Video Article

Visualizing the Beating Heart in *Drosophila*

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Abstract

The *Drosophila* heart has recently emerged as a good model system for examining the genetic, cellular, and molecular mechanisms underlying function in myogenic hearts. A key step in examining heart function in the fly is finding a way to access the heart in a manner that preserves its myogenic function while still allowing the beating heart organ to be observed and recorded. Two different methods for observing and recording the beating heart in both larva and adult *Drosophila* are described here. Our semi-intact preparation using adult flies allows clear visualization of the abdominal heart without interference from the pigmented cuticle and overlying fat bodies. To record larval heart beats it is necessary to immobilize the larva, which minimizes body wall movements thereby reducing heart movements that are not associated with myocardial contractions. Our methodologies produce stable adult and larval heart preparations that can beat for hours at rates of 1-3 Hz.

Video Link

The video component of this article can be found at https://www.jove.com/video/1425/

Protocol

Before you start

Adult hearts

1. Freshly prepare artificial hemolymph (AH) solution containing 108mM Na^+, 5mM K+, 2mM Ca^{2+}, 8mM $MgCl_2$, 1mM NaH_2PO_4, 4mM $NaHCO_3$, 10mM sucrose, 5mM trehalose, 5mM HEPES (pH 7.1, all reagents from Sigma Chemicals). The sucrose and trehalose should be added to the AH from refrigerated stock solutions just prior to use in order to prevent bacterial contamination.
2. Bring AH to room temperature and oxygenate the solution by air-bubbling for at least 15 min.
3. Pull several fine capillaries (e.g. Glass Capillaries, 100ul, VWR) required for removal of fat.

Larval hearts

1. Sylgard-coated coverslips: using a toothpick, transfer about 50-70µl of Sylgard solution onto a 22x22mm coverslip. Let the Sylgard harden at 65°C over night.
2. Prepare the Broadie and Bate's buffer (135mM NaCl, 5mM KCl, 4mM $MgCl_2$, 2mM $CaCl_2$, 5mM TES, 36mM Sucrose; pH 7.15. See Broadie and Bate, 1993).
3. Prepare the tools required to apply the histoacryl glue. First, pull several fine capillaries (e.g. Science Products GB100T8P). Connect a 1.5ml pipette tip with a small plastic tubing (Tygon, 1/32") by inserting the smaller end into the tube. Insert a capillary with its wide opening to the other end. Load the capillary with Histoacryl glue by dipping the capillary into a drop of glue and gentle suction into the plastic tip. When working with the glue one should have a second glass capillary at hand to remove excessive glue from the tip of the glue capillary. The glue is liquid until it comes into contact with buffered saline where it will harden within a couple of seconds. However, it usually remains plastic long enough to be removed from the glue capillary tip or to while applying it to the specimen.

Semi-intact Drosophila Heart from the Adult Fly

1. Adult Drosophila flies are anesthetized with Fly Nap (Carolina Biological Supply Co.) for 2-5 minutes. Care must be taken not to leave the flies in the Fly Nap for longer exposures. Short term exposure to cold can also be used to anesthetize flies but CO_2 is not recommended as it has longer lasting effects on heart rate and rhythmicity.
2. Anesthetized flies are placed, dorsal side down, into a Petri dish coated with a thin layer of petroleum jelly. The hydrophobic cuticle in the wings and body reversibly "stick" to the jelly but the fly can be repositioned if needed. This becomes especially important during subsequent manipulations.

3. An initial cut is made using a curved pair of spring scissors (Roboz #5611). The scissor blades are placed under the legs and angled down toward the dorsal surface of the thorax near the neck. The head, ventral nerve cord, and legs are removed with a single cut. The preparation is then submerged in an oxygenated, artificial hemolymph (AH) solution.
4. Using spring scissors the posterior tip of the abdomen (comprised of abdominal segments 7 and 8) is removed with a single cut. This provides access for making lateral cuts along both edges of the abdomen and serves to sever the connection between the posterior gut and the abdominal cuticle. The freed flap of ventral abdominal cuticle is then removed.
5. In steps 3 and 4 the anterior and posterior connections of the gut are severed so the abdominal organs are now held in place only by tracheols. The gut and other abdominal organs can usually be removed as a single mass using jeweler's forceps (Roboz, #55) and gentle tugging. Removing the internal organs reveals the beating heart tube still attached to the dorsal cuticle surrounded by fat bodies.
6. The fat bodies are quite opaque in the light microscope consequently it is often necessary to remove some of this fat in order to see the heart tube clearly. This is accomplished by a liposuction technique using finely drawn glass capillaries. Capillaries are made using a standard pipette puller (E.g. Sutter P-97 Pipette Puller) and are then inserted into plastic tubing (Tygon, 1/16") attached to a vacuum source. This system is used to suction off excess fat from around the heart tube. Suction force is proportional to tip diameter so pipettes with tips larger than 40 microns in diameter should not be used. Extreme care must be taken to avoid touching the heart itself and because the posterior portion of the heart is very fragile that region should be avoided altogether.
7. The adult Drosophila heart tube is now exposed and should be beating. If necessary the cuticle and attached heart can be repositioned by gently lifting the cuticle out of the petroleum jelly and carefully tamping it back down, again avoiding any contact with the heart tissue. At this point the solution bathing the preparation should be replaced with fresh AHL. This preparation should be allowed to equilibrate for 20-30 minutes with oxygenation prior to any manipulations. If the preparation is to be maintained for longer than 60 minutes it should be perfused with fresh AHL.

Immobilizing Drosophila larvae for optical recording

1. Place a drop of Histoacryl glue close to each corner of the Sylgard coverslips. Add 50µl of B&B buffer to the glue drops which will harden instantly. Fill the space between the glued spots with B&B – the glue will keep the buffer on the slide.
2. Take a wandering L3 larva and place it on a piece of wet tissue paper on ice for 2 min. This will slow down its movement. Then transfer the larva into the buffer and orient it with the dorsal side up (this is the side of the larva that lacks the denticle belts). Quickly apply some glue between the head region and the Sylgard. The glue will harden very quickly and prevent the head of the larva from moving.
3. Using forceps carefully grab the animal by the posterior spiracles and gently stretch it. Fix the larva in this position by applying the glue along both sides of the larva at the posterior half. To reduce further movement of the cuticle by the body wall muscles, add more glue to the flanks of the animal. The larva is now fixed in this position. The coverslip can be placed into a small Petri dish filled with AHL or PBS. Heart beats can now be recorded without interference from body wall movements.

Representative Results:

The semi-intact adult Drosophila heart will beat rhythmically for hours following dissection when maintained in fresh, oxygenated AH (see Supplemental Data, Ocorr, et al., 2007). A representative example is shown in Movie 1. Hearts from 3rd instar larva and young flies (1 – 3 weeks post eclosion) generally exhibit a regular heart beat at rates between 1 – 3 Hz. Flies older than 3 weeks generally are more arrhythmic; nevertheless, these hearts are still able to beat spontaneously for hours in oxygenated AHL. Hearts in adult flies that are damaged as a result of the dissection procedure typically show localized regions of extreme constriction that are unable to relax. Heartbeat rates slower than 1 Hz are rarely observed in flies younger than 3 weeks of age; consequently such preparations are considered to be damaged and are discarded.

Click here to download Movie 1.- A one week old adult wildtype fly (w^{1118} laboratory strain) showing the exposed abdominal heart tube (anterior to the left). Note regular, rhythmic contractions. Opaque regions in the upper right hand corner are the remaining abdominal fat bodies; round cells on either side of the heart are pericardial cells.

Discussion

The Drosophila model has proven to be a powerful genetic tool that has been used to address a variety of scientific questions ranging from embryological development to learning and memory. Recently this versatile model organism has been used to investigate the genetics of heart function in the fly. A number of attempts to quantify heart physiology in adult Drosophila have relied on observations made in intact flies through the abdominal cuticle. Most of these approaches have relied on visual observation or recordings of changes in light intensity transmitter through the abdomen to quantify a single parameter, heart rate. These methods have several limitations: typically only the anterior end of the heart can be observed, what is being observed is the movement of fat bodies secondary to the heart contractions, and the nervous input to the heart is intact. The adult semi-intact preparation provides a more detailed view of a large portion of the functioning heart permitting quantification of a number of parameters in addition to heart rate. It can be used for electrophysiological or optical (see JoVE article, 1435) recording procedures. In addition, this preparation is suitable for histological manipulations (see JoVE article, 1435). It can also be extracted as a relatively clean heart tissue sample for PCR, Western blotting, microarray analysis, etc.

The adult heart in Drosophila is, for the purposes of this dissection, conveniently fixed to the dorsal cuticle of the abdomen by a network of alary muscles. Thus it is possible to dissect away the head and ventral lying nerve cord, and then to remove the internal organs without damaging the heart. However, extreme care should be taken in step 3 to cut only the very tip of the abdomen as the heart extends into abdominal segment 5/6 and a pacemaker cell or cells is undoubtedly present in this region.

Care must also be taken to avoid touching the heart during the dissection. If contact with the heart is suspected the preparation should be discarded. Moderate suction usually suffices to remove some of the fat and tracheols that surround the heart for better visualization of the

heart tube but excessive suction can also damage the heart. The suction force is controlled primarily by the size of the pipette tip used for the liposuction; tips larger than approximately 40 microns should be avoided.

Unlike in the adult, the heart tube in Drosophila larva is relatively free to move around the body cavity. Thus a semi-intact larval heart preparation presents difficulties for optical recording techniques. Due to the translucency of the integument it is possible to visualize the heart in the intact larva using brightfield microscopy. The glue technique described here immobilizes larva of any developmental stage and keeps the heart in a fixed position. This permits recording of a relatively stationary heart tube. Heart contractions recorded by such immobilized larva can be subsequently analyzed by the optical recording technique described in JoVE 1435.

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References

1. Ocorr K, Reeves N, Wessells RJ, Fink M, Chen H-SV, Akasaka T, Yasuda S, Metzger J, Giles W, Posakony JW, and Bodmer R. KCNQ potassium channel mutations cause cardiac arrhythmias in Drosophila that mimic the effects of aging. *Proc Natl Acad Sci U S A* 104:3943-8. (2007)
2. Broadie, K. S. and Bate, M. Development of the embryonic neuromuscular synapse of Drosophila melanogaster. *J Neurosci* **13**, 144-66. (1993)

