Enhanced assessment of contractile dynamics in *Drosophila* hearts

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The *Drosophila* heart has gained considerable traction as a model of cardiac development and physiology. Previously we described a semiautomatic optical heartbeat analysis (SOHA) method for quantifying functional parameters from the fly heart that facilitated its use as an organ system and disease model. Here we present an extensively rewritten version of the original SOHA program that takes advantage of additional information contained in high-speed videos of beating hearts. Program updates allow more precise quantification of cardiac contractions, increase the signal-to-noise ratio, and reduce the overall cost and time required to analyze recordings. This new SOHA version permits relatively rapid and highly accurate determination of subphases of contraction and relaxation. Importantly, the improved functionality enables the calculation of novel physiological data, suggesting that the fly model system may also be practical for screening drugs and alleles that modulate cardiac repolarization and force production.

Drosophila has proven to be a superb model for delineating molecular events involved in embryonic development. The extensive *Drosophila* genetic toolbox currently available has aided in identifying genes that determine embryonic polarity, patterning, and cell fate specification that are conserved in humans. We previously published the SOHA method for quantifying functional parameters in the fly cardiac tube, allowing its use as an organ system and disease model (1–6). These and other studies have shown significant conservation in cardiac genes and gene functions between flies and humans, including many muscle structural components and ion channel proteins (1,7,8). The ability to rapidly and precisely quantify physiological parameters from the fly heart is fundamentally important to comprehensively tap the power of this genetic model.

SOHA uses two algorithms to identify systolic and diastolic intervals from movies of beating hearts (Supplementary Video S1) (1,9). The darkness algorithm uses frame-to-frame changes in mean overall darkness to determine the contractile state of the heart wall when not moving (i.e., completely contracted versus completely relaxed); however, this output is typically noisy, and the beginning and end of a contraction interval are not clearly defined (Figure 1). The pixel-by-pixel algorithm analyzes individual pixels for darkness changes above a threshold, permitting a more precise delineation of when movement starts and stops (Figure 1). Used together, these algorithms make identification of all the intervals in a movie virtually automatic. (For detailed information on the algorithms used, see Reference 9.)

Here we present a rewritten SOHA program (SOHA version 3, or SOHA v.3) as a stand-alone application available for free download at (http://sohasoftware.com/). This new version runs independently of MatLab; thus, it does not require an annual MatLab subscription, which may be cost prohibitive for smaller laboratories or for educational use. We improved the graphical user interface and added modifications that substantially enhance the array of parameters that can be extracted from high-speed videos, permitting accurate quantification of automatically or user-derived subphases of the cardiac cycle. As discussed below, these modifications allow additional functional measurements to be made that would otherwise be impossible, expanding the repertoire of physiological indices that can be investigated in the genetically malleable fly heart.

The improved precision of interval delimitation can be attributed to several factors. First, we added the ability to directly analyze output files

METHOD SUMMARY

Our semi-automatic optical heartbeat analysis (SOHA) method for assessing function in small hearts has facilitated the use of *Drosophila* as a cardiac model. The SOHA program has been rewritten providing more precise quantification of motion, enhanced signal/noise ratio, and reduced time needed for analysis. These improvements enable calculation of novel physiological parameters, suggesting this model system may be practical for in situ screening of drugs and mutant alleles that modify cardiac performance.

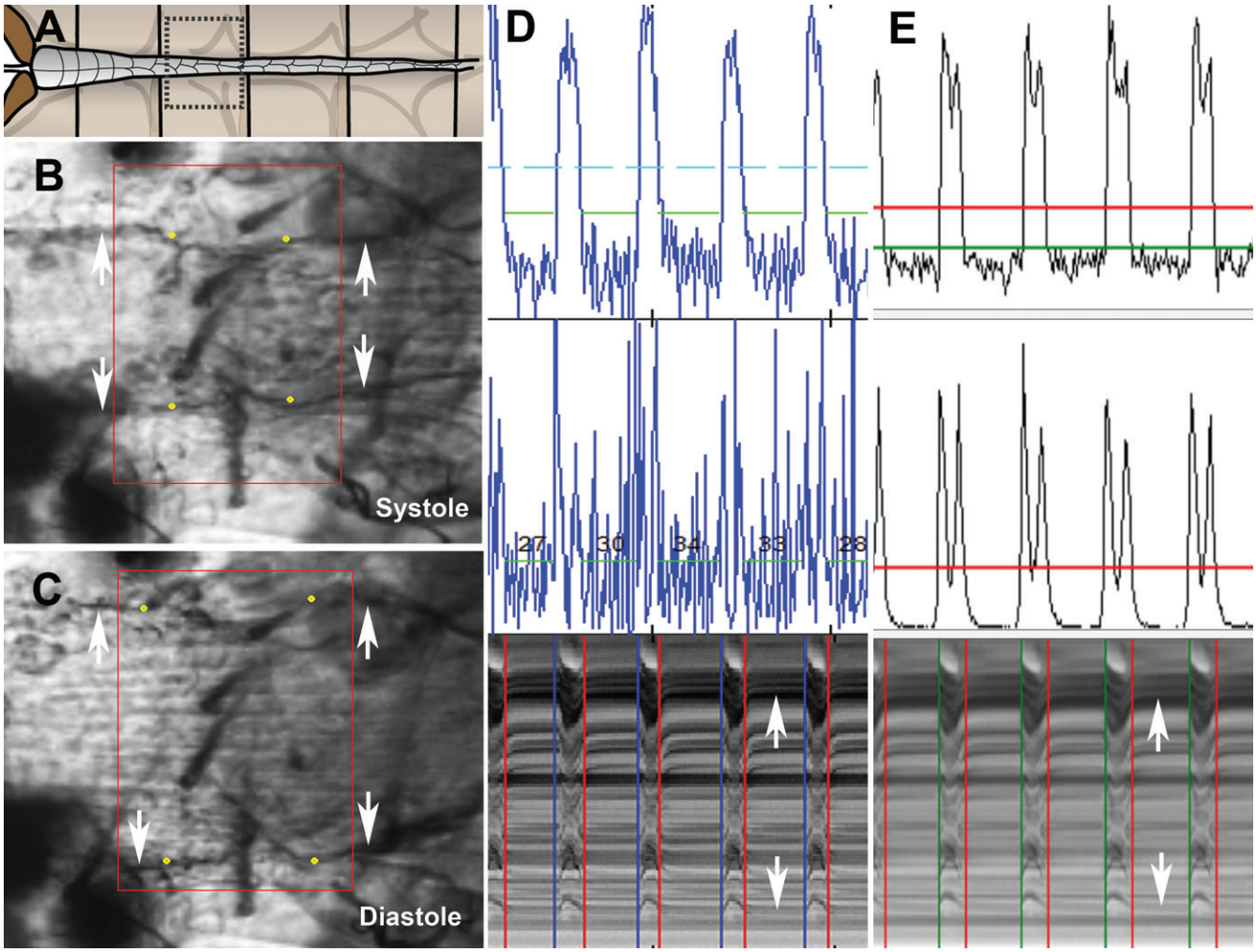

Figure 1. SOHA v.3 program upgrades improve and augment data quantification. (A) Schematic representation of a heart in a semi-intact *Drosophila* preparation (adapted from Reference 6); dashed lines enclose the region of the heart tube displayed in the pictures below. (B,C) New marking interface allows specification of a region of interest (red box) to be analyzed for movement. Heart wall positions for diameter measurements are marked in pairs, with the possibility of making (B) two pairs of systolic diameter marks (yellow dots) and (C) two pairs of diastolic diameter marks (yellow dots). Horizontal measurements are also possible. (D,E) Side-by-side comparison of algorithm output for the same AVI formatted movie of a heart from a wild-type fly (w^{1118}). (D) Output for four contractions from the original, MatLab-based SOHA program obtained via the darkness algorithm (top) and pixel-by-pixel algorithm output (middle) and an M-mode (bottom) from the corresponding movie frames. (E) Output for the same four contractions from SOHA v.3. Note the clear resolution by SOHA v.3 of two discrete peaks, corresponding to contraction and relaxation, for each individual systolic interval. In all figures, arrows indicate the position of the heart wall.

Table 1. Comparison of original and new capabilities available in SOHA v.3.

Features	SOHA (original version)	SOHA (version 3)
Platform	Requires MatLab	Stand alone
Analyzable files	Black and white *.avi files only	Black and white *.avi and *.cxd files (HCImage)
Timing	• Based on average fps	• For AVI files: average fps • For CXD files based on each frame's time stamp
Noise reduction	• Threshold setting • High- and low-pass filters for average darkness algorithm only	• Threshold setting • High- and low-pass filter for both average darkness and pixel-by-pixel algorithms • Selectable ROI eliminates noncardiac tissue from analysis
Interval identification	• Automatic; modifiable by average darkness algorithm	• Automatic; modifiable by average darkness algorithm • Ability to correct individual intervals if needed • Adjustable threshold for ignoring periods with no movement (minimum interval)
New phase analysis	NA	• Systolic interval precisely parsed into contraction and relaxation phases
Diameter measurements	• Two sets of vertical measurements	• Selectable vertical and horizontal measurements
AVI movie output with movement highlighted	• AVI movies to AVI output only • Threshold not changeable	• Both AVI and CXD files to AVI output • Includes adjustable movement threshold (0%–100%)
New output		• Contraction phase • Relaxation phase • Automatic detection of unusually long intervals • Manually selectable shortening and lengthening times

NA: not applicable; ROI: region of interest; SOHA: semiautomatic optical heartbeat analysis.

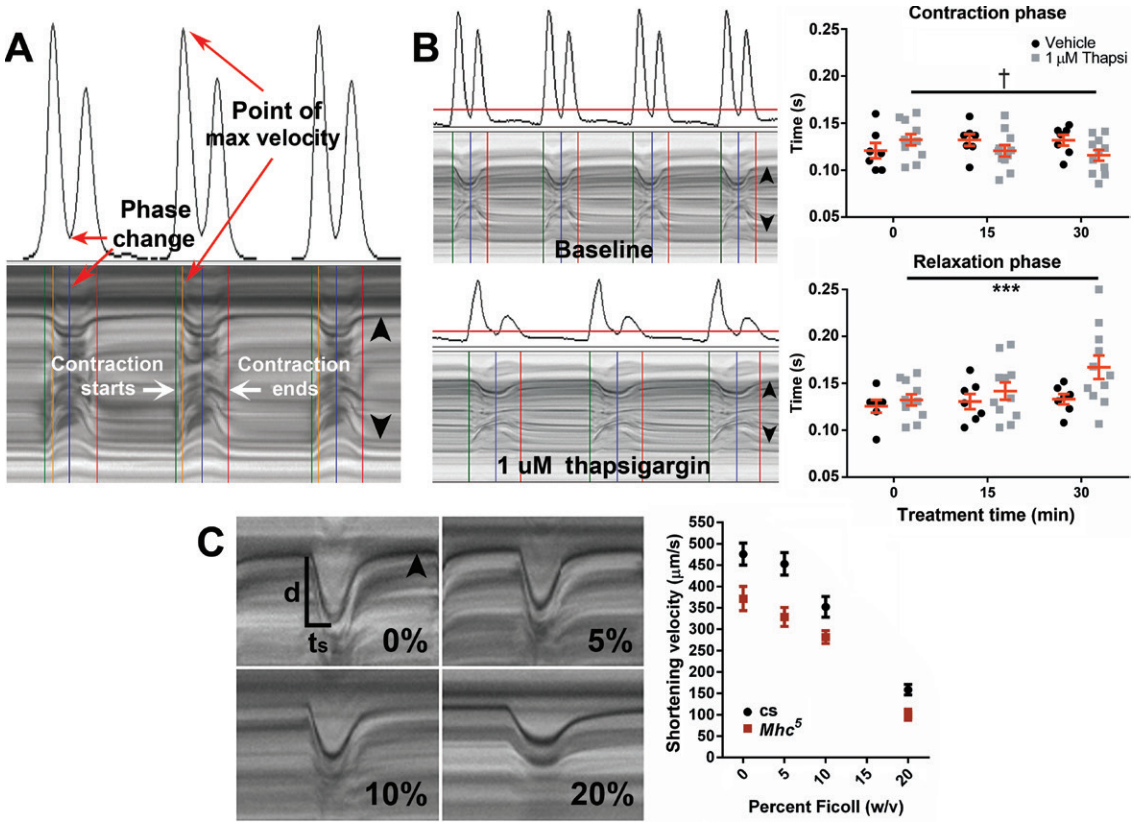

Figure 2. Enhanced SOHA v.3 software features permit aquisition of novel in situ *Drosophila* cardiac data. (A) New contraction phase and relaxation phase determination. Movement peak maxima and minima are used to determine these subphases in a semiautomated fashion. The beginning and end of individual contractile events are delineated by green and red lines, respectively, and are determined as previously described by the original SOHA movement detection algorithms (9). SOHA v.3 now permits quantification of a contraction phase, which is the time interval between the start of systole (green line) and the point at which no further movement occurs (blue line), to be discerned (Supplementary Figure S1). A relaxation phase, the time interval between the blue and red lines, can also be resolved. The point at which shortening velocity is greatest coincides with the first peak maxima (yellow line). (B) M-mode traces and phase analysis output from high-speed movies of semi-intact fly heart preparations before and after treatment with 1 μM thapsigargin. Graphs show quantification of contraction phases (top) and relaxation phases (bottom) before treatment (T = 0) and at 15 and 30 min after exposure to either vehicle (DMSO, black circles) or 1 μM thapsigargin (gray boxes). Note that the relaxation phase is selectively lengthened after 30 min thapsigargin treatment. Mean ± SEM are indicated in orange. Significance is determined by two-way repeated-measures ANOVA with Sidak's multiple comparison post-hoc test; $^{\dagger}P < 0.01$; $^{***}P < 0.001$. (C) Incubating semi-intact *Drosophila* hearts in artificial hemolymph solutions that contain increasing amounts of Ficoll 400 (w/v) reduces wall movement distance (d) and prolongs time of shortening (t_s) (left) (also compare Supplementary Videos S1 and S2). Thus, shortening velocity decreases with increasing viscous loads. Here, the contraction phase was further partitioned into t_s in a user-dependent manner (Supplementary Figure S1) to demarcate only the time interval during which movement occurs, for a more precise calculation of shortening velocity. Increased viscosity significantly reduced shortening speeds of both genotypes ($P < 0.0001$) with *Mhc*^{5} mutant *Drosophila* hearts performing significantly worse over the defined range of loads relative to Canton S (cs) control hearts ($P = 0.0019$; mean ± SEM are indicated, significance determined by two-way repeated-measures ANOVA) (right). These data suggest that *Mhc*^{5} hearts generate lower force over the span of loads examined, and they are consistent with the reduced flight ability of the mutants caused by myosin-induced muscle destruction (2).

(in CXD format) from a commonly used image capture software program, HCImage (Hamamatsu Corporation, Bridgewater, NJ), in addition to black-and-white AVI formatted movies, to make use of time stamp information embedded in each CXD movie frame. This improves accuracy even in the event of fluctuating frame rates (e.g., because of computer RAM and hard drive memory limitations), allowing short-duration events (i.e., contraction/relaxation subphases) to be resolved. Moreover, quantification of *all* cardiac cycle intervals in CXD formatted videos is substantially more precise compared with AVI movies, for which an average frame rate is used to determine timing. Second, we upgraded noise reduction by (*i*) allowing selection of a user-defined region of interest that is then used for movement analysis, allowing specific detection of heart movements versus movement of attached tissue such as fat (Figure 1, B and C); (*ii*) adding high- and low-pass filters to normalize baseline fluctuations in light levels for both algorithms; and (*iii*) introducing the ability to manually remove intervals that are incorrectly identified and replace them using actual heart wall movements (displayed in corresponding M-modes) as a guide. These advances significantly reduce the percentage of movies discarded due to excessive noise. Overall, the refinements greatly improve the speed at which videos can be analyzed and the quality of data output. Table 1 presents a summary of the new capabilities.

Importantly, the SOHA v.3 upgrades permit accurate division of the systolic interval into discrete subphases in a semi-automated (Figure 2A) or user-specified fashion (Supplementary Figure S1), enabling additional functional parameters to be assessed. The movement traces, which are the

graphical representation of the pixel-by-pixel analysis output, can now be accurately parsed into a contraction phase and relaxation phase (Figure 2, A and B). We can also calculate shortening velocity [shortening distance (obtained from systolic and diastolic diameter marks, e.g., Figures 1, B and C)/contraction phase time] and, similarly, lengthening velocity (Figure 2C).

To examine the relative effects of experimental manipulations on the contraction or relaxation phases, it is necessary to acquire images at very high frame rates. At 200 fps, 40 frames will minimally be captured during a single systolic interval, making an accurate division into subphases (Figure 2A) or a more precise partitioning of shortening and lengthening periods (Supplementary Figure S1) possible. To demonstrate this capability, we applied the sarco/endoplasmic reticulum Ca^{2+}-ATPase blocker thapsigargin, which prevents calcium re-uptake after initiation of contractions, to the fly heart (Figure 2B). We hypothesized that this should differentially affect the relaxation phase relative to the contraction phase. Semi-intact heart preparations were perfused with saline containing either 1 μM thapsigargin or vehicle (DMSO). Phase analysis showed thapsigargin treatment selectively prolonged the relaxation phase and slightly reduced the contraction phase at 30 min compared with controls (Figure 2B). These data are consistent with the increased Ca^{2+} transient observed in response to similar thapsigargin treatments by Lin et al. (10) and suggest that SOHA v.3 upgrades, in combination with the semi-intact heart preparation, may be useful to screen for drugs that modulate repolarization reserve in cardiac tissue.

To date, there is no way to assess force production in the isolated fly heart in a nondamaging way. However, our refined ability to calculate shortening velocity can be used to provide an index of relative force produced by cardiac tubes of flies of distinct genotypes or after application of small molecules that influence contractility. Shortening velocity varies inversely with the load on muscle. Thus, mechanical performance is frequently evaluated by measuring how fast a muscle contracts against a range of applied loads. We monitored changes in cardiac shortening velocity under "loaded" conditions using Ficoll 400 solutions of differing viscosities for control versus *Mhc*^{5} mutant *Drosophila* (Figure 2C). The significant decreases in shortening velocities for mutant relative to wild-type hearts are consistent with impaired force generating properties across the span of viscous loads. This protocol provides a novel and relatively simple in situ approach to impose a load and to monitor relative changes in output. In conclusion, we present the latest version of SOHA, SOHA v.3, which has been extensively refined to permit the extraction of new data from the powerful *Drosophila* heart model.

Author contributions

A.C. and K.O. conceived, designed, and executed experiments, analyzed data, and wrote the manuscript. K.O. conceived the programmatic elements and S.O. wrote the computational code.

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Competing interests

The authors declare no competing interests.

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