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# GABA-antagonist inverts movement and object detection in flies

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Movement detection is one of the most elementary visual computations performed by vertebrates as well as invertebrates. However, comparatively little is known about the biophysical mechanisms underlying this computation. It has been proposed on both physiological[1,8,21] and theoretical[2,15,23] grounds that inhibition plays a crucial role in the directional selectivity of elementary movement detectors (EMDs). For the first time, we have studied electrophysiological and behavioral changes induced in flies after application of picrotoxinin, an antagonist of GABA. The results show that inhibitory interactions play an important role in movement detection in flies. Furthermore, our behavioral results suggest that the computation of object position is based primarily on movement detection.

Male flies chase female flies. In order to do it successfully, they seem to process at least two different kinds of information — the movement and position of the female being chased. The associated optomotor and fixation responses have been investigated thoroughly at both the behavioral and computational level (reviews see refs. 4, 20).

Stationarily walking or flying flies with head fixed to the thorax follow actively angular displacement of their visual surrounding by turning their body. This optomotor response allows freely moving flies to counteract involuntary deviation from their straight course. It has been shown that a single object leaving the fly's frontal visual field is followed more strongly than an object moving into the frontal position from a lateral position[10,19]. This characteristic asymmetry of the optomotor response could lead to the dynamic fixation of objects in the fly's frontal visual field (fixation behavior or object detection). The hypothesis that fixation behavior is based on movement detection was first proposed by Reichardt[19]. It has been later shown that computation of the position of an object can also be performed independently of movement detection, but through separate computational channels called position detectors (flicker detectors whose outputs are parametrized according to their position in the eye)[17]. These two alternative hypotheses of object detection have been the subject of some controversy[6,9,24].

Although elementary movement detectors (EMDs) have been described at the computational level[18], they have not yet been identified at the cellular level. Torre and Poggio[23] proposed a cellular model for movement detection (Fig. 1b) consistent with the computational models of Hassenstein and Reichardt[12], and of Barlow and Levick[2], which are based on insect behavior and vertebrate neurophysiology, respectively. To account for the non-linearity in these models, Torre and Poggio suggest a cellular mechanism for shunting inhibition based on a non-linear interaction between excitatory and inhibitory synapses on the postsynaptic direction-selective cell. An inhibitory synapse from one photoreceptor (or group of photoreceptors) successfully cancels the signals produced by a neighboring excitatory synapse from the adjacent photoreceptor(s)[15] when stimulation is in the null direction. In the preferred direction, excitation is not simultaneous with, but precedes in-

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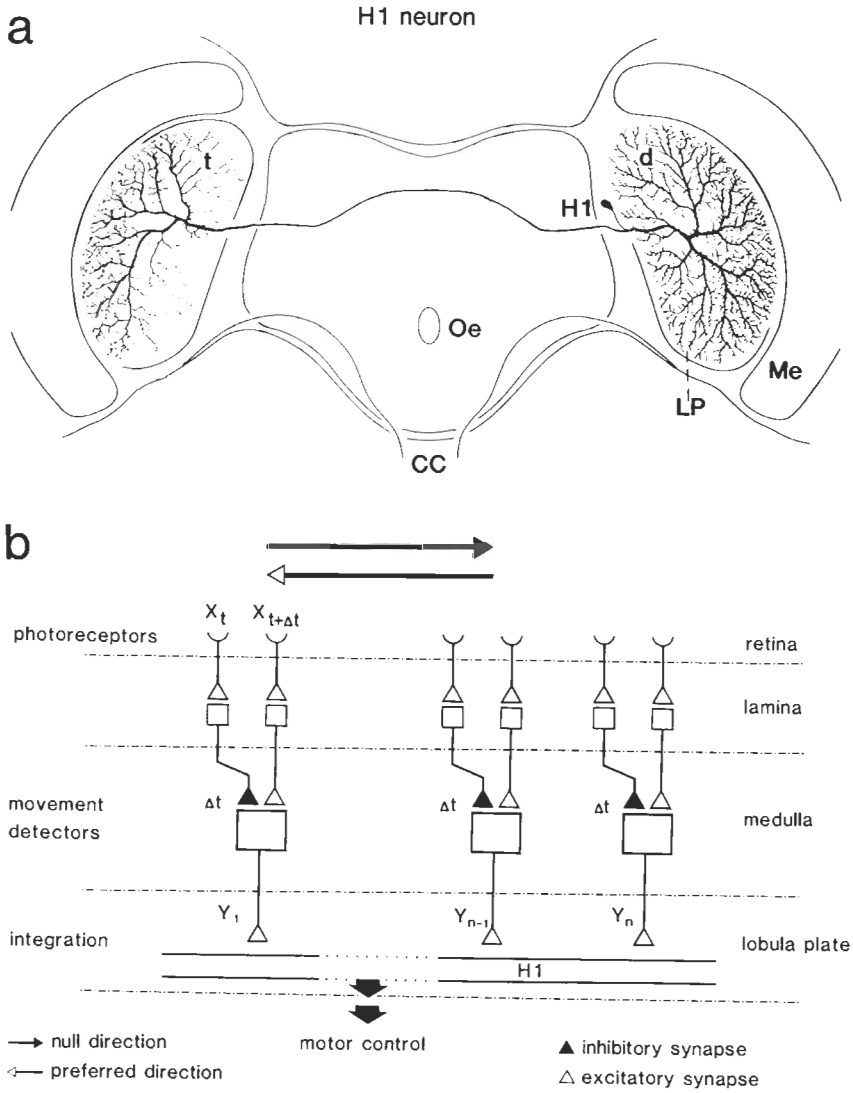



Fig. 1. a: frontal view of the direction-selective H1-neuron in the brain; courtesy of K. Hausen. Me, medulla; LP, lobula plate; CC, cervical connective; Oe, oesophagus; d, dendrite; t, terminal. b: minimal scheme (one synaptic layer per neuropil) presenting the processing of information relevant to movement detection. The elementary movement detectors (EMDs) distal to the giant cells (e.g. H1-cell) of the lobula plate are shown here in the medulla. The excitatory and delayed inhibitory synapses of each EMD belong to two neighboring photoreceptors or group of photoreceptors. By stimulation in the null direction, the asymmetrical delay ($\Delta t$) allows the inhibitory element stimulated first to shunt successfully the signals induced by the excitatory synapse. In the preferred direction, the inhibitory synapse is activated after the excitatory one. Only EMDs with the same preferred direction as the H1-neuron are shown. The H1-neuron integrates the direction-sensitive outputs ($Y_1 \ldots Y_n$) of the EMDs of the ipsilateral eye and projects into the contralateral lobula plate. Other giant cells (e.g. HS-neurons for horizontal movement) transmit the information toward the motor center. $X_t$, $X_{t+\Delta t}$ inputs to the EMD, $Y$ output of the EMD.

hibition, and therefore remains uneffected. This model predicts that drugs interfering with inhibition should induce modification of directional selectivity of the EMDs.

In the fly *Calliphora erythrocephala*, intra- and extracellular recordings in the lobula plate (posterior part of the lobula complex, third visual neuropil in dipteran optic lobe) have characterized giant cells which are sensitive to the direction of movement[14]. The effect of picrotoxinin on the spike activity of one of them, the H1-neuron (Fig. 1a), is reported in this study. This neuron integrates movement detector

outputs (Fig. 1b) over the whole ipsilateral visual field, and projects into the contralateral lobula plate[13,14]. Its spike rate is enhanced for movement in the preferred (back-to-front) direction and reduced for movement in the null (front-to-back) direction. In Fig. 1b, only EMDs with the same preferred direction as the H1-neuron are shown. They feed their output to this cell through excitatory synapses. EMDs with an opposite preferred direction (i.e. the null direction of the H1-neuron) are most probably connected to H1 via inhibitory synapses[13].

We used picrotoxinin, an antagonist of the inhibitory neurotransmitter, $\gamma$-aminobutyric acid (GABA), in vertebrates and invertebrates[22] in order to assess the importance of inhibitory interaction for directional selectivity in flies. We assume that the picrotoxinin-induced changes reflect indeed a blockage of the GABA receptors and not other picrotoxinin effects such as dopamine release[16]. Similar experiments with the GABA antagonists picrotoxinin and bicuculline have shown that directional selectivity is abolished in the retina and cortex of mammals[1,8,21]. Bicuculline, a more specific antagonist of GABA, has proved to be ineffective in flies (Schmid and Bülthoff, in preparation).

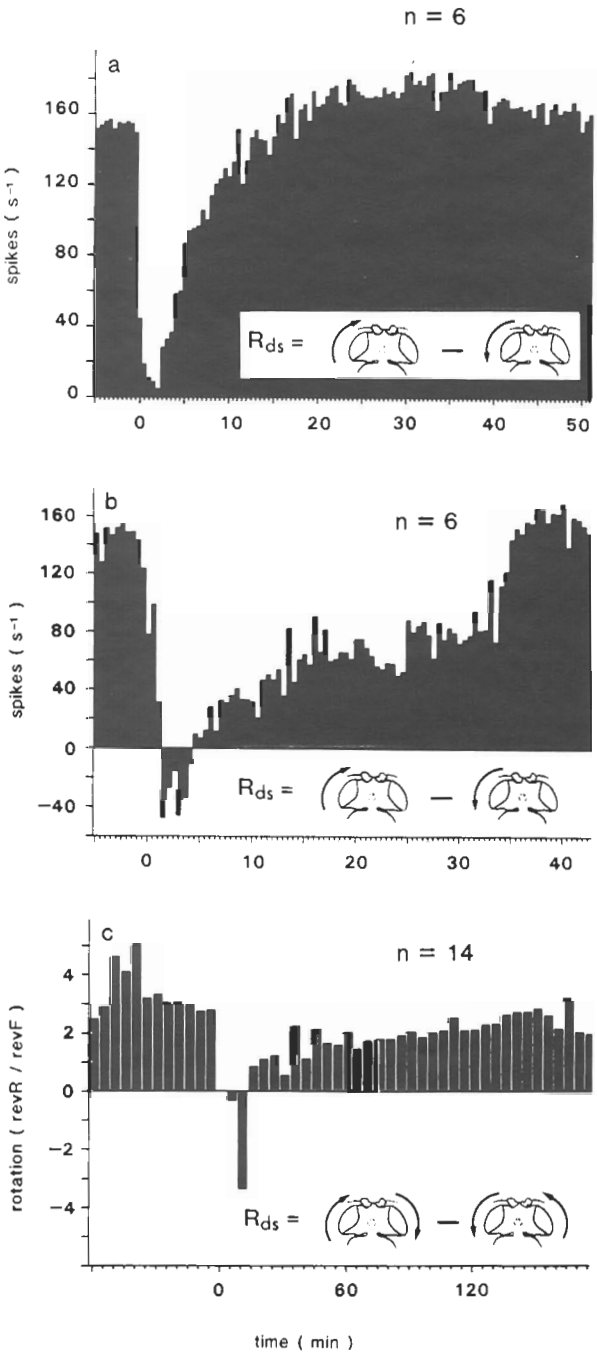
Extracellular recordings of the H1-neuron in visually stimulated flies show a strong modification of its directional selectivity immediately after application of picrotoxinin. The activity of the direction-selective H1-neuron of female *Calliphora erythrocephala* was recorded extracellularly with tungsten electrodes. After stable recording conditions were obtained, picrotoxinin was injected into the haemolymph (100–200 pmol) with a $\mu$l-syringe (Hamilton) or pressure-injected (Neurophore PPM2, Medical Systems) into the brain (1–5 pmol) of the fly through the opened rear side of the head capsula. Spikes with an amplitude above a threshold chosen so as to eliminate background noise were recorded. The directional selectivity $R_{ds}$ of the H1-neuron was calculated as the difference between the spike rate for movement in the preferred (back-to-front) and the null (front-to-back) direction.

The visual stimuli were presented on a CRT monitor (Tektronix 608, P31 phosphor) placed in front of the eye ipsilaterally to the recorded H1-neuron. Periodic gratings of bright and dark bars could be moved or flickered in counterphase (screen diameter 39°; spatial wavelength of grating 13°; contrast 30%; stimulus frequency 3 Hz). The flies were stimulated repetitively with a constant sequence of visual stimuli. This consisted of motion from back to front, counterphase flicker, motion from front to back and again counterphase flicker. Each stimulus lasted for 3 s and the stimuli were displayed in intervals of 10 s.

The direction-specific response ($R_{ds}$) drops dramatically after pressure-injection of 1–5 pmol picrotoxinin into the brain and then recovers within 15–45 min (average of 6 flies in Fig. 2a). Some flies show in the recovery period a slightly higher response than in the pretest period. The picrotoxinin-induced reduction of $R_{ds}$ is in agreement with models invoking inhibition as a crucial element in movement detection. The excitation induced by the stimulus in the null direction can no longer be shunted after removal of the inhibition, and therefore the movement detectors respond equally to both directions. After injection of a larger dose (up to 8 pmol) into the brain, or application of a much higher amount (100–200 pmol) of this drug to the haemolymph of the head, we measured a reduction of $R_{ds}$ followed by an inversion of $R_{ds}$, which becomes negative for 5–10 min (Fig. 2b). Immediately after application of this high dosage of picrotoxinin, movement in the preferred direction results in a very high spike frequency (above 200 Hz) but the spike amplitude is strongly reduced (50–80%). Therefore the PST-histograms (Fig. 3) show a transient reduction of the spike frequency for movement in the preferred direction because many spikes fall below the detection threshold. The reduction of the spike amplitude itself cannot be shown in the PST-histograms but has been observed on-line on the oscilloscope which monitors the extracellular signal before feeding it into the threshold of the spike-counter. In the null direction, the frequency of spikes is lower but the spike amplitude is less reduced, allowing more spikes to stay above the detection threshold of the recording device. This suggests that the inverted $R_{ds}$ is due to a picrotoxinin-induced alteration of the H1-neuron and/or its environment — for example, an increase of the extracellular potassium concentration[25] — but is not due to inversion of the EMDs. As shown by the behavioral experiment presented below, the inversion of the directional selectivity of the H1-cell is also detected by the cells postsynaptic to the neurons of the lobula plate. After

injection of 20–30 pmol picrotoxinin into the abdomen, stationary-walking *Drosophila melanogaster* stimulated binocularly show an inverted optomotor response, i.e. a negative $R_{ds}$ for about 10 min (Fig. 2c).

The optomotor following response of *Drosophila* walking on a small ball free to rotate was registered



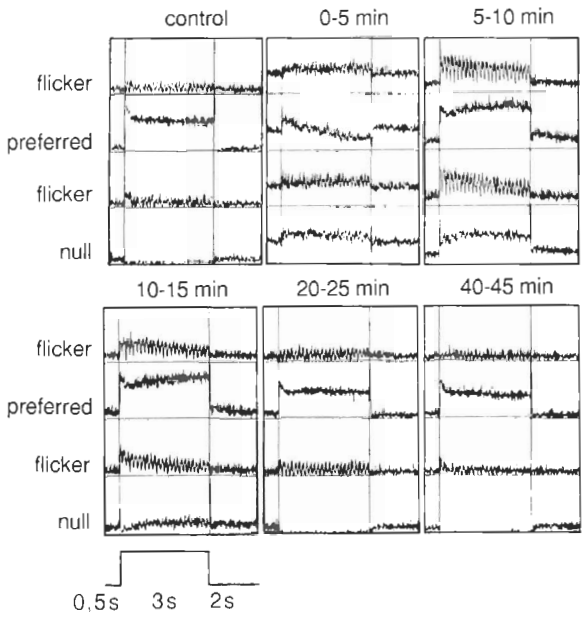




Fig. 3. PST-histogram for a single H1-cell stimulated repetitively with counterphase flicker, motion in the preferred direction, flicker again and then motion in the null direction. To show the time dependence of the responses after application of picrotoxinin PST-histograms were averaged into groups of 5 min. Inversion of the directional selectivity is visible in the first group (0–5 min) in which the response to the preferred direction becomes transient and for most of the stimulus period is lower than the response to movement in the null direction. This implies that the directional selectivity is reversed. Note also the increased flicker sensitivity after picrotoxinin.

Fig. 2. Effect of picrotoxinin on movement detection in flies. As picrotoxinin has a reversible action, the modifications induced by this drug are transient. a: after injection of 1–5 pmol picrotoxinin into the brain of 6 *Calliphora erythrocephala*, the directional selectivity $R_{ds}$ of the H1-neuron is first reduced and, when the drug produces its maximal effect, it is almost suppressed. During the next 45 min, $R_{ds}$ returns to values measured prior to the injection with a small overshoot between 15 and 45 min. b: after application of a much higher dose (100–200 pmol picrotoxinin) into the haemolymph of 6 *Calliphora*, $R_{ds}$ is reduced and then inverted for 5–10 min before slow recovery. c: optomotor walking response (revR/revF) of 14 *Drosophila melanogaster* walking stationarily on a small ball. A fixed fly intending to turn produces rotatory revolutions of the ball (revR) which are counted positively (turn to the right) or negatively (turn to the left). revF, forward revolutions produced by a fly walking straight ahead. The direction-sensitive response, $R_{ds}$, is computed as for the electrophysiological experiments as the difference of the response to opposite directions of motion. After injection of 20–30 pmol picrotoxinin into the abdomen $R_{ds}$ is inverted for the initial 10 min and then recovers slowly within 1 h.

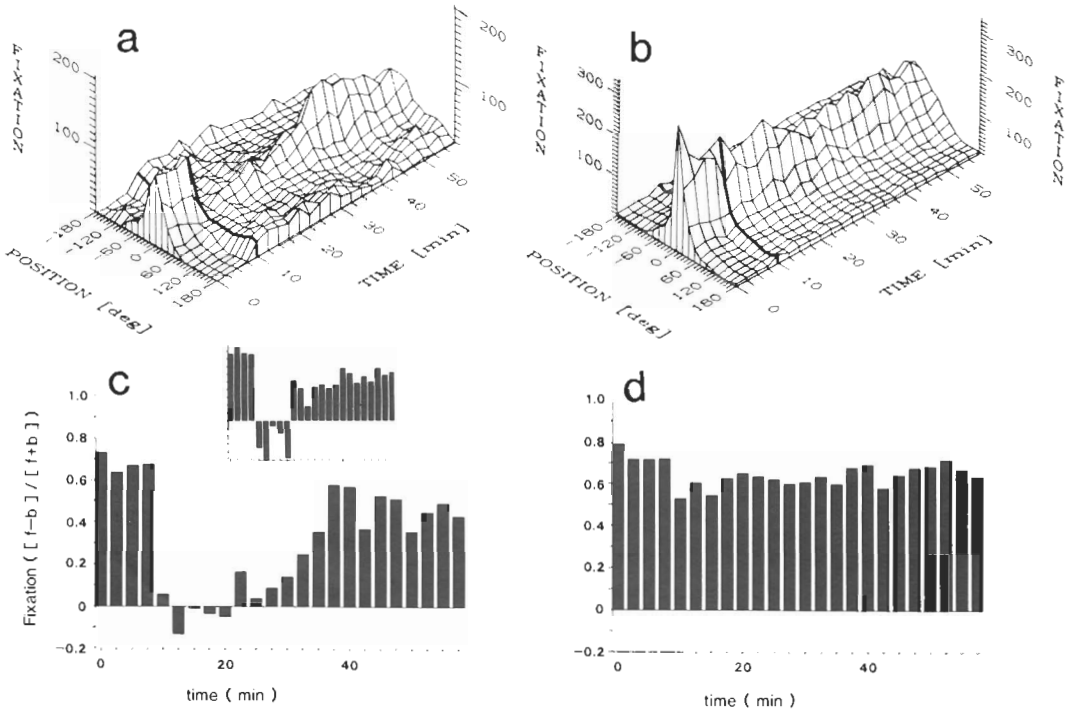



Fig. 4. Effect of picrotoxinin on the fixation behavior of stationarily flying *Drosophila melanogaster* in a flight-simulator in which the fly is able to control its visual surrounding by the difference of its wingbeat amplitudes. The histograms in a and b show the position of an object (20° black stripe) relative to the fly for successive periods of 2.5 min. Before injection the most frequent position of the object is in front of the fly (around 0°). a: about 5 min after injection of 20–30 pmol picrotoxinin into the abdomen, 11 of 22 flies fixate the object more frequently in the back (around ± 180°), i.e. they show antifixation. b: the other 11 flies do not seem to modify their fixation behavior. The heavy black line in a and b represents the first results obtained after application of the drug. In c and d, fixation is calculated from the results shown in a and b as (f–b)/f+b), where f is the integral of object position in front of the fly (between 0° and ±90°) and b the integral of object position behind the fly (between ±90° and ±180°). In c the negative values of (f–b)/(f+b) correspond to the period during which the flies show antifixation. The results of a strongly antifixating single fly are shown in the inset of c.

before and after injection of picrotoxinin. $R_{ds}$ was calculated as the difference between the optomotor following response to clockwise and counterclockwise moving sine-wave gratings with the same size and wavelength as in the electrophysiological experiments. The locomotion recorder is described elsewhere[3,5]. This method is not applicable to measure the optomotor response of *Calliphora*, because these flies walk only very poorly under these conditions. On the other hand, electrophysiological recordings of *Drosophila* are too difficult for obvious reasons. Therefore the electrophysiological and behavioral experiments had to be carried out in different species.

Instead of following the displacement of the visual surroundings, the turning tendency of drug-injected flies is directed in the opposite direction. This result suggests, moreover, that the inversion of directional selectivity does not occur for the H1-neuron alone, but more generally for the movement-sensitive cells of the visual system whose outputs project toward the motor center.

The analysis of the optomotor response of flies stimulated monocularly with a moving grating (data not shown) shows that, contrary to untreated flies, picrotoxinin-injected flies respond more strongly to back-to-front than to front-to-back movement[7]. This should elicit antifixation of an object — i.e. the fly should apparently avoid fixation of an object in its frontal visual field if the fixation behavior relies on the asymmetry in movement detection described above. For measuring fixation behavior, the flying *Drosophila* was suspended in the center of a cylinder consisting of a homogeneous background and a single vertical black bar (20° wide). The position of the black bar was controlled indirectly by the difference between the left and right wing beat amplitude (method described by K.G. Götz)[11]. The position of

the black bar was monitored before and after the injection of 10–30 pmol picrotoxinin. Controls were done with injections of water. Since the range between the minimum quantity of picrotoxinin required to induce inversion in fixation and the maximum dose allowing the insect to fly properly is very small, only 50% of the flies able to fly after injection developed the expected transient antifixation of an object (Fig. 4a, c). The others showed no significant modification of their fixation response presumably because the injected dose was too low (Fig. 4b, d). The fact that the application of picrotoxinin can result in the inversion of both optomotor and fixation response suggests that detection of object position requires movement detection as an intermediate processing stage. The electrophysiological results show that the picrotoxinin-induced changes observed in behavior are caused by an inversion at the level of motion integration and not at the motor output.

At present, mapping of neuronal activity with the deoxyglucose method and mapping of GABAergic cells with immunochemical staining methods are in progress. These experiments should allow us to localize the sites of inhibitory interactions in the visual neuropil where picrotoxinin is expected to interfere. Preliminary results suggest that picrotoxinin modifies the intensity of neuronal activity in the lobula plate where potential GABAergic cells have been mapped (Buchner, personal communication; Bülthoff, unpublished results).

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1 Ariel, M. and Daw, N.W., Pharmacological analysis of directionally sensitive rabbit retinal ganglion cells, *J. Physiol. (London)*, 324 (1982) 161–185.
2 Barlow, H.B. and Levick, W.R., The mechanism of directionally selective units in rabbit's retina, *J. Physiol. (London)*, 178 (1965) 477–504.
3 Buchner, E., Elementary movement detectors in an insect visual system, *Biol. Cybern.*, 24 (1976) 85–101.
4 Buchner, E., Behavioural analysis of spatial vision in insects. In M.A. Ali (Ed.). *Photoreception and Vision in Invertebrates*, Plenum, New York, London, 1984, pp. 561–621.
5 Bülthoff, H., *Drosophila* mutants disturbed in visual orientation. II. Mutants affected in movement and position computation, *Biol. Cybern.*, 45 (1982) 71–77.
6 Bülthoff, H. and Wehrhahn, C., Computation of motion and position in the visual system of the fly (*Musca*). Experiments with uniform stimulation. In D. Varjú and U. Schnitzler (Eds.), *Localization and Orientation in Biology and Engineering*, Springer, Berlin-Heidelberg, 1984, pp. 149–152.
7 Bülthoff, H., Bülthoff, I., Combining neuropharmacology and behavior to study motion detection in flies, *Biol. Cybern.*, in press.
8 Caldwell, J.H., Daw, N.W. and Wyatt, H.J., Effects of picrotoxin and strychnine on rabbit retinal ganglion cells: lateral interactions for cells with more complex receptive fields, *J. Physiol. (London)*, 276 (1978) 277–298.
9 Geiger, G., Is there a motion-independent position computation of an object in the visual system of the housefly? *Biol. Cybern.*, 40 (1981) 71–75.
10 Götz, K.G., Hirnforschung am Navigationssystem der Fliegen, *Naturwissenschaften*, 62 (1975) 468–475.
11 Götz, K.G., Genetischer Abbau der visuellen Orientierung bei *Drosophila*, *Verh. dt. Zool. Ges.*, 1983 (1983) 83–99.
12 Hassenstein, B. and Reichardt, W., Systemtheoretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers *Chlorophanus*, *Z. Naturforsch.*, 11 (1956) 513–524.
13 Hausen, K., Monocular and binocular computation of motion in the lobula plate of the fly, *Verh. dt. Zool. Ges.*, 1981 (1981) 49–70.
14 Hausen, K., The lobula-complex of the fly: Structure, function and significance in visual behaviour. In M.A. Ali (Ed.), *Photoreception and Vision in Invertebrates*, Plenum, New York, London, 1984, pp. 523–559.
15 Koch, C., Poggio, T. and Torre, V., Nonlinear interaction in a dendritic tree: localization, timing and role in information processing, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 2799–2802.
16 O'Connor, P., Dorison, S.J., Watling, K.J. and Dowling, J.E., Factors affecting release of $^3$H-dopamine from perfused carp retina, *J. Neurosci.*, 6 (1986) 1857–1865.
17 Pick, B., Visual flicker induces orientation behaviour in the fly *Musca*, *Z. Naturforsch.*, 29 (1974) 310–312.
18 Poggio, T. and Reichardt, W., Considerations on models of movement detection, *Kybernetik*, 13 (1973) 223–227.
19 Reichardt, W., Musterinduzierte Flugorientierung. Verhaltens-Versuche and der Fliege *Musca domestica*, *Naturwissenschaften*, 60 (1973) 122–138.
20 Reichardt, W. and Poggio, T., Visual control of orientation behaviour in the fly. Part I. A quantitative analysis, *Q. Rev. Biophys.*, 9 (1976) 311–375.
21 Sillito, A.M., Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex, *J. Physiol. (London)*, 271 (1977) 699–720.
22 Takeuchi, A. and Takeuchi, N., A study of the action of picrotoxin on the inhibitory neuromuscular junction of the crayfish, *J. Physiol. (London)*, 205 (1969) 377–391.

23 Torre, V. and Poggio, T., A synaptic mechanism possibly underlying directional selectivity to motion, *Proc. R. Soc. London Ser. B*, 202 (1978) 409–416.
24 Wehrhahn, C. and Hausen, K., How is tracking and fixation accomplished in the nervous system of the fly? A behavioural analysis based on short-time stimulation, *Biol. Cybern.*, 38 (1980) 179–186.
25 Yarom, Y., Grossmann, Y., Gutnick, M.J. and Spira, M.E., Transient extracellular potassium accumulation produced prolonged depolarizations during synchronized bursts in Picrotoxin-treated cockroach CNS, *J. Neurophysiol.*, 48 (1982) 1089–1097.