Outdoor performance of a motion-sensitive neuron in the blowfly

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Abstract

We studied an identified motion-sensitive neuron of the blowfly under outdoor conditions. The neuron was stimulated by oscillating the fly in a rural environment. We analysed whether the motion-induced neuronal activity is affected by brightness changes ranging between bright sunlight and dusk. In addition, the relationship between spike rate and ambient temperature was determined. The main results are: (1) The mean spike rate elicited by visual motion is largely independent of brightness changes over several orders of magnitude as they occur as a consequence of positional changes of the sun. Even during dusk the neuron responds strongly and directionally selective to motion. (2) The neuronal spike rate is not significantly affected by short-term brightness changes caused by clouds temporarily occluding the sun. (3) In contrast, the neuronal activity is much affected by changes in ambient temperature.

Keywords: Adaptation; Light; Motion; Ecology; Natural scenes

1. Introduction

There are large changes in light intensity between day and night. Although only few animals are active for the whole day–night cycle, most animals are confronted with changes in light intensity of at least five to six orders of magnitude during their activity period. This fact poses a problem to visual systems, since photoreceptors as well as neurons have a limited range of response amplitudes and have to deal with often considerable noise as a consequence of their biophysical properties. Although adaptational mechanisms are known to exist in the peripheral visual system, the properties of photoreceptors and their postsynaptic elements still depend significantly on light intensity (for review see Laughlin, 1994; Rodieck, 1998). So far, relatively little is known about the consequences of the enormous range of light intensities, as they are encountered during the course of the day, on the performance of neurons at more central levels of the visual system. Other characteristics of the environment also change during the course of the day. Examples are ambient temperature, polarisation and the spectral content of the light. Again, little is known about the effect of these variables on the responses of higher order visual neurons.

We investigated an identified motion-sensitive interneuron in the visual system of the blowfly, the so-called H1-neuron (Eckert, 1980; Hausen, 1976). We asked to what extent the spike rate is affected by brightness and temperature changes as they occur during the course of the day. To stimulate the H1-neuron under outdoor conditions, retinal image motion was induced by moving the animal in a natural habitat. We expected a strong dependence of the performance of the H1-neuron on temperature, because under precisely controlled laboratory conditions both the spike rate as well as the latency of this neuron have been found to depend strongly on the ambient temperature in a behaviourally relevant range (Warzecha, Horstmann, & Egelhaaf, 1999). The blowfly was used as an experimental animal, because it served in many laboratory studies as a model system for the investigation of the neuronal mechanisms underlying visual information processing (for review see Egelhaaf & Borst, 1993; Egelhaaf & Warzecha, 1999; Juusola, French, Uusitalo, & Weckström, 1996; Hausen & Egelhaaf, 1989; Laughlin,

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1994). It will be shown that the mean amplitude of the responses to motion is little affected by brightness changes of five orders of magnitude, but depends strongly on changes in temperature.

The main results have previously been summarised in abstract form (Egelhaaf, Grewe, Warzecha, & Kern, 2000).

2. Methods

The experiments were carried out with blowflies of the genus *Calliphora* in a rural setting (Fig. 1). The animals were positioned to view the same scene throughout the whole study. The blowfly was oscillated sinusoidally on a turntable with electrodes inserted into its brain. The oscillation frequency was 0.5 Hz, the oscillation amplitude 30°. The blowfly was oscillated for six cycles, i.e. for 12 s. Then the turntable was stopped for 4 s, before the motion started again. Thus one trial lasted for 16 s. These motion parameters were chosen because, on the one hand, the responses of the H1-neuron were varied over large parts of its activity range, and on the other hand, the frequency of changes in the direction of motion (0.5 Hz) as well as a the maximal velocity (approximately 100°/s) were still low enough to ensure sufficiently stable recordings in many preparations. The turntable was controlled via a PC by a servomotor whose shaft carried a ring potentiometer and a gear. The turntable was driven from the servomotor by a transmission belt. The ring potentiometer voltage was fed back to the input of the servomotor electronics so that the servomotor was operated under position control from the input provided by the PC.

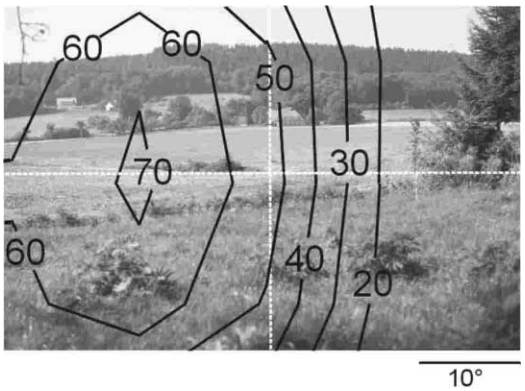

Fig. 1. View of the environment as seen by the blowfly. The environment is a rural setting where blowflies of the genus *Calliphora* have been frequently observed during the course of the experiments. Hence the environment where the experiments were done represents a natural habitat of blowflies. A contour plot of the spatial sensitivity distribution of the H1-neuron, which has its input region in the left visual field, is superimposed on the view of the environment. The sensitivity distribution was redrawn from Karmeier et al. (2001). The neural responses analysed for the spatial sensitivity distribution were obtained by local motion stimulation. The numbers along the contour lines give the mean absolute motion sensitivity in spikes/s. The maximum motion response obtained under the conditions of local motion stimulation amounts to 70 spikes/s. The sensitivity decreases towards the receptive field margins to less than 10 spikes/s. Note that the receptive field of the H1-neuron is much larger than the view of the environment shown in the photograph (for details see Karmeier et al., 2001). The white horizontal and vertical lines indicate the horizontal and vertical midline of the blowfly's visual field.

The experiments were performed during August and September, 1999. A total number of 37 flies contributed to the data of this paper. Experiments were done at any time of the day between 7:30 and 20:30 h. The duration of the experiments ranged between 25 min and about 3 h. Experiments were terminated after 25–30 min, if during a clear and sunny day the brightness and temperature changed only little during the recording session. Then experiments were carried out with additional flies to obtain more independent data. Evening recording sessions had to be terminated when it became so dark that it was hardly possible to control, without artificial light, the electronic equipment.

Spike activity was recorded extracellularly in the right lobula plate from the output arborisation of the H1-neuron. The receptive field of the H1-neuron covers almost the entire contralateral visual field (i.e. the visual field of the left eye). The rear part (approximately 30°) of the visual field of the left eye was partially occluded by recording equipment (micromanipulator, electrode holder etc.), which was also placed on the turntable and thus oscillated together with the fly. Most likely the responses of the H1-neuron were not affected by this inevitable consequence of the recording situation. First, the H1-neuron is not very sensitive to motion in the rear part of its receptive field (Karmeier, Egelhaaf, & Krapp, 2001). Second, objects that do not move relative to the eye (as was the case for the recording equipment) do not significantly affect the response of the H1-neuron, because it is mainly activated by visual motion.

The animals were dissected according to the procedure used in our laboratory for extracellular recording (for details see e.g. Warzecha, Egelhaaf, & Borst, 1993). In brief, after the head capsule was opened, the brain was supplied with Ringer's solution (for composition see Hausen, 1982) to avoid desiccation. Electrolytically sharpened tungsten electrodes insulated with varnish were used to record from the H1-neuron. The electrodes had resistances between 2 and 8 MΩ. Recorded signals were amplified by standard electrophysiological equipment. The H1-neuron can be identified unambiguously by the location of its receptive field in the visual field contralateral to the recording electrode as well as by its preferred direction of motion (back-to-front). After the spikes of the H1-neuron could be discriminated unambiguously against the background noise, the spikes were transformed into pulses of fixed duration and height. The pulses were fed to a PC through an I/O

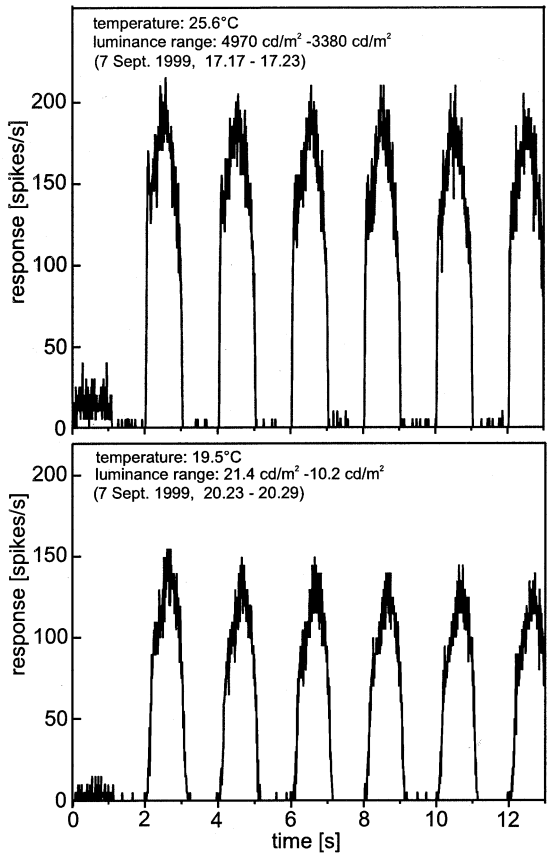

Fig. 2. Spike frequency histograms of the responses of an H1-neuron obtained from a continuous recording during two time intervals, one in the afternoon (17:17–17:23 h, 7 September, 1999, upper diagram) and the other during dusk (20:23–20:29 h, bottom diagram). Each histogram was obtained from 20 consecutive trials. The time resolution of the histograms is 10 ms. The cell was recorded in its output terminal in the right half of the visual system. Hence, it was sensitive to visual motion in the left visual field. The cell was excited during counter-clockwise rotation of the turntable, the preferred direction of the cell, and was inhibited below the resting activity during clockwise rotation, the null direction of the cell. During the two 6-min recording intervals the temperature changed by less than 0.2 °C, whereas the luminance changed very much. The mean temperature and the luminance range are given in the figure.

card (2801A, Data Translation). The data acquisition rate was 1 kHz. Throughout an experiment the signal-to-noise ratio of the spikes was checked. If the spikes of the H1-neuron could no longer be discriminated reliably from the background noise, the experiment was terminated. Usually, the recordings were sufficiently stable even while moving the fly on the turntable to allow us to monitor the activity of the H1-neuron as long as was required by the experimental design.

The temperature and the brightness were determined twice in each trial, 1 s before motion onset and directly after motion offset. Since the temperature and brightness did not change much during this time, the two measurements were averaged. Preliminary tests had shown that even the fastest brightness changes that occurred (i.e. fast moving clouds covering the sun) took several seconds. Temperature changes were even slower. Since the responses to each of the six oscillation cycles of one trial were very similar (see Fig. 2), the motion-induced activity was calculated by averaging the spike rate during those stimulus phases where the retinal image of the environment moved in the preferred direction of the H1-neuron. The spontaneous spike rate was determined for 1 s just before the turntable started to move. Hence, for each trial we obtained one spike rate value for the spontaneous activity, one spike rate value for the mean activity during preferred direction motion as well as one brightness and one temperature value.

During the experiments the ambient temperature ranged between 17 and 29 °C, covering most of the blowfly's activity range. The temperature was measured with a thermometer which provided a voltage output proportional to temperature that could be sampled by a PC. Temperature was measured with an accuracy of 0.1 °C. The temperature range that was encountered during the experiments was subdivided into four temperature classes: $17 \leq T \leq 20$, $20 \leq T \leq 23$, $23 \leq T \leq 26$ and $26 \leq T \leq 29$ °C. The sensor of the thermometer was about 10 cm below the blowfly underneath the turntable. It was thus shaded from direct illumination by the sun. For technical reasons, the thermometer could not be inserted into the brain of the animal. Hence our temperature readings may somewhat differ from the actual temperature of the nervous system. Since the dependence of the responses of the H1-cell on the ambient temperature is very pronounced (see Section 3), the basic results of our study are likely to be robust with respect to potential differences between the head temperature and the temperature recorded by our sensor.

The brightness varied by five logarithmic units between bright sunlight and dusk. The brightness was determined with a photodiode that was fixed above a white sheet of paper close to the blowfly. The luminance of the green grass which covered part of the blowfly's visual field was about one order of magnitude smaller than the luminance of the white paper. The photodiode was calibrated with a luminance meter (Minolta LS-100) by measuring the luminance of a sheet of white paper at different times of the day and, thus, over a wide range of light intensities. The brightness range encountered during the experiments was subdivided into five logarithmic luminance classes: $0.6 \leq L \leq 6$, $6 \leq L \leq 60$, $60 \leq L \leq 600$, $600 \leq L \leq 6000$, and $6000 \leq L \leq 60000$ cd/m^2.

Statistical significance of the results was tested by a subset analysis of variance for 'some-cells empty data' (Edwards, 1993).

3. Results

The H1-neuron responded directionally selective to motion under all stimulus conditions that were encountered during the course of our experiments. This is illustrated by the spike frequency histograms in Fig. 2. The histograms were obtained from the same cell during two different time intervals of the same recording session. For each time interval of approximately 6 min duration the mean time-dependent spike rates were determined for 20 consecutive trials. The first time interval started at 17:17 h, the second at 20:23 h (September 7, 1999). During the first time interval the entire environment was brightly illuminated by the sun and it was warm (luminance range: 4970–3380 cd/m^2; average temperature: 25.6 °C). The luminance decreased by more than a factor of two during the second time interval and it was hard to read even the capital letters of a newspaper towards its end; moreover, it was noticeably cooler than during the first time interval (luminance: 21.4–10.2 cd/m^2, average temperature: 19.5 °C). During motion of the retinal image in the cell's preferred direction (i.e. during counter-clockwise motion of the turntable), the spike rate strongly increased for both time intervals. The activity decreased below the resting activity and the cell almost ceased firing during motion in the opposite direction. Irrespective of the time of the day, and thus irrespective of the brightness and temperature, the spike rate approximately followed the velocity of oscillation of the turntable. This finding is representative for all experiments carried out in the present study under outdoor conditions and is in accordance with earlier results obtained under laboratory conditions at relatively low light levels (Bialek, Rieke, Ruyter van Steveninck, & Warland, 1991; Eckert, 1980; Haag & Borst, 1997; Warzecha et al., 1999; Warzecha, Kretzberg, & Egelhaaf, 2000; Warzecha & Egelhaaf, 1996). Although the overall response pattern of the H1-neuron was independent of the environmental conditions, the response amplitude was affected to some extent. In the sample record shown in Fig. 2 it was smaller during dusk than in the afternoon. Since both temperature and brightness changed between these recording intervals, further analysis was required to find out which of these parameters is relevant in determining the response amplitude. Since there was no or only little activity of the H1-neuron during motion in its null direction under all environmental conditions (Fig. 2), only the spontaneous activity of the cell as well as the responses to preferred direction motion were assessed quantitatively and related to the environmental brightness and temperature.

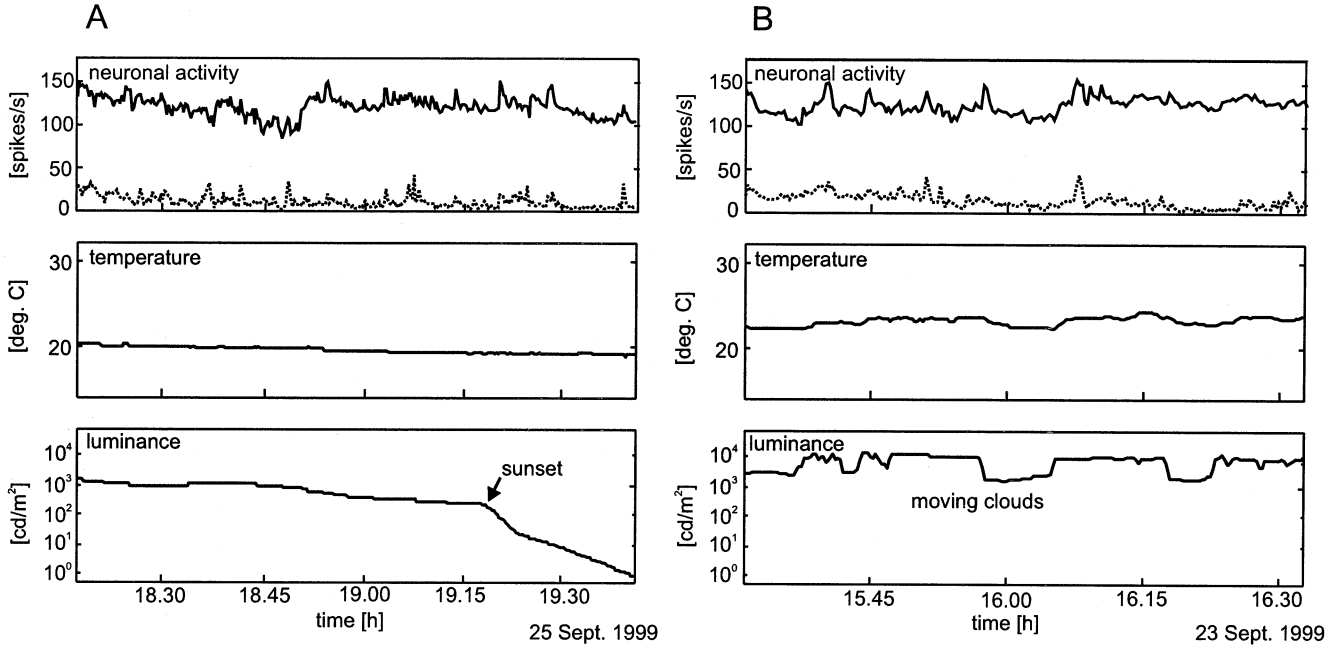

Fig. 3. Time course of the spike rate of the H1-neuron induced during preferred direction motion (solid line in upper panel), of the mean spontaneous activity (dotted line in upper panel), of the temperature (middle panel) and of luminance (bottom panel) recorded during two experiments at different times of the day. (A) The experiment was performed on a sunny day with only few clouds in the sky. Note the decline in luminance after sunset. This decrease in light intensity is not accompanied by an obvious decline in the spike rate. (B) The experiment was performed on a sunny day with the sun being occasionally occluded by rapidly moving clouds. The resulting changes in light intensity did not induce any obvious changes in neuronal activity.

3.1. Time course of light intensity, temperature and neuronal spike rate during the day

During most of the day the light intensity changes only slowly as a consequence of changes in the position of the sun. The brightness changes are somewhat faster after sunset, as is shown by the example in Fig. 3A. Much faster changes in brightness occur only when the sun is occluded by rapidly moving clouds. However, even then the fastest changes in light intensity usually take at least some seconds. These relatively fast changes in light intensity are usually smaller than 1 logarithmic unit. One such example is shown in Fig. 3B. Hence, we may distinguish between brightness changes on two different timescales; slow ones attributable to positional changes of the sun, with time constants in the order of minutes up to hours, and fast ones, attributable to moving clouds, with time constants in the order of seconds up to minutes.

Brightness changes may be followed by changes in temperature. This is quite obvious during the course of the day, but also on a shorter timescale when the sun is occluded by clouds. However, the latter changes in temperature are usually slower than the corresponding brightness changes and may be relatively modest, as is exemplified in Fig. 3B (compare middle and bottom traces; see also below). Since for technical reasons we did not measure the temperature directly in the head capsule of the fly, but only close to the animal, the recorded temperature changes represent only an approximation to the actual temperature changes in the nervous tissue.

Both the spontaneous activity as well as the motion induced spike rate of the H1-neuron fluctuate considerably. There are fluctuations occurring between consecutive trials as well as over intervals of some minutes and even tens of minutes. These fluctuations in spike rate could usually not be attributed to any obvious change in the sensory stimulus situation. Similar activity fluctuation can also be found under well defined laboratory conditions (Warzecha, unpublished results). Given these fluctuations, it is hardly possible, just from scrutinising the time-dependent response traces, on the one hand, and the brightness and temperature traces, on the other hand, to infer any pronounced influence of brightness and temperature on the neuronal activity (see Fig. 3A, B). Therefore, a more quantitative analysis was required to search for such potential influences. Since brightness changes at different timescales, these potential influences will be investigated in different ways.

3.2. Consequences of brightness and temperature changes on a large timescale

It was known from previous work done under well-defined laboratory conditions that the response properties of blowfly motion sensitive neurons strongly depend on changes in the ambient temperature and that the nervous system of the blowfly does not acclimatise even during prolonged exposure to a given temperature (Warzecha et al., 1999; Warzecha & Egelhaaf, 2000). Potential brightness dependent activity changes of the H1-neuron had, therefore, to be separated from temperature effects. Since temperature changes tend to covary to some extent with brightness changes, this separation was not possible for a given H1-neuron. Rather the data of 37 H1 neurons recorded at different times of the day and under a wide range of illumination conditions had to be pooled. The temperature range that was encountered during the experiments was subdivided into four equally sized temperature classes, and the brightness range encountered during the experiments was subdivided into five equally sized logarithmic luminance classes (for details see Section 2). The resting spike rate and the average motion induced spike rate were determined for each trial and allotted to one of the 20 brightness/temperature classes. Since each cell could contribute more than one spike rate value to a particular brightness/temperature class, the average over these values was determined for each cell. These mean values obtained for each cell and brightness/temperature class were then averaged over cells. Note that each cell contributed only to a limited range of brightness/temperature classes, because, during a particular recording session, usually only a small part of all possible combinations of temperature and brightness occurred. For instance, it was never bright and hot as well as bright and cool during a given day. Moreover, it was not possible to obtain data at high temperature during dusk, because such a condition is rare in German summers.

To test whether the variability in the neuronal activity can be attributed to changes in temperature and/or luminance, a subset two-factor analysis of variance was performed for data sets where not all combinations of the two factors (i.e. temperature and luminance) are available (Edwards, 1993). Three subsets of data were tested (subset 1: all luminance classes and the two lowest temperature classes; subset 2: the four highest luminance classes and the three lowest temperature classes; subset 3: the three highest luminance classes and all temperature classes). The statistical analysis was done for the spontaneous activity of the H1-neuron, for its average response evoked by preferred direction motion as well as for the response increment during preferred direction motion relative to the spontaneous activity.

The mean spontaneous activity and motion induced spike rates were plotted both as a function of luminance with the temperature as parameter (Fig. 4A) and

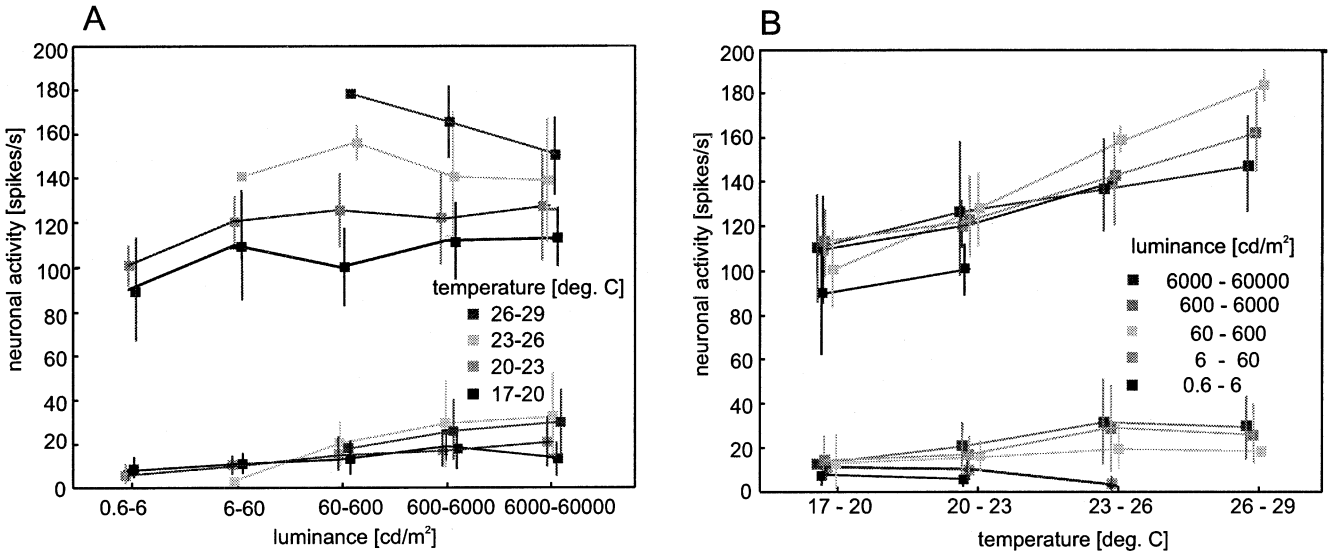

Fig. 4. Consequences of luminance changes (A) and temperature changes (B) for the mean motion induced spike rate of the H1-neuron (data in upper part of the diagrams) as well as for its spontaneous activity (data in lower part of the diagrams). Data of 37 H1-neurons recorded at different times of the day and under a wide range of brightness and temperature conditions. The temperature range that was encountered during the experiments was subdivided into four temperature classes, whereas the brightness range encountered during the experiments was subdivided into five equally sized logarithmic luminance classes (for details see Section 3). The average spike rate induced by preferred direction motion as well as the spontaneous activity were determined for each trial and allotted to one of the 20 luminance/temperature classes. Averages and standard deviations over cells are displayed. The number of cells contributing to a particular class varied between 3 and 15.

as a function of temperature with the luminance as parameter (Fig. 4B). The data reveal that, in accordance with a recent laboratory study (Warzecha et al., 1999), the mean spike rate of the H1-neuron increases considerably with increasing temperature. For a temperature increase from about 17 °C to about 29 °C the mean spike rate elicited during preferred direction motion increased by approximately 50 spikes/s and thus by about 50%. The dependence of the motion induced spike rate on temperature is significant for all tested subsets ($P < 0.01$). The spontaneous activity changes inconsistently with temperature, again in accordance with the laboratory study (Warzecha et al., 1999). Whereas the spontaneous activity increases slightly with increasing temperature at high brightness levels (subset 3: $P < 0.01$), no such increase could be observed if moderate and low light levels were also taken into account (subsets 1 and 2: $P > 0.05$).

The average spike rate induced by preferred direction motion does not depend consistently on luminance. Whereas the spike rate did not depend significantly on luminance for values above 6 cd/m^2 (subsets 2 and 3, $P > 0.05$), the spike activity slightly decreased with decreasing luminance at low luminances (subset 1, $P < 0.05$). However, even this decrease in spike activity was relatively small and could hardly be discerned in the individual time-dependent responses against the activity fluctuations not correlated with brightness changes (for an example see Fig. 3). In contrast to the motion induced responses, the spontaneous activity increased slightly but consistently with light intensity ($P < 0.05$ for all subsets).

If the motion induced activity is not taken in absolute terms but if the response increment with respect to the spontaneous activity level is taken into account, no principally different conclusions emerge. The response still increases greatly with increasing temperature for all tested luminance ranges (subset 1, $P < 0.01$; subsets 2 and 3, $P < 0.05$), but does not depend significantly on luminance ($P > 0.05$ for all subsets). The weak significance observed at low light levels if the spontaneous activity is not subtracted from the motion induced responses thus disappears if the spontaneous activity is subtracted.

It can be concluded from this analysis that much of the variability found in the spontaneous activity of the H1-neuron during the course of the day can be attributed to changes in both luminance and temperature. In contrast, much of the variability found in the mean motion induced responses can be attributed to changes in temperature but not to changes in luminance. This means that the motion induced spike rate increases consistently with increasing temperature in a temperature range corresponding to the normal operating conditions of blowflies. In contrast, the motion induced response of the H1-neuron does not depend on luminance over four orders of magnitude. If at all, it is only during dusk at light levels below 6 cd/m^2, that we see a weak decrease of the H1 discharge with decreasing luminance.

3.3. Consequences of brightness and temperature changes on a short timescale

Potential consequences of short-term brightness changes caused by rapidly moving clouds were analysed in a different way. Irrespective of the temperature, changes in brightness of at least 0.2 log-units (corresponding to brightness changes by a factor of approximately 1.58) between consecutive trials were selected. The average changes in the mean spontaneous activity, in the mean motion induced spike rate and in the mean temperature were then determined that are concomitant with these brightness changes. This was done separately for increments and decrements in light intensity. To assess the significance of the resulting average neural activity changes they were compared with the standard deviation of the corresponding fluctuations in average spike rate as determined over 100 consecutive trials *prior* to the threshold brightness change.

The data shown in Fig. 5 reveal that even a sudden increment or decrement in brightness of more than 0.2 logarithmic units does not lead to any consistent change of the resting activity and of the motion induced spike rate of the H1-neuron. The fluctuations observed in the mean responses after the brightness change are, for most of the time, within the limits of the standard deviation of the corresponding activity fluctuations before the brightness change. Even these fluctuations in spike rate were, on average, hardly larger than five spikes/s, which is less than 5% of the mean motion induced activity of the H1-neuron under our experimental conditions. There might be slight average increases and decreases in temperature concomitant with brightness increments and decrements. Even if these temperature changes were statistically significant, they were too small (approximately 0.01 °C) to cause noticeable changes in spike activity on their own.

In conclusion, there is no evidence that relatively rapid changes in brightness as they occur in a blowfly's natural environment by rapidly moving clouds affect the spike rate of the H1-neuron.

4. Discussion

Most experiments on visual information processing are done in the laboratory. Working in the laboratory has the great advantage that the visual stimulus condi-

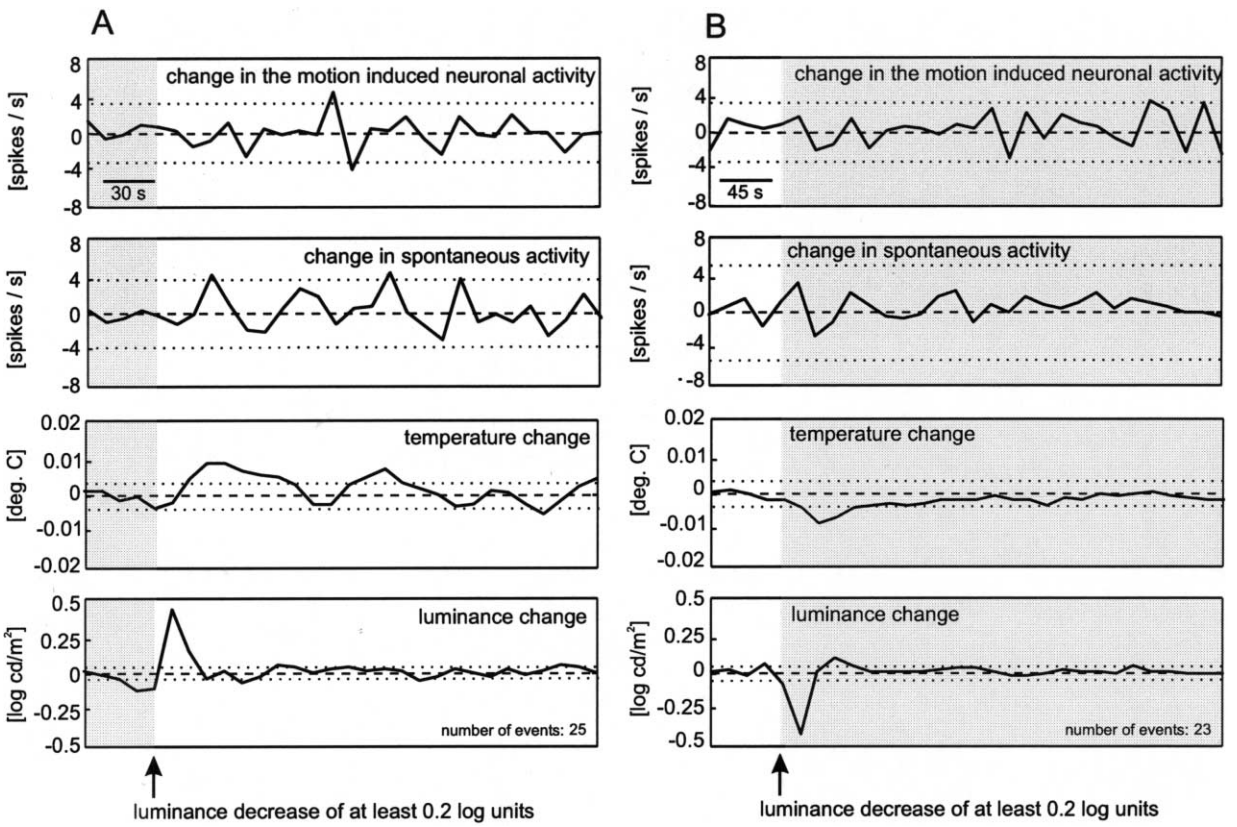

Fig. 5. Consequences of luminance increments (A) and decrements (B) on a short timescale. Changes in luminance of at least 0.2 log units between consecutive trials were selected. The average changes in the mean motion induced spike rate, in the mean spontaneous activity and in mean temperature were determined that go along with these brightness changes. Dotted lines represent the corresponding standard deviations of the average responses as determined over 100 trials prior to the threshold luminance change. The dashed line corresponds to the zero-change level. Data obtained from six flies and 25 supra-threshold luminance increments and 23 decrements.

tions can be designed precisely by the experimenter. Moreover, experiments can be done under exactly the same stimulus conditions on a large number of animals. However, most visual stimulus devices have the disadvantage that they do not cover the range of light intensities with which an animal is confronted in its normal environment. For this reason, we decided to analyse the performance of the H1-neuron, an identified motion sensitive neuron in the visual system of the blowfly under outdoor conditions. The blowfly has served for many years as a model system for analysing the mechanisms underlying visual motion computation (e.g. Egelhaaf & Borst, 1993; Egelhaaf & Warzecha, 1999; Hausen & Egelhaaf, 1989; Rieke, Warland, Ruyter van Steveninck, & Bialek, 1997).

By oscillating the blowfly with electrodes inserted into its brain during different times of the day in a rural setting, we found that the direction selectivity of the H1-neuron did not change throughout the day, i.e. its activity increased during preferred direction motion and decreased below the resting activity during motion in the opposite direction. The mean spike rate of the H1-neuron elicited by motion in the preferred direction does not depend on the luminance over a range of several orders of magnitude, at least for most times of the day. Only during dusk, i.e. at luminance levels below 6 cd/m^2, when it was hardly possible for a human observer to read even the headlines of a newspaper, the response amplitude may be slightly smaller than during the rest of the day. This slight decrease in the motion induced response may be attributable to a concomitant decrease in the spontaneous activity of the cell at low light levels. Moreover, not only the luminance but also other stimulus parameters such as the contrast of structures in the environment change during dusk. The decrease in contrast on its own may have contributed to the slightly decreased response rate during dusk (Egelhaaf & Borst, 1989; Lenting, Mastebroek, & Zaagman, 1984). In addition, the response amplitude during dusk might have been affected by circadian rhythms that modulate the sensitivity of the H1-neuron. It has been shown that both the spontaneous activity of the H1-neuron and its sensitivity to image motion decrease in the evening (Bult, Schuling, & Mastebroek, 1991). However, apart from experiments at very low light levels which were always performed in the evening (because only then was it warm enough; early in the morning it was always cooler than 17 °C), the data were pooled from experiments done in the morning and in the afternoon. Hence, circadian modulation of the electrical activity of the H1-neuron might have increased the overall variability of the responses obtained with different cells, but is unlikely to have affected our results in a systematic way.

The near independence from light intensity of the motion induced spike rate of the H1-neuron under outdoor conditions between bright sunshine and dusk is in accordance with previous findings obtained under laboratory conditions: The motion induced responses of blowfly motion sensitive neurons started to decrease only at luminances well below 0.7 cd/m^2 (Hausen, 1981). Above 0.7 cd/m^2 the response amplitude stayed largely independent of the luminance of the stimulus pattern. Although under laboratory conditions no luminances higher than 70 cd/m^2 were employed, this independence is in accordance with our findings obtained under the much broader range of light intensities characteristic of outdoor conditions. The motion induced responses of the H1-cell are not only relatively insensitive to changes in light intensity on a timescale of minutes and hours but also on a timescale of seconds or some tens of seconds, as they occur when the sun is occluded by clouds. On the basis of the present data, which were, for methodological reasons, obtained at a given location, it cannot be assessed whether brightness changes encountered by the blowfly while flying, for instance, from a sunny meadow into a shady forest, are sufficiently large and rapid to affect the activity of motion sensitive neurons.

In contrast to the relative insensitivity of the motion induced responses of the H1-neuron to naturally occurring changes in light intensity, its response amplitude was found to depend strongly on the ambient temperature. Since brightness changes as they occur during the course of the day usually go along with changes in temperature, care has to be taken to distinguish between both factors with respect to potential consequences on the neuronal responses. The temperature dependence of the H1 response as observed under outdoor conditions is in accordance with previous results obtained under precisely controlled laboratory conditions (Warzecha et al., 1999). Here a temperature increase of about 10 °C led to an increase in the motion induced spike rate of around 40–60% which is in the same range as observed in the present study under outdoor conditions. Both studies reveal that in a temperature range between 17 and 29 °C the responses of the H1-neuron to motion stimulation are greatly affected by the ambient temperature. The temperature range that was tested here is likely to represent the operating range of the blowfly *Calliphora*. We are not aware of any systematic study on the behavioural ecology of blowflies in which the temperature range was monitored in which this genus is active. Our own observations suggest that at temperatures below approximately 17 °C flying blowflies can be observed only rarely. It should be noted that the temperature of the nervous system of insects is not only determined by the ambient temperature, but also by the animal's own muscular activity. Although the head temperature of insects including blowflies was shown to increase during flight (Heinrich, 1993; Stavenga, Schwering, & Tinber-

gen, 1993), it is not clear so far to what extent the head temperature is affected during the different types of flight manoeuvres (for a more detailed discussion see Warzecha et al., 1999).

The influence of temperature on the response properties of the H1-neuron can be attributed, at least to a large extent, to the temperature dependence of the response properties of the photoreceptors (for a detailed discussion see Warzecha et al., 1999). In brief, the light induced responses of photoreceptors were found to increase in light-adapted animals by about 65% when the temperature was raised from 17 to 34 °C (Tatler, O'Carroll, & Laughlin, 2000), i.e. in the likely behavioural operating range of flies.

Light adaptation in the peripheral visual system is obviously sufficiently efficient to ensure that the H1-neuron is largely insensitive to brightness changes, at least for the brightness conditions during most of the day. The peripheral visual system was concluded to be optimised to encode contrast largely independent of light intensity. However, light adaptation does not only change the gain of the system but also its dynamical properties (for review see Juusola et al., 1996; Laughlin, 1994). Therefore, without detailed modelling, it is hard to make any sound prediction of how these changes in the peripheral visual system may affect the responses of downstream motion sensitive neurons, such as the H1-cell. In any case, these adaptational changes make the motion induced spike rate of the H1-neuron largely independent of brightness changes, whatever additional consequences they may have.

In our present study we only used the spike rate of the H1-neuron as averaged over several seconds as an indicator of the cell's performance under outdoor conditions and did not take into account the exact timing of spikes or the reliability of the responses. The functional significance of the timing of spikes in the H1-neuron and the variability of its responses is currently under intense debate (Ruyter van Steveninck, Borst, & Bialek, 2001; Warzecha & Egelhaaf, 2001) and has been analysed in many studies (for review see Bialek & Rieke, 1992; Egelhaaf & Warzecha, 1999; Ruyter van Steveninck et al., 2001; Warzecha & Egelhaaf, 2001). We refrained from studying outdoors the reliability of neuronal coding, because the environmental conditions frequently change much too fast to allow for such an analysis. For instance, during dusk (but also, though to a lesser extent during other times of the day) the brightness changes greatly within minutes (for examples see Figs. 2 and 3). However, an analysis of neuronal variability requires many presentations of the same stimulation sequence and thus much longer time intervals than when the light intensity is stationary. Apart from the light intensity many other environmental factors continually change, such as temperature, wind, spectral composition of the light, humidity etc. All these factors may influence the neuronal responses and need to be stationary to allow us to analyse the reliability of neural coding. Otherwise changes in the neuronal activity induced by changes in the experimental conditions are incorrectly attributed to intrinsic noise of the system. Interestingly, wind does not appear to be a relevant cause of this large variability, since strong gusts of wind (which even overturned a heavy umbrella protecting the electronic equipment from the sun) did not alter the activity of the H1-neuron in any obvious way.

After submission of our paper a study has been published which addresses the reliability of neural coding under outdoor conditions (Lewen, Bialek, & Ruyter van Steveninck, 2001). It is concluded that the reliability of neural coding increases as the light level increases over the natural range. This conclusion needs to be qualified for various reasons. (i) As outlined above, it is critical for an analysis of neuronal reliability that the experimental conditions remain stationary for a sufficiently long time interval. However, in the experiments of Lewen et al. (2001), light intensity changed by approximately one order of magnitude within the time intervals of analysis shortly before and after dusk (see their Fig. 3). (ii) The conclusions of Lewen et al. (2001) are based on experiments on only two flies. Given the documented large variability in neuronal performance obtained even under exactly controlled laboratory conditions (Schneidman, Brenner, Tishby, Ruyter van Steveninck, & Bialek, 2001; Warzecha et al., 1999; Warzecha et al., 2000), conclusions based on such a small number of experiments can hardly be accepted as scientifically valid. (iii) It is questionable whether the observed decrease in neuronal reliability can be attributed to changes in light intensity as has been claimed by Lewen et al. (2001). The most obvious change in the responses obtained between noon and after sunset (the time interval during which the experiment was performed) is a decrease in spike rate. In our analysis based on 37 flies we did not find such an activity decrease as a consequence of brightness decreases, but only as a consequence of temperature decreases. Therefore, it appears likely that the changes in the H1-response observed by Lewen et al. (2001) are the consequence of temperature changes which go along with brightness changes between noon and dusk rather than of brightness changes. No notion can be found in the paper about the temperature during the experiments. Although we do not want to exclude that the light level affects the reliability of neural coding in the fly's visual motion pathway, the study of Lewen et al. (2001) does not provide compelling evidence in this regard.

Various features of neuronal responses, other than the spike rate, strongly depend on the visual stimulus parameters and on the environmental conditions. For

instance, the response latency at the onset of motion stimulation decreases with increasing ambient temperature or increasing pattern contrast (Warzecha & Egelhaaf, 2000). Moreover, the signal-to-noise ratio of H1 responses was found to increase with increasing temperature (Warzecha et al., 1999). Finally, the response variance as determined across responses elicited by identical stimuli was found to increase with decreasing pattern contrast (Warzecha et al., 2000). Given this stimulus dependence and taking into account that the signal-to-noise ratio and the dynamical properties of photoreceptors and of first-order visual interneurons depend on light intensity (e.g. Juusola et al., 1996; Laughlin, 1994), it cannot be excluded that changes in the visual stimulus parameters as they occur under natural operating conditions of blowflies may also affect the temporal fine structure of the response of the H1-cell without affecting the mean spike rate.

In conclusion, motion stimuli used in the laboratory that are brighter on average than about 6 cd/m^2 lead to approximately the same spike rate in motion sensitive neurons of the blowfly as do much brighter motion stimuli that are encountered outdoors. Since, however, various aspects of the neuronal responses strongly depend on temperature changes in the behavioural operating range of flies, utmost care must be taken not to confound effects elicited by visual stimuli with those that are attributable to changes in temperature.

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