Submission ID #: 61070
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18626398

Title: Planarian Motility Assay to Gauge the Biomodulating Properties of Natural Products

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Y**

If **Yes**, can you record movies/images using your own microscope camera?

N

If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope:
A custom stereomicroscope will be available. It has a camera port

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Evelyn B. Voura**: The planarian locomotor velocity test is a means for quickly assessing stimulant and possible withdrawal effects for a variety of biological modifying agents that might affect motility **[1]**.

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

REQUIRED:

1.2. **Elisa J. Livengood**: This assay is easily controlled and provides a straightforward and accessible method for generating reliable behavioral data in even the most basic research settings **[1]**.

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

OPTIONAL:

1.3. **Evelyn B. Voura**: Since the planarian nervous system has a centralized rudimentary brain, a bilateral symmetry, and analogous neurotransmitter systems, behavioral studies using these organisms offer pre-clinical insight into possible vertebrate responses **[1]**.

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

OPTIONAL:

1.4. **Angela M. Pratt**: Experiments can be further affected by physiological pathway modifiers, drugs, or even genetically modified planarians to provide insight into the associated biological mechanisms **[1]**.

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

OPTIONAL:

1.5. **Robyn A.M.V. Fong**: It is important to handle the planarians so that the transfers between containers do not stress them and to standardize the gridline counts between lab members **[1]**.

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

OPTIONAL:

1.6. **Carlos H. Pulquerio**: The best way to obtain consistent results from these behavioral assay experiments is by seeing how experienced users perform them **[1]**.

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

Protocol

2. Planarian Husbandry

2.1. After setting up a planarian culture, feed the worms ad libitum on a regular, twice-a-week schedule of chopped organic boiled eggs or blended organic beef liver for 1-2 hours **[1-TXT]**.

2.1.1. WIDE: Talent adding egg to culture **TEXT: See text for full planarian culture setup details**

2.2. After feeding, remove any soiled water from the container **[1]** and use a flat watercolor paintbrush to transfer any food debris and slime adhering to the container to a paper towel **[2]**.

2.2.1. Water being removed

2.2.2. Food and/or slime being wiped with brush, with paper towel visible in frame

2.3. Use fresh spring water and gentle swirling or agitation to dislodge the planarians **[1]** and decant the worms into a clean container **[2]**.

2.3.1. Plate being swirled/planarians being dislodged

2.3.2. Planarians being added to container

2.4. Slide a wide bore pipette tip under any planarians remaining adhered to the old container **[1-TXT]** to facilitate their transfer into the new dish **[2]** and carefully decant the transfer water **[3]**.

2.4.1. Tip being slide under planarian **TEXT: Optional: Transfer planarians with round, no. 3-6 watercolor paintbrush**

2.4.2. Planarian being set into new dish

2.4.3. Water being decanted

2.5. Then cover the planarians in the new container with fresh spring water **[1]**.

2.5.1. Water being added to dish

3. Planarian locomotor velocity (pLmV) Test

3.1. To perform a planarian locomotor velocity test, first ensure that the 5-10-day starved planarians are fully formed **[1]**, with complete and pigmented heads and tails **[2]**.

3.1.1. WIDE: Talent at microscope, looking at planarians

3.1.2. SCOPE: Shot of planarian with pigmented head and tail *Videographer: Important step*

3.2. If the planarians are ready, place one clean 10-centimeter diameter Petri dish onto one piece of prelaminated grid paper with 0.5-centimeter squares per experimental group **[1]** and add 20 milliliters of unadulterated spring water for the control group **[2]** or 20 milliliters of spring water containing the appropriate concentration of the natural product being tested to each dish **[3]**.

3.2.1. Talent placing dish onto grid

3.2.2. Talent adding spring water onto dish

3.2.3. Talent adding water + compound, with compound container visible in frame

3.3. Position a camera above the prepared locomotor velocity stimulation dish **[1]** and add 5-10 milliliters of unadulterated spring water **[2]** or spring water containing the appropriate concentration of the experimental compound to an appropriately sized habituation container **[3]**.

3.3.1. Talent placing camera above planarian locomotor velocity dish *Videographer: Important step*

3.3.2. Talent adding water to vial or 5-cm dish

3.3.3. Talent adding water + compound to vial or dish, with compound container visible in frame

3.4. Next, use a small, clean watercolor paintbrush to gently transfer one planarian from the culture container to the prepared habituation container **[1-TXT]** and allow the planarian to habituate to the solution in the container for 2 minutes **[2]**.

3.4.1. Planarian lifted with paintbrush *Videographer: Important/difficult step* **TEXT: Optional: Transfer with wide bore pipette; See text for details**

3.4.2. Talent setting timer, with habituation container visible in frame

3.5. **Carlos H. Pulquerio**: Be sure to allow the planarians to move onto the brush and to carefully lift the animals without forcing them **[1]**.

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

3.6. At the end of the habituation period, use the brush to carefully transfer the planarian to the center of the prepared locomotor velocity stimulation dish containing the same solution as the habituation container **[1]** and start the camera to record 10-11 minutes of movement of the planarian **[2]**.

3.6.1. Planarian being placed into dish, with compound container visible in frame

3.6.2. Talent starting camera

3.7. To test the effects of stimulation withdrawal, at the end of the habituation period, rinse the planarian in a 5-centimeter dish containing spring water only **[1]** before transferring the worm to the center of the prepared locomotor velocity dish containing fresh spring water **[2]** and start the camera to record 10-11 minutes of movement **[3]**.

3.7.1. Talent adding planarian to the 5-centimeter dish, with spring water container visible in frame *Videographer: Important step*

3.7.2. Talent placing planarian 10-centimeter dish, with spring water container visible in frame

3.7.3. SCOPE: Shot of planarian activity in dish

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

3.1.2., 3.3.1., 3.4.1., 3.7.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.4.1 (as with 3.6.1 and 3.7.1; having the planarian move onto the brush)

Results

4. Results: Representative Planarian Biomodulating Property Assessment

4.1. The camera setup allows a clear view of the planarian and the grid paper **[1]** and permits an accurate assessment of the progress of the animal for the duration of the experiment **[2]**.

4.1.1. LAB MEDIA: Figure 3A
4.1.2. LAB MEDIA: Figure 3B

4.2. The data collection should include the total number of grid lines crossed **[1]** as well as the cumulative total number of lines crossed per minute of the experiment **[2]**.

4.2.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize Grid Count*
4.2.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize Accumulated Totals row*

4.3. To standardize the results from each trial, the planarian locomotor velocity runs can be calculated and plotted as the number of boxes crossed relative to the progress of the matching control worm **[1]**.

4.3.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize Relative to Control*

4.4. As a benchmark, planarians in spring water typically cover approximately 24 boxes in 3 minutes **[1]**.

4.4.1. LAB MEDIA: Figure 4A *Video Editor: please emphasize 3 min data bar*

4.5. A range of test reagent concentrations can be examined to determine planarian motility in response to the test compounds of interest **[1]**.

4.5.1. LAB MEDIA: Figure 4B Video Editor: please sequentially emphasize data bars from 0.001-10

4.6. Withdrawal data can be assessed by quantifying the number of grid lines crossed relative to the spring water control as the planarians move through the planarian locomotor velocity container **[1]**.

4.6.1. LAB MEDIA: Figure 4D *Video Editor: please emphasize blue data line in graphs*

4.7. Spring water control planarians move over grid lines for the duration of the

experiment **[1]**.

4.7.1. LAB MEDIA: Figure 5A

4.8. At times, however, the test planarians cease crossing grid lines during the assay **[1]**.

4.8.1. LAB MEDIA: Figure 5B

4.9. The data can be illustrated as the percentage of the total number of animals exposed to a particular reagent concentration **[1]**.

4.9.1. LAB MEDIA: Figure 5C *Video Editor: please sequentially add/emphasize Control and 0.03 mM circle graphs*

Conclusion

5. Conclusion Interview Statements

5.1. **Angela M. Pratt**: It is essential to handle the planarians so that they are not damaged during the transfer between dishes **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.7.1)

5.2. **Robyn A.M.V. Fong**: After determining the effects of different concentrations of a natural product, biological pathway modifiers can be added to observe any dynamic changes to the initial results **[1]**.

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

5.3. **Elisa J. Livengood**: The planarian locomotor velocity test was first used to test for stimulation and withdrawal using known pharmacological agents. We adapted the system to study these same concepts with natural products **[1]**.

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