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Directionally Selective Motion Detecting Units in the Optic Lobe of the Honeybee

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Summary. We have found four classes of neurons in the honeybee optic lobe. These neurons respond to changes in light intensity and selectively to movement of objects within the entire acceptance angle of a compound eye. We suggest that these neurons are part of the neural system that controls flight, for example the optomotor response. Properties of these units are described in this paper. To our knowledge this is the first report of recording from interneurons of the honeybee.

Introduction

Since the basic work of v. Frisch (1914), several investigators have studied the visual system of the honeybee by means of behavioral experiments. These workers have provided us with the knowledge that the honeybee has the ability to discriminate the wavelength and the plane of polarization of light.

Recent workers have reported some aspects of the physiological basis of these capabilities (for example see Autrum and v. Zwehl, 1964; Goldsmith, 1961, 1962; Gribakin, 1969; Dethier, 1963). These efforts have concentrated upon the structure and the spectral sensitivity of the individual photoreceptors of the compound eye.

Nothing is known about the processing of visual information in the central nervous system of the bee. In a complex structure such as the insect compound eye information is abstracted from the environment by means of interactions among groups of photoreceptors. Given the distribution and arrangement of primary photoreceptor types and their fine structure, one must study the properties of aggregates of photoreceptors in order to elucidate the principles by which the eye processes information and to determine the role the individual photoreceptors play in this processing. One way to do this is to monitor the activities of interneurons whose output reflect an integration of primary photoreceptor

activity. We feel that such a study is particularly relevant if the neurons studied are part of the neural system underlying a functional behavioral response.

We were able for the first time to record from high order interneurons of the visual system of the honeybee. Properties of these units are described in this paper.

Materials and Methods

Experimental Animals. Two colonies of honeybees (*Apis mellifera*) were maintained in an open field under natural conditions. One colony contained only wild-type animals; the other colony contained a pigment gene mutation "snow" which is expressed as an absence of shielding pigments in the retinas of some worker and drone bees. Only worker bees were used. Foraging wild-type worker bees were collected at the hive entrance and kept in a small cage until used in an experiment. White-eyed (snow mutant) workers do not forage. They were occasionally collected at the hive entrance, but usually one must open the hive to find them. While in the cage, all animals had a mixture of powdered sugar and honey available to them.

Preparation. For experiments the animals were removed from the holding cage and held in a glass test tube where they were temporarily immobilized with carbon dioxide gas. The legs and antennae were removed and the animal was affixed (using a hot wire) to a ball-joint stand with a glue made by melting and mixing (by weight) three parts resin (for violin) to one part beeswax. The head, thorax, and abdomen were held rigidly in place. Some respiratory movement was possible. The animal was allowed to recover for at least $^1/_2$ hour from the CO_2 treatment before the preparation was made. Access to the optic lobe was achieved either by removing a small portion of the exoskeleton from the back of the head or from the front of the head between the compound eyes. Air sacs were encountered in either approach; we removed as few air sacs as possible. Laboratory temperature was approximately 24° C in all experiments.

Electrodes and Recording. All recording was extracellular. Two types of metal electrodes were used: etched stainless steel insect pins (number 00) coated with Insul-x (Insl-x E33, Insl-x Products Corp., Yonkers, N. Y.), or etched tungsten wire (5 mil.) coated with Hysol (CG7-4225, Dexter Corp., Orleans, N. Y.). A platinum indifferent electrode was placed in a pool of Ringer solution (in 1 l H_2O: 0.2 g KCl, 0.2 g $CaCl_2$, 4.0 g dextrose, 9.0 g NaCl) in the opening made for recording. The diameter of the recording electrode was 1 micron or less at the tip.

Standard techniques were used for signal amplification and the data were either abstracted on line or stored on magnetic tape (Ampex FR-1300 FM recorder) for subsequent analysis.

Analysis. Average spike firing frequency was taken as indicative of neural response. Spikes were counted during certain post-stimulus intervals as indicated in the figure legends. This was achieved on-line with a counter (HP-524 C) or off-line with a general purpose computer (Lockemann and Knutsen, 1967).

Stimulation. A moving black and white striped pattern was used to stimulate the experimental animals. In most cases it consisted of a precision-made striped drum, 24 inches in diameter (61 cm) and 10.5 inches (26.6 cm) high. The animal was mounted in the center of this drum. The animal saw the black bars of the pattern and, through the openings between the bars, the inner white surface of a big globe (74 inches diameter, 1.88 m). The globe surface was illuminated by hundreds of

small incandescent lamps to provide a uniform illumination. The spatial wavelength of the pattern was 14.5 degrees in most cases. In order to stimulate only one eye a circular black screen between pattern and animal reduced the horizontal visual field to 150 degrees. Different drum speeds were presented non-systematically. Except for starting and stopping accelerations the drum speed was constant. In some cases (intensity curves) the moving pattern was provided by projecting a moving striped film onto the globe surface. The striped pattern then had a circular boundary. The globe surface was illuminated in these experiments only by the moving pattern. In this case light and movement were presented simultaneously, as the striped film was moving at the time the light was turned on (by a rotary solenoid and vane arrangement). Light flashes were provided by an arrangement of an incandescent lamp, electronically-controlled rotary solenoid and vane, and a fiber optic "light pipe".

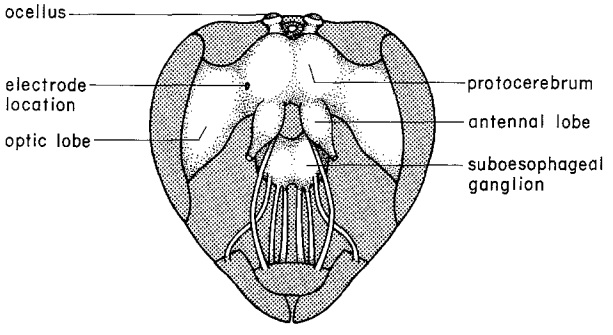

Fig. 1. Schematic frontal view of the head of the worker honeybee. Mark indicates approximate point of entrance of horizontal recording electrode in experiments in which the frontal approach was used. Drawing after Dade (1962)

Results

The units described below can be approached either from the front or back of the head. They were recorded in the wild type and in the white-eyed mutant worker honeybee. In Fig. 1 is shown a schematic frontal view of the head of the worker honeybee. A dot has been placed approximately at the point of electrode entrance in the frontal approach, which was the approach used most often. From the angle of entrance and depth of penetration in frontal and rear approaches we estimate that the active region of the recording electrode was just central to the lobula in the region between the lobula and the protocerebrum. We found recording difficult in the honeybee in two ways: (1) single unit recording was difficult to achieve, and (2) the units described here were difficult to find. On the average we made four preparations to achieve one useful single unit recording. Other units were recorded. As yet the properties of these units have not been determined.

The distinguishing feature of the responses of the units reported here is that they are excited by objects moving in certain directions in their

visual fields and inhibited by objects moving in opposite directions. All of these units were spontaneously active with a dark frequency of approximately 5—10 per second. The directional property of these units was contrast insensitive. For each unit there is an excitatory direction for which the response is maximum. Object movement in other directions exerts less excitatory (or inhibitory) effect. Movement response was not observed for object movement perpendicular to the direction of maximum response. With respect to their direction of maximum excitatory response four types of units were observed: (1) anterior to posterior, or into the page in Fig. 1, (2) posterior to anterior, or out of the page, (3) ventral to dorsal, and (4) dorsal to ventral. We refer to these as outward, inward, upward and downward units respectively. The visual fields of these units are monocular and either ipsilateral or contralateral. Units with contralateral fields were recorded most often and, by far, the unit recorded most frequently was the contralateral, inward unit. Hence, the detailed studies on intensity responses and dependence upon drum velocity were made with this unit.

The characteristics of the responses to flashes of light and to movement were similar for all types of units. These units responded phasically to light flashes. At intensity threshold the units responded at *on*. As light intensity was increased the *on* response increased (more spikes, longer burst) and an *off* response appeared. With further increase in light intensity the *on* response decreased and the *off* response increased. At high intensities the small *on* response remained, while the *off* response was intense and lasted several seconds.

The more interesting response is that to movement (Figs. 2 and 3). A multiunit recording is shown in Fig. 2. The larger unit was an inward unit; the smaller unit was opposite in response to the larger, i.e. it was an outward unit. A post-stimulus-time histogram of a single unit recording is shown in Fig. 3. In every case the globe was continously illuminated and the drum rotated with a constant velocity. All units responded in the manner shown in these two figures. After an initial transient increase in spike firing rate, there was maintained, but slowly decreasing activity. On a less compressed time scale the firing rate in any five or ten second period was relatively constant. At the cessation of motion in the excitatory direction there was a depression of activity followed by a slow return to the spontaneous rate. The duration of this depression increased with the intensity, and/or duration of the stimulus. Motion in the null direction reduced the spike activity, sometimes abolishing it at first. Following such depression, i.e. when the drum stopped moving, a rebound increase in activity occurred. Following this the activity was still slightly depressed and returned slowly to the spontaneous rate.

The dependence of response with light intensity is indicated in the data shown in Fig. 4. In this experiment the image of a moving striped

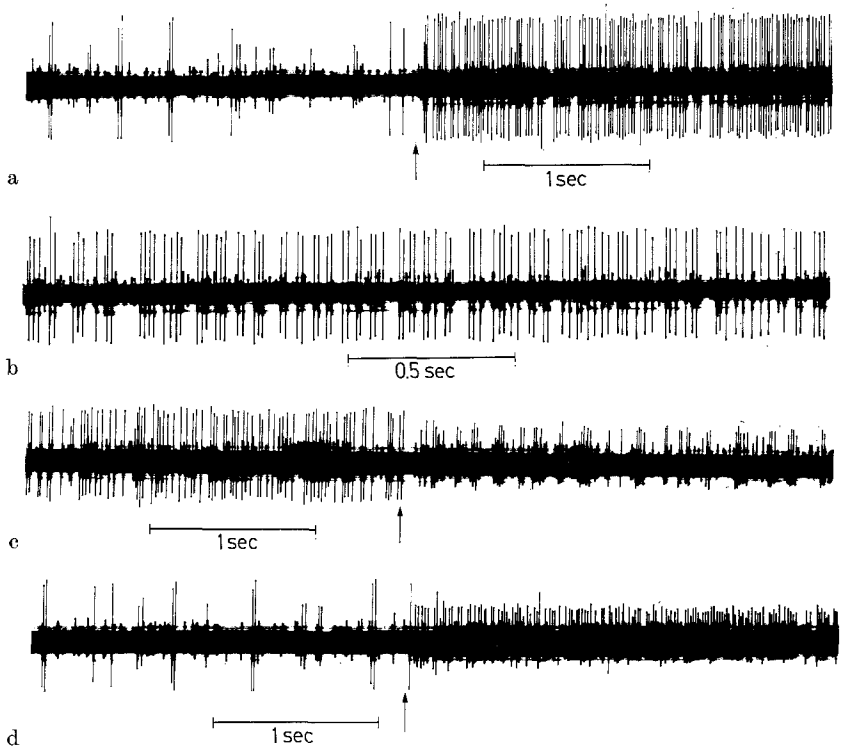

Fig. 2 a—d. Photograph of oscilloscope trace from a recording from a "medium" quality preparation. This record was chosen because it is a multiple unit recording. Both units had contralateral visual fields that extended over the entire eye. Luminance of globe surface was 2 lux. Spatial wave length of drum pattern: 36 degrees; contrast frequency: 2 c/sec. Simultaneous recording of inward (see text) unit (large spike) and outward unit (small spike): (a) Section of recording showing transition from pattern-still/light-on to pattern-moving/light-on conditions. Note grouping in spontaneous activity. Larger unit was excited, and smaller unit was inhibited; (b) Section of record during stimulation at contrast frequency of 2 c/sec.; different time scale due to higher film speed. Grouping into 2's, 3's, and occasionally 4's was consistantly observed; (c) Transition from pattern-moving/light-on to pattern-still/light-on condition. The large spike became smaller after firing at high frequency. Note post-stimulus inhibition of large unit. Post-inhibition excitation of smaller unit occurs, but cannot be seen very well on this time scale (see Fig. 3); (d) Transition from pattern-still/light-on to pattern-moving/light-on condition with pattern moving in opposite direction to that in (a). Smaller unit was excited and larger unit was inhibited

film was projected onto the dark surface of the globe. A shutter arrangement was used, so the stimulus was a combination of an increase in light intensity and the movement of a pattern. The response was taken as the number of spikes occurring during the stimulus period. The response-log

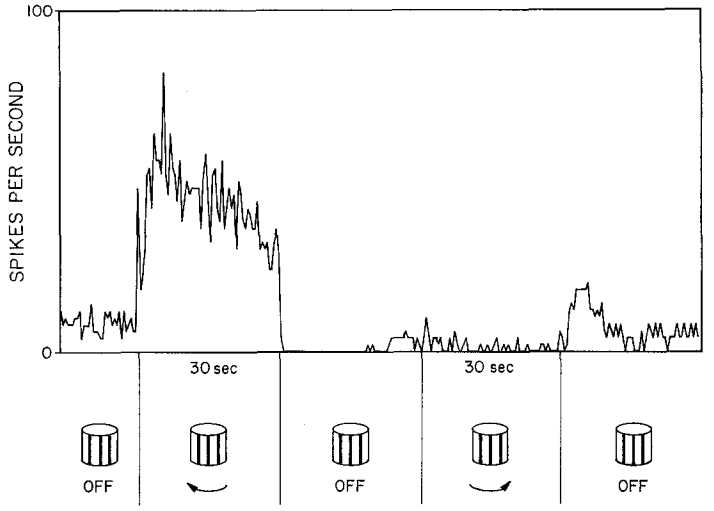

Fig. 3. Post-stimulus time histogram taken from single unit recording of a contralateral inward unit. Ordinate is number of spikes per second determined by counting the number of spikes in successive 0.5 sec intervals. Abscissa: time. Arrows on patterns indicate constant pattern (drum) velocity in direction shown. Light was on at all times. Spatial wave length, 14.5 degrees; contrast frequency, 3.6 c/sec; luminance at globe surface, 88 lux. All units showed the characteristics illustrated here: spontaneous activity, phasic response to motion, slowly declining response to constant velocity motion, post-excitation inhibition, inhibition in the null direction (sometimes total), post-inhibition excitation

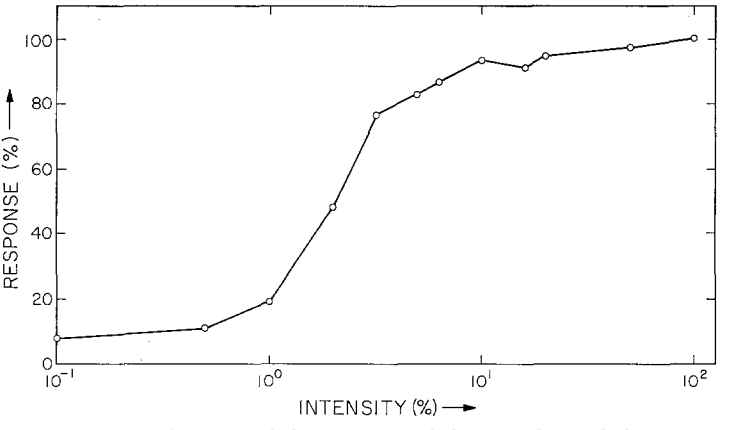

Fig. 4. Response as a function of the intensity of the stimulating light. Average of two curves from same unit. Animal dark adapted 15 min or longer. Contralateral inward unit. Stimulus was projected striped pattern with circular boundaries. Monochromatic light. $\lambda = 535$ nm. Light was turned on with pattern already in motion. The stimulus program was: 5 sec light-on, 15 sec light-off. Measurements were made from low to high light intensity. Ordinate: number of spikes during light-on, normalized to maximum response. Abscissa: log light intensity (relative units)

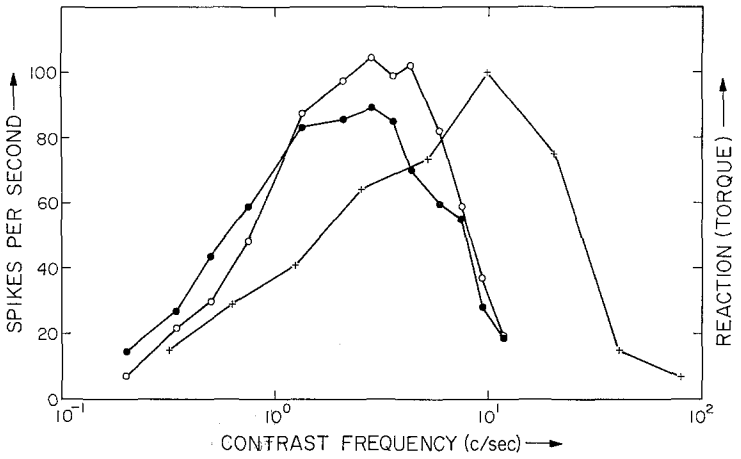

Fig. 5. Comparison of unit response with behavioral response. Pattern moved for 30 sec in excitatory direction and number of spikes counted in the last 20 sec; 30 sec pattern stationary. Globe illuminated continuously. —●— Average of 3 curves from the same stable unit. Globe luminance at surface 22 lux. —○— Single curve from the same unit, globe luminance at surface 88 lux. — + — Behavioral data (Kunze, 1961): Worker honeybee in tethered flight. Turning torque measurements on optomotor response. Spatial wavelength: 20 degrees.

intensity relationship is sigmoidal, and has a 1 log unit wide region in which the relationship is approximately linear.

In an exploratory investigation such as this, where there is no prior information concerning the function of single neurons, we feel it is more profitable, at least at first, to concentrate upon those units which appear to be part of the nervous system mediating a known behavioral response. For this reason, and since these units respond selectively to moving objects, we performed "optomotor" experiments with these units. For these experiments the globe was continuously illuminated. The drum was used as the stimulus; it was rotated at constant velocity for 30 sec in the excitatory direction at each of several velocities in a non-systematic way. Spike rate was averaged over the last 20 seconds. Typical results from such experiments are shown in Fig. 5 along with torque measurements made upon a worker honeybee held in tethered flight and presented a similar stimulus regime (Kunze, 1961). Kunze used several spatial wavelengths. His result for a spatial wavelength of 20 degrees is reproduced here because it is closest to the condition we used (14.5°). The curves obtained from single unit responses were all similar to each other and similar in shape to the torque curve. However, they all were displaced from the torque curve by the same amount. The torque curve has a maximum at a contrast frequency of 10 c/sec (Kunze, 1961) while the

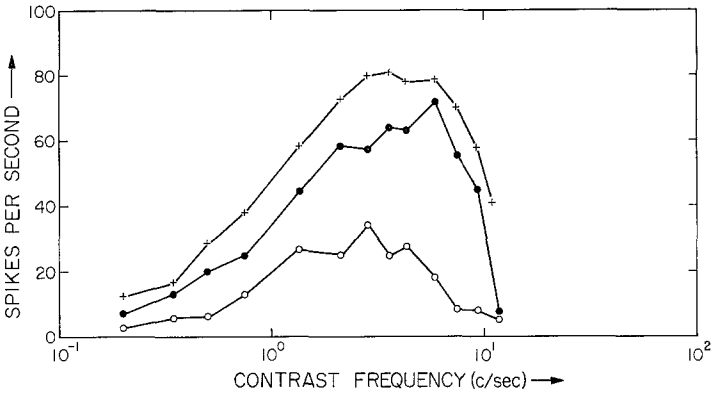

Fig. 6. Increase of response with time. All curves from same unit. Pattern moved for 35 sec in the excitatory direction. Spikes were counted for the last 20 sec. 55 sec pattern stationary. Continuous illumination at 2 lux at globe surface. Lower curve: measurement begun after 30 min adaptation. Middle curve: measurement begun 115 min after beginning of lower curve. Spontaneous firing rate during measurements of this curve was more than 2 times higher than the spontaneous rate during measurements of lower curve. Upper curve: measurement begun 150 min after beginning of measurement of lower curve. Spontaneous rate not measured

curves obtained from the single unit activity have a maximum at 2 to 3 c/sec.

If the responses of a unit are to be taken as an indicator or measure of a property or capability of a nervous system, it is important that the stability in time of the responses is known. Hence, we measured variability of the responses of these units. We found that the responses did change in time. This change was particularly evident under conditions of stimulation that did not drive the units very hard. Such a situation is illustrated in Fig. 6. The experimental situation for this experiment was the same as that in the experiment illustrated in Fig. 5, except that the light intensity was very low (2 lux at the globe surface). Under this light condition the system was not driven hard as evidenced by a low spontaneous rate and a low response initially. As time passed both the spontaneous rate and the response increased. This was not due to adaptation (see time values in legend of Fig. 6). It appears also that the point of maximum response drifted slightly toward a higher value of contrast frequency. These phenomena were present to a smaller degree at higher light levels.

Discussion

It appears that in the optic lobe of the worker honeybee there is a system of at least four different units that are specialized to encode the *direction of a moving object*. Each of these units has a continuous

spontaneous activity that is augmented maximally by movement in one direction and is inhibited by movement in the opposite direction. They are then two-way movement detectors. Movements in directions of the main two-way axis elicit lesser effects (excitatory and inhibitory). Hence, movement in any given direction is signalled simultaneously by four types of units, i.e. by increased activity in two types of units and decreased activity in two types of units. Since the response of the individual unit is a function of three known variables: direction of movement, light intensity, and speed of pattern movement, the only accurate information an *individual* unit can give is whether the movement occurred in the excitatory or inhibitory direction. Yet, the animal can get precise information about the direction of the movement by a simultaneous comparison of the responses of the four different types of directionally sensitive units.

Each of these units retains its unique directional selectivity over one entire compound eye. Each of these units has been recorded with a contralateral or ipsilateral visual field. Further, each has been recorded by placing the recording electrode in the same general area of the optic lobe, often observing more than one type simultaneously, including those with contralateral and ipsilateral visual fields. From our experiments we cannot tell whether information from each of the two compound eyes is integrated at this point in each optic lobe, or whether we were recording from an area in which traffic was ongoing in two directions.

A system of four directionally selective units such as this must certainly be involved in the control of flight. But, are these units "optomotor" units ? Certainly they signal the kind of information necessary to the optomotor response. However, the dependence upon contrast frequency of these units (Fig. 5) is displaced toward shorter contrast frequencies compared to the contrast frequency dependence on torque measured by Kunze. Kunze's experiments were different from ours in the following ways: the animals were flying (for short periods only), the pattern presentation was different (a moving striped pattern was viewed through slits in a stationary drum), there was less pattern contrast and higher light intensity. According to Kunze (personal communication) the increase in body temperature caused by flying has a negligible effect, but the displacement of our curves can be at least partially accounted for by the physical effects of lower light intensity and greater pattern contrast. We cannot say definitively whether these are important differences in experimental conditions.

On the other hand, the difference in the two results is suggestive. If these units are involved in encoding the optomotor response, as it is defined by torque measurements, they apparently signal only partial information, since they do not respond over the entire range of contrast frequencies pertinent to the behavioral response. In fact, the reponse of these

units is almost zero at the contrast frequency that evokes maximum response in the torque experiments. Another set (or sets) of units with response to higher contrast frequencies is needed to cover the range of contrast frequencies to which the animal can respond. Further exploration in the nervous system may reveal such a set. The presence of such a set would provide the system with unique *determination of object velocity.* There is experimental evidence that the worker honeybee can determine groundspeed (Heran, 1955, 1957).

Kaiser (1968) and Bishop (1969) could not find specific *color effects* as well in the flying optomotor reaction as in the responses of motion detecting interneurons of the fly. Since the color vision of the bee is very well known from behavioral experiments (v. Frisch, 1914; Daumer, 1956; Menzel, 1967), it would be of interest to look for encoding of color information in the motion detecting units described in this paper. Experiments concerning color and polarization information are in progress.

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