Video Article

March 2012: This Month in JoVE

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Abstract

The March issue of the Journal of Visualized Experiments (JoVE) begins with an article that focuses on three fundamentally important assays for measuring *Drosophila* locomotor behavior. Specifically, these assays include the larval crawling assay (for larval motor function), the RING assay (for adult motor function), and the courtship assay (to assess coordination and sensory abilities).

The larval crawling assay involves collection of larva and exposure of the juvenile flies to an experimental drug of interest. Animals are then transferred to a petri dish laid atop a sheet of graph paper, and the number of gridlines crossed per minute can be calculated.

The Rapid Iterative Negative Geotaxis, or RING, protocol, involves placing adult flies in specialized vials where they are forced to the bottom by gentle tapping. Fruit flies instinctively will crawl upward, against gravity. Through image analysis, the extent of adult locomotor ability can be determined by measuring how far upward the flies can climb in a short period of time.

To assess a more complex sensory-guided behavior, the courtship assay is used and begins by placing sexually-naïve males and females into a specialized apparatus known as the mating wheel. During experimental sessions, investigators record the amount of time in which the male fly exhibits several characteristic courtship behaviors. Impressively, within a matter of minutes, the male fly will orient himself toward the female, tap her abdomen, generate a song with his wing, lick the female and ultimately copulate.

Taken together, these assays represent a series of powerful behavioral tests that can help elucidate the genetic and environmental factors that contribute to drosophila behavior.

Later in the month, JoVE shifts its focus to exercise physiology, where investigators from the University of Sau Paulo present a method designed to uncover the contribution of the various energy systems to complex exercise, like sports. As an example, our authors have chosen judo for their demonstration. Using a carefully positioned portable gas analyzer, oxygen consumption can be measured during judo exercise under controlled conditions. By comparing oxygen consumption before, during, and after exercise, the energy contribution of aerobic and alactic anaerobic metabolism can be determined. Blood collection before and after exercise, followed by electrochemical analysis is used to determine the energy contribution of lactic anaerobic metabolism to the sport of judo. When combined, oxygen consumption and plasma lactate concentration data, can be used to determine the relative contributions of the different energy systems to specific aspects of complex exercise, like one's favorite judo throw.

Also in March, JoVE plans the release of a developmental study involving alcohol exposure in zebrafish. Once embryos develop to the desired timepoint, the effects of alcohol exposure on gene expression can be assessed by purifying mRNA and performing qPCR and by whole mount in situ hybridization. Using light microscopy, morphological changes such as somite shape can also be assessed. If a gene of interest is observed to decrease or increase by ethanol exposure, either capped mRNA or morpholino can be injected into the embryo, somewhere around the two-cell stage, in order to rescue the alcohol-induced phenotype. Our authors show that alcohol exposure in the developing zebrafish results in similar morphological phenotypes and gene expression profiles to that observed in mammals, and they also demonstrate that mRNA injection can reverse the effects of alcohol on developmental abnormalities.

In Immunology and Infection, JoVE presents an experiment in plant parasitology, from UC Riverside. In particular, our authors are interested in the root knot nematode - an obligate plant parasite that moves freely through wet soil to infect the roots of plants, and feed. First, methods are described for processing infected plants and collection of root knot nematodes. Once juvenile worms develop to a stage with high infectivity, soil can be inoculated. Later, roots can be stained and assessed for the presence of the characteristic root knots generated by these parasites. In addition to experiments with potted plants, our authors show how root knot assays can be conducted with plants grown in clear pouches, allowing for easy viewing of roots, and facilitating high throughput screening for the presence of this devastating infection that accounts for 5% of global crop loss, yearly.

This summary of JoVE's content for March highlights four of over fifty articles scheduled for release in the month. Other notable experiments involve methods for imaging odor-evoked responses in the antenna of fruit flies, using an electronic nose to analyze the volatile compounds from fruit, and the repurposing of ordinary office equipment for the delivery of macromolecules into living cells.

Video Link

The video component of this article can be found at https://www.jove.com/video/4336/

Protocol

Calcium imaging of odor-evoked responses in the *Drosophila* antennal lobe

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We describe an established technique to measure and analyze odor-evoked calcium responses in the antennal lobe of living *Drosophila melanogaster*.

Determining the Contribution of the Energy Systems During Exercise

Guilherme G. Artioli[1], Rômulo Bertuzzi[2], Hamilton Roschel[1, 3], Sandro H. Mendes[1], Antonio H. Lancha[1], Emerson Franchini[4]
[1]Laboratory of Applied Nutrition, School of Physical Education and Sport, University of Sao Paulo, [2]Aerobic Performance Research Group, School of Physical Education and Sport, University of Sao Paulo, [3]Laboratory of Neuromuscular Adaptations to Strength Training, School of Physical Education and Sport, University of Sao Paulo, [4]Martial Arts and Combat Sports Research Group, School of Physical Education and Sport, University of Sao Paulo

This protocol allows researchers focused on exercise and sports sciences to determine the relative contribution of three different energy systems to the total energy expenditure during a large variety of exercises.

High and Low Throughput Screens with Root-knot Nematodes *Meloidogyne spp.*

Hagop S. Atamian, Philip A. Roberts, Isgouhi Kaloshian
Department of Nematology, University of California, Riverside

Two distinct methods to screen plants with root-knot nematodes are described. The described approaches include high-throughput screens with nematodes in a nondestructive manner facilitating the use of these plants in breeding programs.

Creating Transient Cell Membrane Pores Using a Standard Inkjet Printer

Alexander B. Owczarczak, Stephen O. Shuford, Scott T. Wood, Sandra Deitch, Delphine Dean
Department of Bioengineering, Clemson University

A description of the methods used to convert an HP DeskJet 500 printer into a bioprinter. The printer is capable of processing living cells, which causes transient pores in the membrane. These pores can be utilized to incorporate small molecules, including fluorescent G-actin, into the printed cells.

Assessing Teratogenic Changes in a Zebrafish Model of Fetal Alcohol Exposure

Evyn Loucks[1], Sara Ahlgren[1, 2]
[1]Program in Developmental Biology, Children's Memorial Research Center, [2]Department of Pediatrics, Northwestern University

In order to understand the molecular mechanisms of the ethanol-induced developmental damage, we have developed a zebrafish model of ethanol exposure and are exploring the physical, cellular, and genetic alterations that occur after ethanol exposure[1]. We then seek to find potential interventions and rapidly test them in this animal model.

Methods to assay *Drosophila* behavior

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Drosophila melanogaster is a genetically and behaviorally tractable model system that has been used to understand the molecular and cellular basis of many important biological processes for over a century[1]. Drosophila has been well exploited to gain insights into the genetic basis of fly behavior.

Fruit Volatile Analysis using an Electronic Nose

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A rapid method for volatile compound analysis in fruit is described. The volatile compounds present in the headspace of a homogenate of the sample are rapidly separated and detected with ultra-fast gas chromatography (GC) coupled with a surface acoustic wave (SAW) sensor. A procedure for data handling and analysis is also discussed.

Disclosures

No conflicts of interest declared.