** window, select **Show** and **Masks** to open a new stack showing all the objects that were detected with the chosen parameters **[1]**. Set a size filter to remove unwanted noise by entering the approximate area of the planarian, then check the boxes for **Display results** and **Clear results** and click **OK [2]**.

3.8.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 4:06 – 4:29.

3.8.2. SCREEN: 61549_12.2-1.2.10_t1.mp4. 4:30 – 4:41.

3.9. Pan through the mask image stack using the slider at the bottom of the panel to make sure that there is no noise or frames without a planarian **[1]**.

3.9.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 4:42 – 4:49.

3.10. On the **Results** window, save the data using **File** and **Save As**. Add the .csv extension to the filename to save data as comma-separated values. Once the data for the image stack is saved, close the respective image stack, **Results**, and **Mask** windows **[1]**.

3.10.1. SCREEN: 61549_12.2-1.2.10_t1.mp4. 4:50 – 5:19.

4. Scrunching Induction

4.1. To induce scrunching via noxious temperature, heat planarian water in a glass beaker to 65 degrees Celsius on a hot plate **[1]**. Place a planarian in the center of the arena and wait until it orients itself upright and begins gliding **[2]**, then begin recording **[3]**.

4.1.1. Water heating on a hot plate.

4.1.2. Talent placing the planarian in water and the planarian orienting itself. Videographer NOTE: take 1 was probably the least useful take of the multiple takes so should use any of the three other takes *Videographer: Obtain multiple usable takes, this will be reused in 4.4.1.*

4.1.3. Talent starting the recording. *Videographer: Obtain multiple usable takes, this will be reused in 4.4.2.*

4.2. Use a P-200 pipette to slowly and consistently pipette 100 microliters of the pre-heated planarian water post-pharyngeally onto the tail end of the planarian to induce scrunching **[1]**. Stop the recording once scrunching has ceased **[2]**. *Videographer: This step is difficult and important!*

4.2.1. Talent pipetting the heated water on the planarian tail and the planarian scrunching.

4.2.2. Scrunching ceasing. NOTE: shot together with 4.2.1. Videographer NOTE: Planarian stopped scrunching towards the end.

4.3. Place the planarian in a recovery container **[1]** and exchange the media in the petri dish with fresh, room temperature planarian water if running more experiments **[2]**.

4.3.1. Talent placing the planarian in the recovery container.

4.3.2. Talent exchanging the bath water.

4.4. To induce scrunching via amputation, transfer a planarian to the center of the arena and wait until the planarian orients itself upright and begins gliding **[1]**, then begin recording **[2]**. Amputate the planarian with a clean razor blade, making sure that the cut location is consistent across experiments **[3]**. *Videographer: This step is important!*

4.4.1. *Use 4.1.2.*

4.4.2. *Use 4.1.3.*

4.4.3. Talent amputating the planarian. NOTE: Use 2nd take

4.5. Stop the recording once the anterior piece has ceased scrunching **[1]**. Remove both pieces, place them in a separate container, and allow them to regenerate for 7 days. Amputated planarians can be reincorporated into the home container once they have regenerated **[2]**.

4.5.1. Anterior piece scrunching ceasing. NOTE: this was recorded as part of a single take with shot 4.4.3

4.5.2. Talent transferring pieces in a separate container.

Results

5. Results: Species Specific Responses to Near UV Light and Cinnamaldehyde Exposure

5.1. This protocol was used to test whether near-UV light exposure induces scrunching in *S. mediterranea* and *D. japonica* planarians **[1]**. While *D. japonica* planarians scrunch when exposed to near-UV light **[2]**, *S. mediterranea* planarians either exhibit tail thinning or no response **[3]**.

5.1.1. LAB MEDIA: Figure 4.

5.1.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the D. japonica images in A and data in B.*

5.1.3. LAB MEDIA: Figure 4. *Video Editor: Emphasize the S. mediterranea images in A and data in B.*

5.2. A quantification of the scrunching parameters for the *D. japonica* planarians that exhibited at least 3 consecutive straight-line scrunches reveals characteristic scrunching parameters for this species **[1]**.

5.2.1. LAB MEDIA: Figure 4 B. *Video Editor: Show the following text somewhere on the screen.* **TEXT: ν_m = 0.84 ± 0.14, $|\Delta\varepsilon|_{max}$ = 0.56 ± 0.06, ν_m^* = 0.47 ± 0.07, and f_{elong} = 0.56 ± 0.03, values reported as mean ± standard deviation for N=7**

5.3. In contrast, exposure to 250 micromolar cinnamaldehyde, a known TRPA1 agonist in mice, causes scrunching in *S. mediterranea* **[1]**, whereas *D. japonica* planarians display a mixture of snake-like and oscillatory motion, interrupted by gliding or vigorous head turns **[2]**.

5.3.1. LAB MEDIA: Figure 5 A. *Video Editor: Emphasize the S. mediterranea data.*

5.3.2. LAB MEDIA: Figure 5 A. *Video Editor: Emphasize the D. japonica data.*

5.4. A quantification of the samples with at least three consecutive oscillations yielded significantly lower values for 3 out of 4 parameters than expected for scrunching in *D. japonica*, indicating that the observed oscillatory motion is not scrunching **[1-TXT]**.

5.4.1. LAB MEDIA: Figure 5 A. *Video Editor: Show the following text somewhere on the screen.* **TXT: ν_m = 0.43 ± 0.08, $|\Delta\varepsilon|_{max}$ = 0.39 ± 0.03, ν_m^* = 0.17 ± 0.02, and f_{elong} = 0.54 ± 0.06, values reported as mean ± standard deviation for N=8**

5.5. RNAi confirms the specificity of scrunching in response to cinnamaldehyde exposure in *S. mediterranea* **[1]**. Within 180 seconds of exposure in planarian water all control RNAi planarians scrunched **[2]**, compared to none of the *SmTRPA1 (spell out 'S-M-T-R-P-A-one')* RNAi planarians, demonstrating that *S. mediterranea* scrunching in cinnamaldehyde requires *SmTRPA1* **[3]**.

5.5.1. LAB MEDIA: Figure 5 B.

5.5.2. LAB MEDIA: Figure 5 B. *Video Editor: Emphasize the control data (black).*

5.5.3. LAB MEDIA: Figure 5 B. *Video Editor: Emphasize the SmTRPA1 RNAi data (blue).*

Conclusion

6. Conclusion Interview Statements

6.1. **Ziad Sabry:** When attempting this protocol, it is essential to be consistent in how the animals are manipulated to reduce noise and ensure reproducibility in the behavioral measurements.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1.*

6.2. **Kevin Bayingana:** This protocol can be expanded to include body shape analysis, which would allow for identification and quantification of other planarian behaviors that are not captured here.

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