Automated Analysis of Feeding Behavior in Small Animals.

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Abstract

We discuss the implementation of an automated apparatus for recording and quantifying food choice behavior by small animals. A computer controls the apparatus by positioning a video camera over each one of twelve petri dishes. When the camera is in position, an image of the underlying dish is captured and the digitized image is returned to the computer for further processing. Within the computer a digital image processing procedure analyzes and quantifies the observed behavior.

INTRODUCTION

In this paper we describe the implementation and operation of an apparatus that is designed to record and analyze the feeding behavior of a small animal such as a caterpillar. The behavior studied here is driven by input from taste receptors; changes in peripheral sensory input will induce observable behavioral changes. There are a number of peripheral sensory modalities that can influence behavior, including chemosensory, the tactile, and visceral. Chemosensory inputs, particularly gustatory (taste), are of prime importance.

We have implemented a system capable of modeling the chemosensory induced behavioral changes of a plant-feeding caterpillar, the larval *Manduca sexta* [1, 2]. Eight taste neurons provide primary input to the feeding decision center of the CNS which produces the observable behavior response. A mathematical model encodes the relationship between the activity levels of peripheral taste receptors and the observable feeding behavior.

In previous work we developed subsystems that provide an estimate of the activity levels of the taste neurons. Extracellular recordings are obtained from two taste organs [3] containing the chemoreceptive neurons. The taste organs are termed sensilla, and each sensillum contains four different taste neurons. The electrophysiological recordings from these neurons spike trains that are analyzed by the signal processing subsystem. The signal processing subsystem is composed of a spike detection module [2] and a spike classification module [4], the output of which provides an estimate of the activity levels of the eight taste neurons.

To complete the mathematical model we must relate the estimated neuronal activity levels with behavior. This requires that we be able to quantify behavior. The current method for recording food-choice behavior [modified from 5] involves an operator periodically observing an insect food choice arena and estimating the amount eaten. Specifically, the insect is placed inside a large (12 cm) petri dish with samples of the test compound and a control applied to glass fiber disks that simulate leaf samples. At fixed time intervals, the operator determines consumption by visually estimating the surface area of each disk that has been eaten. The experiment is terminated when a criterion amount has been consumed or when a certain amount of time has expired. The natural variability of biological systems requires that a large number repetitions (~20) of each behavioral experiment be performed.

To eliminate the tedium of repeated visual observations and to minimize operator error, we have developed a device which automatically records and analyzes food choice behavior. The implementation of this device is the focus of this paper.

METHODS

The automated behavior analysis apparatus is composed of a microcomputer, a camera positioning device, image capturing hardware, and software which controls a CCD camera position, image capturing, and digital image processing (see Figure 1). We use an Apple Macintosh although any system with a SCSI port and a serial port can be used.

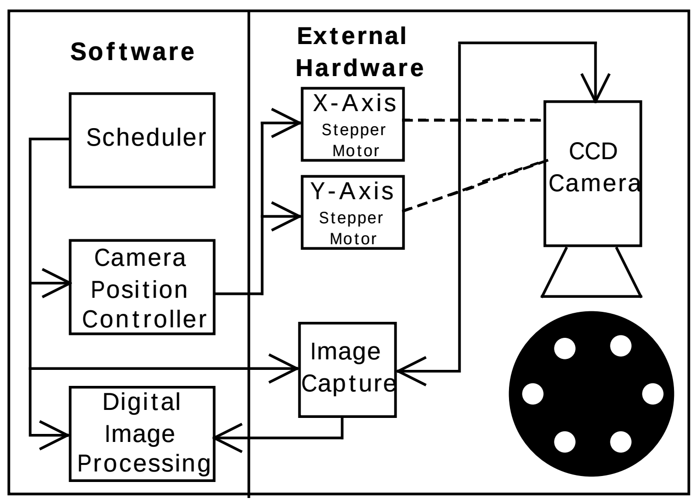

Figure 1: The behavior analysis apparatus is constructed from both software and hardware components; hardware is located outside the computer. The scheduling software module determines the timing of camera positioning, image capture, and processing of the digitized image. The camera is moved over each one of twelve dishes in the arena for image capture and processing.

The camera positioning device is essentially an X-Y table. A CCD camera is mounted on a movable platform which can be moved to any X-Y coordinate. Movement of the camera table is accomplished by two stepper motors, one to effect movement along each axis. The controller software communicates via a serial port with an Alpha DR-173 stepper motor driver. The stepper motor driver relays the desired number of steps to each of the two Alpha PD-123 power driver boards which then drive the two Oriental Motor PH-264 stepper motors. One of the stepper motors moves the platform along the X-axis while the second stepper motor provides the Y-axis positioning.

The current implementation permits observation and analysis of twelve experiments at the same time. The observation plane consists of an array of three rows (X-axis) of petri dishes with each row containing four columns (Y-axis) of dishes. Each dish contains six disks arranged in an alternating pattern of control and experimental compound just inside the perimeter of the dish (see Figure 2). The insect is initially placed in the center of the dish and is free to move to any location within the covered petri dish as it feeds on the discs.

Once the camera has been positioned over the correct dish an image can be captured and digitized. The image is captured by a Digital Vision ComputerEyes/RT image digitizer. The controller software communicates with the image digitizer through a SCSI port. The image is captured and digitized within the ComputerEyes/RT device which is external to the computer. The digitized image is transferred, via the SCSI port, to the computer's RAM for further processing. The first step is to apply a threshold algorithm to the gray scale image. The value of the threshold is obtained by analyzing the gray level histogram of the digitized image. The histogram will be bimodal since the background of the arena is flat black and the disks are white. This provides a maximal contrast between disk and background. The value of the threshold is set to the lower wing of the distribution that is associated with the white pixels. The gray level of each pixel is compared with the threshold value. If the pixel's gray level is greater than or equal to the threshold, the pixel's gray level is set to the maximum value of the gray scale; if the pixel's gray level is below threshold it is set to the minimum. The transformed image is now a binary image, with white pixels associated with the disk surfaces and black pixels assumed to be background.

Following the thresholding step, the image processing procedure estimates the remaining surface area of the disc, and the process is repeated for each of the six disks located within the petri dish being analyzed. Each disk is located at a known position within the dish. All of the disks' centers are located on a circle of fixed radius from the center of the dish. The first disk to be examined is located at the twelve o'clock position within the dish. The area of the disk is estimated by enclosing the disk within a square window and counting the number of white pixels within the area of the window. The length and width of the window are both equal to one and a half times the diameter of the disk. The window is large enough to permit some error in positioning but small enough to minimize noise (i.e., spurious white pixels). When the procedure has estimated remaining area of the current disk, the window is moved clockwise in 60 degree increments until all six disks have been analyzed.

A pass over all of the dishes is made at fixed time intervals (e.g., 10 min.). The areas of all disks are stored in an array during each pass and upon completion of the pass, the area of each disk is written to a data file for further processing. When the area of one of the disks has been diminished to a prescribed level the scanning process is terminated. The scanning process will also terminate after a given amount of time has expired irrespective of the surface areas of the disks.

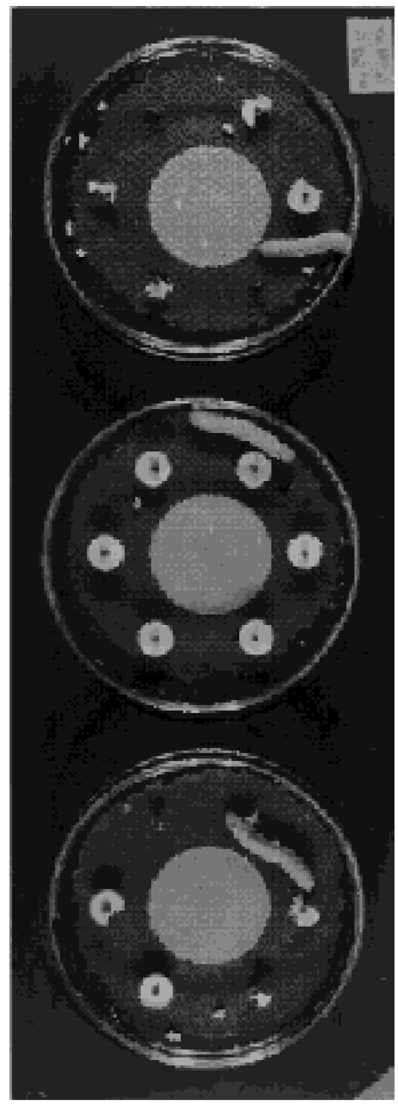

Figure 2: One of four columns each of which is composed of three rows of petri dishes. Each of the dishes contains six discs located at fixed positions; these can be seen in the middle dish. All three dishes contain caterpillars. The top and bottom dishes contain discs which have been consumed to various degrees.

Three of the six disks within a dish are saturated with a solution of the experimental compound and these are alternated among the three discs which are coated with the control compound. The relative amount of consumed surface area of the experimental versus the control is used to quantify the feeding behavior.

RESULTS

The performance of the automated behavior analysis apparatus has been evaluated for positioning accuracy and the repeatability of disk surface estimation. The accurate positioning of the camera platform is a function of the step size of the stepper motors. Each stepper motor's shaft rotates 1.8 degrees per step. Mounted on the shaft of each stepper motor is a gear which engages a drive chain which in turn is connected to the camera platform. This arrangement functions similarly to a rack-and-pinion, transforming the circular motion of the gear into the lateral motion of the platform. With this arrangement we are able to move the platform from the origin to within two millimeters of the desired location in a 60 x 45 cm field, sufficiently close for this application.

The second test evaluated the repeatability of the disk area estimation. This evaluation repeatedly positioned the camera platform over a dish containing discs but no insect, and calculating the area of the six disks within the dish. The camera platform was initially positioned at the origin and then moved to the dish near the middle of the array. An image of the dish was captured and the area of each of the disks was calculated. The platform was returned to the origin following the completion of the image processing procedure. This cycle was repeated 100 times, while processing the same dish containing the same disks. The disk area estimations were very consistent; the worst case was a deviation from the mean value of less than 0.3%.

DISCUSSION

We have described an apparatus that is designed to automate the process of quantifying food selection behavior of small animals. Although we are using large (3 - 5 cm long) caterpillars, the system could be easily scaled up or down to accommodate a wide range of sizes of animals. The apparatus improves the accuracy and reliability of the measurements and minimizes the between-operator variability.

The proper operation of the apparatus is highly dependent upon the ambient lighting conditions. Direct overhead lighting produces glare on the lids of the petri dishes resulting in erroneous estimates of the area for the underlying disks. We are in the process of designing and testing enclosures that will provide diffuse side-lighting to prevent glare.

Another source of error is that partially eaten discs are deflected downwards by the animal and thus is out of view of the camera. This leads to overestimation of consumption; methods to correct this problem are being evaluated.

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