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A portable system for monitoring the behavioral activity of *Drosophila*

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ABSTRACT

We describe a low-cost system for monitoring the behavioral activity of the fruit fly, *Drosophila melanogaster*. The system is readily adaptable to one or more cameras for simultaneous recordings of behavior from different angles and can be used for monitoring multiple individuals in a population at the same time. Signal processing allows discriminating between active and inactive periods during locomotion or flying, and quantification of subtler movements related to changes in position of the wings or legs. The recordings can be taken continuously over long periods of time and can thus provide information about the dynamics of a population. The system was used to monitor responses to caffeine, changes in temperature and g-force, and activity in a variable size population.

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1. Introduction

Complex animal behavioral patterns are controlled by the nervous system; precisely how they are controlled remains a fundamental topic of neuroscience research (Chronis et al., 2007; Martin, 2003). Locomotor activity is a fundamental measure of behavior and has complex patterns. Because it is a complicated trait, highly dependent on physiological, diurnal, and environmental factors, quantifying locomotor activity is challenging (Martin, 2003). An additional difficulty stems from practical concerns: most experimental approaches to monitoring behavior are time consuming, and the required instrumentation is expensive and large.

These practical concerns can partially be mitigated by a convenient choice of animal: *Drosophila* (the fruit fly) is often used in behavioral activity studies due to its short life span, small size, and ease of maintenance. Monitoring fly activity can allow study of many traits such as the brain control of physiological adaptations, the effects of environmental stress factors or genetic mutants, behavioral responses to pharmaceuticals, and aging or disease-associated behavior.

Existing systems for monitoring fruit fly activity in the laboratory range from visual observation (Balakireva et al., 1998; Diagana et al., 2002; Gargano et al., 2005; Martin and Grotewiel, 2006) to elaborate video-based electronic solutions (Cole, 1995; Fry et al., 2008; Ramazani et al., 2007; Reiser and Dickinson, 2008) combined with multiple-parameter analysis (Martin and Grotewiel, 2006; Martin, 2004). Some of the experimental approaches are limited to individual flies or are specifically designed to capture particular behavioral events (Card and Dickinson, 2008; George et al., 2005; Sharma et al., 2009).

We present a novel setup that is simple, low-cost and can discriminate between different behavioral traits (general locomotor activity versus subtle movement). The system can monitor activity within a population and allows continuous recording over long periods of time, providing statistical power to the analysis of the behavioral pattern.

2. Materials and methods

2.1. System description

Two complementary setups were constructed based on a prototype developed previously (Inan et al., 2009): one for monitoring flies walking in a flat Petri dish, and one for monitoring flies walking and flying in a cubic volume. The second setup was an extension of the first, using identical signal processing and analysis steps.

2.1.1. Monitoring walking

Fig. 1A illustrates the first basic setup used for data collection. Four white LEDs, mounted in a custom light diffuser, illuminated a 5 cm diameter Petri dish from below. The dish was imaged from above using a miniature, monochrome CMOS video camera (166XS, Ingram Technologies, Price, UT). The field of view was set to 1.5 cm × 1.5 cm. The video signal output of this camera was input to an analog circuit for filtering and amplification.

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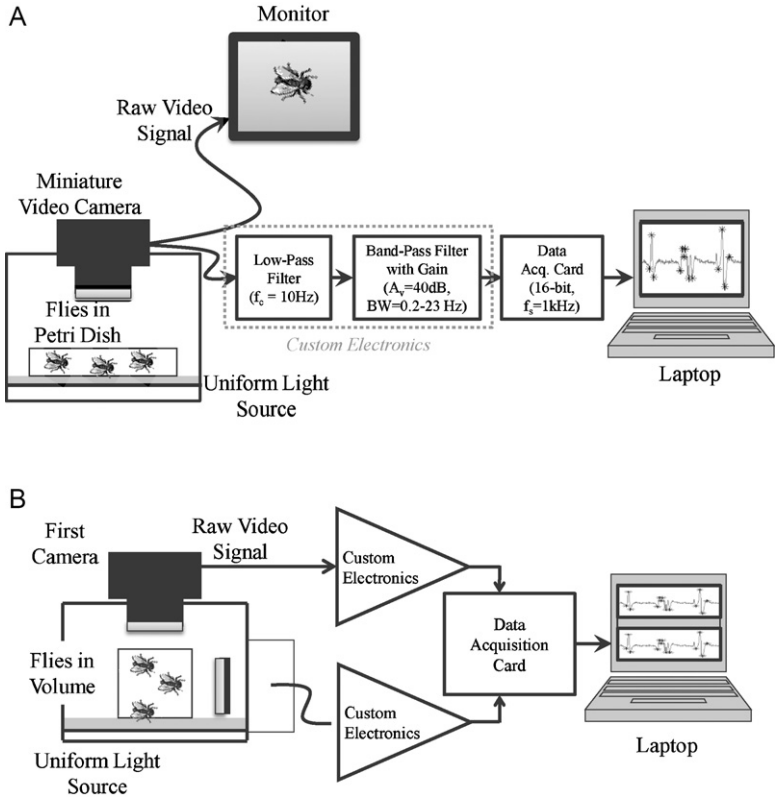

Fig. 1. Block diagram of the system. (A) Basic measurement setup for imaging flies. The analog circuit constituted a band-pass filter with gain, extracting only the low frequency light-level variations incident on the camera. A data acquisition card sampled the output of this circuit and the signals were stored on a laptop computer. The output from the camera was simultaneously viewed on a monitor. (B) Two-camera approach to measuring vertical and horizontal movements in a cubic volume. Two copies of the analog circuit used in (A) were made and both signals were stored on the laptop.

Spatial and temporal information describing the image is encoded in this video signal. This information can be decoded by a monitor or display to reconstruct the video. The bandwidth required is on the order of several MHz. In some applications, it is advantageous to significantly lower the data bandwidth by intentionally discarding some of the video content.

In this study, by passing the video signal through a low frequency band-pass filter (0.3–10 Hz), the average image intensity over time was extracted. The resulting signal had a bandwidth of several Hz, a 10^6-fold reduction compared to the raw video signal. The spatial information, describing which areas of the image were changing in intensity over time, was lost in the filtering. By observing average intensity over time, aggregate fly movement was detected. Analyzing this signal, as described below, provides several quantitative measures of locomotor behavior.

2.1.2. Monitoring walking and flying simultaneously

Fig. 1B illustrates the second basic setup for data collection. The setup mirrored the first, except that a second camera was mounted horizontally to monitor the vertical activity of the flies. Two identical circuits were used for amplification and filtering, one for each camera. All experiments detailed below used the first setup unless otherwise noted.

2.2. Circuit description and technical characterization of the system

The circuit consisted of three filtering and gain stages, and some basic power regulation. The first stage was a second-order Sallen-Key low-pass filter with unity gain and a cutoff frequency of 100 Hz. From this stage, the output was connected to an LTC1064 eighth-order switched capacitor low-pass filter with a cutoff frequency of 10 Hz—the cutoff frequency was set by an op-amp relaxation oscillator (f_{clk} = 1 kHz). The final stage was a band-pass filter with 40 dB of gain and a bandwidth from 0.3 to 10 Hz.

Powered by a single 9 V supply, the current consumption of the circuit was 20 mA. The 9 V supply voltage was regulated down to 5 V using a linear regulator, and this regulated 5 V output was then inverted using a switched capacitor inverter resulting in ±5 V supplies. The output noise of the circuit was less than 10 mV_{rms}, and was dominated by the output noise of the LTC1064 filter IC (typically 80 μV_{rms}).

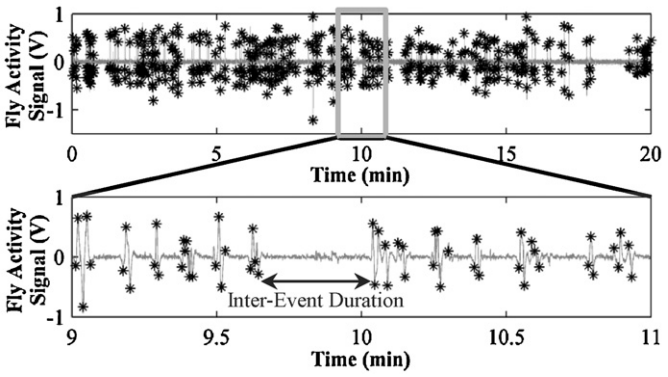

Fig. 2. Sample fly activity signal output of analog circuit. "Events" are denoted by black asterisks. The inter-event-durations were extracted from this signal, and examined to determine an overall activity level for the flies. The bottom trace shows a zoomed-in version of the signal, where the signal-to-noise-ratio can be observed. The activity peaks are 3–10× higher in peak amplitude than the peak-to-peak noise levels in the system.

Using an LED in the field of view as a light source, the linearity and total harmonic distortion (THD) of the system was characterized for varying light levels and frequencies. The system was found to be linear ($R^2 = 0.99$) for light levels ranging from 1 to 125 μW of light power, and the THD was found to be sufficiently low (<1%) for the levels and frequencies relevant to this work.

For more information on the engineering details of the circuit design, characterization and performance, the reader is referred to previous work (Inan et al., 2009). Briefly, the major advantages of the method are low operating power, high signal quality, ultra-low bandwidth, and the ability to simultaneously provide video imaging. These advantages make the system well-suited for portable applications, where data must be transmitted wirelessly via a low bandwidth data link.

2.3. Signal analysis and feature extraction

The movement signal output of the circuit was sampled by a data acquisition card (6063E, National Instruments, Austin, TX) and stored on a laptop computer using software (Matlab® v2007b, The Mathworks, Natick, MA). The signal processing and analysis steps were implemented in software after acquisition.

This movement signal represents the average light level changes versus time. As a fly moves into the image, the average light level decreases slightly, and as a fly moves out of the image, the light level increases. Fig. 2 shows an example movement signal with peaks caused by flies entering and exiting the image.

The first signal processing step was event detection, achieved by digital filtering operations combined with a fixed threshold peak detection algorithm. The details of this algorithm are provided in (Inan et al., 2009). The events are annotated in Fig. 2 with black asterisks. The durations in between events, termed *inter-event durations*, are one of the features extracted from the signal. These durations are representative of the aggregate locomotor rate of the flies in the image, which, as shown below, are characteristic of the genetic background and can be modulated by pharmacological and environmental factors. Using the statistics of these durations, such as the mean duration for a given recording, different levels of activity can be accurately quantified.

An important feature of the recorded signal is that the amplitude of the events could vary with the speed of movement and/or the size of the object that moves in or out of the image. The effects of object size were found to be much more prominent than speed of movement in modulating signal amplitude, and were used to distinguish general locomotor activity versus subtle movements. For example, if the wing of one fly moved in or out of the image, or moved from behind the body to its side, a small peak was observed in the movement signal. Similar peaks were also observed for flies that were shaking on the edge of the image, since only part of their bodies entered and exited the field of view. The amplitude of these peaks was much smaller than the corresponding amplitude for a fly entering or exiting the image. The fixed threshold used in the peak detection algorithm was empirically set such that these subtler movements could be differentiated from flies moving their whole bodies in and out of view.

To quantify the level of these subtler movements, the root-mean-square (RMS) power of the baseline was computed for segments of the trace intercalated between the larger fly movement events. This RMS power provided a *shaking index*, which was used as a complementary feature to the *inter-event-durations* for quantifying the locomotor behavior of the flies.

2.4. Animals and media

Oregon-R (wild type) strain *Drosophila* were grown at 25 °C in a 12 h light/dark cycle and 4-day old adult males were used. The medium was a modified version of the one described by Lewis (1960), composed of 1 L water, 61 g cornmeal, 129 g dextrose, 32 g yeast, 9.3 g agar, and 11.8 mL of phosphoric and propionic acid mix. Each Petri dish contained 15 flies unless otherwise noted.

2.5. Correlation of automatically extracted features to raw video

A basic validation of the system was carried out by comparing the features that were detected automatically using the methods described above to features that were observed and annotated manually from the raw video recording. Two flies were placed in the dish and a twenty second recording was taken—this recording is provided online for the reader's convenience. The data extracted automatically was plotted against the data gathered manually to compare the results.

2.6. Demonstrative experiments

Several biologically relevant experiments were conducted to validate the system performance and demonstrate measurement versatility. More importantly, these experiments were selected to illustrate how these novel signal analysis techniques can allow a simple hardware set-up to thoroughly describe the locomotor behavior of the flies including subtle wing movements. The experimental methods are described below, classified as pharmaceutical studies, environmental studies, and population studies.

2.6.1. Pharmaceutical studies

The system can be used to establish a locomotor behavior dose response for various pharmaceuticals, including a quantitative view of pharmacokinetics. To demonstrate this capacity of the system, the flies were exposed to caffeine, in two non-lethal concentrations (10 mg/mL and 20 mg/mL). Three hours after the addition of caffeine to the food, the spontaneous and stimulated responses of the flies were monitored and compared to a control group.

To evaluate the spontaneous response to caffeine, the flies were monitored for a 45-min period, and the mean inter-event-durations were measured and compared to each other and the control. The statistical significance of each comparison was evaluated using Student's *t*-test, and a *p*-value was accordingly computed. The dose response was composed of the mean inter-event-duration (±standard error, SE) of the flies plotted against the caffeine concentration.

To evaluate the stimulated response of the flies, the Petri dish containing the animals was softly tapped on the table then immediately moved into the field of view of the camera. The movement activity signal was recorded for 20 min following this stimulus, and the events were detected for this period. A cumulative distribution function (CDF) plot was used to visualize the response of the flies, and an exponential was fitted to the data. The goodness of fit was analyzed using a Kolmogorov–Smirnov (KS) test and the best fit was determined accordingly. The time constant for this best fit was compared for the two groups exposed to caffeine, and to the control.

To validate the dual-camera setup, the spontaneous response experiment was repeated for a control (flies with no caffeine) and a test group (flies dosed with caffeine). The mean inter-event-durations (±SE) were measured for both groups and compared. Statistical significance was assessed using Student's *t*-test.

2.6.2. Environmental studies

The system is practical for evaluating the effects of environmental changes on a fly population. Temperature was used as a common environmental stimulus since the normal behavioral movement of flies is dependent on daily and seasonal changes in temperature

(Mikasa and Narise, 1983; Zhang et al., 2010). The details of the temperature experiment results are provided in (Inan et al., 2009).

The apparatus was housed in a closed metallic container with a custom heating coil, and the temperature was varied as the fly movement was monitored. A closed container was used to minimize the heat lost during the temperature variation. A second Petri dish was placed next to the dish holding the flies, and a thermistor (8502-16, Cole Parmer, Vernon Hills, IL) was positioned such that the temperature could be measured. The temperature output of this thermistor was recorded simultaneous with the fly movement signal output of the circuit.

The chamber temperature was varied over a period of 80 min. First, for 50 min, the temperature was ramped from 22 °C to 37.5 °C at varying rates. The rate for the first 10 min (22 °C < temperature < 30 °C, rate: 0.8 °C/min) was higher than the second 10 min (30 °C < temperature < 32 °C, rate: 0.2 °C/min). Then, for 30 min, the temperature was decreased back to 22 °C passively at an approximate rate of −0.5 °C/min. The upper threshold, 37.5 °C, was set as a biologically safe limit.

The mean inter-event-durations were computed for low (22 °C < temperature < 33 °C) and high (33 °C < temperature < 37.5 °C) temperatures, and low (0.8 °C/min) and high (0.2 °C/min) rates of increase and compared to each other and the control (room temperature). To determine the effect of rate of temperature change on activity, event frequency versus temperature was extracted from the data.

Hypergravity was selected as a second environmental stimulus because of our interest in elucidating the mechanism of response and adaptation to gravity. We used two fly strains, wild-type and a mutant line in the succinate dehydrogenase B gene, sdhB (SdhB^{EY12081} was obtained from the Bloomington Drosophila Stock Center). The mutant flies have an abnormal geotaxic behavior, therefore we expected that their response to a hypergravity (3 g) stimulus would be impaired. The hypergravity exposure was for 30 min in a low-g centrifuge. Four recordings were obtained: wild type flies at 1 g (control) and after exposure to 3 g, and mutant sdhB flies at 1 g and 3 g. Because the analysis of the hypergravity data included the shaking index as well as inter-event durations, the data is presented in its own section below (3. 5.).

2.6.3. Population studies

The system can be used to statistically estimate the number of living flies in the dish. This information is relevant, for example, in lethality studies of drug or environmental effects. For demonstrating this application, the number of flies contained in the Petri dish was varied and the spontaneous activity levels were observed for 30 min. The mean inter-event-durations were computed for each group with different numbers of flies in the dish, and the results were compared. The p-value obtained from Student's t-test was used as threshold of significance.

3. Results and discussion

3.1. Correlation of automatically extracted features to raw video

Fig. 3 shows the fly activity signal (top plot) and the manually annotated events from the raw video recording (bottom plot). The asterisks on the top trace correspond to automatically detected "events"—positive valued events correspond to increased light level and, thus, a fly leaving the image while negative valued events correspond to decreased light level from a fly entering the image.

Except for the second automatically detected peak, the manually detected events are closely in sync with the automatically detected ones. Furthermore, the polarity is consistent for both. The second event is, in fact, quite interesting as it does not correspond to a fly moving in or out of the image, but rather to a fly falling into the hole in the food and squirming to climb out, as can be seen in the video recording provided online.

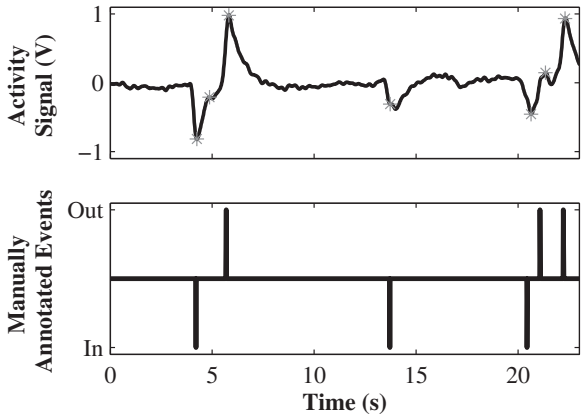

Fig. 3. Correlation of automatically and manually detected events. Top: fly activity signal measured using the methods described here. The asterisks denote automatically detected events corresponding to fly movement. Positive valued events correspond to flies moving out of the image (increased light level) and negative valued events to flies moving into the image (decreased light level). Bottom: manually detected events from a simultaneously recorded video file. These events corresponded to flies moving in and out of the image. For convenience, flies moving into the image are plotted as negative values, and moving out are positive values.

3.2. Spontaneous and stimulated response to caffeine

3.2.1. Spontaneous responses

The spontaneous response of the flies to caffeine (shown in Fig. 4A) was as follows: the mean (±SE) inter-event-durations for the three groups (control, 10 mg/mL caffeine, and 20 mg/mL caffeine) were 5.92 (±1.04), 3.12 (±0.21), and 1.29 (±0.037) s, respectively.

These baseline activity levels show that the caffeine-dosed flies were significantly hyperactive: the mean inter-event-duration decreased more than five times compared to the control with a concentration of 20 mg/mL. A dose response was successfully established: the activity level monotonically increased with higher caffeine concentrations. This is consistent with results from the literature, which show that resting time decreases with increasing caffeine dosage for flies (Andretic et al., 2008; Wu et al., 2009).

The differences among all three sets of flies were statistically significant ($p \ll 0.001$), indicating that this method of detection, combined with the statistical analysis of large numbers of events, can be used reliably for dose–response curves to pharmacological stimuli.

3.2.2. Stimulated responses

The stimulated response of the flies is shown in Fig. 4B and it is also dependent on the concentration of caffeine. The y-axis of these plots represents the fraction of the total events that have occurred before a given time. The time constant for the best fit exponential is 7, 9, and 34 min for the control, 10 mg/mL caffeine-treated, and 20 mg/mL caffeine-treated, respectively. The corresponding KS-test statistics for the three exponential fits were 1.358, 1.674, and 1.076. As a result, the null hypothesis that an exponential fits the data can be rejected at the 1% level of significance for the 10 mg/mL group, but cannot be rejected at the 5% level of significance for the control and 20 mg/mL groups.

The stimulated response experiment showed that flies given higher concentrations of caffeine took longer to recover from a mechanical stimulus. Caffeine is naturally produced by plants as an anti-feeding and pesticide agent for insects, and in *Drosophila* it was shown to exert its effects through the dopamine receptor, cAMP

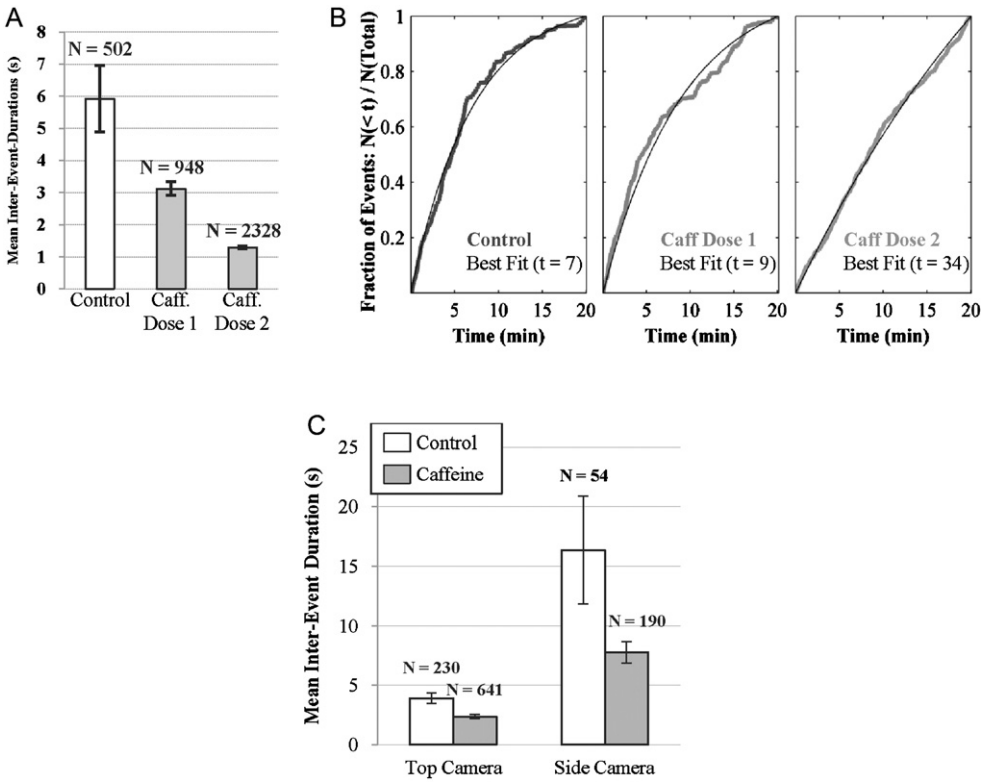

Fig. 4. Spontaneous and stimulated response to caffeine. Caff Dose 1 corresponds to 10 mg/mL, and Caff Dose 2 to 20 mg/mL. N = Total number of events. (A) The spontaneous locomotor response to caffeine increases and is dose-dependent. The mean inter-event durations decrease with the amount of caffeine, corresponding to increased locomotor activity of the flies. (B) The stimulated response to mechanical vibration is delayed in caffeine-treated flies. The best exponential fit is shown for each data set, demonstrating that as the caffeine dose increases, the relaxation period required for the flies to return to their basal activity level is longer. (C) Simultaneous recordings with the 2-camera system. Movements in both the horizontal (top camera) and vertical plane (side camera) are significantly affected by caffeine (p-values < 0.01 for both sets of data).

pathway and protein kinase A activity in the brain (Andretic et al., 2008; Bhaskara et al., 2008; Wu et al., 2009), which decrease the lag-time of response to a secondary stimulus. The system described here provides a way to monitor behavior over time, through the automated continuous collection of data points.

3.2.3. *Two-camera recordings*

The two-camera setup described above was used to test that walking and flying can be recorded simultaneously and more behavioral information can be extracted from the same population of flies. The data in Fig. 4C shows a significant difference ($p < 0.01$) in the activity levels of control and caffeine treated flies, for both types of movement, walking (recorded with the top camera) and flying (recorded with the side camera).

3.2.4. *Summary of results for caffeine study*

The caffeine study demonstrates that the system can be used to measure behavior changes as responses to drug dosage or to test toxic substances. Since the recordings are taken continuously, both stimulated and spontaneous activity can be measured using the same system. Moreover, using a two-camera system allows recording horizontal and vertical movement simultaneously, and provides additional information on behavior. Recordings taken immediately after administering the drug and followed over a period of time can indicate pharmacokinetic effects on locomotor activity.

3.3. *Frequency and hysteresis of locomotor events are temperature-dependent*

The measured temperature profile versus time is shown in Fig. 5A. The mean (±SE) inter-event-durations for low temperature, high temperature, low rate of temperature increase, and high rate of temperature increase were 1.12 (±0.049), 0.56 (±0.018), 1.45 (±0.11), and 1.17 (±0.085) s, respectively.

The event frequency as a function of time (calculated from the moving average inter-event-duration) is plotted versus temperature in Fig. 5B for both increasing and decreasing temperatures. The points for the rising temperature curve are shown in black and for the falling curve in gray.

The results of the temperature experiment are consistent with the effect of temperature on fly locomotion (Watson et al., 2001). Additionally, as shown in Fig. 5B, the activity levels had a hysteretic response to temperature, since the rate of change of temperature increase was different from the rate of decrease. The system can be also applied to measuring activity levels during circadian rhythm, which are temperature-dependent (Busza et al., 2007; Glaser and Stanewsky, 2007; Matsumoto et al., 1998; Miyasako et al., 2007; Wheeler et al., 1993; Yoshii et al., 2005).

3.4. *Discriminating power in a variable size population*

The mean (±SE) inter-event-durations are plotted versus number of flies in the dish in Fig. 6. All comparisons were statistically significant ($p \ll 0.001$) except for differentiating one fly from five flies in the dish.

This is a useful method to quantify the number of live and active animals housed together especially for counting in real time and eliminates the need for anesthetizing procedures, which are not ideal for behavioral analyses.

The population recordings can be used to monitor population behavior or size in either a local or remote, field environment. For example, a stimulus could be applied daily and the response of the

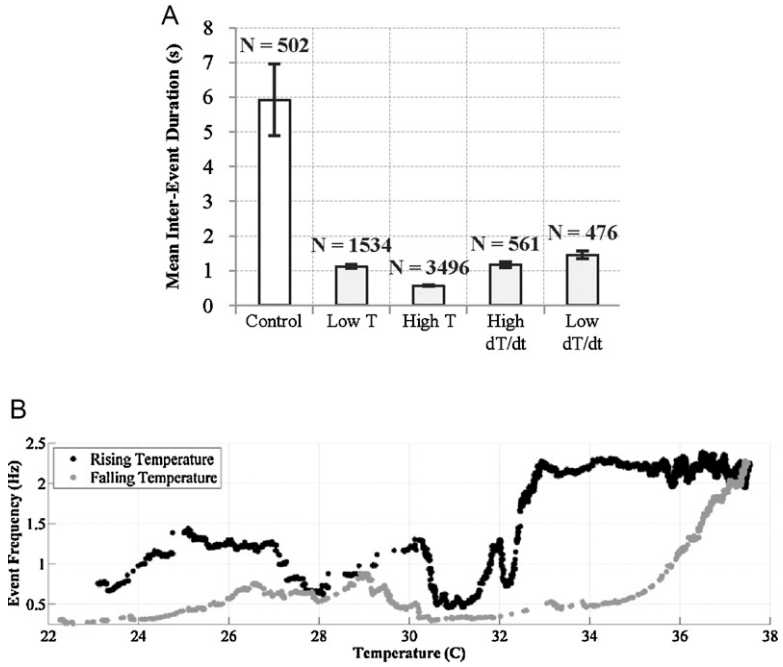

Fig. 5. Temperature-dependent locomotor activity. (A) The locomotor activity increases at low and high temperature and is dependent on the rate of change of temperature. Low temperature corresponded to 22 °C < T < 33 °C, and high temperature to 33 °C < T < 37.5 °C. High rate of temperature change corresponded to dT/dt = 0.8 °C/min and low rate of change to 0.2 °C/min. N = Total number of events. (B) Event frequency versus measured temperature for the flies. The rising and falling temperatures are shown in separate shades of gray to illustrate the hysteresis in the response to temperature.

flies could be measured for the subsequent 30–45 min. The stimulated responses over a period of time can be used for example to monitor mating behavior, or induced aging and lethality in a population of flies.

3.5. Multiple behavioral traits

We demonstrate that concomitant with detecting low-resolution locomotor events, this system can also discriminate high-resolution movements, which we label as "shaking" behavior (Fig. 7). The shaking index is calculated post-recording, and is defined as the RMS power of each baseline segment of the trace longer than 20 s and the overall shaking index is computed from the mean and standard deviation of all such segments from the entire recording file. This allows for the detection of more subtle movements than aggregate locomotor rate. Flies mutant for sdhB do not change their locomotor rate after hypergravity (Fig. 7A), but do respond by a decrease in the shaking index (Fig. 7B). The large number of signals computed from one recording allows us to calculate the statistical significance of this response, which would otherwise not be detectable or would require a large population of flies.

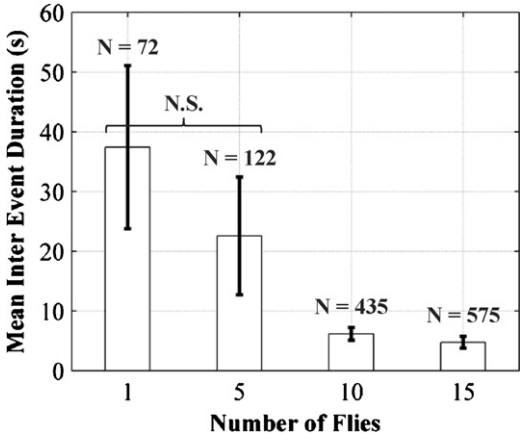

Fig. 6. The activity level is dependent on the population size. Mean inter-event-durations versus number of flies in the dish. Recordings were taken for 45 min. N = Total number of events. All differences were significant ($p \ll 0.001$) except the comparison of one fly to five (indicated as N.S. above).

3.6. Comparison to previous systems

The system presented here is a simple alternative to the existing options for monitoring *Drosophila* behavioral activity. The system is particularly well suited for applications requiring portable, standalone hardware, including laboratory and field experiments which require automated recordings. In these settings, the primary advantages in terms of practicality are inexpensive hardware, low cost, small size, and low data bandwidth.

It should be noted that the primary focus of this paper was to validate this system, and present several different measurement and analysis techniques that could be used to monitor fly behavior. Disclosing new scientific findings observed using this new system is a goal of future investigations. To confirm the data values obtained for each individual experiment (such as the response to hypergravity) multiple biological replications of the experiment would need to be carried out with the system described here.

Two key limitations of the current system are that individual flies cannot be tracked and speed of movement cannot be extracted. Tracking individual flies is possible either using sophisticated video processing algorithms available in expensive, bench-top systems or by segregating the flies such that only one fly is in a chamber. Neither of these options would be conducive to a portable application targeting population studies. Regarding the speed of movement, this variable may be encoded in the amplitude or the rise time of the peaks measurable with this system. Future work will focus on calibrating the system to determine the speed of movement of the flies using more advanced signal processing techniques.

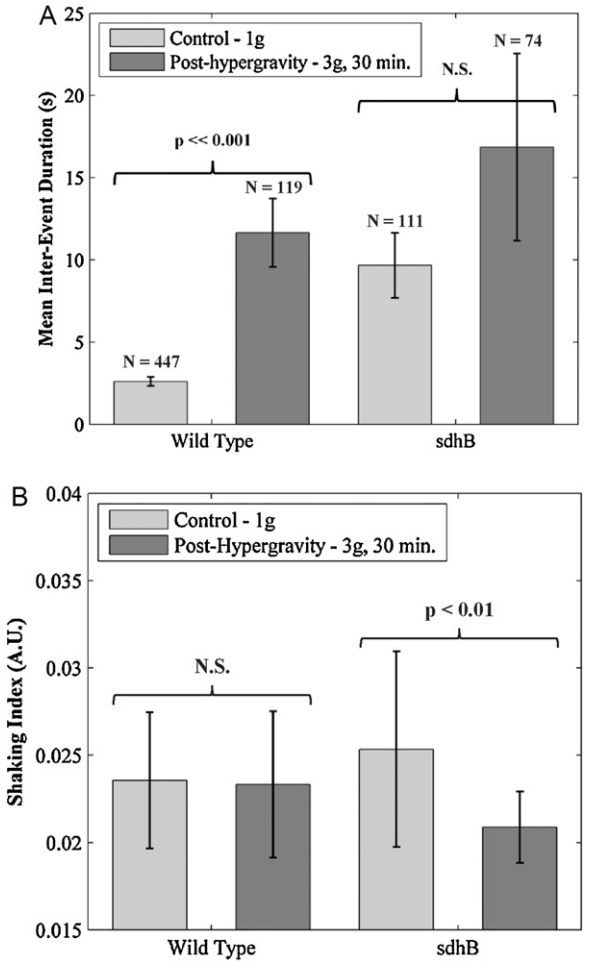

Fig. 7. Locomotor activity and high-resolution shaking activity. Post-recording analysis of the same flies can discriminate between the low-resolution locomotor events (A) versus high-resolution movements of individual flies (B). Hypergravity does not affect the locomotor activity of the sdhB mutants, but has a statistically significant effect ($p \ll 0.01$) on their shaking behavior. Shaking index is defined as the RMS power of each baseline segment of the trace longer than 20 s. Mean and standard deviation shaking index are computed over all such segments in the file to determine an overall shaking index for the file. Flies were exposed to 30 min of centrifugation at $3 \times g$.

Another potential limitation is that the light source must be held on for the entire duration of the recording. Because of the sensitivity of flies to light/dark cycles, this could pose a problem for longer duration recordings. However, to address this limitation, infrared (IR) LEDs can be used. These would still be detected by the camera, but not by the flies. Moreover, the system could be designed with both white and IR LEDs, with the circuit switching from white to IR at night, and from IR to white light in the morning.

The detection of locomotor (but not shaking) behavioral changes is dependent on flies entering/exiting the field of view of the camera, which thus needs to be smaller than the fly enclosure. However, the position of this field of view can be changed, e.g. the operator can position the camera towards the edge of the dish, rather than directly in the center, if the fly population aggregates towards the edge. Ideally, the results from one experiment would always include a control. It should be noted that in our experience with the system, positioning the camera either in the center, or at the edge of the dish, always provided high quality recordings.

This system provides the ability to extract biologically relevant quantitative measures of behavioral locomotor activity. The nervous system control of behavior is determined by the genetic inheritance and can be modified by external stimuli. This simple system is useful for recording behavioral traits which occur as adaptations to environmental cues, or behavioral changes which indicate metabolic activity, drug abuse, disease state or associated with mutations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2011.08.039.

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