JOURNAL OF NEUROPHYSIOLOGY
Vol. 69, No. 2, February 1993. *Printed in U.S.A.*

Neural Circuit Tuning Fly Visual Interneurons to Motion of Small Objects. I. Dissection of the Circuit by Pharmacological and Photoinactivation Techniques

ANNE-KATHRIN WARZECHA, MARTIN EGELHAAF, AND ALEXANDER BORST
Max-Planck-Institut für biologische Kybernetik, W-7400 Tübingen, Germany

SUMMARY AND CONCLUSIONS

1. Visual interneurons tuned to the motion of small objects are found in many animal species and are assumed to be the neuronal basis of figure-ground discrimination by relative motion. A well-examined example is the FD1-cell in the third visual neuropil of blowflies. This cell type responds best to motion of small objects. Motion of extended patterns elicits only small responses. As a neuronal mechanism that leads to such a response characteristic, it was proposed that the FD1-cell is inhibited by the two presumably GABAergic and, thus, inhibitory CH-cells, the VCH- and the DCH-cell. The CH-cells respond best to exactly that type of motion by which the activity of the FD1-cell is reduced. The hypothesis that the CH-cells inhibit the FD1-cell and, thus, mediate its selectivity to small moving objects was tested by ablating the CH-cells either pharmacologically or by photoinactivation.

2. After application of the γ-aminobutyric acid (GABA) antagonist picrotoxinin, the FD1-cell responds more strongly to large-field than to small-field motion, i.e., it has lost its small-field selectivity. This suggests that the tuning of the FD1-cell to small moving objects relies on a GABAergic mechanism and, thus, most likely on the CH-cells.

3. The role of each CH-cell for small-field tuning was determined by inactivating them individually. They were injected with a fluorescent dye and then ablated by laser illumination. Only photoinactivation of the VCH-cell eliminated the specific selectivity of the FD1-cell for small-field motion. Ablation of the DCH-cell did not significantly change the response characteristic of the FD1-cell. This reveals the important role of the VCH-cells in mediating the characteristic sensitivity of the FD1-cell to motion of small objects.

4. The FD1-cell is most sensitive to motion of small objects in the ventral part of the ipsilateral visual field, whereas motion in the dorsal part influences the cell only weakly. This specific feature fits well to the sensitivity of the VCH-cell to ipsilateral motion that is most pronounced in the ventral part of the visual field. The spatial sensitivity distribution of the FD1-cell matches also the characteristics of figure-ground discrimination and fixation behavior.

INTRODUCTION

When an animal passes a nearby object, the retinal images of the object and its background move relative to each other, leading to motion discontinuities in the retinal motion field. These discontinuities can be used to discriminate objects from their background. The importance of relative motion as a visual cue for figure-ground discrimination is illustrated by the fact that many animal species are able to detect objects that differ from their surroundings only by the velocity at which they move (e.g., flies: Egelhaaf et al. 1988; bees: Srinivasan et al. 1990; locusts: Collett and Paterson 1991; primates: Miles and Kawano 1987; Regan and Beverley 1984). Accordingly, neurons that respond best to small objects moving relative to their background are found in various phylogenetically divergent animal groups (e.g., hawkmoths: Collett 1971, 1972; dragonflies: Olberg 1981, 1986; hoverflies: Collett and King 1975; blowflies: Egelhaaf 1985b; toads: Tsai 1990; pigeons: Frost and Nakayama 1983; Frost et al. 1988; cats: von Grünau and Frost 1983; monkeys: Allman et al. 1985; Tanaka et al. 1986). Despite the widespread significance of object detection by relative motion, not much is known so far about the underlying cellular mechanisms. Here we investigate the synaptic interactions leading to the selectivity of a particular visual interneuron in the fly for small-field and relative motion.

Flies are well suited for such a project. *1*) They are able to track moving objects in free flight (Land and Collett 1974; Wagner 1986; Wehrhahn 1979). They also detect and fixate objects that differ from their background only by motion under tightly controlled stimulus conditions in tethered flight (Bülthoff 1981; Egelhaaf 1985a; Reichardt et al. 1983, 1989; Reichardt and Poggio 1979; Virsik and Reichardt 1976). *2*) The major neurons in their main center of motion computation are amenable to electrophysiological analysis and can be identified individually on the basis of their invariant anatomic structure and response characteristics (Hausen 1981, 1984; Hausen and Egelhaaf 1989). *3*) Moreover, by comparing behavioral and electrophysiological data obtained under the same stimulus conditions, some of these neurons, the so-called figure-detection or FD-cells, have been concluded to play a decisive role in mediating figure-ground discrimination behavior (Egelhaaf 1985a–c, 1987; Egelhaaf et al. 1988; Reichardt et al. 1989).

The main center of motion computation in the fly is the posterior part of the third visual neuropil, the lobula plate. Like the other ~50 so-called tangential neurons in this neuropil, the FD-cells are presumed to spatially integrate with their almost planar dendritic arborization over a large array of retinotopically organized local motion-sensitive elements (Egelhaaf 1985b). Owing to this input, they respond directionally selective to motion in large parts of the visual field. The FD-cells, however, differ from the other tangential cells in that they respond more strongly to small objects than to spatially extended stimulus patterns. Thus the FD-cells' response is reduced during large-field motion, al-

though a larger number of local motion-sensitive elements is activated by this type of stimulus than when only a small object moves in their receptive fields. Consequently, spatial integration over retinotopically organized local motion-sensitive elements does not suffice to explain the response characteristics of the FD-cells.

To investigate the mechanisms leading to small-field selectivity, only one type of FD-cell, the FD1-cell, is considered here. This cell type responds best to unilateral front-to-back motion of objects that are relatively small (angular width, 10–40°) compared with the fly's panoramic visual field (Egelhaaf 1985b). However, this cell type is not only activated when an object moves in front of a stationary background but also, depending on stimulus parameters such as object size direction and velocity, when the ground moves relative to the object (Egelhaaf 1985b). The specific tuning of the FD1-cell to small-field and relative motion has been concluded to be mediated by the inhibitory action of one or several elements that respond best to binocular clockwise large-field motion and are inhibited by motion in the opposite direction (Egelhaaf 1985b,c). Two cells were found with the same receptive fields and the same tuning to small-field and relative motion, which, however, differ with respect to their output regions (Egelhaaf 1985b). Because they can only be discriminated anatomically but not by their physiological characteristics, they will both be collectively referred to as FD1-cell.

There are several lobula plate tangential cells that have the appropriate preferred direction to act as the elements that inhibit the FD1-cell during large-field motion. Two HS-cells (HSN-, HSE-cell) as well as the two CH-cells (DCH- and VCH-cell) in the right half of the brain respond strongest to binocular clockwise large-field motion (Eckert and Dvorak 1983; Hausen, 1976a,b; 1982a,b). Of these the CH-cells are the most plausible candidates because they have been suggested to be GABAergic (Meyer et al. 1986) and, thus, most likely inhibitory elements.

In the present study the hypothesis was tested that the CH-cells represent the large-field inhibitor of the FD1-cell and thus mediate its small-field selectivity. Primarily, this was done by inactivating the CH-cells in two ways. *1*) Extending preliminary experiments (Egelhaaf 1990), the γ-aminobutyric acid (GABA) antagonist picrotoxinin (Benson 1988; Rauh et al. 1990) was injected into the hemolymph, and it was investigated whether this eliminates the sensitivity of the FD1-cell to small moving objects. Application of picrotoxinin, however, was expected to change, in addition, the direction selectivity of the FD1-cell, because GABA is involved in the mechanism of motion detection in flies (Egelhaaf et al. 1990; Schmid and Bülthoff 1988). *2*) Candidates for the large-field inhibitor were individually ablated by the photoinactivation technique (Miller and Selverston 1979; Selverston and Miller 1980). Here, single neurons are injected with a fluorescent dye and then killed by irradiating the preparation with the dye's excitation wavelengths. The almost planar arborization of the tangential cells in the lobula plate of the fly close to the surface of the brain allows their exposure to intense light without much tissue dissection and thus to easily apply the photoinactivation technique.

Part of the results have already been published in a brief contribution (Warzecha et al. 1992).

METHODS

Preparation

Experiments were performed with 1- to 5-day-old female blowflies (*Calliphora erythrocephala*). The animals were anesthesized briefly with CO_2 and mounted ventral side up with wax on a small piece of glass. The legs were amputated and the wounds sealed with wax. The head capsule was opened from behind, and the trachea and air sacs were removed to gain access to the lobula plate. To avoid desiccation of the brain, the head capsule was supplied with Ringer solution (for a formula see Egelhaaf 1985b). For intracellular recording, movements of the brain caused by peristaltic contractions of the esophagus were prevented by cutting away the proboscis and pulling out the gut. The animals were adjusted in the stimulus apparatus according to the optics of their eyes by using the symmetry of the deep pseudopupil (Franceschini and Kirschfeld 1971).

Electrophysiological recording

For extracellular recording, glass capillaries (Hilgenberg; 1.5 mm OD, 1.17 mm ID) were pulled on a vertical puller (Getra). Filled with 1 M KCl, they had resistances of 3–14 MΩ. For intracellular recording, glass micropipettes (Clark, Electromedical; GC100F-10) were pulled on a Brown-Flaming puller (P-80, Sutter Instruments). The tips of these electrodes were filled with a saturated solution of the fluorescent dye 6-carboxy-fluorescein (Sigma) dissolved in 1 M potassium-acetate. Electrode shafts were filled with 1 M potassium-acetate resulting in resistances between 40 and 90 MΩ.

Recorded signals were amplified by the use of standard electrophysiological equipment. Extracellularly recorded spikes were transformed into pulses of fixed height and duration. For further data analysis these digital signals were fed into an IBM AT computer through the digital input of a I/O-card (DT 2801-A, Data Translation) at a rate of 1.62 kHz. The graded potentials of the HS- and CH-cells were recorded intracellularly, and, after being amplified, signals were fed to the computer through an analog-to-digital converter at a sampling rate of 930 Hz. The programs for data acquisition and evaluation as well as for the control of the stimulus movements were written in ASYST (Keithley Instruments).

Visual stimulation

For visual stimulation, vertically oriented square-wave gratings were generated by an image synthesizer (Picasso, Innisfree) at a frame rate of 200 Hz and displayed on monitors (Tektronix 608). In all experiments cells were recorded in the right half of the brain so that clockwise motion corresponds to motion in the preferred direction for the FD1-cell as well as for the HS- and CH-cells. The screen (or screens, see below) in front of the right eye of the fly was subdivided along its horizontal axis into two areas that could be controlled independently: a window ("figure") with a horizontal extent between 13° and 44° corresponding to the stimulus area where small-field motion was presented to the fly and the remaining part of the screen ("background"). For each recorded FD1-cell, that figure width was chosen that excited the cell most strongly. Only figures with a width of integral multiples of one spatial period were used to prevent modulation of the mean intensity. Throughout the text, "ipsilateral," "contralateral," or "binocular" large-field motion means that the entire grating pattern of

the right, left, or both monitors moved coherently. Patterns were moved for 1 s at a temporal frequency of 2 Hz.

In the following, all specifications of the azimuthal position refer to the frontal midline of the fly in the sagittal plane that is defined as 0°. Positions with positive (negative) values lie on the right (left) side of this reference point as seen from the fly. Specifications of the elevation refer to the equatorial plane (0°). Positions that are above or below the equatorial plane are given as positive or negative values, respectively. Monitors were mounted asymmetrically to the sagittal plane to position the figure in the area between −10 and +40° where the FD1-cell is most sensitive (Egelhaaf 1985b). Three stimulus conditions were used in the different experiments. By all of them, the DCH-, VCH-, and HSE-cell could be stimulated strongly [compare position of the screens with receptive fields of the VCH- and DCH-cell (Egelhaaf et al. 1993) and the HSE-cell (Hausen 1982b)]. In *stimulus condition 1* two CRT screens were mounted in front of the fly's eyes, one on each side. Position of the center of each screen was as follows (azimuth, elevation): right screen: +25°, 0°; left screen: −45°, 0°. Orientation of the screens was perpendicular to the horizontal plane of the animal. The angular extent of each screen was 68 and 81° (horizontal and vertical, respectively). The horizontal position of the small-field stimulus was −4 to +9° or −4 to +16°. Spatial wavelength at the center was 7.8°, and contrast was 92.5%. In *stimulus condition 2*, three monitors were used, one in front of the left and two in front of the right eye mounted one on top of the other. Position of the left monitor was as follows: position of its center was −59 and −6° (azimuth and elevation, respectively); orientation was perpendicular to the horizontal plane; angular extent was 60 and 50° (horizontal, vertical respectively); spatial wavelength at the center was ~12°; and contrast was 81%. For the right screens, the position of their centers (azimuth, elevation) was as follows: upper screen: +24°, +44°; lower screen: +24°, −36°. The right upper screen was tilted forward out of the vertical by 16°; the lower screen was tilted backward by 18°. The angular extent (horizontal, vertical) for the upper monitor was 55°, 41°; the lower monitor was 57°, 45°. The horizontal size of the small-field stimulus was as specified in the figure legends. Spatial wavelength at the center was ~12°, and contrast was 81%. *Stimulus condition 3* was as stimulus condition 2, only the position of the right screens was changed. Position of their centers was as follows (azimuth, elevation): upper screen: +24°, +4°; lower screen: +24°, −58°. Orientation of the upper screen was tilted forward out of the vertical by 6.5°; lower screen was tilted backward by 32°. The angular extent (horizontal, vertical) of the upper monitor was 42°, 58°; that of the lower monitor was 54°, 39°. In the experiments where the spatial sensitivity distribution is measured (Fig. 5), both screens were subdivided into two equal areas one on top of the other. The resulting four windows were centered at the vertical positions +15, −5, −47, and −67.

Experimental procedure

IDENTIFICATION OF THE FD1-CELL. In all experiments the activity of the FD1-cell in the right lobula plate was recorded extracellularly to obtain recording times that would suffice for the ablation experiments. Consequently, no anatomic criteria could be used for identification. To distinguish the FD1-cell from other lobula plate neurons, only those cells were taken into account that met all of the following functional characteristics (Egelhaaf 1985b): *1*) maximum sensitivity to small-field motion from the front to the back in the frontolateral part of the ipsilateral visual field (i.e., approximately −10° to +40°); *2*) reduction of the response to small-field motion during binocular clockwise large-field motion; and *3*) no reduction in response amplitude during contralateral counterclockwise motion. Some of the recorded cells were excluded because they met only part of these strict requirements but, nevertheless, might have been FD1-cells (for a discussion of this point, see Egelhaaf et al. 1993). This identification procedure does not allow to discriminate between the different morphological types of FD1-cells. As the results will demonstrate, this has no functional impact on the neuronal mechanism tuning FD1-cells to small-field motion (see DISCUSSION).

PHARMACOLOGICAL EXPERIMENTS. One hundred to 150 nl of a 1-mM picrotoxinin solution (Sigma) were injected into the hemolymph above the right lobula plate. The response of the FD1-cell to small-field and large-field motion together with its spontaneous activity was recorded before and after application of picrotoxinin until the signal-to-noise ratio was too small to discriminate spikes reliably. The mean recording time was 25 min, the longest recording lasted for ~70 min.

PHOTOABLATION. One of three identified tangential neurons (HSE-, VCH-, DCH-cell) in the right half of the brain was iontophoretically filled with 6-carboxy-fluorescein (−1.7 to −4 nA for 11–45 min). At this stage of the experiment, the identification of the cells was based on their characteristic physiological responses to motion stimuli. Then, in the same fly, the FD1-cell was probed, and, after characterization of its normal response to small-field and large-field motion, the injected cell was killed by illuminating part of the right lobula plate and the protocerebrum with a laser beam (Argon laser, Ion Laser Technology ILT Model 5425 AWC, 25 mW, between 459 and 515 nm) for ~2 min. The laser beam was directed on the fly's brain with the use of two mirrors and was focused by a convex lense (f = 40 mm, mirrors and lense from Spindler & Hoyer). An orange filter (OG 590, Schott) was mounted in front of the dissection microscope (Zeiss, OPM1). This allowed us to view the cells during their photoinactivation and to identify them also anatomically.

In control experiments the specificity of the technique was tested. *1*) Laser illumination of the lobula plate up to 10 min did not lead to a persistent change in the response of unstained lobula plate tangential cells (n = 2). *2*) Changes in membrane potential of tangential neurons filled with the dye were recorded intracellularly while illuminating the brain with the laser beam. During and after laser illumination that lasted ~2 min, cells depolarized and stopped responding to motion stimuli (n = 4). To ensure that the tip of the electrode was still inside the cell after photoinactivation, the dye was injected once more. In two experiments, cells could be filled again demonstrating that they actually had been inactivated.

Data analysis

The small-field selectivity of the FD1-cell is defined as

$$I_{SF} = (R_{SF} - R_{LF})/(R_{SF} + R_{LF} - 2 * R_{Spont})$$

where R_{SF} is the response to small-field motion from front to back, R_{LF} is the response to binocular clockwise large-field motion, and R_{Spont} is the spontaneous activity.

Under all three stimulus conditions, the small-field selectivity of the FD1-cell was not very pronounced [I_{SF} between 0.18 and 0.4 as compared with a mean I_{SF} ~0.53 as found in a previous study (Egelhaaf 1985b)]. This difference is likely due to the smaller size of the CRT screens on which the large-field stimulus was displayed. Despite this limitation, CRT screens were used, instead of an oscillatory drum (Egelhaaf 1985b), because they allowed to control the stimulus parameters electronically and thus make them more easily changeable.

Spike-frequency histograms shown in Figs. 1 and 3 were smoothed with a Gaussian-like function [Blackman's "lucky-guess" function, cut-off frequency: 14.3 Hz (Astheimer 1989)].

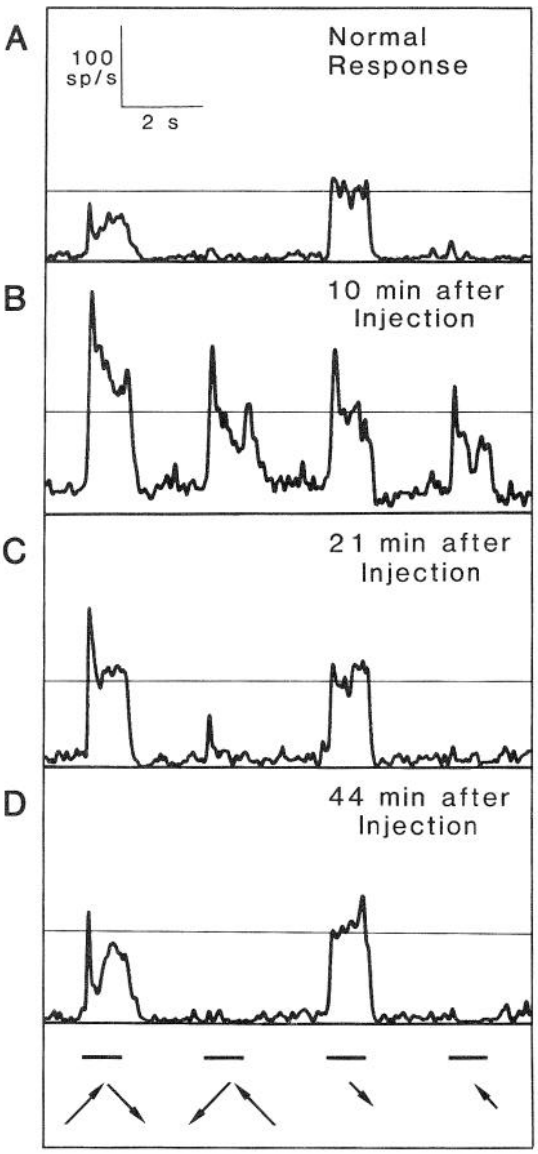

FIG. 1. Effect of picrotoxinin on the motion-induced responses of the FD1-cell in the right half of the brain. The fly was stimulated either by binocular large-field motion or by ipsilateral small-field motion within a 20°-wide window in the preferred (clockwise) or null direction as is indicated by the arrows in the stimulus trace at the *bottom*. Exact position of the screens is given by stimulus condition 1 (see METHODS). Horizontal scale bars in the stimulus trace specify the duration of motion (1 s). Spike-frequency histograms were averaged over 5 stimulus presentations and are shown before and in 3 time intervals after picrotoxinin application (the beginning of the interval is indicated in the figure). The horizontal line in the histograms indicates the mean response amplitude to small-field motion in each time interval. *A*: under normal conditions the FD1-cell responds more strongly to small-field than to binocular large-field motion in the preferred direction. Motion in the opposite direction does not activate the cell. *B*: 10 min after injection of picrotoxinin, the cell responds with a larger amplitude during clockwise large-field than during small-field motion. The cell is also activated by motion in its null direction. Spontaneous activity as well as maximum activity are increased. *C*: FD1-cell has recovered its normal directional selectivity and spontaneous activity level but still responds with a larger amplitude to large-field than to small-field motion. *D*: FD1-cell responds more strongly again to small-field motion. Its overall activity is still slightly increased. This experiment suggests that a GABAergic mechanism is responsible for the small-field tuning of the FD1-cell.

RESULTS

Elimination of small-field tuning by picrotoxinin

The mechanism that is responsible for tuning the FD1-cell to motion of small objects was tested first by injection of the GABA antagonist picrotoxinin into the hemolymph above the lobula plate. This was expected to block the inhibitory action of the GABAergic CH-cells and thus to eliminate the small-field tuning of the FD1-cell. The spike-frequency histograms in Fig. 1 show a characteristic example of the responses of the FD1-cell in the right lobula plate before (Fig. 1*A*) and after picrotoxinin has been applied (Fig. 1, *B–D*). Before injection of picrotoxinin, the cell responds in its normal way, i.e., most strongly to ipsilateral small-field motion from front to back. Simultaneous clockwise large-field motion reduces the response. During small-field back to front motion as well as during binocular counterclockwise motion, no inhibition of the FD1-cell can be seen, although it is known from intracellular recordings that the FD1-cell receives inhibitory input during motion in its null direction (Egelhaaf 1985b). This inhibitory input is likely to remain hidden in the present extracellular recordings because of the low spontaneous activity of the FD1-cell (<15 Hz). As a consequence of picrotoxinin application, the response profile of the FD1-cell changes considerably (Fig. 1*B*). *1*) The cell is also excited during motion in its

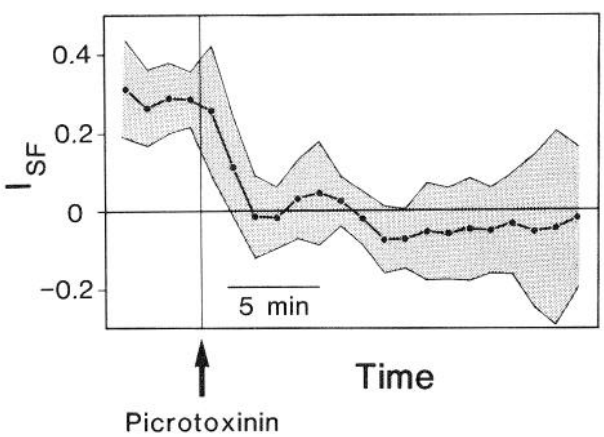

FIG. 2. Mean time course of the small-field selectivity (I_{SF}) of the FD1-cell after picrotoxinin application. Responses to small-field and large-field motion and the spontaneous activity were averaged over 5 successive stimulus presentations. Together they lasted for ~1 min. Small-field stimulus was presented within a 13- or 20°-wide window. Exact position of the stimuli is given by stimulus condition 1 (see METHODS). The index for small-field selectivity is defined as the relative difference between the responses to small-field and large-field motion (see METHODS) and was calculated for each animal and time interval. Values of $I_{SF} > 0$ (< 0) mean that the cell responds more (less) strongly to small-field than to large-field motion in the preferred direction. Mean index values were calculated from a varying number of experiments, depending on the duration from which the cells could be recorded. (Until 8 min after injection, values were obtained from 9 animals decreasing to 5 animals from the 16th to the 20th minute). Grey area indicates the mean time course ±SE. Picrotoxinin was applied at the time indicated by the arrow and the vertical line. Before application of picrotoxinin, the FD1-cell responds with a larger amplitude to small-field motion than to large-field motion ($I_{SF} > 0$). Small-field selectivity vanishes already 3 min after application of picrotoxinin. Seven to 20 min after application, the effect of picrotoxinin gets even more pronounced ($I_{SF} < 0$).

null direction but to a lesser extent than during motion in the preferred direction. Hence, as was expected, the direction selectivity is greatly reduced. *2*) The spontaneous activity as well as the overall response amplitudes during motion in any direction are increased. *3*) Most importantly in the current context, the cell now responds with a somewhat larger amplitude to large-field than to small-field motion. Hence the small-field tuning of the FD1-cell vanishes. All effects are reversible with the direction selectivity and activity level returning first to the normal conditions (Fig. 1*C*) followed by the recovery of the small-field tuning (Fig. 1*D*).

After picrotoxinin application the small-field tuning of the FD1-cell was eliminated in each of the nine flies tested. The mean time course of the small-field selectivity averaged over all experiments is shown in Fig. 2. Positive or negative values of the index of small-field selectivity (I_{SF}) indicate that the cell responds with a higher or lower spike frequency, respectively, to small-field than to large-field motion. Index values depend on the time after application of picrotoxinin. After picrotoxinin is injected, the selectivity for small-field motion decreases rapidly. The small-field tuning is already eliminated 3 min after the injection. Interestingly, the small-field tuning is not only eliminated, but also the FD1-cell responds with a significantly higher spike frequency to large-field than to small-field motion for a time interval from the 7th to the 21st minute [Wilcoxon signed-rank test, $\alpha < 0.006$ (Rohlf and Sokal 1981; Sokal and Rohlf 1981)]. Consequently, the small-field tuning is not eliminated because of picrotoxinin just driving the cell's response to its maximum level. This can also be inferred from the spike-frequency histogram shown in Fig. 1*B*. The recovery of the small-field selectivity that already was demonstrated in the example of Fig. 1 can also be confirmed statistically in those cells ($n = 4$) that could be recorded long enough. This was tested for the interval between the 31st and 39th minute after picrotoxinin application ($\alpha < 0.004$).

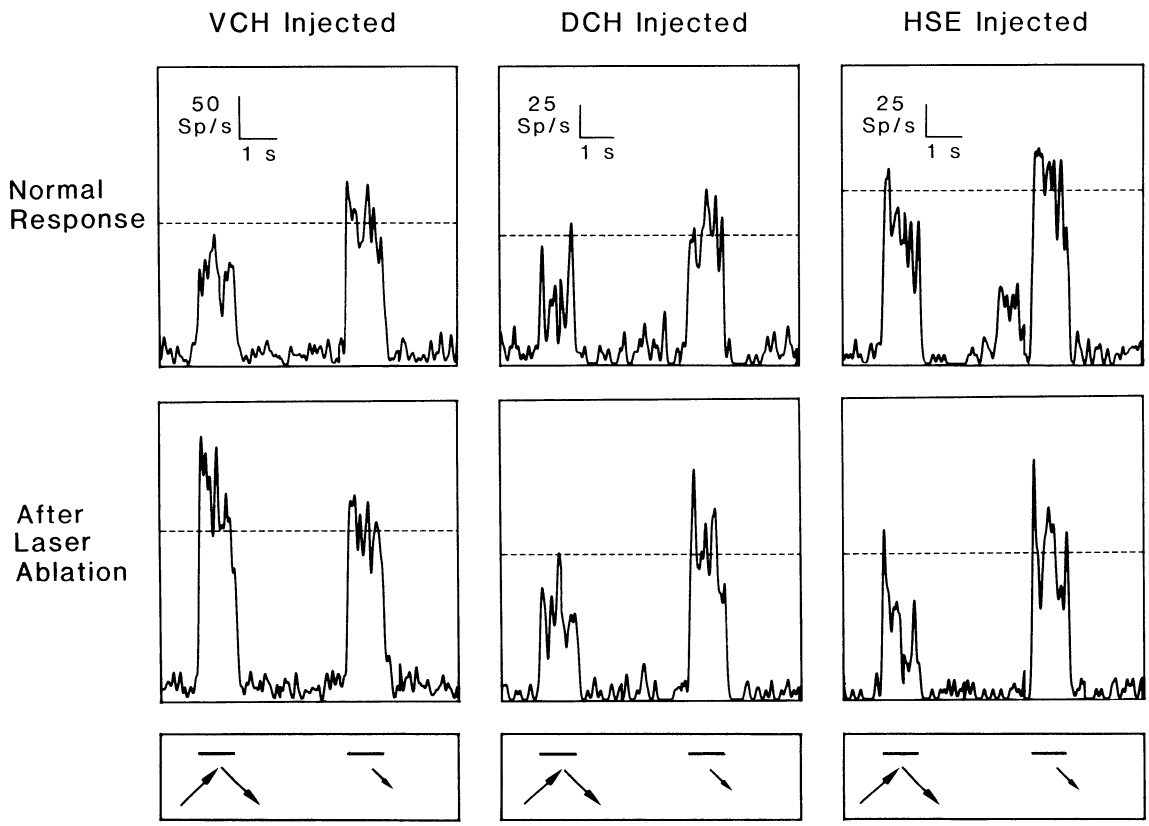

FIG. 3. Responses of FD1-cell to large-field and small-field motion before and after photoinactivation of the VCH-, DCH-, or HSE-cell. Time-dependent responses of 3 different FD1-cells that were averaged over 5 stimulus presentations are shown as spike-frequency histograms. Width of small-field stimulus amounted to 44° (*left* and *right column*) or 20° (*middle column*). Horizontal scale bars in stimulus traces indicate the duration of motion (1 s); arrows specify size and direction of the moving pattern. Dotted horizontal lines in histograms indicate mean response amplitudes during small-field motion. Position of stimuli is given by stimulus condition 2 (see METHODS) for experiments where the VCH-cell or the HSE-cell was inactivated. During the inactivation of the DCH-cell, stimuli were presented further ventrally (stimulus condition 3, see METHODS). Under both stimulus arrangements the VCH-, DCH-, and HSE-cell could be excited strongly. Before photoinactivation the FD1-cell responds in its normal way, i.e., with a larger response amplitude to small-field than to large-field motion (*top row*). Spike-frequency histograms in the *bottom row* were recorded 4, 6, or 2 min after photoinactivation (*left* to *right*, respectively). Only after photoinactivation of the VCH-cell, the selectivity of the FD1-cell for small-field motion is eliminated; the cell now responds with a larger response amplitude to large-field than to small-field motion. In contrast, inactivation of the DCH-cell or HSE-cell has no significant effect on the response. The FD1-cell still responds with a larger amplitude to small-field motion.

Elimination of individual neurons by photoinactivation

The results presented so far provide evidence that a GABAergic mechanism is responsible for the small-field tuning of the FD1-cell. Because the CH-cells are the only known GABAergic tangential cells with the appropriate functional characteristics, it is most likely that they are responsible for the large-field inhibition of the FD1-cell. However, the experiments presented above do not allow to assess the role of each of the two CH-cells. To find out whether both CH-cells or only one of them tune the FD1-cell to small-field motion, we applied the photoinactivation technique that allows to ablate individual neurons. Besides the two CH-cells, a third tangential cell in the right half of the brain, the HSE-cell, that also responds best to clockwise large-field motion was inactivated as a control.

The cell to be eliminated was filled iontophorctically with the fluorescent dye 6-carboxy-fluorescein. At that stage the cell was identified by using physiological response characteristics. Then the FD1-cell was probed, and its normal response to small-field and large-field motion characterized. While continuously recording from the FD1-cell, part of the right lobula plate and lateral brain was illuminated by a laser beam for ~2 min, leading to the ablation of the filled cell. During the illumination the filled cell could also be identified anatomically, corroborating the previous physiological identification. Responses of the FD1-cell before and after laser ablation of the VCH-, DCH-, and HSE-cell, respectively, are shown as spike-frequency histograms in Fig. 3. Before the laser was switched on, the FD1-cell responds with a larger amplitude to small-field than to large-field motion (Fig. 3, *top row*). After photoinactivation of the DCH-cell or HSE-cell, this response characteristic does not change (Fig. 3, *middle* and *right panel* of *bottom row*). The FD1-cell is still more sensitive to small-field than to large-field motion. The situation is much different when the VCH-cell is ablated (Fig. 3, *left panel* of *bottom row*). Now the response of the FD1-cell to large-field motion becomes about three times as large as before. The large-field response even exceeds the response to small-field motion. Thus, after photoinactivation of the VCH-cell, the FD1-cell has lost its specific small-field sensitivity.

These effects of photoinactivating the VCH-, DCH-, or HSE-cell were found to be qualitatively the same for all experiments carried out on a total of nine flies. The results are summarized in Fig. 4, where the index of small-field selectivity (I_{SF}) of the FD1-cell is shown before and after photoinactivation of the respective cell. Index values were found to be normally distributed for $\alpha = 0.05$. Student's t test was used to determine whether there is a significant change in the small-field tuning of the FD1-cell after photoinactivation of a specific neuron (Sokal and Rohlf 1981). In all experiments I_{SF} is positive before photoinactivation ($\alpha < 0.0005$), indicating that the FD1-cell responds stronger to small-field than to large-field motion (Fig. 4, *left column*). After photoinactivation of the DCH-cell ($n = 2$) or HSE-cell ($n = 3$), index values do not differ significantly from those before photoinactivation ($\alpha < 0.05$). Thus the DCH- and HSE-cell could not be proven to participate in the mechanism tuning the FD1-cell to small-field motion, although there is a slight decrease in the mean index of the small-field selectivity after ablation of the DCH-cell (Fig. 4, compare *left* and *right columns* of *middle panel*). In contrast, photoablation of the VCH-cell leads to index values that are significantly smaller than 0 ($\alpha < 0.001$) and thus abolishes the specific sensitivity of the FD1-cell to small-field motion.

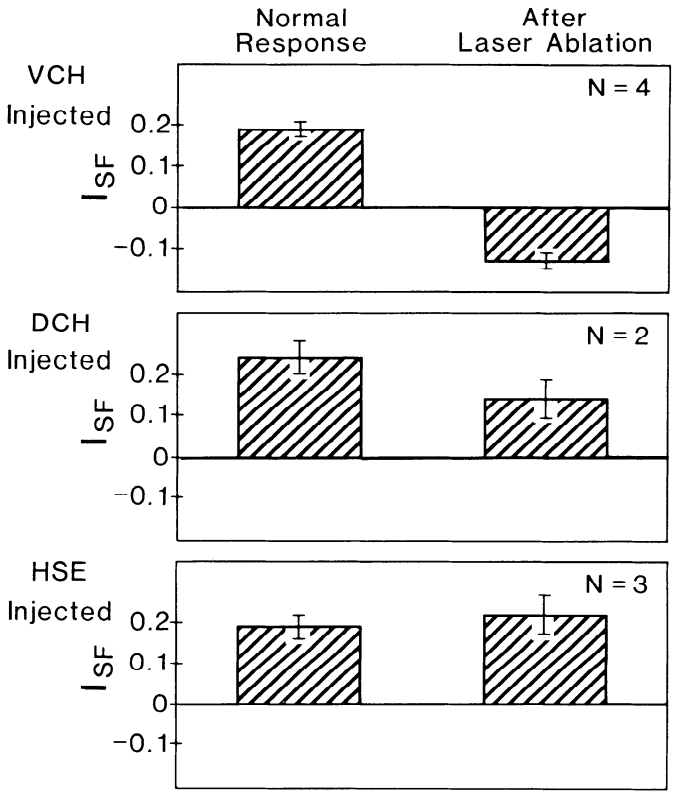

FIG. 4. Mean selectivity of the FD1-cell to small-field motion before and after photoinactivation of the VCH-, DCH-, or HSE-cell. From the individual reactions to 15–100 stimulus presentations before and after the inactivation, mean responses to large-field and small-field motion, the spontaneous activity, and the corresponding SEs were determined. From these values I_{SF} and SE were calculated for each experiment. The indexes were averaged over the number of experiments (N) and are shown here together with the corresponding SE. The position of the stimuli during the ablation experiments of both DCH-cells and one of the VCH-cell are given by stimulus condition 3; stimulus positions during the other experiments are given by stimulus condition 2 (see METHODS). The width of the small-field stimulus amounted to 20° (1 DCH-cell), 32° (3 VCH-cells, 1 DCH-, and 1 HSE-cell), or 44° (1 VCH-cell and 2 HSE-cells). Before photoinactivation the index of selectivity for small-field motion is >0 in all experiments. Only after photoinactivation of the VCH-cell, I_{SF} assumes values <0. Hence the VCH-cell is decisive for tuning the FD1-cell to small moving objects.

Hence, out of all the candidates, the VCH-cell is the only one that appears to be responsible for tuning the FD1-cell to the motion of small objects.

Receptive-field characteristics of the FD1-cell

Because the VCH-cell responds to motion within the ventral part of the ipsilateral visual field but is not excited by motion in the dorsal part (Egelhaaf et al. 1993), the receptive-field properties of the FD1-cell along the vertical axis of the eye need to be determined. Figure 5 shows mean response amplitudes of the FD1-cell during small-field mo-

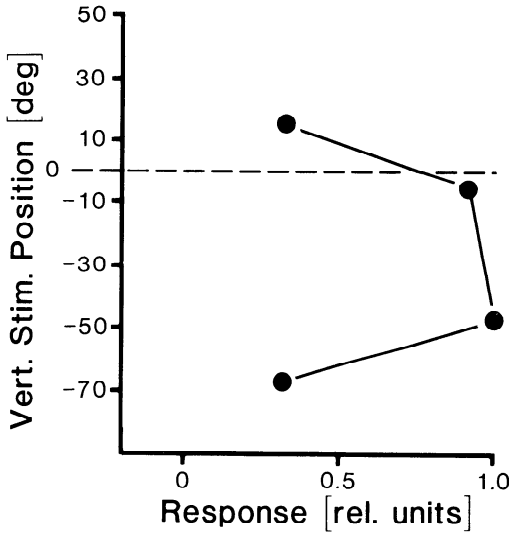

FIG. 5. Spatial sensitivity distribution of the FD1-cell along the vertical axis of the eye. The FD1-cell was activated by small-field motion within a 32°-wide and 20°-high window at 4 different vertical positions. The position of the center of the respective window is plotted on the ordinate. Response amplitudes (abscissa) are normalized to the largest response that corresponds to a spike frequency of 62 spikes/s. SE (not shown) is <0.01 units. Results were obtained from 2 flies and a total of 6 and 8 stimulus presentations, respectively. Stimulus duration amounted to 2 s, which was followed by an interval of ~2.5 s without pattern motion. The exact position of the stimuli is given by stimulus condition 3 (see METHODS). The FD1-cell responds with a much larger amplitude to small-field motion in the ventral part of the visual field than in the dorsal part.

tion within a window of ~20° vertical extent placed subsequently at four different vertical positions. Maximum responses are induced below the equator line of the eye, i.e., within an area with an elevation of 0 to −50°. Small-field motion in the dorsal part of the visual field leads to small responses only. Interestingly, large-field motion in the dorsal part of the visual field exhibits neither a strong excitatory nor an inhibitory action on the FD1-cell. This shows that the FD1-cell is most sensitive in that part of the visual field where the VCH-cell is excited most strongly.

The asymmetric sensitivity distribution of the FD1-cell appears surprising when taking the cell's anatomy into account. In the published reconstructions (Egelhaaf 1985b) its dendritic branches appear quite homogeneously distributed from the dorsal to the ventral border of the distal lobula plate. However, closer inspection of the original photographs that were the basis for the reconstructions indicate that the ventral branches are slightly thicker and thus might conduct postsynaptic potentials with less decrement than the dorsal ones. This feature might not have been realized in the previous study because, on the available Lucifer yellow–filled material, the size of the dendrites cannot be measured with sufficient accuracy. Therefore this aspect needs to be further investigated with the use of other non-fluorescent dyes.

DISCUSSION

In the present study we investigated the neural circuit by which a particular cell in the fly visual system, the FD1-cell, becomes selectively responsive to small objects moving relative to their background. On the basis of pharmacological and photoablation experiments, we concluded that the FD1-cell acquires this particular feature through the inhibitory input from another identified cell, the VCH-cell, which is most sensitive to large-field motion. Before discussing details of the circuit and the possible role of the FD1-cell in behavior, we first want to ask about the specificity of the techniques on which our conclusions are based.

Specificity of the network analysis

How specific are the techniques used to ablate the CH-cells? Picrotoxinin is commonly used as a blocker of GABAergic chloride channels (Benson 1988; Simmonds 1983). In insects, there is no evidence that picrotoxinin interacts with other inhibitory receptors (Benson 1988; Rauh et al. 1990). Hence, from the effect of picrotoxinin on the FD1-cell, it can be inferred that, in fact, a GABAergic mechanism is responsible for the small-field tuning of this cell. Does this conclusion also allow the interpretation that the CH-cells rather than other inhibitory elements are the neuronal basis for the small-field tuning of the FD1-cell? Four tangential cells in the lobula plate were suggested to be GABAergic as a result of immunohistochemical labeling. From these, only the CH-cells have the appropriate direction selectivity to act as large-field inhibitor of the FD1-cell. The other GABAergic neurons in the visual system of the fly are local retinotopically organized elements (Meyer et al. 1986). Some of them are likely to be involved in motion detection because direction selectivity is reduced by picrotoxinin (Egelhaaf et al. 1990; Schmid and Bülthoff 1988). Local GABAergic elements have therefore been concluded to constitute part of the retinotopic input elements of the FD1-cell. Nevertheless, inhibitory lateral interactions between these retinotopic elements alone cannot mediate the small-field tuning of the FD1-cell because it is also inhibited by motion outside its excitatory receptive field (Egelhaaf 1985b). Therefore our conclusion concerning the mechanism of tuning the FD1-cell to small moving objects is not affected by the interference of picrotoxinin with direction selectivity. Thus we conclude from the pharmacological experiments that most likely the CH-cells mediate the small-field tuning of the FD1-cell.

A more specific method to test for the involvement of the CH-cells in tuning the FD1-cell to small moving objects is the photoablation technique (Miller and Selverston 1979), because here it is possible to assess the role of each of the CH-cells individually. How specific is this method, or does laser illumination of the brain also damage neurons that were not previously injected with a fluorescent dye? The response amplitudes of tangential cells that were not filled with the dye did not decrease after laser illumination for up to 10 min (see METHODS). In addition, the response characteristics of the FD1-cell did not change after photoablation of the DCH-cell and the HSE-cell, a further tangential neuron that has the same preferred direction as the CH-cells. This indicates that the changes in the response of the FD1-cell after laser illumination of the VCH-cell are, in fact, a consequence of ablating the VCH-cell rather than of unspecific damage of nervous tissue. It is possible that illumination of a cell filled with a fluorescent dye does not inactivate this cell? Intracellular recordings of two dye-filled lobula

plate tangential neurons during and after laser illumination showed, in accordance with reports on other systems (Fraser and Heitler 1991; Miller and Selverston 1979; Selverston et al. 1985), that cells depolarize and, in the case of the fly's tangential neurons, stop responding to motion after laser illumination (see METHODS). Moreover, the small-field tuning of the FD1-cell was abolished after photoablation of the VCH-cell in each of the four experiments, whereas the small-field tuning remained normal in all five experiments where other cells were injected with the dye. This makes it rather unlikely that one of the filled cells had not been inactivated by laser illumination. Consequently, the VCH-cell appears to play the main role in tuning the FD1-cell to small moving objects.

However, by taking the spatial sensitivity distribution of the FD1-cell and the CH-cells into account, it might still be possible that also the DCH-cell inhibits the FD1-cell. Both CH-cells are complementary elements concerning their dendritic arborization in the lobula plate and the position of their receptive fields in the dorsal (DCH-cell) and ventral (VCH-cell) part of the ipsilateral visual field (Egelhaaf et al. 1993; Hausen 1976b). The FD1-cell, as the VCH-cell, responds mainly to motion in the ventral part of the ipsilateral visual field. Neither small-field nor large-field motion in the dorsal part influences the FD1-cell much. Hence, even if the DCH-cell inhibits the FD1-cell, the consequences of photoablating the DCH-cell for the response properties of the FD1-cell would be only small and therefore difficult to detect reliably. Notwithstanding, the FD1-cell is mainly inhibited by the VCH-cell, and the still possible inhibition by the DCH-cell is of no obvious functional significance for the small-field tuning of the FD1-cell.

As was already mentioned in the INTRODUCTION, two different anatomic types of FD1-cells have been identified that show the same physiological characteristics (Egelhaaf 1985b). These two types could not be distinguished in the present study because, as a consequence of the extracellular recording techniques that had to be employed, the FD1-cells were only identified on the basis of their characteristic functional properties. Nevertheless, no qualitative differences in the response of FD1-cells identified in this way could be detected in the various experiments after ablating the CH-cells either pharmacologically or by photoinactivation. Hence either the same anatomic type of FD1-cell was recorded from in all experiments reported here, or both types of FD1-cells have the same input organization.

Mechanism for small-field selectivity of the FD1-cell and of other small-field sensitive cells

According to our analysis the FD1-cell becomes selectively responsive to small moving objects through two kinds of input. *1*) It receives retinotopic input from an array of local motion-sensitive elements that it spatially integrates by its large dendritic arbor as is characteristic of lobula plate tangential cells (Borst and Egelhaaf 1990; Haag et al. 1992). Thereby the FD1-cell becomes directionally selective to motion within its ipsilateral receptive field, i.e., it is excited by front-to-back motion and inhibited by back-to-front motion. *2*) In addition, the FD1-cell receives inhibitory input from the VCH-cell. The VCH-cell is stimulated by contralateral back-to-front motion and ipsilateral front-to-back motion. It is inhibited by motion in the opposite direction in front of either eye. The inhibitory input from the VCH-cell is responsible for the FD1-cell being most sensitive to a particular object size.

From the directionality of the VCH-cell, one might expect, at first sight, that the response of the FD1-cell to a small object moving from the front to the back is only reduced when an extended background pattern moves in phase with the object, i.e., in the preferred direction of the VCH-cell. When the background moves in the opposite direction and thus in antiphase to the object, the VCH-cell is inhibited or at least does not show large responses. Accordingly, it may be expected that it does not inhibit the FD1-cell, which, therefore, should respond with a large amplitude. This expectation, however, is in contrast to the experimental findings (Egelhaaf 1985b). Nevertheless, there is no inconsistency between the proposed input organization of the FD1-cell and the experimental data, because the VCH-cell does not represent the only inhibitory input of the FD1-cell. In addition to the excitatory retinotopic input, the FD1-cell also receives input from a set of retinotopic inhibitory elements that enhance its direction selectivity. Because the optimal stimulus width of the FD1-cell is usually smaller than the excitatory receptive field (Egelhaaf 1985b), the inhibitory retinotopic input elements are usually activated by the background pattern when it moves in antiphase to an optimally sized object. Hence the retinotopic inhibitory elements are responsible for the decrease in response amplitude during antiphase motion. It should be noted that such an antiphase inhibition can hardly be avoided if the optimal object size is smaller than the excitatory receptive field of the small-field element.

Does the mechanism underlying small-field tuning of the FD1-cell also account for the response properties of the other FD-cells? In the lobula plate, three further FD-cells have been described that differ from the FD1-cell in their preferred direction, the position and size of their receptive fields, as well as the directions of large-field motion that lead to a reduction of their responses (Egelhaaf 1985b). These characteristic responses can be explained by assuming that all FD-cells are inhibited by one or several large-field cells (Egelhaaf 1985c). However, from the properties of the different FD-cells, it is clear that the VCH-cell cannot represent the only large-field inhibitor in the fly visual system tuning cells to small moving objects. At least one further large-field inhibitor with opposite polarity to the VCH-cell has been postulated (Egelhaaf 1985c), whose cellular identity, however, is not yet known.

At the processing stage further downstream from the lobula complex, descending neurons have recently been described in the fly that are selectively activated by local motion and inhibited by wide-field motion (Gronenberg and Strausfeld 1992). However, the mechanism tuning these neurons to small-field motion was not investigated. Moreover, the small-field stimulus used in these experiments to measure the response of the descending neurons to small objects did not only differ from the large-field stimulus in its size but also in its structure and velocity (hand-held dots

and gratings vs. stimuli consisting of consecutively flashed photodiodes). Because the response amplitude of motion-sensitive neurons in the fly's brain does not only depend on the size of a stimulus but also on several other stimulus parameters, it is not possible from these experiments to tell whether the characterized descending neurons are really tuned to small-field motion.

A similar mechanism as is proposed here for the FD1-cell could well be responsible for the small-field tuning of other nerve cells in a variety of different species (e.g., hawkmoth: Collett 1971; hoverfly: Collett and King 1975; toad: Tsai 1990; pigeon: Frost et al. 1981; cat: Grünau and Frost 1983; monkey: Allman et al. 1985; Davidson and Bender 1991). They all receive inhibitory input from outside their excitatory receptive field. However, the mechanism tuning the FD1-cell to small-field motion is not just based on the inhibition by a neuron with a larger receptive field than that of the FD1-cell. Instead, the FD1-cell is excited most strongly by objects that are usually smaller than its excitatory receptive field. This has been accounted for by a nonlinearity in the synaptic transmission characteristic between the VCH- and the FD1-cell (see Reichardt et al. 1983; Egelhaaf 1985c; Egelhaaf et al. 1993). Thus, by employing a nonlinearity, it is possible to obtain a neuron that is tuned to small objects by the inhibition of another cell whose receptive field is not larger than that of the small-field neuron itself. It is not clear to us, whether the above-mentioned small-field sensitive cells of the various animals show a similar nonlinear transmission characteristic as well.

There is one example of a small-field sensitive neuron where a mechanism different from the one described from the FD1-cell has been proposed. Lateral inhibitory interactions between retinotopically arranged input elements as well as feed-forward inhibition by two kinds of large-field sensitive elements have been concluded to tune locust lobula giant movement detector (LGMD) neurons to small objects (Rowell et al. 1977). However, the neuronal mechanism underlying this specific spatial tuning has not been unraveled so far at the level of identified neurons and their synaptic interactions.

Role of FD-cells in behavior

Female as well as male flies are able to detect and fixate objects when relative motion is the only cue to discriminate these objects from their background (Bülthoff 1981; Egelhaaf 1985a; Reichardt et al. 1983, 1989; Reichardt and Poggio 1979; Versik and Reichardt 1976). What is the neuronal correlate for this behavior? The neural machinery that is believed to control male chasing maneuvers (see Land and Collett 1974; Wagner 1986; Wehrhahn 1979) cannot account for the aforementioned fixation response because it is sex specific (Gilbert and Strausfeld 1991; Hardie et al. 1981; Hausen and Strausfeld 1980; Strausfeld 1991). Interestingly, the male specific neurons that are assumed to play a decisive role in female chasing have extended receptive fields and, on the basis of the available experimental data, do not seem to be specifically tuned to small targets at all (Gilbert and Strausfeld 1991). Hence these neurons do not seem to have the adequate properties to discriminate objects from their background by motion cues alone.

The FD-cells, on the other hand, can signal the presence of small objects within their excitatory receptive fields and thus could be involved in fixation behavior. It is hardly possible to assess the functional role of the FD1-cell or any other FD-cell individually, because their receptive fields overlap, and therefore the cells cannot be activated in isolation. However, comparison of electrophysiological experiments with investigations of behavioral responses of tethered flying flies indicate that the FD-cells, in general, play a major role in detecting and fixating objects, as has been discussed in detail in several previous papers (Egelhaaf 1985c; 1987; 1989; Reichardt et al. 1989).

This view has been challenged in a recent study where a group of directionally unselective retinotopic output cells of the lobula plate with small receptive fields was claimed to play this role (Gilbert and Strausfeld 1992). It was argued that the ability of the fly to discriminate between two stripes (Reichardt and Poggio 1975) cannot be based on the FD-cells because of their relatively large receptive fields. Instead, it has been assumed that this discrimination performance can only be attributed to cells with much smaller receptive fields. This argument is not conclusive for the following reason. Turning responses leading to fixation behavior can be elicited from everywhere in the visual field (Reichardt and Poggio 1976). Hence, many retinotopic input channels have to converge on a single output. This implies that spatial pooling has to take place somewhere in the visual pathway. Thereby the retinotopic input channels have to be weighted appropriately according to their position in the visual field to enable the fly to discriminate between two stripes (Reichardt and Poggio 1976). These considerations show that it is not possible to discard a spatially integrating neuron such as an FD-cell from playing a role in fixation behavior simply for its receptive-field size. The other argument against the view that the FD-cells play an important role in mediating the detection and fixation of objects is that the fixation response contains a strong directionally insensitive component, whereas the FD-cells are directionally selective (Gilbert and Strausfeld 1992). Because any directionally insensitive behavioral response component can be formally decomposed into two directionally selective ones with opposite polarity, there is no reason why the neuronal elements controlling fixation behavior need to be directionally insensitive (for discussion see Egelhaaf 1985a,c). In this context it is interesting to note that there are not only FD-cells responding to object motion from front to back (FD1- and FD4-cell) but also FD-cells with the opposite preferred direction (FD2- and FD3-cell) (Egelhaaf 1985b). Hence, they jointly could well lead to a completely directionally insensitive fixation system, if their output is weighted appropriately. Although the responses of the FD1-cell and the other FD-cells characterized so far have not been analyzed for all situations that have been employed to characterize the fly's fixation behavior, the known properties of the FD-cells suffice to explain all that we know so far about how flies detect moving objects. Of course, this does not exclude that other elements may also be involved in the control of the fly's fixation behavior.

A decisive role of the FD1-cell in figure-ground discrimination is further suggested by the correspondence of the spatial sensitivity distribution of the FD1-cell and the fly's fixation behavior. Under both tethered and free flight conditions, female flies detect and fixate small objects better in the ventral than in the dorsal part of the visual field (Reichardt 1973; Wagner 1986; Wehrhahn 1979). In general, the ventral part of the visual field should be especially important for flying animals, because they encounter objects in front of a structured environment more often in the ventral than in the dorsal part of their visual field. Therefore, in this part of the visual field, mechanisms sensitive to relative motion are particularly needed. In fact, bees flying freely over an artificial meadow are able to evaluate the relative height of objects that differ from their background only by relative motion (Srinivasan et al. 1990). If a normal flight posture is assumed, this suggests that bees obviously possess a mechanism in the ventral part of their visual field that extracts information on relative motion. However, from the available data it is not possible to assess whether this mechanism is related to the mechanism tuning the FD1-cell to relative and small-field motion.

We thank S. Flecks and A. Wildemann for technical assistance and C. Gilbert, K. G. Götz, R. Kern, and W. Reichardt for comments on the manuscript.

Address reprint requests to A.-K. Warzecha.

Received 6 April 1992; accepted in final form 30 September 1992.

REFERENCES

ALLMAN, J., MIEZIN, F., AND MCGUINNESS, E. Direction- and velocity-specific responses from beyond the classical receptive field in the middle temporal visual area (MT). *Perception* 14: 105–126, 1985.

ASTHEIMER, J. P. *Asyst: Module2–Analysis.* Rochester, NY: Keithley Instruments, 1989.

BENSON, J. A. Transmitter receptors on insect neuronal somata: GABAergic and cholinergic pharmacology. In: *Neurotox '88: Molecular Basis of Drug and Pesticide Action,* edited by G. G. Lunt. Amsterdam: Elsevier, 1988, p. 193–205.

BORST, A. AND EGELHAAF, M. Direction selectivity of fly motion-sensitive neurons is computed in a two-stage process. *Proc. Natl. Acad. Sci. USA* 87: 9363–9367, 1990.

BÜLTHOFF, H. Figure-ground discrimination in the visual system of *Drosophila melanogaster. Biol. Cybern.* 41: 139–145, 1981.

COLLETT, T. S. Visual neurones for tracking moving targets. *Nature Lond.* 232: 127–130, 1971.

COLLETT, T. S. Visual neurones in the anterior optic tract of the privet hawk moth. *J. Comp. Physiol. A* 78: 396–433, 1972.

COLLETT, T. S. AND KING, A. J. Vision during flight. In: *The Compound Eye and Vision of Insects,* edited by G. A. Horridge. Oxford, UK: Clarendon, 1975, p. 437–466.

COLLETT, T. S. AND PATERSON, C. J. Relative motion parallax and target localization in the locust, *Schistocerca gregaria. J. Comp. Physiol. A* 169: 615–621, 1991.

DAVIDSON, R. M. AND BENDER, D. B. Selectivity for relative motion in the monkey superior colliculus. *J. Neurophysiol.* 65: 1115–1133, 1991.

ECKERT, H. AND DVORAK, D. R. The centrifugal horizontal cells in the lobula plate of the blowfly, *Phaenicia sericata. J. Insect Physiol.* 29: 547–560, 1983.

EGELHAAF, M. On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. I. Behavioural constraints imposed on the neuronal network and the role of the optomotor system. *Biol. Cybern.* 52: 123–140, 1985a.

EGELHAAF, M. On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. II. Figure-detection cells, a new class of visual interneurones. *Biol. Cybern.* 52: 195–209, 1985b.

EGELHAAF, M. On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. III. Possible input circuitries and behavioural significance of the FD-cells. *Biol. Cybern.* 52: 267–280, 1985c.

EGELHAAF, M. Dynamic properties of two control systems underlying visually guided turning in house-flies. *J. Comp. Physiol. A* 161: 777–783, 1987.

EGELHAAF, M. Visual afferences to flight steering muscles controlling optomotor response of the fly. *J. Comp. Physiol. A* 165: 719–730, 1989.

EGELHAAF, M. How do fly FD-cells acquire their sensitivity to small-field motion. *Naturwissenschaften* 77: 182–185, 1990.

EGELHAAF, M., BORST, A. AND PILZ, B. The role of GABA in detecting visual motion. *Brain Res.* 509: 156–160, 1990.

EGELHAAF, M., BORST, A., WARZECHA, A.-K., FLECKS, S., AND WILDEMANN, A. Neural circuit tuning fly visual neurons to motion of small objects. II. Input organization of inhibitory circuit elements revealed by electrophysiological and optical recording techniques. *J. Neurophysiol.* 69: 340–360, 1993.

EGELHAAF, M., HAUSEN, K., REICHARDT, W., AND WEHRHAHN, C. Visual course control in flies relies on neuronal computation of object and background motion. *Trends Neurosci.* 11: 351–358, 1988.

FRANCESCHINI, N. AND KIRSCHFELD, K. Les phénomènes de pseudopupille dans l'oeil composé de *Drosophila. Kybernetik* 9: 159–182, 1971.

FRASER, K. AND HEITLER, W. J. Photoinactivation of the crayfish segmental giant neuron reveals a direct giant-fiber to fast-flexor connection with a chemical component. *Neuroscience* 11: 59–71, 1991.

FROST, B. J., CAVANAGH, P., AND MORGAN, B. Deep tectal cells in pigeons respond to kinematograms. *J. Comp. Physiol. A* 162: 639–647, 1988.

FROST, B. J. AND NAKAYAMA, K. Single visual neurons code opposing motion independent of direction. *Science Wash. DC* 220: 744–745, 1983.

FROST, B. J., SCILLEY, P. L., AND WONG, S. C. P. Moving background patterns reveal double-opponency of directionally specific pigeon tectal neurons. *Exp. Brain Res.* 43: 173–185, 1981.

GILBERT, C. AND STRAUSFELD, N. J. The functional organization of male-specific visual neurons in flies. *J. Comp. Physiol. A* 169: 395–411, 1991.

GILBERT, C. AND STRAUSFELD, N. J. Small-field neurons associated with oculomotor and optomotor control in muscoid flies: functional organization. *J. Comp. Neurol.* 316: 72–86, 1992.

GRONENBERG, W. AND STRAUSFELD, N. J. Premotor descending neurons responding selectively to local visual stimuli in flies. *J. Comp. Neurol.* 316: 87–103, 1992.

VON GRÜNAU, M. AND FROST, B. J. Double-opponent-process mechanism underlying RF-structure of directionally specific cells of cat lateral suprasylvian visual area. *Exp. Brain Res.* 49: 84–92, 1983.

HAAG, J., EGELHAAF, M., AND BORST, A. Dendritic integration of visual motion information in the fly. *Neurosci. Lett.* 140: 173–176, 1992.

HARDIE, R. C., FRANCESCHINI, N., RIBI, W., AND KIRSCHFELD, K. Distribution and properties of sex-specific photoreceptors in the fly *Musca domestica. J. Comp. Physiol.* 145: 139–152, 1981.

HAUSEN, K. Functional characterization and anatomical identification of motion sensitive neurons in the lobula plate of the blowfly *Calliphora erythrocephala. Z. Naturforsch.* 31c: 629–633, 1976a.

HAUSEN, K. *Struktur, Funktion und Konnektivität bewegungsempfindlicher Interneurone im dritten optischen Neuropil der Schmeissfliege Calliphora erythrocephala* (PhD dissertation). Tübingen, Germany: Univ. of Tübingen, 1976b.

HAUSEN, K. Monocular and binocular computation of motion in the lobula plate of the fly. *Verh. Dtsch. Zool. Ges.* 74: 49–70, 1981.

HAUSEN, K. Motion sensitive interneurons in the optomotor system of the fly. I. The horizontal cells: structure and signals. *Biol. Cybern.* 45: 143–156, 1982a.

HAUSEN, K. Motion sensitive interneurons in the optomotor system of the fly. II. The horizontal cells: receptive field organization and response characteristics. *Biol. Cybern.* 46: 67–79, 1982b.

HAUSEN, K. The lobula-complex of the fly: structure, function and significance in visual behaviour. In: *Photoreception and Vision in Invertebrates,* edited by M. A. Ali. New York: Plenum, 1984, p. 523–559.

HAUSEN, K. AND EGELHAAF, M. Neural mechanisms of visual course control in insects. In: *Facets of Vision,* edited by D. Stavenga and R. Hardie. Berlin: Springer-Verlag, 1989, p. 391–424.

HAUSEN, K. AND STRAUSFELD, N. J. Sexually dimporphic interneuron arrangements in the fly visual system. *Proc. R. Soc. Lond. B Biol. Sci.* 208: 57–71, 1980.

LAND, M. F. AND COLLETT, T. S. Chasing behaviour of houseflies (*Fannia canicularis*). A description and analysis. *J. Comp. Physiol.* 89: 331–357, 1974.

MEYER, E. P., MATUTE, C., STREIT, P., AND NÄSSEL, D. R. Insect optic lobe neurons identifiable with monoclonal antibodies to GABA. *Histochemistry* 84: 207–216, 1986.

MILES, F. A. AND KAWANO, K. Visual stabilization of the eyes. *Trends Neurosci.* 10: 153–158, 1987.

MILLER, J. P. AND SELVERSTON, A. I. Rapid killing of single neurons by irradiation of intracellularly injected dye. *Science Wash. DC* 206: 702–704, 1979.

OLBERG, R. M. Object- and self-movement detectors in the ventral nerve cord of the dragonfly. *J. Comp. Physiol.* 141: 327–334, 1981.

OLBERG, R. M. Identified target-selective visual interneurons descending from the dragonfly brain. *J. Comp. Physiol.* 159: 827–840, 1986.

RAUH, J. J., LUMMIS, S. C. R., AND SATTELLE, D. B. Pharmacological and biochemical properties of insect GABA receptors. *Trends Pharmacol. Sci.* 11: 325–329, 1990.

REGAN, D. AND BEVERLEY, K. I. Figure-ground segregation by motion contrast and by luminance contrast. *J. Opt. Soc. Am. A* 1: 433–442, 1984.

REICHARDT, W. Musterinduzierte Flugorientierung. Verhaltens-Versuche an der Fliege *Musca domestica. Naturwissenschaften* 60: 122–138, 1973.

REICHARDT, W., EGELHAAF, M., AND GUO, A. Processing of figure and background motion in the visual system of the fly. *Biol. Cybern.* 61: 327–345, 1989.

REICHARDT, W. AND POGGIO, T. A theory of the pattern induced flight orientation of the fly *Musca domestica* II. *Biol. Cybern.* 18: 69–80, 1975.

REICHARDT, W. AND POGGIO, T. Visual control of orientation behaviour in the fly. Part I. A quantitative analysis. *Q. Rev. Biophys.* 9: 311–375, 1976.

REICHARDT, W. AND POGGIO, T. Figure-ground discrimination by relative movement in the visual system of the fly. Part I. Experimental results. *Biol. Cybern.* 35: 81–100, 1979.

REICHARDT, W., POGGIO, T., AND HAUSEN, K. Figure-ground discrimination by relative movement in the visual system of the fly. Part II. Towards the neural circuitry. *Biol. Cybern.* 46, *Suppl.*: 1–30, 1983.

ROHLF, F. J. AND SOKAL, R. R. *Statistical Tables.* New York: Freeman, 1981.

ROWELL, C. H. F., O'SHEA, M., AND WILLIAMS, J. L. D. The neuronal basis of a sensory analyzer, the acridid movement detector system. IV. The preference for small field stimuli. *J. Exp. Biol.* 68: 157–185, 1977.

SCHMID, A. AND BÜLTHOFF, H. Using neuropharmacology to distinguish between excitatory and inhibitory movement detection mechanisms in the fly *Calliphora erythrocephala. Biol. Cybern.* 59: 71–80, 1988.

SELVERSTON, A. I., KLEINDIENST, H.-U., AND HUBER, F. Synaptic connectivity between cricket auditory interneurons as studied by selective photoinactivation. *J. Neurosci.* 5: 1283–1292, 1985.

SELVERSTON, A. I. AND MILLER, J. P. Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. I. Pyloric System. *J. Neurophysiol.* 44: 1102–1120, 1980.

SIMMONDS, M. A. Multiple GABA receptors and associated regulatory sites. *Trends Neurosci.* 6: 279–281, 1983.

SOKAL, R. R. AND ROHLF, F. J. *Biometry.* New York: Freeman, 1981.

SRINIVASAN, M. V., LEHRER, M., AND HORRIDGE, G. A. Visual figure-ground discrimination in the honeybee: the role of motion parallax at boundaries. *Proc. R. Soc. Lond. B Biol. Sci.* 238: 331–350, 1990.

STRAUSFELD, N. J. Structural organization of male-specific visual neurons in calliphorid optic lobes. *J. Comp. Physiol. A* 169: 379–393, 1991.

TANAKA, K., HIKOSAKA, K., SAITO, H., YUKIE, M., FUKADA, Y., AND IWAI, E. Analysis of local and wide-field movements in the superior temporal visual areas of the macaque monkey. *J. Neurosci.* 6: 134–144, 1986.

TSAI, H.-J. Response of toad's tectal neurons to in-phase and anti-phase movements of object and textured background. *J. Comp. Physiol. A* 167: 857–863, 1990.

VIRSIK, R. AND REICHARDT, W. Detection and tracking of moving objects by the fly *Musca domestica. Biol. Cybern.* 23: 83–98, 1976.

WAGNER, H. Flight performance and visual control of the flight of the free-flying housefly (*Musca domestica*). II. Pursuit of targets. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 312: 553–579, 1986.

WARZECHA, A.-K., BORST, A., AND EGELHAAF, M. Photo-ablation of single neurons in the fly visual system reveals neural circuit for the detection of small moving objects. *Neurosci. Lett.* 141: 119–122, 1992.

WEHRHAHN, C. Sex-specific differences in the chasing behaviour of houseflies (*Musca*). *Biol. Cybern.* 32: 239–241, 1979.