Video Article

Acquisition of High-Quality Digital Video of *Drosophila* Larval and Adult Behaviors from a Lateral Perspective

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Abstract

Drosophila melanogaster is a powerful experimental model system for studying the function of the nervous system. Gene mutations that cause dysfunction of the nervous system often produce viable larvae and adults that have locomotion defective phenotypes that are difficult to adequately describe with text or completely represent with a single photographic image. Current modes of scientific publishing, however, support the submission of digital video media as supplemental material to accompany a manuscript. Here we describe a simple and widely accessible microscopy technique for acquiring high-quality digital video of both *Drosophila* larval and adult phenotypes from a lateral perspective. Video of larval and adult locomotion from a side-view is advantageous because it allows the observation and analysis of subtle distinctions and variations in aberrant locomotive behaviors. We have successfully used the technique to visualize and quantify aberrant crawling behaviors in third instar larvae, in addition to adult mutant phenotypes and behaviors including grooming.

Video Link

The video component of this article can be found at https://www.jove.com/video/51981/

Introduction

The common fruit fly *Drosophila melanogaster* is a powerful experimental model system for studying the function of the nervous system[1-3]. Evolutionary conservation of structure and function of the nervous system with humans, as well as ease of genetic manipulation and a vast array of genetic tools makes *Drosophila* the premiere organism to model human neurodegenerative diseases[4]. Gene mutations that cause dysfunction of the nervous system often result in viable mutant larvae and adult *Drosophila* with impaired locomotion. Phenotypes observed in nervous system defective mutants include reduced rate of locomotion, aberrant coordination, and spastic movements in adults, as well as deficits in peristaltic contraction of the body wall musculature, and partial paralysis of larvae. These phenotypes have been exploited in the development of high-throughput genetic screens and locomotion assays of mutant larvae[5,6] and adult[7-10] *Drosophila* aimed at quantifying the locomotion impairment and identifying genes necessary for function of the nervous system. While these approaches are extremely useful for quantifying larval and adult locomotive behaviors, they fail to convey qualitative information about each specific aberrant behavior. For example, while mutant third instar larvae may exhibit altered locomotion parameters in a behavioral assay, it may be unclear if this is the result of alterations in rhythmic peristaltic contractions during the crawling cycle, general lack of coordination, or partial paralysis of the posterior body wall musculature. Here we describe a simple and widely accessible microscopy technique for acquiring high-quality digital video of *Drosophila* adult and larval locomotive phenotypes from a lateral perspective. Digital video acquired from a lateral perspective allows the direct observation and analysis of subtle distinctions in locomotive behaviors from a more informative side-view orientation.

Protocol

1. The Stereo Microscope System

Note: Although this protocol is easily adaptable to virtually any stereo microscope system coupled to a digital camera with the capability of acquiring video, details are provided on the system used in our lab (Table of Materials/Equipment).

1. Acquire digital video using a trinocular stereo microscope coupled to a commercial digital camera.
2. In order to couple the commercial digital camera to the trinocular port of the stereo microscope, remove the ½x C-mount of the phototube port of the stereo microscope and replace it with a 1X C-mount.
3. Mount a digital camera coupler (43 mm thread) to the 1X C-mount.
4. Mount two step-down rings, 58 mm to 48 mm, and 48 mm to 43 mm, to the camera coupler to bridge the connection from the digital camera coupler to a lens adapter kit for the digital camera.
5. Mount the digital camera to the lens adapter kit.

6. Acquire video with the microscope magnification and optical zoom of the digital camera set for a combined magnification of approximately 12X (30 frames per sec, 640 x 480 pixels). Note: The magnification of the stereo microscope must be compensated in accordance with the newly reconfigured 1X C-mount of the trinocular port.

2. Imaging *Drosophila* Third Instar Larvae

1. Tape a permanent marker to the black stage plate of a stereo microscope coupled to a digital camera so that the side of the marker cap occupies approximately ⅓ to ¼ of the vertical field of view observed in the camera LCD monitor. Use marker tops as the stage to perform larval imaging because they come in an assortment of colors that can be used to color code and differentiate the genotypes of larvae being imaged.
2. Demarcate the field of view observed in the digital camera LCD monitor on the surface of the marker top with a fine point marker.
3. Select a third instar larva to image. The criteria for selecting third instar larvae was body length, emergence from the food source during the larval phase of the life cycle, the presence of anterior and posterior spiracles, and the structure of the mandibular hooks of the mouth apparatus[11]. Ensure the larva is clean by washing it thoroughly in water.
4. Illuminate the permanent marker top stage from above with light from a fiber optic lighting system. Adjust the angle of incident light to provide optimal illumination.
5. Focus the microscope on the edge of the permanent marker top. Begin acquiring digital video.
6. Place the larva on the side of the marker cap approximately 75° away from the vertical axis, just outside the field of view, with the anterior of the larva facing towards the field of view (**Figure 1**). Note: Placement of the larva on the side of the marker cap allows the camera to record movement of the larva from a lateral perspective. It helps to keep the larva moist with water so they don't fall off the side of the marker cap. Care must be exercised, however, to not use too much water as excessive amounts will adhere to the larva as it crawls across the field.
7. Gently poke and prod the larva with a small paintbrush to coerce it to crawl across the field of view. Be patient as the larvae rarely cooperate and often have to be returned to the starting point many times before they crawl straight across the field.
8. Record approximately 10-15 min of uninterrupted digital video footage and crop and remove all unnecessary footage post-acquisition with digital video editing software.

3. Imaging Adult *Drosophila*

1. Place a single adult *Drosophila* in a disposable 1.5 ml spectroscopic polystyrene cuvette.
   Note: CO_2 anaesthetization of adult *Drosophila* immediately before a behavioral analysis protocol can compromise results[12]. It is recommended that adult *Drosophila* be given a 24-hr period to recover from CO_2 anaesthetization before performing in a behavioral test[13].
2. Plug the end of the cuvette with a small cotton ball. Ensure the cotton ball is packed tight enough to occupy the large cap space and confines the fly to the reduced volume compartment of the cuvette.
3. Place the cuvette on the white stage plate of a stereo microscope and properly align the reduced volume compartment of the cuvette with the field of view observed in the digital camera LCD monitor.
4. Illuminate the cuvette from above with light from a fiber optic lighting system. Adjust the angle of incident light to provide optimal illumination.
5. Focus the microscope and begin acquiring digital video.
6. Record approximately 30-45 min of uninterrupted digital video footage and crop and remove all unnecessary footage post-acquisition with digital video editing software.

Representative Results

We have successfully used this technique to acquire and quantify the larval behavioral phenotype associated with loss of function of the *stathmin* gene (**Figure 2**)[14]. The *stathmin* gene encodes a microtubule regulatory protein that partitions tubulin dimers from pools of soluble tubulin, and binds microtubules and promotes their disassembly[15,16]. Stathmin function is required to maintain the integrity of microtubules in the axons of peripheral nerves[14]. Disruption of stathmin activity in *Drosophila* third instar larvae results in a phenotype in which the posterior body segments flip upward after each peristaltic wave of muscle contraction during the crawling cycle. This posterior paralysis or 'tail-flip' phenotype is a hallmark of defective axonal transport. We quantified the penetrance and severity of the posterior paralysis phenotype in third instar larvae of seven different *stathmin* mutant genotypes by measuring the angle above horizontal the tail was raised during the crawling cycle (**Table 1**). Larvae were determined to exhibit a *robust tail-flip* if the tail was raised greater than 40° above horizontal when crawling, a *mild tail-flip* if the tail was raised less than 40° above horizontal, and *no tail-flip* if the larvae exhibited a normal crawling behavior.

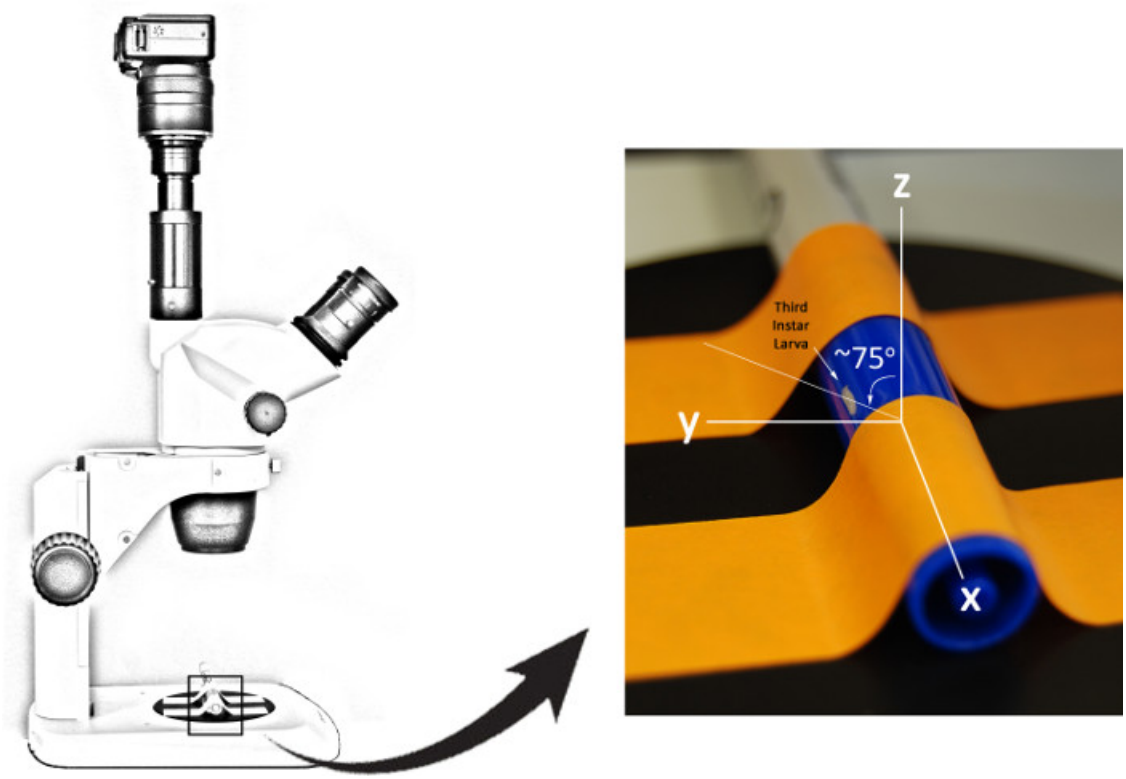

Figure 1. Position of third instar larva on a permanent marker cap stage for acquisition of digital video from a lateral perspective using a stereo microscope. Side-view of a basic stereo microscope system with digital camera mounted at the trinocular port. The inset magnification shows the orientation of a permanent marker taped to the microscope stage and the position of the third instar larva on the marker cap for acquisition of digital video of aberrant behavior from a lateral perspective. In the image the three-dimensions of space are defined; the x-axis runs the length of the permanent marker and is parallel to the microscope stage, the y-axis is perpendicular to the x-axis and parallel to the microscope stage, and the z-axis is vertical from the marker cap to the objective lens and perpendicular to the microscope stage. A third instar larva is placed on the side of the marker cap approximately 75° away from the vertical z-axis towards the y-axis, just outside the field of view of the digital camera, with the anterior of the larva facing towards the field of view. Placement of the larva on the side of the marker cap allows the digital camera of the stereo microscope to record movement of the larva across the field from a lateral perspective.

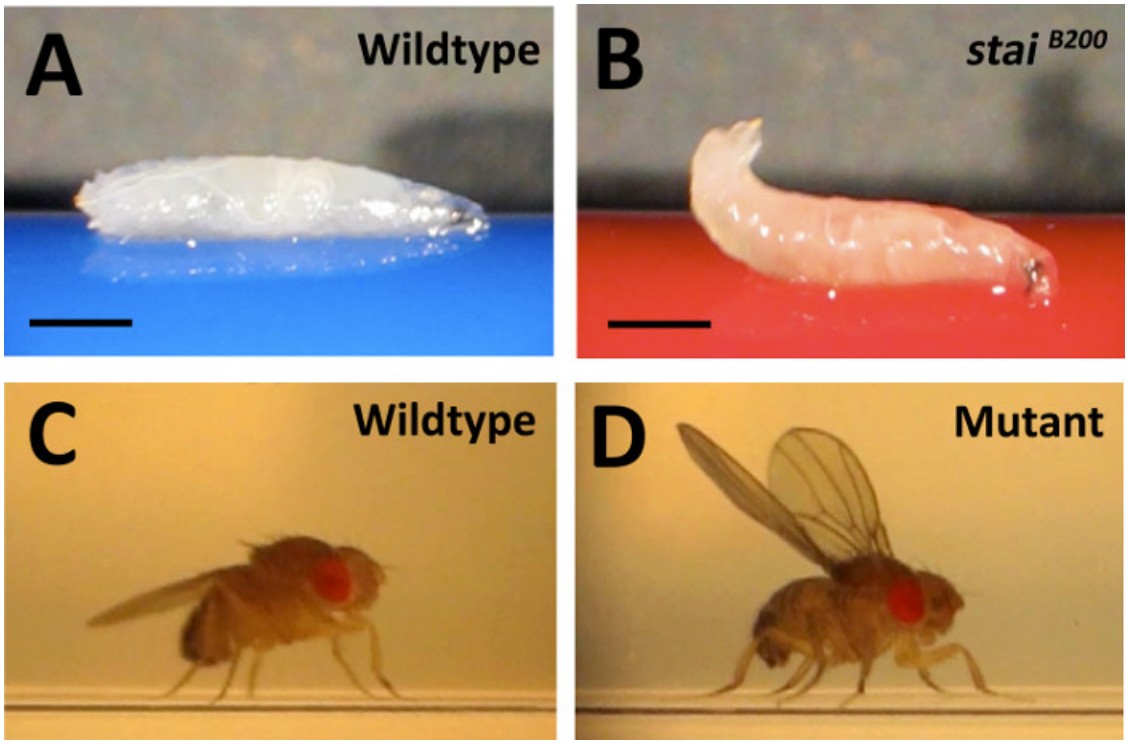

Figure 2. Images of representative results. Representative images from digital video of *Drosophila* larva **(A, B)** and adult **(C, D)** phenotypes and behaviors acquired from a lateral perspective. Each image is a video still frame extracted from acquired digital video files. **(A)** Wildtype third instar larva exhibit a flat body posture when crawling along a substrate. **(B)** Third instar larva homozygous for a mutation in the *stathmin* gene exhibit an aberrant crawling tail-flip behavior, indicative of a paralysis of the posterior musculature. **(C)** The wings of wildtype adult *Drosophila* are held flat against the body as the fly walks. **(D)** Adult *Drosophila*, homozygous for an unknown mutation, hold their wings at angles approximately 45° above normal. Both aberrant larval and adult phenotypes described are best observed and communicated with digital video acquired from a lateral side-view perspective. In panel A and B the scale bar = 1 mm. This figure has been modified from Duncan *et al*., 2013.

		Severity of the Posterior Paralysis Phenotype		
Genotype	n	No Tail-Flip	Mild Tail-Flip (<40°)	Robust Tail-Flip (>40°)
wildtype	150	100.0% (n=150)	0.0% (n=0)	0.0% (n=0)
$stai^{B200}$/+	130	100.0% (n=130)	0.0% (n=0)	0.0% (n=0)
$stai^{rdtp}$/+	140	100.0% (n=140)	0.0% (n=)	0.0% (n=0)
Df(2L)Exel6015/+	120	100.0% (n=120)	0.0% (n=0)	0.0% (n=0)
$stai^{B200}$	120	23.3% (n=28)	23.3% (n=28)	53.4% (n=64)
$stai^{B200}$/Df(2L)Exel6015	101	10.9% (n=11)	21.8% (n=22)	67.3% (n=68)
$stai^{rdtp}$	125	16.0% (n=20)	32.0% (n=40)	52.0% (n=65)
$stai^{rdtp}$/Df(2L)Exel6015	140	7.7% (n=11)	23.7% (n=33)	68.6% (n=96)

Table 1. Penetrance and severity of the posterior paralysis phenotype observed in *stathmin* (*stai*) mutant *Drosophila* third instar larvae. The penetrance and severity of the posterior paralysis phenotype of *stathmin* mutant *Drosophila* third instar larvae was scored and quantified by acquiring digital video of the behavior from a lateral perspective and measuring the angle that the tail was raised above the horizontal crawling plane during the crawling cycle. Larvae were scored as having a robust tail-flip if the tail was raised greater than 40° above the horizontal plane and a mild tail-flip if the tail was raised less than 40° above the horizontal plane. Larvae exhibiting a normal crawling behavior were scored as having no tail-flip. The crawling behavior of at least one hundred larvae was analyzed for each genotype tested. This table has been modified from Duncan *et al.,* 2013.

Discussion

Drosophila melanogaster's strength as a model system for studying nervous system function stems largely from the convergence of the powerful genetic tools available and the broad array of robust behavioral assays developed. Here we present a simple and widely accessible microscopy technique for acquiring high-quality digital video of *Drosophila* adult and larval locomotive phenotypes from a lateral perspective. We have successfully used this approach to characterize and quantify the severity of posterior paralysis 'tail-flip' phenotypes observed in neurologic third instar larval mutants by directly measuring the maximum angle that the tail was raised from the horizontal axis during the crawling cycle[14]. The benefit of the approach presented here is that video is acquired from a lateral perspective, allowing the direct observation and analysis of aberrant locomotive behaviors, often observed in neurologic larval and adult mutants, from a more informative 'side-view' orientation. Consequently, visualization of peristaltic muscle contractions in larval *Drosophila*, and aberrant gait phenotypes in adult *Drosophila* are more readily observed and analyzed. One limitation of this technique is that it is not a high-throughput approach. In addition, specific *Drosophila* larval and adult behaviors can only be analyzed for short durations of time due to the restrictive tracking area afforded by the field of view of the stereo microscope. This can be particularly problematic when acquiring video of adult *Drosophila* behaviors, as the volume of the cuvette chamber is significantly larger than the field of view of the stereo microscope. We have addressed this problem by using cotton and cardboard inserts to minimize the cuvette chamber volume and restrict the movement of the adult fly to a space contained within the field of view. While the majority of our imaging has focused on neurologic larval mutants, we have also used the technique to observe adult mutant phenotypes and behaviors, including grooming, suggesting that the technique can be easily expanded to include analysis of other *Drosophila* behaviors such as courtship, copulation, and aggression. It is possible that this technique could be useful for imaging other Drosophilidae family members, as well as other insects of similar size. Additionally, minor modification of the technique would allow imaging of larger insect species.

Disclosures

The authors have declared that no competing interests exist.

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