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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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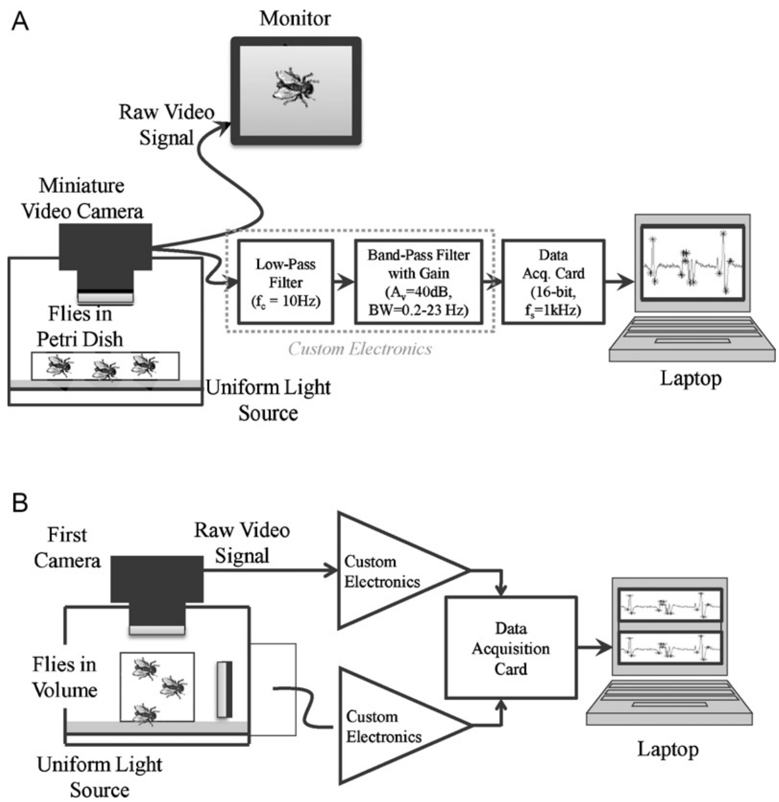

Fig. 1. Block diagram of the system. (A) Basic measurement setup for imaging flies. The analog circuit constituted a band-pass filter with gain, extracting only the low frequency light-level variations incident on the camera. A data acquisition card sampled the output of this circuit and the signals 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}).

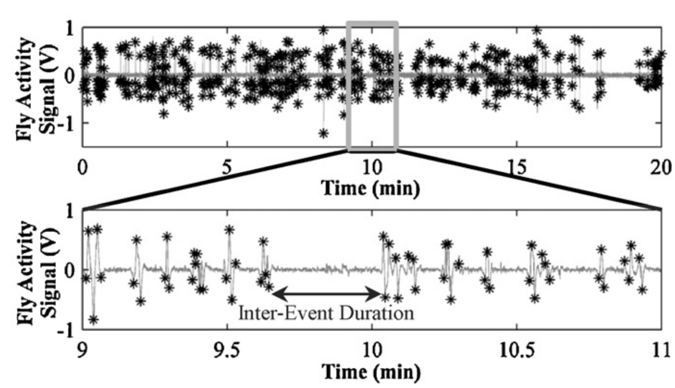

Fig. 2. Sample fly activity signal output of analog circuit. "Events" are denoted by black asterisks. The inter-event-durations were extracted from this signal, and examined to determine an overall activity level for the flies. The bottom trace shows a zoomed-in version of the signal, where the signal-to-noise-ratio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