Submission ID #: 61323
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18703563

Title: Quantification of Macronutrients Intake in a Thermogenetic Neuronal Screen Using Drosophila Larvae

Authors and Affiliations:

Gonçalo M. Poças[1,2], Pedro M. Domingos[2], Christen K. Mirth[1]

[1]School of Biological Sciences, Monash University, Melbourne, Victoria, Australia
[2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Lisbon, Portugal

Corresponding Authors:

Gonçalo M. Poças (goncampocas@itqb.unl.pt / goncalo.pocas@monash.edu)

Email Addresses for All Authors:

christen.mirth@monash.edu
domingp@itqb.unl.pt
goncampocas@itqb.unl.pt
goncalo.pocas@monash.edu

Author Questionnaire

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

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

   No

   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.

   Nikon SMZ-745 stereo microscope

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? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Gonçalo M. Poças:** Our protocol enables the identification of larval neuronal populations encoding nutritional choices related to the control of the intake levels of two major macronutrients: proteins and carbohydrates.

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

1.2. **Gonçalo M. Poças:** The simplicity and relatively high throughput of our method allows the quantification of food intake for several genotypes exposed to different nutritional conditions.

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

Protocol

2. Preparation of Third-instar Larvae (L3)

2.1. To genetically cross the parental lines, set up 60-millimeter embryo collection cages with L3 rearing diet plates, supplemented with some active yeast paste **[1]**.

2.1.1. WIDE: Establishing shot of talent setting plates down on a lab bench.

2.2. Transfer the adult Trip A1 female virgins and Janelia Gal4 males, aged 5 to 8 days, to the embryo collection cages **[1-TXT]** and allow the mating to occur for 24 to 48 hours at 25 degrees Celsius with 60% humidity and a 12-12 light-dark cycle **[2]**.

2.2.1. Talent transferring flies to the embryo collection cages. **TEXT: 100 females and 30 males per cage**

2.2.2. Talent putting the embryo collection cages in the incubator and closing the door.

2.3. After the mating period, transfer the mated adult flies to fresh L3 rearing diet plates and allow the egg-laying to occur for 3 to 4 hours at 25 degrees Celsius **[1]**. Make sure that all plates are labeled with the genotype and date of the egg lay **[2]**.

2.3.1. Talent transferring the flies to fresh plates.

2.3.2. Properly labeled plate.

2.4. When the egg laying period is finished, remove the plates from the cages **[1]** and estimate the number of eggs per plate by counting the number of embryos in one quarter of the plate. Keep the larval density to a maximum of 200 embryos per plate **[2]** and cover the plates with plastic lids **[3]**.

2.4.1. Talent removing plates from the cages.

2.4.2. Talent counting embryos and covering the plates.

2.5. Incubate the L3 rearing plates at 18 degrees Celsius, 60% humidity, and a 12-12 light-dark cycle for 9 days **[1]**.

2.5.1. Talent putting the plates in the incubator and closing the door.

2.6. After the incubation, collect 3 groups of 10 L3 from each of the genotypes to be tested as well as one group of 10 L3 for the “zero-dye food” control. Use number 5 or featherweight forceps, performing the larvae collection as gently as possible **[1]**. Transfer the collected larvae to plastic dish weight boats containing 1 milliliter of water **[2]**. *Videographer: This step is important!*

2.6.1. SCOPE: Talent collecting larvae.

2.6.2. Talent placing the larvae into the plastic dish weight.

3. Thermogenetic Activation and No-choice Feeding Assay

3.1. Set up a 30-degree Celsius incubator and keep humidity levels high to avoid larval dehydration during the assay **[1]**. Equilibrate the temperature of the assay plates by warming them to 30 degrees Celsius prior to starting **[2]**. Make sure that all plates are labeled with the genotype and protein to carbohydrate ratio of the diet **[3]**.

3.1.1. Talent setting the temperature of the incubator.

3.1.2. Talent putting a tray with water (to keep humidity levels high) and the plates in the incubator.

3.1.3. Properly labeled plates

3.2. Heat-shock the experimental larvae for 2 minutes **[1]** in a 37-degree Celsius water bath, keeping the animals in the plastic weight boats with water **[2]**. Set the required number of timers for 1 hour **[2]**, then carefully drain the water from the plastic boats **[3]** and transfer the L3 groups from the boats to the center of the assay plates **[4]**. *Videographer: This step is difficult and important!*

3.2.1. Talent setting a timer for 2 minutes.

3.2.2. Added shot: Talent heat shocking the larvae NOTE: Might be slated differently

3.2.3. Talent setting a timer for 1 hour.

3.2.4. Talent draining water from a boat.

3.2.5. Talent transferring larvae to an assay plate.

3.3. Cover the plates and start the timer **[1]**. Allow the larvae to feed for 1 hour at 30 degrees Celsius in the dark **[2]**. Stop the feeding assay by transferring the plates to an ice bath **[3]**. *Videographer: This step is important!*

3.3.1. Talent covering a plate and starting a timer.

3.3.2. Talent putting the plate in the incubator.

3.3.3. Talent putting plates on an ice bath.

4. Food Dye Extraction and Colorimetric Quantification of Food Consumption

4.1. Prepare 2-milliliter microtubes for each L3 group tested, containing enough 0.5-millimeter glass beads **[1]** to fill the bottom portion of the microtube and 300 microliters of ice-cold methanol **[1-TXT]**. Use a bench cooler to keep the microtubes cold **[2]**.

4.1.1. Added shot: Transfer the beads and add the methanol to one 2-milliliter microtube NOTE: Might be slated differently

4.1.2. Prepared tubes in a tube rack, with the glass beads visible. **TEXT: Caution! Methanol is highly flammable and toxic**

4.1.3. Talent putting the tubes in a bench cooler.

4.2. Carefully recover the L3 groups from the feeding assay plates and transfer them to the lids of the plates with some water **[1]**. Rinse the larvae to remove food debris from their bodies, taking care to avoid any injuries **[2]**. *Videographer: This step is important!*

4.2.1. SCOPE: Talent transferring larvae to the lid with water.

4.2.2. Talent rinsing larvae.

4.3. Transfer the larvae to the prepared microtubes **[1]** and lyse them with a tissue lyser to extract the food dye from the guts **[2]**. *Videographer: This step is important!*

4.3.1. Talent transferring larvae to the prepared tubes.

4.3.2. Talent lysing the larvae.

4.4. Transfer the extracts to clean 1.5-milliliter microtubes by directly inverting the 2-milliliter microtubes onto the new tubes **[1]**. Do so gently so that most of the glass beads stay at the bottom of the 2-milliliter tube **[2]**.

4.4.1. Talent transferring the extracts to a clean tube.

4.4.2. Old tube with the beads still inside.

4.5. Clear the cellular debris by centrifuging the extracts at a maximum speed and 4 degrees Celsius for 10 minutes **[1]**, then transfer the supernatants to clean 1.5-milliliter tubes **[2]**.

4.5.1. Talent putting tubes in the centrifuge and closing the lid.

4.5.2. Talent transferring the supernatant to a clean tube.

4.6. To perform colorimetric quantification of food consumption, prepare standard solutions of blue dye by serially diluting it 1 to 2 in methanol. **[1-TXT]**. Transfer 100 microliters of the experimental samples, standards, and blank to the wells of a 96-well microplate **[2]** and measure the absorbance at 600 nanometers **[3]**.

4.6.1. Talent making serial dilutions of the blue dye. **TEXT: Use methanol only as a blank**

4.6.2. Talent transferring the samples to the 96-well plate.

4.6.3. Talent using a plate reader.

Results

5. Results: Amount of Food Eaten per Larva under Neuronal Thermogenetic-activation Conditions in Three Macronutrient Balancing Diets

5.1. This protocol was used to quantify the relative amounts of macronutrients consumed for animals under thermogenetic activation of specific neuronal populations in the larval nervous system **[1]**.

5.1.1. LAB MEDIA: Figure 2.

5.2. Activating distinct populations of neurons significantly affected macronutrient balancing in third-instar larvae **[1]**. The feeding pattern observed for the control line showed an expected decrease of food intake in higher protein to carbohydrate ratio diets, demonstrating the effectiveness of the method **[2]**.

5.2.1. LAB MEDIA: Figure 4.

5.2.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the grey dots and line.*

5.3. The 5 phenotypic classes that were established for the experimental animals were **[1]** "Eat a lot and overcompensate for protein dilution" **[2]**, "Eat a lot but compensate normally" **[3]**, "Eat little but compensate" **[4]**, "Eat little and do not compensate" **[5]**, and "Eat aberrantly" **[6]**.

5.3.1. LAB MEDIA: Figure 5.

5.3.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize the section of the plot labeled 1.*

5.3.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize the section of the plot labeled 2.*

5.3.4. LAB MEDIA: Figure 5. *Video Editor: Emphasize the section of the plot labeled 3.*

5.3.5. LAB MEDIA: Figure 5. *Video Editor: Emphasize the section of the plot labeled 4.*

5.3.6. LAB MEDIA: Figure 5. *Video Editor: Emphasize the section of the plot labeled 5.*

5.4. For each of these phenotypic classes and genotypes, the GFP patterns in the central nervous systems of third-instar larvae are shown **[1]**.

5.4.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize the fluorescence images, maybe even enlarge them.*

Conclusion

6. Conclusion Interview Statements

6.1. **Gonçalo M. Poças:** When attempting this protocol, it is important to generate developmentally synchronized early third-instar larvae in order to minimize the variation associated to animal behavior.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3, 2.4.*

6.2. **Gonçalo M. Poças:** Following this protocol, radiolabeling of the food would allow the confirmation and further dissection of the hits found using this method.

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