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Computer automated movement detection for the analysis of behavior

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Abstract

Currently, measuring ethanol behaviors in flies depends on expensive image analysis software or time intensive experimental observation. We have designed an automated system for the collection and analysis of locomotor behavior data, using the IEEE 1394 acquisition program dvgrab, the image toolkit ImageMagick and the programming language Perl. In the proposed method, flies are placed in a clear container and a computer-controlled camera takes pictures at regular intervals. Digital subtraction removes the background and non-moving flies, leaving white pixels where movement has occurred. These pixels are tallied, giving a value that corresponds to the number of animals that have moved between images. Perl scripts automate these processes, allowing compatibility with high-throughput genetic screens. Four experiments demonstrate the utility of this method, the first showing heat-induced locomotor changes, the second showing tolerance to ethanol in a climbing assay, the third showing tolerance to ethanol by scoring the recovery of individual flies, and the fourth showing a mouse's preference for a novel object. Our lab will use this method to conduct a genetic screen for ethanol-induced hyperactivity and sedation, however, it could also be used to analyze locomotor behavior of any organism.

Keywords: Ethanol; *Drosophila melanogaster*; Tolerance; Locomotor behavior; Movement analysis; Genetic screen; Mammal

1. Introduction

Behavioral phenotypes are thought to be an emergent property of the nervous system. The measurement of animal behavior offers us a glimpse into the neural activity of the animal without the invasive drawbacks of inserting electrodes into the brain. We can observe movement to determine circadian rhythms, exploratory behavior, anxiety, ability and/or motivation to learn a link between two cues, ability to navigate a maze, and changes in locomotor behavior resulting from pharmacological manipulations. Although human observation can quantify such behavior, it is time-consuming, labor intensive and carries the risk of experimenter bias. To this end, using computers to automate the collection and analysis of data can be useful.

Our interest in movement analysis stems from our study of ethanol sedation in the fruitfly *Drosophila melanogaster* as a model for human intoxication. Initially upon exposure to ethanol vapor, flies exhibit a hyperactive phase, followed by incoordination and sedation (Moore et al., 1998). Lower doses of ethanol can elicit the hyperactive response without consequent sedation. Withdrawing the source of ethanol vapor allows the flies to gradually recover. This biphasic response (hyperactivity then sedation) seems to parallel humans, who show a loss of inhibition at low doses of ethanol that is overshadowed later by depressive effects. Flies can also develop rapid tolerance to ethanol sedation; with prior exposure 24 h earlier, a group of flies will recover from a sedating dose of ethanol faster than their naive counterparts (Cowmeadow et al., 2005).

Multiple techniques have been employed to measure ethanol intoxication in flies. Perhaps the most widespread is the inebriometer (Weber, 1988). It consists of a long vertical tube with a series of slanted mesh baffles; the flies cling to the baffles to avoid falling. As they become intoxicated, they lose postural control and fall down until they elute out the bottom of the apparatus. The mean elution time represents the ethanol sensi-

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tivity for a population of flies. Another method that has been used is the inebri-actometer (Parr et al., 2001). This apparatus consists of a set of 128 narrow tubes, equipped with photodiode emitter/detectors and connected in a grid to a computer. Ethanol vapor is pumped through the system and when a fly crosses the midpoint of its tube, the computer records the movement. A third method is to expose groups of flies to ethanol vapor while in vertical tubes and visually count the number of intoxicated flies at regular intervals (Wen et al., 2005; Cowmeadow et al., 2005). A fourth method, developed by the Heberlein lab, involves a sophisticated program called Dynamic Image Analysis System (DIAS). Flies are placed in a clear, shallow box and ethanol is pumped into the box while a camera above videotapes the flies. DIAS calculates the position of the flies and computes aspects of their movement such as bouts of activity, velocity and turning behavior (Wolf et al., 2002). These methods have identified a number of candidate genes that affect the actions of ethanol on flies, including *amn*, *barfly*, *tipsy*, *cex*, *ccb*, *vap*, *fasII*, *TβH*, *iav*, and *slo* (Moore et al., 1998; Singh and Heberlein, 2000; Scholz et al., 2000; Cheng et al., 2001; Scholz, 2005; Cowmeadow et al., 2005). In addition, these methods have identified the *npf* circuit and the cAMP pathway in insulin producing cells as being involved in ethanol behavior (Wen et al., 2005; Corl et al., 2005).

Though past work has yielded many tolerance and sensitivity mutants, the methods used have limitations. The inebriometer has been used most commonly in the past and is the best suited to screening large numbers of mutations. However, it can only measure the knockdown phase of intoxication (Leibovitch et al., 1995; Moore et al., 1998; Singh and Heberlein, 2000; Berger et al., 2004). As has been demonstrated with other assays, flies become hyperactive when exposed to ethanol before becoming sedated (Moore et al., 1998). The inebriometer is unable to separate the two effects; a fly may fall through the apparatus because it has lost consciousness or it may fall because its hyperactivity leaves it unable to grip the baffles. Hyperactivity and sedation phases likely represent an important distinction in the human ethanol response. The inebri-actometer (Parr et al., 2001) solves this problem but introduces another. Because there are multiple tubes feeding into the apparatus, extreme care must be exercised to ensure that each tube is conducting the same flow rate of ethanol vapor. In its first published study, one of the trial runs showed a significant row effect (Parr et al., 2001). Direct visual observation of the negative geotactic response and postural control has been used by multiple labs, including ours (Berger et al., 2004; Ghezzi et al., 2004; Wen et al., 2005; Cowmeadow et al., 2005). While this is certainly a thorough way to quantify sedation, it is also labor-intensive and therefore not well suited to the large volume of measurements inherent in a genetic screen. Thus, the greatest strength of *Drosophila* as a model system, the ability to perform high-throughput genetic screens, can be difficult to utilize in the study of ethanol responses because the assays are time-consuming and require individual attention. A natural solution to this problem is computer monitoring of behavior. To be effective, the approach should be inexpensive and scalable.

We have created a system that could be adapted to large screens and that has the longevity to be used by other labs in the future. For most responses to alcohol (sedation, tolerance, hyperactivity), a computer need only to detect whether movement has occurred or the relative amount of movement among a population in order to be useful. Other activity monitoring programs have been described in the literature. The image analysis programs DIAS and EthoVision have been used to document complex responses but unfortunately, these are not readily scalable (Wolf et al., 2002; Martin, 2004). Although developed independently, the proposed method is similar to these older methods in that all use the digital subtraction of images to determine when the animal moves (Hasegawa et al., 1988; Hoy et al., 1996; Cole and Cheshire, 1996). Some of these previous methods might have been able to meet our needs. Unfortunately, these previous programs are no longer available and all use proprietary software and/or hardware that no longer exists. The methods that we describe use only open source software tools and run interchangeably on different hardware platforms (we have used Mac OSX, Windows XP and Linux, although the data in this paper was all analyzed with a computer running Linux). Open source tools tend to have greater permanence than closed source since they are maintained by communities and they can be modified by the end user. It also is not limited to a single camera system or computer platform. It is readily available to the public, and can be modified by future users, provided that they have a general understanding of the programming language Perl.

In the proposed method, a camera records images of a group of flies at a regular interval and the images are analyzed to provide an estimate of the population movement at any given moment. The collection and analysis of data can proceed in an automated fashion. Unlike visual observation, a much larger quantity of flies can be tested with a relatively small investment of time and effort. The technique offers the ability to measure various aspects of ethanol intoxication, such as the hyperactivity phase, the knockdown to sedation, and the recovery from sedation. It can be implemented in a lab with relatively low start up costs; the software is free and the only required equipment is a standard computer and any camera capable of interfacing with that computer. The number of groups of flies that can be observed concurrently is limited only by the visual field of the camera. We plan to apply it towards a genetic screen, but with minor modifications it could be adapted to many situations where analysis of locomotor activity is needed, including studies with mammals.

2. Methods

2.1. Fly maintenance

Flies were raised on cornmeal/agar medium and newly eclosed flies were collected over a 2 day period and tested 5 days later unless otherwise noted. No anesthesia was used prior to behavioral experiments; transfer of flies was done using mouth-applied suction through a flypette (a trimmed yellow pipet tip shoved into a section of plastic tubing, with a small piece of nylon mesh acting as a barrier to prevent flies from being sucked through).