A Fast Stimulus Procedure to Determine Local Receptive Field Properties of Motion-sensitive Visual Interneurons

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We present a method to determine, within a few seconds, the local preferred direction (LPD) and local motion sensitivity (LMS) in small patches of the receptive fields of wide-field motion-sensitive neurons. This allows us to map, even during intracellular recordings, the distribution of LPD and LMS over the huge receptive fields of neurons sensing self-motions of the animal. Comparisons of the response field of a given neuron with the optic flow fields caused by different movements in space, allows us to specify the particular motion of the animal sensed by that neuron.

Visual system Motion detection Directional selectivity Visual stimulation Visual interneurons Optic flow *Calliphora erythrocephala*

INTRODUCTION

The coordination of locomotion in a given environment requires information about momentary self-motion. For visually oriented animals, including man, the optic flow that is generated over both eyes during locomotion in a visually structured environment is an adequate source of such information (Gibson, 1950; Nakayama & Loomis, 1974; Koenderink & van Doorn, 1987). It is a question of increasing interest how the optic flow is exploited by the visual system at the neuronal level. In electrophysiological investigations in both vertebrates and invertebrates, motion-sensitive wide-field neurons have been found that are thought to be involved in optic flow processing (reviewed by Albright, 1993; Hausen, 1993). There are at least two experimental approaches to studying such neurons. The first is to present complex motion stimuli or drifting gratings approximating certain global aspects of optic flow. These stimuli can be generated in different ways using moveable slide projections (e.g. Tanaka & Saito, 1989), computer-generated "cartoons" displayed on a screen (e.g. Lemmnitz & Gewecke, 1992; Duffy & Wurtz, 1991), computer-controlled oscilloscope screens (Srinivasan & Dvorak, 1980), image synthesizers (e.g. Borst, 1991) or pattern projectors (e.g. Hengstenberg, 1982; Hausen, 1982). In all of these cases it is possible to classify the neurons with respect to their general responsiveness to translatory and/or rotatory optic flow components. The second approach is the application of local motion stimuli in order to determine the directional tuning curves and motion sensitivities at many positions within the receptive field. This approach permits a detailed investigation of the particular input organization of the recorded wide-field neurons. In addition, if the locally determined directional tuning curves and motion sensitivities at many positions in the visual field are known, the resulting response field can be quantitatively compared with calculated optic flow fields.

The duration of stable recordings from single neurons is often limited, especially when intracellular recording techniques are applied. If, therefore, the directional tuning and motion sensitivities are to be determined at many positions in the visual field, a rapid measuring procedure is required. Inspired by visual stimuli used in electrophysiological experiments on the visual system of the cat (Schoppmann & Hoffmann, 1976) and behavioural experiments on free-flying fruitflies (David, 1985), we developed such a fast procedure. We tested its reliability with recordings from some well known motion-sensitive wide-field neurons in the third visual neuropil of the blowfly *Calliphora* (H1 and V1; Hausen, 1984). These neurons spatially integrate the signals of extended retinotopic arrays of elementary movement detectors (EMDs; reviewed by Reichardt, 1987; Borst & Egelhaaf, 1989; Hausen, 1993), where each single EMD analyses the local motion along its respective preferred direction. In a crude approximation, the H1 neuron integrates the signals of EMDs with horizontally oriented preferred directions. The V1 neuron, however, receives its input from some (contralateral) wide-field neurons which

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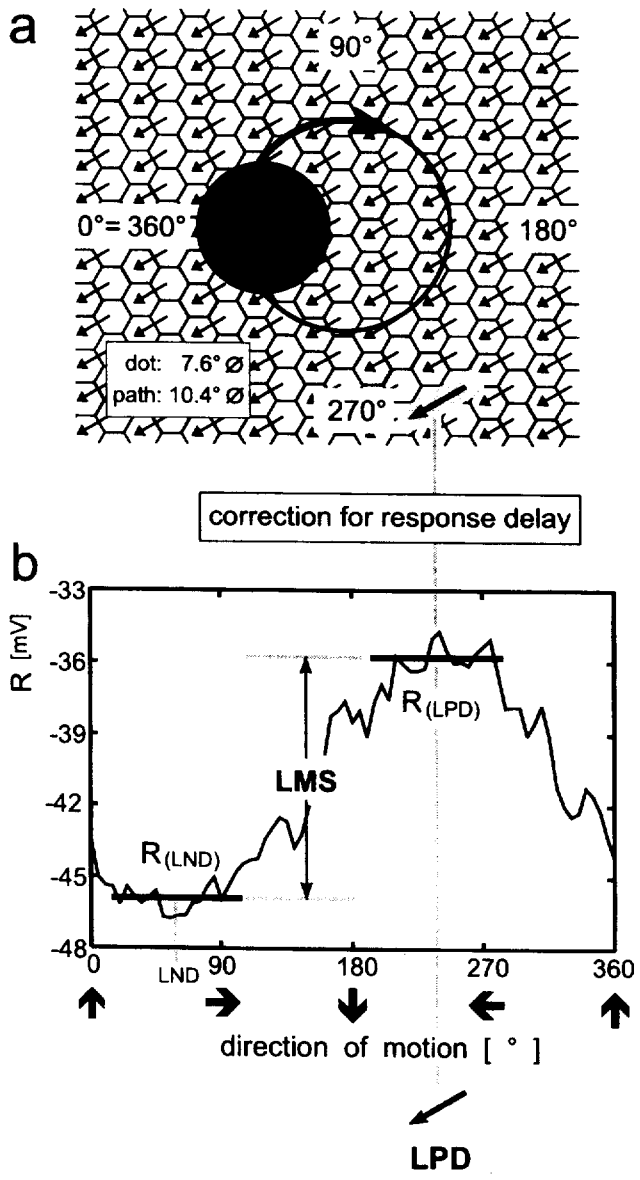

FIGURE 1. Determination of the local preferred direction (LPD) and local motion sensitivity (LMS) of a visual interneuron. (a) A small area (*c.* 4%) of the compound eye of the fly is shown by the hexagonal pattern. Thin arrows indicate the preferred directions of elementary movement detectors (EMDs). The visual stimulus is a black dot (diameter: 7.6 deg; solid angle as seen by the fly) that is moved clockwise (thick curved arrow) at a constant speed on a circular path (diameter: 10.4 deg). The direction of dot motion changes continuously from vertical upwards (= 0 deg = ↑) to horizontal front-to-back (= 90 deg = →) to vertical downwards (= 180 deg = ↓) to horizontal back-to-front (= 270 deg = ←) and to vertical upwards again (= 360 deg = 0 deg = ↑). If the instantaneous direction of dot motion coincides with the preferred direction of the EMDs whose signals are integrated by a (e.g. intracellularly) recorded neuron, the measured response reaches its maximum (b). The influence of the response delay is corrected by comparing responses to cw and ccw stimulation (see text). The LPD is given by the mean vector of the corrected response curve as determined by circular statistics. The LMS is defined as the difference between the averaged response of the intervals LPD $\pm$ 45 deg and LND $\pm$ 45 deg, where LND denotes the local null direction: $LMS = R_{(LPD \pm 45\ deg)} - R_{(LND \pm 45\ deg)}$ (see bottom part).

predominatly integrate the signals from EMDs with vertically oriented preferred directions. There is good neuroanatomical, neurogenetic, electrophysiological and behavioural evidence that some of the wide-field neurons of the third visual neuropil are involved in the visual control of posture and locomotion (Hausen, 1993).

MATERIALS AND METHODS

Determination of the LPD and the LMS

To determine the LPD and LMS over a small area of the fly's eye, a black dot (diameter = 7.6 deg) is moved clockwise (cw) at constant speed on a circular path (diameter = 10.4 deg). Thus, during one cycle the dot continuously runs through all possible directions of motion [Fig. 1(a)]. When the instantaneous direction of dot motion coincides with the preferred direction of the EMDs whose signals are integrated on the dendrites of the simultaneously recorded neuron, the measured response reaches its maximum [Fig. 1(b)]. To improve the signal-to-noise ratio the response of the neuron is subdivided into 72 bins per stimulus period, thus pooling data over 5 deg wide segments of direction of stimulus motion.

The response of the neuron is phase-shifted with respect to the instantaneous direction of dot motion by the latency and temporal filter properties of the visual system. The response may also be distorted if the response depends upon the position of the dot in the stimulated array. The phase shift can be corrected and the distortion reduced by comparing responses to clockwise (cw) and counterclockwise (ccw) dot motion.

One of the two responses (e.g. the ccw response) is reversed and shifted in phase by 180 deg because the sense of dot motion has been reversed but not the preferred direction of the neuron. This transformation converts the phase delay of the ccw response into a phase advance in the ccw* response. Consequently, the two response curves are separated by twice the unknown phase shift. This can be determined by calculating the direction of the mean vector of the two response curves (Batschelet, 1981). The results are equivalent to the arguments of the first harmonics obtained by discrete Fourier transformation of the responses. The two response curves are each shifted by half the phase difference in the appropriate direction and then averaged. From this corrected local motion tuning curve the local preferred direction (LPD) is calculated, again by circular statistics or discrete Fourier transformation. The local motion sensitivity (LMS) is defined as the difference between the mean response of the interval LPD $\pm$ 45 deg and the mean response of the interval LND $\pm$ 45 deg, where LND (local null direction) is assumed to be 180 deg apart from LPD [see Fig. 1(b)].

Our procedure has been designed to map relative motion sensitivity over the receptive field, and to compare response maps of different neurons, or different recordings from the same neuron in different individuals. To facilitate such comparisons we normalize the local responses relative to the maximum response in the receptive field. For other purposes a different normalization e.g. relative to the local mean response may be useful.

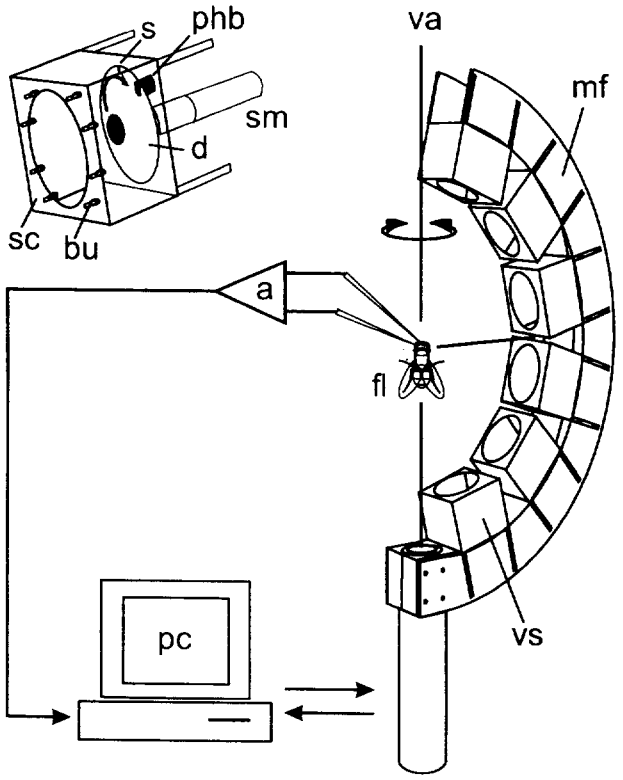

FIGURE 2. Experimental set-up. Six visual stimulators (vs) are mounted at elevations of ±15, ±45 and ±75 deg on a meridional frame (mf) that is centred at the fly's (fl) head and can be turned manually around its vertical axis (va). The equator of the fly's eyes is aligned with the horizontal plane of the apparatus (in the schematic drawing the thorax and abdomen are bent ventrally by 90 deg with respect to the head). Each visual stimulator consists of a servo motor (sm) turning a disk (d) with a single excentric black dot. A photoelectric barrier (phb) activated by a slit at the edge of the disk gives a phase reference pulse for the stimulation cycle. The pattern is illuminated by eight small electric bulbs (bu) on the inner side of the screen (sc) which confines the fly's view to the moving dot. Signals from single neurons are recorded extra- or intracellularly and are processed together with stimulus signals by a personal computer (pc) that is also used to control the visual stimuli.

LMS could also be determined by twice the amplitude of the first harmonic from the discrete Fourier transformation of the response or, equivalently, by the mean vector length from circular statistics. All three methods utilize most or all data contained in the local motion tuning curve. Compared to just reading the minima and maxima of the tuning curve, the accuracy of the determination of LPD and LMS is improved by a factor $m^{-1/2}$, where m denotes the number of statistically independent samples per stimulus cycle. This advantage is most important for intracellular recordings, where the number of possible stimulus repetitions n is limited by the stability of the penetration.

Experimental set-up

Figure 2 shows the apparatus for determining the LPD and LMS at many positions within the visual field. It consists of a meridional frame that can be rotated manually around its vertical axis. The respective position along the azimuth is measured by a circular potentiometer and displayed to allow defined adjustment under visual control. The frame carries six local stimulators at elevations of ±15, ±45 and ±75 deg relative to the horizontal plane; for stimulation one of the six can be selected by a computer (see below). Each stimulator contains a pivoted disk carrying a visual object (e.g. a black dot on white background; see inset Fig. 2). This pattern is rotated by a servo motor and illuminated by eight small electric bulbs. In the centre of the apparatus the fly is mounted in an adjustable holder (see Fig. 2). Its frontal eye equator is aligned exactly with the horizontal plane of the apparatus. A screen ensures that the fly can only see the pattern. Once per stimulus cycle a slit at the edge of the disk passes a photoelectric barrier (see inset Fig. 2), and produces a phase reference pulse, defining phase zero in the stimulus cycle and allowing us to reconstruct the instantaneous direction of dot motion.

Signals from visual interneurons were recorded either by extra- or intracellular techniques and were sampled at a frequency of 0.7 kHz by a personal computer (IBM, PC 486). For extracellular recordings electrolytically shaped tungsten electrodes with a tip diameter <1 μm and an impedance of about 2 MΩ were used. Intracellular electrodes were pulled from capillaries (Clark, GC 100F-10 500 PCS) on a Flaming/Brown micropipette puller (Sutter Instruments Co., P-87); the resistance of the dye-filled electrodes (Sigma, Lucifer Yellow CH 3% in 1 M LiCl) ranged between 50 and 80 MΩ. The experimental data were also recorded on a Digital-Audio-Tape (Biologic, DTR 1800) for off-line analysis and later rendering of single sweep. The action potentials of spiking neurons were selected by an amplitude window discriminator and converted into standard pulses. Care was taken to avoid under- or oversampling the pulses. For details of preparation see Egelhaaf *et al.* (1989). The experiments were carried out under dim ambient light (40 lx at the position of the fly in the stimulus apparatus). Stimulus patterns were highly visible for the fly (luminance 100–400 cd/m^2, contrast 75–93%; see legend of Fig. 6).

RESULTS

To test the performance of the method, spikes were recorded extracellularly from two individual neurons which have been thoroughly investigated with respect to their directional tuning. The H1 responds strongly to horizontal movements in its receptive field, whereas the V1 is most excited by vertical motion (Hausen, 1984; van Hateren, 1990). The stimuli were placed in the right visual hemisphere at an azimuth of 15 deg and an elevation of −15 deg for the V1 neuron or at an azimuth of 45 deg and an elevation of −15 deg for the H1 neuron. The H1 neuron was recorded in the right lobula plate, which is the third visual neuropil behind the compound eye of the fly, whereas the V1 neuron was recorded in the left lobula plate. As an example, the response of the H1 neuron to a single clockwise stimulus cycle of the black

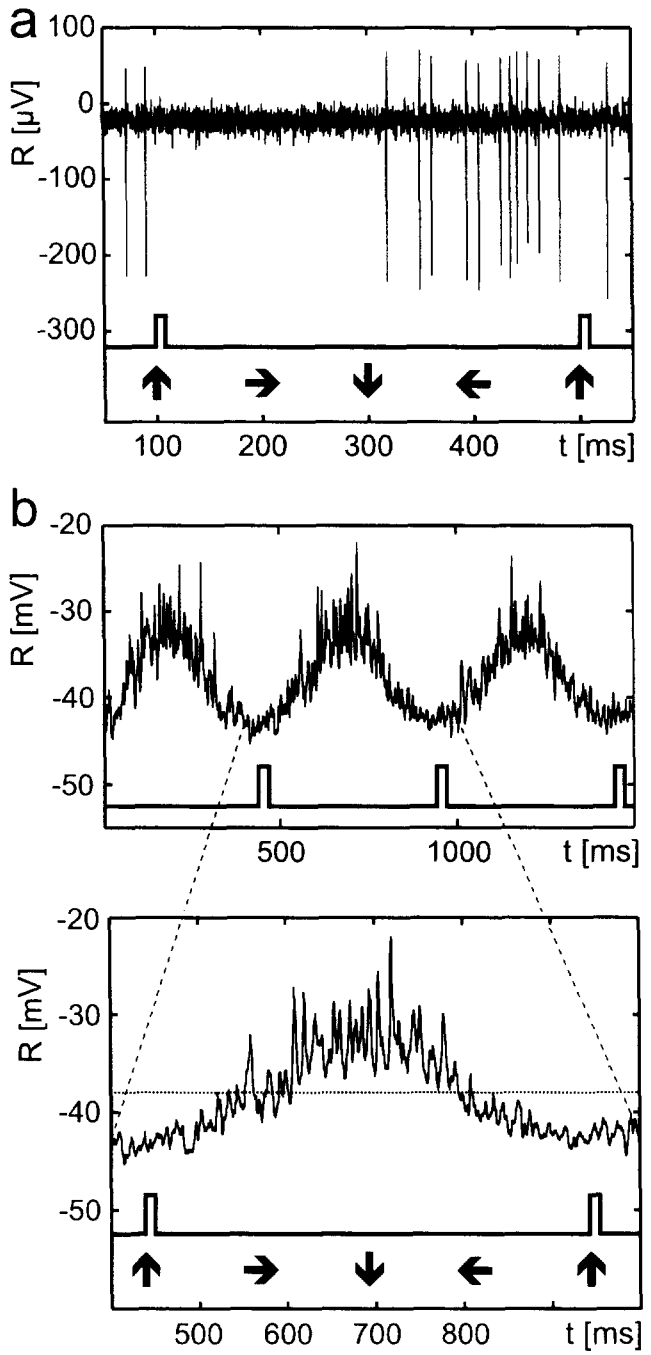

FIGURE 3. Intra- and extracellular responses to the moving black dot. (a) Response of the neuron H1 to a single stimulus cycle (stimulus position: azimuth = 45 deg, elevation = −15 deg). The dot was moved clockwise at a constant speed of 2.5 cps. The recorded spike activity is plotted over time. The pulses in the bottom trace denote the phase reference pulses; arrows below the bottom trace indicate the instantaneous direction of dot motion. When the dot travels from back-to-front (←) the neuron fires several spikes. The spontaneous activity of the neuron (*c.* 15 spikes/sec) is suppressed when the dot travels from front-to-back (→). (b) The upper part shows the intracellularly recorded response of a VS9 neuron to three successive stimulus cycles at 2 cps (stimulus position: azimuth = 150 deg, elevation = −15 deg). In the lower part, the second response cycle is enlarged to show the relationship between the direction of dot motion (see arrows) and the graded membrane potential. At that stimulus position VS9 is excited by downward motion (↓) and inhibited by upward motion (↑). The dotted line represents the potential in the unstimulated state.

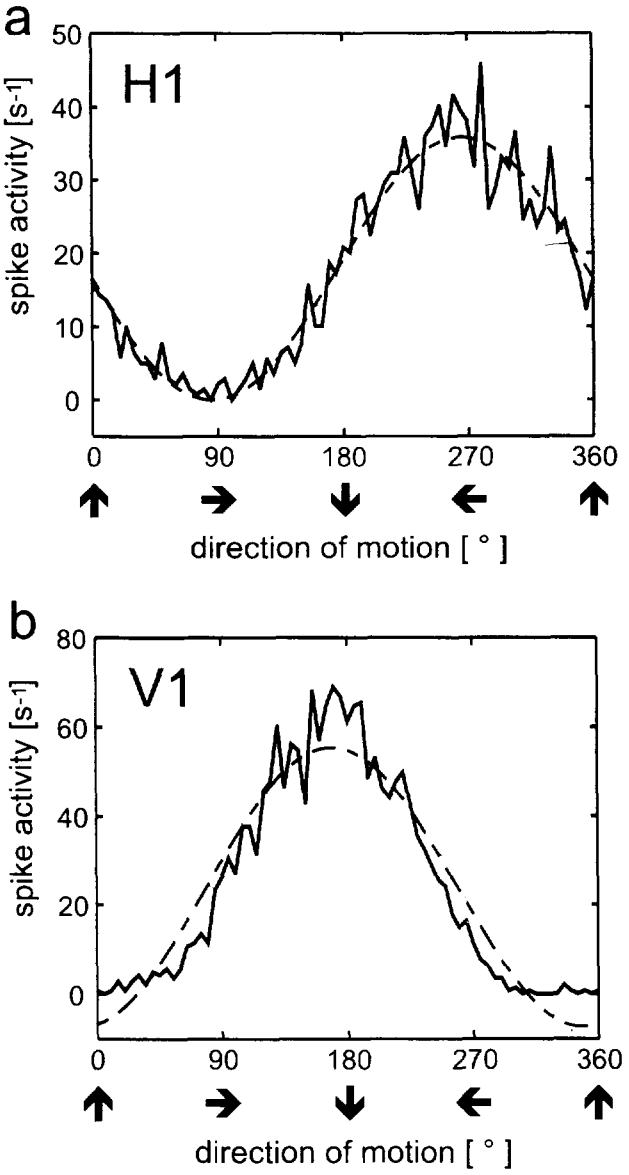

FIGURE 4. Local directional tuning curves of the neurons H1 and V1, respectively. The tuning curves are plotted over the direction of dot motion (see arrows below the abscissa; stimulus parameters as described in the legend of Fig. 3; stimulus position within the right visual hemisphere for H1: azimuth = 45 deg, elevation = −15 deg; for V1: azimuth = 15 deg, elevation = −15 deg). Both curves were obtained by averaging 100 phase-locked response sweeps recorded during dot motion in both cw and ccw and subsequent correction for the response delay (see text). Local preferred directions (LPD) were calculated from successive segments of 10 response curves to stimulation in cw and ccw. (a) For the H1 neuron the mean LPD = 264 deg ± 6 deg SD, i.e., this cell is most excited by almost horizontal back-to-front motion at that particular stimulus position. (b) For the V1 neuron the mean LPD = 169 deg ± 3 deg SD, corresponding to an almost vertical downward motion. At a stimulus speed of 2 cps only 10 sec are required to determine an LPD with sufficient accuracy. The scatter of LPD in successive measurements taken in one fly is surprisingly small (*c.* ±5 deg SD). Dashed lines in (a) and (b) indicate the first harmonics of the Fourier transformation of the respective directional tuning curve.

dot is shown in Fig. 3(a). The spike activity of the neuron is highest when the dot travels from back-to-front. If the dot travels from front-to-back the spike activity of the neuron is inhibited [Fig. 3(a)]. The efficiency of this

stimulus for intracellular recordings is illustrated by the modulation of the membrane potential of an identified VS neuron (Hengstenberg, 1982; Hengstenberg *et al.*, 1982) during three successive stimulus cycles [Fig. 3(b), upper part]. VS neurons respond predominantly with a graded depolarization of their membrane potential to motion in the preferred direction and a graded hyperpolarization to motion in the null direction. At this particular location of the eye the VS9 neuron is strongly depolarized by vertical downward motion of the black dot and hyperpolarized by motion in the opposite direction [Fig. 3(b), lower part].

The phase-locked summation of the responses to 10 stimulation episodes, each comprising 10 cw and ccw stimulus cycles, and the correction for the response delay result in the local tuning curves of the neurons H1 and V1 shown in Fig. 4. They closely resemble the tuning curves obtained with drifting gratings at similar stimulus positions (e.g. Hausen, 1984; van Hateren, 1990). The H1 neuron is maximally excited by horizontal back-to-front motion of the black dot (mean LPD = 264 deg $\pm$ 6 deg SD; $n = 10$) and the LND at 86 deg [see Fig. 4(a)]. In contrast, the V1 neuron responds strongly to vertical downward motion of the stimulus object (mean LPD = 169 deg $\pm$ 3 deg SD, $n = 10$) and is inhibited by vertical upward motion [LND at 349 deg; see Fig. 4(b)]. With 10 cycles of cw and ccw dot motion, the LPD of an extracellularly recorded spiking neuron can be determined accurately. Note the remarkably small SDs of the results. If the stimulus object is moved at 2 cps (cycles per second), the complete determination of a LPD requires little more than 10 sec. In intracellular recordings from neurons that respond to visual stimulation with graded membrane potential changes, the number of cycles can even be reduced to three in each direction. This is nicely illustrated by the similarity of the three individual response cycles of the VS neuron shown in Fig. 3(b). In this case the LPD can be determined within 3 sec.

These results clearly show that the method allows us to determine the LPD and LMS very quickly. However, with respect to general applicability the question is, how reliable are the results when some of the stimulus parameters are changed? To address this question we determined the LPD and LMS (i) at different dot speeds; (ii) with stimulus objects of different shapes and reversed contrast; and (iii) for different path diameters. In addition, we applied such stimuli (iv) at different locations in space in order to map the distribution of LPDs and LMSs within the receptive fields of the neurons H1 and V1.

Speed of dot motion

The response of movement detecting systems depends, among other parameters, on the speed of object motion (Buchner, 1984). By using six different speeds of the stimulus object (0.5, 0.75, 1.0, 1.5, 2.0 and 2.5 cps), we investigated the influence of this parameter on the spike activity of the H1 neuron and the resulting LPD in different flies. Figure 5(a) shows the mean LPD as a function of dot velocity. It is clearly independent of this parameter within the investigated range. In contrast, the mean spike frequency (within the interval LPD $\pm$45 deg) increases with speed [Fig. 5(b)]. The highest spike activity determined from the shortest interspike interval of a single sweep was 150 sec^{-1}, that is, clearly below the neuron's maximum spike activity (about 300 sec^{-1}). This is important because a stimulus eliciting a saturating response would be inappropriate for studying the distribution of LMSs within its receptive field.

The results of these experiments show that the determination of the LPD is independent of the speed in the tested range. The scatter of LPD, measured in different animals, is less than 7 deg SD for all speeds [see Fig. 5(a)]. This is similar in size to the scatter of repeated

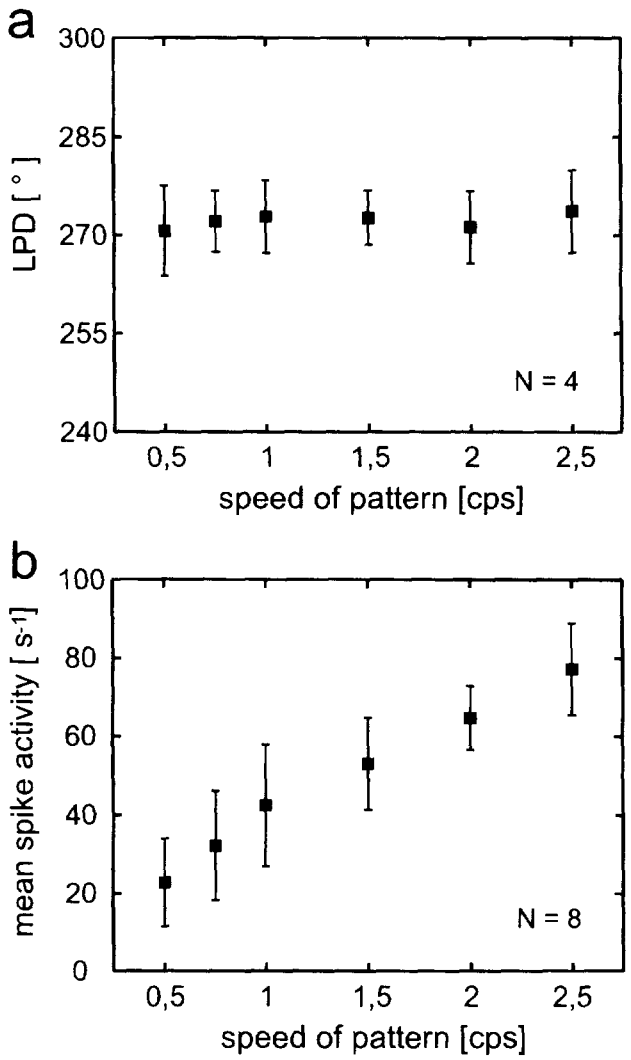

FIGURE 5. (a) LPD and mean spike frequency at different stimulus speeds. The LPDs determined from the responses of the H1 neuron to 10 cw and ccw stimulus cycles recorded with different flies are plotted over the speed of the stimulus. The mean LPD ($\pm$SD, $n = 4$) is clearly independent of the stimulus speed within this range. (b) The mean spike rate (interval LPD $\pm$ 45 deg; $\pm$SD, $n = 8$) increases with stimulus speed, but is never completely saturated. This is important because saturation would lead to an underestimation of the relative LMS, especially in areas of high sensitivity in the receptive field.

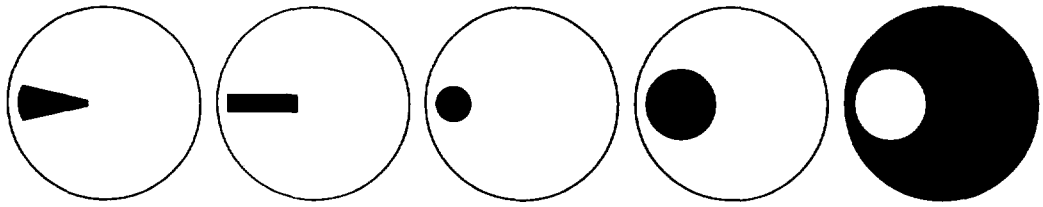

FIGURE 6. The influence of shape, size and contrast of the stimulus pattern on the LPD. Responses of the H1 neuron stimulated at an azimuth of 45 deg and an elevation of −15 deg were recorded. As stimulus objects we used a radially oriented bar (length = 7.6 deg, width = 1.9 deg), a segment of a circle (length = 7.6 deg, outer width = 3.8 deg, inner width = 0.6 deg), a small dot (diameter = 3.8 deg, path diameter = 14.9 deg) and the standard stimulus object (dot, diameter = 7.6 deg, mean luminance = 400 cd/m^2, pattern contrast = 93%). All objects were black on a white background. To test the influence of contrast reversal, a white dot (diameter = 7.6 deg, mean luminance = 100 cd/m^2, pattern contrast = 75%) was mounted on a black disk. The resulting mean LPDs (±SD) are stated below the respective stimulus symbols. Because the LPD is independent of the stimulus speed (see Fig. 5), the results from the six different speeds were averaged for each stimulus pattern. The figure shows that the LPDs are essentially independent of the shape, size and contrast reversal of the pattern.

measurements in a single animal [Fig. 4(a): ±6 deg SD; Fig. 4(b): ±3 deg SD]. These observations prove that at least the fly neurons studied here give exceptionally reproducible and accurate results with this method.

Shape, size and contrast reversal of the stimulus object

Motion responses also depend on the spatial wavelengths and the contrast of stimulus pattern (Buchner, 1984). Because of the complicated dynamic nature of the present visual stimulus we designed experiments to test the influence of stimulus objects of different shapes, sizes and contrasts on the LPD. The stimulus objects we used were a radially oriented bar, the segment of a circle, a smaller dot and the standard stimulus object (dot, diameter = 7.6 deg). All objects were black on a white background. To test the influence of a reversed pattern contrast we also mounted a white dot (diameter = 7.6 deg) on a black disk. The mean LPDs determined from H1 responses are presented below the respective stimulus objects symbolized in Fig. 6. All six speeds were used for each stimulus object. The resulting LPDs were averaged because they are independent of stimulus speed (see section entitled "Speed of dot motion"). As stated above, the amplitude of the tuning curves depends, of course, on several stimulus parameters. Within bounds, however, neither the shape of the tuning curves nor the LPD or LMS depend significantly upon the geometry of the stimulus patterns. The results presented in Fig. 6 show that the LPDs are essentially the same for all the different stimulus patterns used here.

Diameter of motion path

How locally can the LPD be determined? To address this question experimentally we mounted small black dots (diameter = 3.8 deg) at three different eccentricities (3.8, 9.5 and 14.9 deg) on white disks. The standard stimulus was used as a control. In Fig. 7 the LPDs determined from H1 responses are plotted over the path diameters of the respective stimulus pattern. The results show that the LPD is the same for path diameters of 9.5 and 14.9 deg and the standard stimulus (path diameter = 10.4 deg). A small dot moving on a path of 3.8 deg in diameter, however, leads to a strong deviation of the mean LPD and a considerable increase in the standard deviation (see error bar of the respective data point in Fig. 7). The erratic results obtained with the smallest stimulus are not surprising. At 3.8 deg path diameter, the inner edge coincides with the centre of rotation, and is not

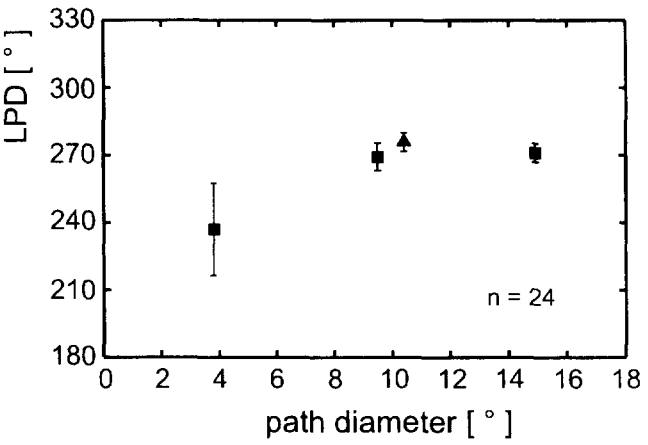

FIGURE 7. Determination of the LPD for different paths of dot motion. Small black dots (diameter 3.8 deg) were mounted on a white pattern carrier at eccentricities resulting in three different diameters of dot motion (3.8, 9.5 and 14.9 deg). The LPDs were determined from the responses of an H1 neuron (stimulus position: azimuth = 45 deg, elevation = −15 deg); six stimulus speeds were applied four times each. The LPDs (squares, ±SD) were averaged for each path diameter (n = 24). The LPD obtained with the standard stimulus (black dot, diameter 7.6 deg) is shown as a control (triangle, ±SD). Path diameters of 9.5 and 14.9 deg led to almost the same mean LPDs as the standard stimulus pattern. The erratic results and large errors at a path diameter of 3.8 deg are to be expected from the size of the stimulus in relation to the sampling base of the movement detectors ($\Delta\varphi \approx 2$ deg; see text).

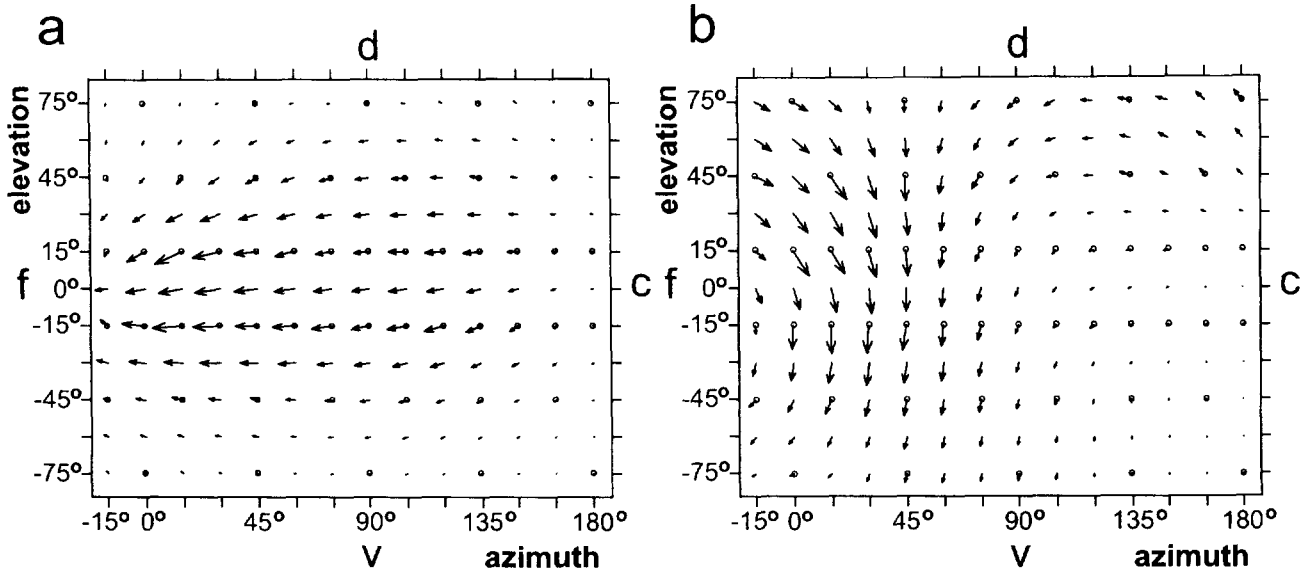

FIGURE 8. Response fields of the neurons H1 (a) and V1 (b) are shown in a Mercator projection (see text) of the right visual hemisphere (f, frontal; c, caudal; d, dorsal, v, ventral). The fly's straight ahead direction would be an azimuth of 0 deg and an elevation of 0 deg. Local motion tuning (obtained with standard stimulus parameters) is represented by arrows. Their direction indicates the local preferred direction (LPD) and their length the normalized local motion sensitivity (LMS). Locations of measurements are marked with little circles; unmarked arrows are interpolated from neighbouring measured responses. The response fields of both neurons extend into the left visual hemisphere (azimuth = −15 deg). The H1 neuron (a) is highly sensitive to horizontal back-to-front motion along the equatorial regions of the visual field. Its motion sensitivity decreases towards the poles of the visual hemisphere. In contrast, the V1 neuron (b) is most sensitive to vertical downward motion in the frontolateral part of the visual field. In the dorsal part of the lateral to caudolateral response field V1 is sensitive to horizontal back-to-front motion and in the dorsocaudal region the neuron responds to slightly tilted upwards motion. The global structure of extended parts of both response fields shows striking similarities with specific rotatory optic flow fields. For the H1 neuron the axis of rotation corresponds to the vertical body axis of the fly. The axis of rotation for the V1 neuron lies approximately in the equatorial plane at an azimuth of about 120 deg. Note the gradual change of LPD and LMS over both response fields.

expected to contribute significantly to the overall response. Moreover, because of the input separation of the fly's movement detectors ($\Delta\varphi \approx 2$ deg) the number of detectors stimulated per cycle becomes too low (see Discussion). Nevertheless, the experiments show that a path diameter of approximately 8 deg ($\approx 4\Delta\varphi$) can be safely used to determine the LPD.

Mapping the response field of the neurons H1 and V1

To demonstrate that our method is suitable for detailed investigations of the receptive field organization of visual interneurons we applied it sequentially at 52 different positions reasonably distributed over more than one visual hemisphere. During extracellular recordings from the H1 and V1, at each position the standard stimulus object (black dot, diameter = 7.6 deg) was moved clockwise and counterclockwise at 2 cps for ten cycles. The LPDs and LMSs were determined from the resulting responses and plotted as arrows in a map of the right visual hemisphere. Due to the kind of projection (Mercator projection) the pole regions are strongly overemphasized with respect to their actual spatial share of the spherical visual field. The orientation of each arrow shows the LPD and its length encodes the normalized LMS. Measuring positions in the map are marked by little circles. Arrows at unmarked positions were obtained by interpolation. Figure 8 shows the response fields of the neurons H1 and V1.

First, it appears that the receptive fields of both neurons extend over large parts of the visual hemisphere, including part of the frontal contralateral visual field (see azimuth = −15 deg in Fig. 8). The H1 neuron responds predominantly to horizontal back-to-front motion. It shows a stripe of high motion sensitivity along the horizontal plane [see Fig. 8(a)]. In contrast, the V1 neuron is very sensitive to vertical downward motion in the frontolateral part of the visual field [see Fig. 8(b)]. In addition, it responds to horizontal back-to-front motion in the dorsal part of the caudolateral visual field. These characteristics of both the H1 and the V1 neurons correspond very well with published results obtained using drifting gratings to determine the general preferred directions of the cells (Hausen, 1976). But it was not known until now that V1 also responds to oblique vertical upward motion in the dorsocaudal region of the visual field [see Fig. 8(b)]. Also, the gradual change of the LPDs from vertical downward in the frontolateral visual field to the almost reversed LPDs in the dorsocaudal region could not be demonstrated using conventional motion stimuli. If a recording is stable enough to map the receptive field several times in one animal, the resulting response fields are indistinguishable in most cases. Similarly, recordings

from the same neuron in different individuals yield almost identical response fields.

The specific structure of the two response fields shown in Fig. 8 has a striking similarity with optic flow patterns that are generated by rotations of the fly around the vertical body axis (in the case of H1) and an oblique horizontal body axis (for V1, at an azimuth of 120 deg and an elevation near 0 deg). The similarity with optic flow patterns is emphasized by the neuron's "belt" of high motion sensitivity along the "equator of rotation", where the rotatory optic flow is also maximal. However, the motion sensitivities are low in the region of the assumed "axis of rotation", where the rotatory flow tends to zero (see Fig. 8).

The results obtained from motion-sensitive interneurons of the fly demonstrate very clearly that this new method of measuring local motion tuning curves is very fast, accurate and reliable. This allows us to map the functional architecture of neurons with very large receptive fields and thus reveal their probable function.

DISCUSSION

General assumptions

The present method yields a *mean* local preferred direction for the small stimulated area within the neuron's receptive field. Due to clockwise and counterclockwise stimulation and comparison of the respective responses the results are independent of the particular input organization in almost every case. (1) The preferred directions of the single EMDs whose signals are integrated at the dendrites of the recorded neuron may scatter considerably without affecting the evaluation of the LPD. (2) The ratio between the excitatory and the inhibitory gain of the EMDs contributing to the local response of the neuron has no effect on the determination of the LPD. (3) Even if the stimulus extends beyond the margin of the receptive field of the recorded neuron, the LPD can be reliably specified. The only trivial requirement that must be fulfilled is that there be any kind of stimulus-induced modulation in the neuron's response at all. In some cases the response modulations might deviate from a sinusoidal function (rising and falling are asymmetrical, e.g. due to a deviation of the preferred and null direction from an antiparallel alignment). Then the phase angle of the maximal response should be determined from more than the first harmonics (from four harmonics, for instance) of the discrete Fourier transformation rather than from circular statistics. Otherwise a phase error in the determination of the LPD will result that amounts to half the angle of divergence from an antiparallel alignment of the preferred and null direction. The tuning curves shown in Fig. 4, however, are almost sinusoidal. Therefore, the respective LPDs could be determined simply by using circular statistics.

Crucial stimulus parameters

The stimulus parameters of this method have to be adapted to the visual system under investigation. The size of the stimulus object must be large enough to be resolved by the optics of the system and sufficient contrast must be transferred at every location within the visual field. As a first approximation in flies the angle between neighbouring ommatidia ($\Delta\varphi$) can be considered as a measure of the spatial resolution of the eye. In *Calliphora* $\Delta\varphi$ ranges between 1.5 and 2.5 deg. Thus, a black dot with a diameter of 7.6 deg is large enough to be seen properly by the fly everywhere within the visual field. As already mentioned above (see section entitled "Speed of dot motion") the response to motion depends on the speed of the stimulus. Between zero and infinite speed an optimum speed is to be expected where the response becomes maximum. With respect to contrast transfer and the optimal speed of motion our stimulus parameters were deduced from the results of behavioural and electrophysiological investigations on the visual system of the fly (Götz, 1965; Hengstenberg, 1982; reviewed by Buchner, 1984; Hausen, 1984).

The results in Fig. 7 show that the preferred direction cannot be determined correctly if both the stimulus object and the path are too small. The combination of a small dot (diameter = 3.8 deg) moving along a path of 3.8 deg in diameter yields erratic results. This, however, is not surprising at all if one takes into account the fact that the inner margin of the dot does not significantly change its location during the whole stimulus cycle (equivalent to zero speed). Furthermore, given an interommatidial angle $\Delta\varphi$ of 2 deg and a path diameter of 3.8 deg, the number of stimulated EMDs that encode the whole range of stimulus directions (360 deg) is reduced to 6. The dot has to change its direction of motion by 60 deg before one EMD is completely stimulated. Therefore, the relative position of the path of the moving dot with respect to the stimulated EMDs becomes critical. An EMD that is stimulated along its preferred direction but at an uneffective speed might contribute less to the modulation of the response than a neighbouring one that is not stimulated along its preferred direction but in the optimal speed range. If these conditions are slightly different during stimulation in the opposite direction this may cause considerable asymmetries in the responses of the neuron to cw and ccw motion that cannot be eliminated by the proposed evaluation procedure.

However, the size of the standard stimulus with a diameter of 7.6 deg (nearly 4 times $\Delta\varphi$) guarantees that the dot fully stimulates 2–3 EMDs simultaneously on its path. Therefore, the effects of relative position between the stimulus area and the stimulated EMDs on the responses are greatly reduced. In addition, due to the path diameter of 10.5 deg (about 5 times $\Delta\varphi$) of the standard stimulus, an increased number of EMDs is stimulated per cycle, resulting in an increased resolution of the directions of object motion. Reliable results are obtained even with small dots if they move on path diameters of 9.5 and 14.9 deg (see small standard deviation of respective data points in Fig. 7). These results correspond to the preferred direction obtained with moving gratings (e.g. Hausen, 1984; van Hateren, 1990).

Sequential vs simultaneous stimulation of optic flow processing neurons

The stimulus procedure described here might not reveal the functional input organization of visual interneurons if they integrate the local motion stimuli in a nonlinear fashion. For example, some visual interneurons found in the cervical connective of the blowfly can only be excited if fairly extended regions of both eyes are stimulated simultaneously, mimicking an image expansion. However, if either the left or the right eye is stimulated alone, no increase in spiking activity is obtained (Borst, 1991). Therefore, our stimulus method can be applied only to visual interneurons whose response increases continually with the extension of the stimulus pattern. Most of the visual interneurons in the lobula plate of the blowfly seem to have this property (Hengstenberg, 1982; Hausen, 1984; Haag *et al.*, 1992).

Another type of neuron has been found in the lobula plate of the blowfly, the so-called figure-detection cells (FD cells; Egelhaaf, 1985b) that are most likely involved in object-fixation behaviour of the fly (Egelhaaf, 1985a). The response of these neurons decreases steadily as the size of the stimulus pattern increases (Egelhaaf, 1985c). Detailed studies showed that the selectivity to small-field motion is mediated by the inhibitory influence of a wide-field neuron that integrates the local motion information over almost all of both visual hemispheres (Warzecha *et al.*, 1993; Egelhaaf *et al.*, 1993). Due to the special design of the input organization of the FD-cells sequential LPD measurements might possibly cause some inconsistencies in the global structure of the resulting response fields. However, abrupt changes with respect to the distribution of the LPDs in a response field can generally be considered as strong evidence that the respective neurons are not involved in the processing of optic flow generated exclusively by self-motion.

An alternative stimulus procedure to investigate the input organization of visual interneurons is the so-called "vector white noise" technique proposed by Srinivasan *et al.* (1993). In this approach, the stimulus consists of a display subdivided into 3 by 3 or 5 by 5 squares, each containing an elongated bar that is moved perpendicular to its long axis. For one stimulus sweep the direction of motion at a certain grid location remains the same; but at different grid locations the bars are moving in different directions. The orientation of the bars is varied randomly from sweep to sweep and the neuronal responses to about 200 – 300 stimulus sweeps are recorded. The LPD and LMS of a certain grid location are calculated from the sum over all the stimulus vectors applied at that location, where each single stimulus vector is weighted by the corresponding response of the neuron to the respective sweep.

However, this elegant approach has its limitations, too. The first one relates to the stimulus area, which is restricted to approximately 100 deg × 100 deg. In several cases the receptive field of visual interneurons, at least in the nervous system of invertebrates, by far exceeds this area (e.g. see. Fig. 8). We stress this point because information best suited to distinguishing between different optic flow fields is obtained when the field of view (i.e. the mapped response field) is equal to or exceeds one visual hemisphere (Koenderink & van Doorn, 1987). In some cases a truncated response field is not sufficient to calculate the set of specific self-motion parameters necessary to describe the particular optic flow field to which the neuron would respond optimally. A second aspect refers to the number of LPDs determined at different locations within the visual field. Accurate calculation of the self-motion parameters from the response field depends on the number of LPDs measured (loc. cit.). This might cause an additional problem for the vector white noise technique. If the number of separated stimulus locations is increased, the accuracy of the determination of each LPD is decreased due to an increased amount of "cross talk" from other locations (Srinivasan *et al.*, 1993). This effect could be compensated for by increasing the number of stimulus sweeps (theoretically, the perfectly accurate LPD is found when the number of stimulus sweeps approaches infinity; loc. cit.). However, a greater number of stimulus sweeps requires more measuring time, which is usually limited in intracellular recordings.

Applicability

The method proposed in this paper proved to be well suited for investigating the distribution of LPD and LMS within the receptive fields of visual interneurons of the blowfly. It is reliable and robust even with considerable variations of the stimulus parameters and fast enough even if intracellular recording techniques are applied. Once the stimulus parameters are adapted to the properties of the investigated visual system, there is only one condition necessary for the application of the method: the recorded neurons have to respond to local motion stimuli. If this criterion is met, sequential measurements are not critical and the response fields of the neurons can be mapped with great accuracy—in principle within the visual system of any species. In addition, if the local responses to motion are integrated almost linearly, specific analyses of the response fields can elucidate the characteristic roles of the neurons in optic flow processing and visually controlled behaviour. The method presented here has been successfully applied to determining the functional adaptation of the 10 VS neurons of the "vertical system" in the lobula plate of the blowfly. They were found to specifically sense rotations of the fly around different horizontal axes (Krapp & Hengstenberg, 1995; Hengstenberg *et al.*, 1996).

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