Implications of Functionally Different Synaptic Inputs for Neuronal Gain and Computational Properties of Fly Visual Interneurons

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Integrating functionally different synaptic inputs

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Abstract

Neurons embedded in networks are thought to receive synaptic inputs that do not drive them on their own, but modulate the responsiveness to driving input. Although studies on brain slices have led to detailed knowledge of how non-driving input affects dendritic integration, its origin and functional implications remain unclear. We tackle this issue using an ensemble of fly wide-field visual interneurons. These neurons offer the opportunity to combine in vivo recording techniques and natural sensory stimulation as well as to interpret electrophysiological results in a behavioral context. By targeted manipulation of the animal’s visual input we find a pronounced modulating impact of non-driving input, whereas functionally important cellular properties like direction tuning and the coding of pattern velocity are left almost unaffected. We propose that the integration of functionally different synaptic inputs is a mechanism that immanently equalizes the ensemble’s sensitivity irrespective of the specific stimulus conditions.
Introduction

Dendritic integration is a central and often highly nonlinear stage of neuronal information processing. The many different presynaptic signals a neuron receives have been classified as drivers and modulators (Sherman and Guillery, 1998). Driving input is thought to carry the relevant information, while the modulating input shapes the neuronal computations without any driving contribution. Shunting inhibition is one example of modulating input, which may even veto the driving input (Koch et al. 1983). Cortical neurons are assumed to be continually bombarded with excitatory and inhibitory inputs induced by background network activity. The impact of background activity leads e.g. to a drop in input resistances *in vivo* as compared to those *in vitro* or in anaesthetized animals when there is only reduced network activity (Destexhe et al. 2003). Accordingly, the background activity has been proposed to be a mechanism that modulates the gain of a neuron (Chance et al. 2002; Mitchell and Silver, 2003; Prescott and De Koninck, 2003). Despite great methodological advances such as the dynamic clamp technique, applied to mimic background activity in slice preparations (Prinz et al. 2004), conclusions about the functional significance and origin of background input are still limited.

Here we address the functional significance of modulating synaptic background input linking cellular physiology with the functional aspects of encoding and representing visual stimuli. We investigate how visual shunting input affects the encoding of visual motion stimuli in fly visual interneurons. The task of these tangential cells (TCs) is to evaluate optic flow patterns, i.e. the retinal image flow evoked during self-motion of the animal (Borst and Haag, 2002; Egelhaaf et al. 2002; Egelhaaf et al. 2005; Hausen, 1984). Optic flow patterns are the only source of information about the environmental layout when airborne. TCs spatially integrate the excitatory and inhibitory outputs of thousands of retinotopically arranged local motion sensitive elements (Brotz et al. 1996). Accordingly, TCs have receptive fields that cover large parts of the visual field and are tuned to optic flow as is generated on the eyes during certain types of translational or rotational self-motion of the animal (Egelhaaf et al. 2002; Kern et al. 2005; Krapp et al. 2001; van Hateren et al. 2005). The activation ratio of excitatory and inhibitory inputs depends on stimulus parameters as pattern contrast and texture or the direction and velocity of visual motion. Even during motion in the preferred direction, which distinctly depolarizes the cell, the inhibitory inputs are activated, though to a much smaller extent than the excitatory input (Borst et al. 1995;
Single et al. 1997). Motion orthogonal to the preferred direction is assumed to activate both types of inputs in approximately equal shares, whereas during null-direction motion the inhibitory input predominates. Fly TCs offer the opportunity to investigate under *in vivo* conditions the influence of selectively manipulated sensory input in a well established functional context. In this study we examined the functional consequences of modulating input on neuronal sensitivity, direction tuning, and the representation of the time course of pattern velocity. Coherent motion as driving input and balanced motion noise as the modulating background input were combined for visual stimulation. This decomposition of the visual input into a driving and a modulating component was done for the sake of systems analysis only, although under natural conditions TCs are exposed to optic flow containing motion vectors in a wide range of directions driving its activity to a different extent or not at all. Thus, in behavioral situations TCs receive inputs that have both driving and modulating impact.

**Methods**

**Electrophysiology**

Experiments were carried out on 1-7 day old female blowflies (*Calliphora vicina*). The animals were prepared as described in Warzecha et al., (1993). In short: the flies were briefly anaesthetized with CO₂ and fixed ventral side up on a small piece of glass. The head was bent down and waxed to the thorax to allow access to the backside of the head. The head-capsule was opened and tissue like air-sacs, fat bodies, and trachea that cover the lobula plate were removed. In some preparations we also removed the gut to eliminate peristaltic movements that can cause disturbances. After preparation, the flies were adjusted according to the symmetry of the deep pseudopupil (Franceschini, 1975) to ensure that different animals received the same visual input.

**Extracellular recordings**

The H1 neuron was recorded extracellulary with self-made tungsten electrodes. A Ringer-filled glass capillary, through which we could provide Ringer solution if necessary (for composition see Hausen, 1982a), was used as indifferent electrode. The recorded signals were amplified by a factor of 3000, bandpass filtered (corner frequencies of 300 Hz and 3 kHz), and subsequently passed through a threshold device that transforms detected spikes into uniform pulses.
The H1 neuron was recorded in the left half of the brain in its output arborization. It can be easily identified by its preferred direction, i.e. back to front motion in front of the right eye.

**Intracellular recordings**
VS neurons were recorded in or close to their main dendrite with sharp electrodes pulled on a Brown/Flaming P-97 Puller (Sutter Instruments) and filled with 2M KCl. The electrodes had resistances between 30 and 40 MΩ and the electrode holder’s silver wire was chlorided before every recording. We used a SEC 10-L amplifier (npi – electronics, Germany) operated in bridge or discontinuous current clamp (DCC) mode. In DCC mode the switching frequency was about 8 kHz.

The raw data traces of intra- and extracellular recordings as well as the thresholded H1 spike trains were sampled at 4 kHz (DaqBoard2000, IOTech Inc., OH) and stored on hard disk for offline analysis. The program for data acquisition was written in Delphi 7 (Borland Software Corporation).

**Visual Stimulation**
Visual stimuli were generated using a VSG 2/3 graphics card (Cambridge Research Systems, Cambridge, UK) and presented on a Joyce Scope DM5 monochrome (P-31 phosphor) monitor (Joyce Electronics Inc., Cambridge, UK). As seen by the fly, the monitor screen (464 x 375 pixel spatial resolution) had a horizontal and vertical extent of 127° and 120°, respectively. The screen centre was positioned at 0° elevation and 20° azimuth. At this point, one pixel had a vertical and horizontal extent of ~0.5°.

While the frame rate was 300 Hz, new stimulus pictures were presented with 150 Hz. We could disprove time-locking of H1 spikes to either frequency by cross-correlating 200 response traces to identical stimulation (data not shown).

The stimuli consisted of two sets of dots moving on the black screen (luminance: 0.001cd/m²). The first set of dots, the motion dots, were randomly positioned in the first frame and moved coherently in the preferred direction of the recorded TC. These served as driving input, forming the sensory signal to be encoded by the TCs. Each dot had a vertical and horizontal extent of 3 pixels resulting in an angular size of approximately 1.5° x 1.5° as seen by the fly; it was
surrounded by a 6° ‘forbidden zone’ in which no other dot was allowed to be placed. By varying
the number of dots (between 4 and 64) different movement strengths were produced. The motion
dots were always presented at full brightness (300 cd/m²). The second set of dots consisted of 112
noise dots, which have the same size and forbidden zone as the motion dots. The noise dots were
designed to constitute the modulating background input. Initially the noise dots were also
randomly placed. In subsequent frames they performed a random walk, in which eight directions
(the horizontal, vertical, and diagonal directions) were possible. For each time step the direction
of each dot was chosen randomly from a predefined distribution. This distribution of directions
was defined so that the directions into which the noise dots moved were balanced, i.e. the
resulting motion noise did not, on average, excite or inhibit the recorded neuron. Balancing was
done in control experiments for one sample VS-cell and H1-cell. The distribution of movement
directions that led to the best balancing, that is, with no clear excitation or inhibition, was used
for all other experiments on the same cell type. The strength of the motion noise was altered by
changing the brightness of the noise dots to ~30 cd/m² (weak motion noise), 150 cd/m² (medium
motion noise), and 300 cd/m² (strong motion noise). Even the lowest brightness value was
sufficient to significantly affect the neuronal responses, increasing the firing rate of the H1-
neuron when dots of this brightness moved in the cell’s preferred direction.

As mentioned above, the dots were surrounded by a “forbidden zone”, in which no other dot
occurred. This zone was introduced to prevent the motion dots and noise dots from interacting on
a local basis, i.e. generating apparent motion. In flies, adapted to total darkness, it is possible to
evoke a weak direction-selective response in H1 when the two stimuli of an apparent motion
paradigm are separated up to 12° (Schuling et al. 1989). Although the flies in our experiments
were not completely dark adapted, we could show in a control experiment that increasing the
forbidden zone to above 12° (with a reduced number of dots) or completely separating motion
and noise dots (coherent motion in the upper third of the screen and motion noise in the lower
third or vice versa) does not significantly alter the effect of motion noise on H1 responses.

**Measurements of TC input resistance**
The input resistances of VS-cells were measured by injecting 200 ms pulses of constant
hyperpolarizing current (-0.75 to -1.5 nA) into the cells. Trials with and without current were
recorded in a pseudo-random order which allows to calculate the input resistances from direct
comparisons of the corresponding response sections (Figure 2a).
**Gain modulation by visual motion noise**
To investigate the consequences of motion noise on the sensitivity of TCs for preferred direction motion, we tested the neuronal responses to motion stimuli of five different strengths, determined by the number of dots moving on the screen (4, 8, 16, 32, and 64 dots) combined with (or without) motion noise. As mentioned above, three different strengths of motion noise were used. Each trial consisted of three test sections: (i) coherent motion with the actual number of motion dots moving, (ii) the motion noise alone at a given intensity, and (iii) coherent motion and motion noise combined. These were alternated with so-called reference sections in which the maximum number of motion dots moved and breaks showing a stationary image (Table 1). This image was the first image of the following section.

**Direction tuning in the presence of motion noise**
A similar stimulus protocol was used to test the direction selectivity of TCs (Table 1, right). In these reference sections the maximum number of motion dots moved in the preferred direction of the cell. In the test sections the coherently moving dots (16 or 64) were moved in one of the 8 tested directions (0-315° in 45° steps). To reduce the number of conditions (in order to have a reasonable number of trials for each condition) we used only the medium and strong motion noise.

**Representation of pattern velocity in the presence of motion noise**
We investigated the representation of pattern velocity with extracellular recordings of the H1-cell, because this analysis requires stable recordings over a longer time than can routinely be accomplished by intracellular recording. The motion dot velocity was dynamically modulated (Figure 6, left column). The underlying velocity profile was built up from sinewaves of 1, 3, 5, 7, and 9 Hz at random phases. The frequencies were matched to the frequency band in which the H1-cell codes pattern velocity with a high gain (Warzecha et al. 1998). Here we used a stimulus protocol consisting of three test sections (dynamic motion alone, motion noise alone, and the combination of both, each of 1000ms duration). The test sections were separated by stationary image sections of 5000ms duration. The interstimulus interval between consecutive trials of any experiment was 5000ms. This time was used to save the data and to provide Ringer solution if necessary.

**Data analysis**
Offline analysis was done with Matlab Release 14 (the Mathworks, MA). To analyze the performance of H1 to encode stimulus velocity (see above) we employed the coherence analysis (Haag and Borst, 1998; van Hateren and Snippe, 2001) using the coherence function distributed with Matlab. For this purpose the responses were aligned with the velocity profile by removing the response delay which was calculated from the cross correlation of the mean response and the velocity profile. Both stimulus and response vectors were zero padded to give 4096 sample points. Due to the rectification non–linearity inherent in the spike generation process, the H1 can only code pattern velocity in its preferred direction. Therefore, we rectified the stimulus velocity accordingly and set it to zero if the dots moved in the null-direction. The coherence between stimulus and response was calculated using a 4096 point Hanning window and an overlap of 2048 points. The coherence analysis quantifies the similarity between the stimulus and the stimulus reconstructed from the responses using the best linear filter. In the case of a noise-free linear encoder the coherence is 1 for all frequencies. Deviations from unity could be a consequence of noise and of non-linearities in the system. To separate these two effects the expected coherence (van Hateren and Snippe, 2001) was used. The expected coherence is defined as the coherence between the individual responses and the “noise free” average response. The deviation of the expected coherence from unity can be traced back to noise in the system. The difference between coherence and expected coherence is due to non-linearities.

To estimate the information that is transmitted by the neuronal responses about the stimulus the coherence rate was calculated from the coherence spectrum according to van Hateren and Snippe (2001).

\[ R_{coh} = -\int_0^{\infty} \log_2(1 - \gamma^2) df \]  

(1)

with \( R_{coh} \) the coherence rate and \( \gamma \) the coherence. The expected coherence rate (\( R_{exp} \)) was calculated accordingly.

Since the interpretation of the coherence rate in terms of transmitted information depends on assumptions that neuronal noise is Gaussian and additive, an assumption which is not strictly correct, we additionally calculated the information rate with the measure developed by Brenner et al. (2000).
\[ I = \frac{1}{T} \int_0^T dt \left( \frac{r(t)}{\bar{r}} \right) \log_2 \left( \frac{r(t)}{\bar{r}} \right) \]  

(2)

with \( T \) the number of time bins, \( r(t) \) the time-dependent spike rate and \( \bar{r} \) the average spike rate.

Both measures implicitly assume that information is encoded by the spike rate of the neuron. This assumption appears justified as a first approximation, since TC spikes were concluded to lock precisely to the stimulus in a frequency range of velocity fluctuations that are only weakly represented in TC responses (Kretzberg et al. 2001, Warzecha et al. 1998).

**Results:**

We analyze the nonlinear dendritic integration of driving and modulating synaptic input in two types of directionally selective, motion sensitive neurons: (1) The H1 neuron (Hausen, 1976) offers the opportunity of stable long lasting extracellular recordings, which is required for a detailed analysis of coding properties (Bialek et al. 1991; Warzecha et al. 2000). The H1-cell is excited by back-to-front motion and inhibited by motion in the opposite direction within almost the entire visual field of one eye. It uses spikes to transmit information from one brain hemisphere to the other. (2) The VS-cells (Hengstenberg, 1982; Krapp et al. 1998) can be recorded intracellularly and respond to downward and upward motion within a broad vertical stripe of the visual field with graded de- and hyperpolarizations, respectively. These graded membrane potential shifts are assumed to represent the summated postsynaptic potentials of the cell and can be recorded even close to the output terminal of the cells (e.g. Egelhaaf and Warzecha, 1999). Since VS graded depolarization may be superposed by spikes, reminiscent of the variable spikes observed in cortical neurons (e.g. Azouz and Gray, 1999), VS-cells can also be employed to study the transformation of postsynaptic potentials into spikes. In this study we concentrate on two types of the ten VS-cells, i.e. VS1 and VS2/3. Since VS2 and VS3 are two cells with largely overlapping receptive fields and virtually indistinguishable functional properties (Krapp et al. 1998), they are lumped here into one category of cells ‘VS2/3’. The analyzed VS-cells have their receptive fields in the frontal part of the visual field.

To test the influence of modulating input on the response to a driving visual signal, two types of stimuli were employed (Fig.1, insets): (1) The driving visual input consists of a variable number of dots moving coherently either in the cell’s preferred or null direction. (2) The modulating input
consists of dots moving in a variety of directions so that excitatory and inhibitory effects cancel each other (‘motion noise’). The driving input and the motion noise can be presented individually or combined. The motion noise component then acts as a modulating background input (see methods for details).

**Balanced motion noise has shunting characteristics**
The motion noise was balanced having no pronounced driving effect on the membrane potential on its own (Figure 1a right shaded response section). To achieve balance, more dots were required with a motion component in the null-direction than into the preferred direction of the respective cell. This is most likely due to the stronger driving force for the excitatory currents than for the inhibitory ones. Although the motion noise neither excites nor inhibits the neuron considerably, it increases the membrane potential fluctuations around the resting potential (Figure 1a, right shaded area). These fluctuations were smaller in some recordings, like the one shown in Figure 2. This might be due to the slight hyperpolarising effect of the motion noise in this experiment or to the different recording sites (close to the dendrites and close to the output terminals for the experiments shown in Figure 1 and 2 respectively).

To determine whether motion noise has shunting characteristics, we measured the changes in input resistance of VS neurons relative to the resting input resistance induced by motion noise stimulation and by null-direction motion stimulation, respectively (Figure 1). The input resistance was estimated by injecting hyperpolarizing current pulses into the cells. The VS-cell input resistances at rest were on average 6.81±2.56 MΩ (n=4 cells) which is in the range reported before (Borst and Haag, 1996). However, the resting input resistances varied considerably. In one VS-cell (shown in Figure 1a, b) an input resistance of 10.5 MΩ at rest was measured deviating much from the average. We did not find any obvious correlation between the difference in input resistance and the visually induced responses of VS-cells. Anyway, during coherent null direction motion, when the cell is hyperpolarized, the input resistance is decreased relative to the resting input resistance [4.71±2.05 MΩ; -32.23 ± 5.86% (Figure 1b, c)]. Motion noise does not depolarize or hyperpolarize the cell significantly but it reduces the input resistance to 5.06±1.95 MΩ [-26.05 ± 6.52% (Figure 1b, c)]. This reduction is in the same range, though slightly smaller, as the reduction of the input resistance during coherent null-direction motion. From these experiments we conclude that visual motion noise can be considered a shunting input comparable
to the background input artificially induced in slice preparations (Chance et al. 2002; Mitchell and Silver, 2003; Prescott and De Koninck, 2003).

**Non-linear integration of motion information**

To analyze the effect of motion noise on the neuronal gain we determined the response amplitudes of VS- and H1-cells as a function of stimulus strength in the presence of motion noise of different levels. Stimulus strength was varied by varying the number of coherently moving dots. The strength of the motion noise was altered by increasing the brightness of the dots constituting the motion noise.

Whereas both graded postsynaptic potentials and spikes can be recorded in VS-cells (Figure 2a), the H1-cell allows only spike responses to be recorded, though for extended periods of time (Figure 3). For both response modes of VS-cells the cell’s sensitivity to motion stimuli is reduced by motion noise, independent of motion strength (Figures 2, 3). Stronger motion noise produces larger reductions in response amplitude. This reduction cannot be explained by a summation of the respective responses to coherent motion and motion noise (Figure 2a left and middle sections). Thus, the reduction in responsiveness is mainly due to the shunting effect of the motion noise. This is true despite the slight hyperpolarization of about -3.5 mV for the strongest motion noise in the example shown in Figure 2 a (middle column). Similar reductions were observed in other recordings in which motion noise led to slight depolarizations. The reduction in sensitivity for the driving input induced by coherent motion is similar for both the graded membrane potential shift and the spiking response of the VS recording (compare Figures 2a and b). However, the threshold nonlinearity of spike generation is apparent, since strong motion noise suppresses completely the spike responses to small and moderate motion strengths. Similar results were found in two other VS recordings and two recordings from other TCs (1 HSE and 1 CH cell).

H1 is a spiking tangential cell which we recorded extracellularly. The responses of one sample recording are shown as PSTHs in Figure 3a. Figure 3b shows the average characteristics measured in 4 cells (similar results were found in 4 additional recordings which are not included since a slightly different stimulus protocol was applied). As for VS cells H1 spike frequency increases with increasing stimulus strength. When the motion stimulus is combined with motion noise the response amplitude is reduced in a similar way as those found in VS-cells. As for VS-
cells, this reduction is present even if the motion noise induces a slight increase of the spike rate on its own. Thus, the interaction of the input mediated by coherent motion, on the one hand, and the input by motion noise, on the other hand, is far from linear. Shunting inhibition resulting from motion noise has severe consequences on the response amplitude and the cell’s sensitivity for preferred direction motion.

**Robust direction tuning**

In which way does motion noise affect the coding properties of blowfly motion sensitive TCs? We used the motion noise to target the robustness of direction tuning of TCs. For this analysis H1 responses were recorded extracellularly to obtain sufficiently long and stable recordings. The coherent motion pattern moved in different directions (45° steps) and was combined with motion noise. We used three conditions: (i) no motion noise (ii) medium and (iii) strong motion noise. The solid lines in Figure 4 show the direction tuning curves for coherent motion alone (64 or 16 motion dots in Figure 4a and 4b, respectively) averaged across 6 H1 recordings. The response amplitude was normalized to the average response amplitude determined in the first reference section at the beginning of the trial where all 64 dots moved coherently in the cell’s preferred direction (see methods). H1 exhibits the typical broad direction tuning, with an abnormal “dent” in the response amplitude at the “preferred direction”. This seemingly surprising finding is in line with the conclusions drawn by Buchner, (1976). Given the hexagonal lattice of the fly’s eye, there are three ways to detect for example horizontal movement involving nearest neighbor interactions (inset Figure 4, shown for left to right movement). In addition to interactions along the horizontal axis of the eye, there are also strong interactions along the oblique axes of the ommatidial lattice (Buchner, 1976; Schuling et al. 1989). If the direction sensitivity of TCs is probed with grating patterns (Hausen, 1976, 1982b) the dent in the tuning curve did not appear. In contrast to stripe patterns, which simultaneously activate all three types of movement detectors when moving horizontally, the dots used here (each dot’s size approximates the acceptance angle of a single photoreceptor) stimulate only one type of detector at a time. Depending on the relative weight of the different types of nearest neighbor interactions, dot stimuli may, thus, lead to the dent in the tuning curve observed here.

When the motion stimulus is combined with motion noise, the peak amplitudes of the direction tuning curve decrease considerably (dashed curves in Figure 4). The tuning width does not significantly change for the combinations of coherent motion with medium and strong motion
noise (Figure 4, dashed and dash-dotted line). Rather than sharpening, the tuning curves become slightly broader. With a very weak motion stimulus of only 16 dots moving in the different directions (Figure 4b) the motion noise reduces the response so much that the cell’s direction tuning almost vanishes. Nevertheless, if we take into account that the very weak motion stimulus consists of only 16 dots and is combined with motion noise consisting of 112 dots moving in random directions, the direction tuning can be concluded to be quite robust against motion noise.

Robust representation of pattern velocity
From analyses with white noise velocity fluctuations it is known that the spike rate of the H1-cell represents the time course of velocity of a moving pattern up to frequencies of about 10 Hz, as long as the velocities are sufficiently small (Bialek et al. 1991; Haag and Borst, 1998; Warzecha et al. 1998). Here we tested the velocity coding of the H1-cell when the dynamic motion signal is corrupted by motion noise. The motion dots moved with a dynamic velocity profile which contained frequencies up to 10 Hz (Figure 5 left column). Motion noise of different strengths was added. To quantify the coding performance we calculated the coherence (Haag and Borst, 1998; van Hateren and Snippe, 2001) between the time-dependent velocity profile of the motion stimulus and the neuronal responses under three conditions, (i) without, (ii) with weak, and (iii) with strong motion noise (Figures 5 upper, middle and bottom plots). The time course and amplitude of the H1 responses to the first and the second condition are very similar. The spike rate reflects the pattern velocity in the preferred direction to a certain extent, while the null direction velocity cannot be resolved due to the rectifying effect of spike generation. Like the response amplitude, the overall response power and the coherence spectra under these two conditions are very similar. The coherence is close to 0.9 for almost the entire tested frequency range (grey solid lines in Figure 5). A noise-free linear system would give a coherence of one. The difference between one and the measured coherence may be due to both the noise and the non-linearities in the fly visual motion pathway. To obtain an estimate of the relative contribution of these two sources, the expected coherences were calculated. The expected coherence (black solid lines in Figure 5) is defined as the coherence between the individual responses and the mean response which can be considered the noise-free response if a sufficiently large number of trials is averaged. The deviation of the expected coherence from one is due to noise in the responses (van Hateren and Snippe, 2001). Consequently, the difference between measured and expected coherence reflects the non-linearity of the system. The data shown in Figure 5 are based on a
single cell with 80 trials per condition. Neither the measured coherence nor the expected coherence changed much when fewer trials were evaluated (not shown). Thus, the responses to dynamically varied pattern velocity are quite reliable and the H1-cell is well able to code stimulus velocity even in the presence of the motion noise.

When the strong motion noise is added the response amplitude drops in accordance with the results obtained with constant velocity motion. Nonetheless, the time course of the response is still very similar to those obtained with weak or without noise (compare the reduction in Figures 3 and 4). With the reduced response amplitude the response power drops as well but the coherence declines only slightly. Though the decrease is much smaller than the corresponding reduction in spike frequency, the coherence remains high (between 0.6 and 0.8). This reflects that the time course of the H1 response does not much change and that the pattern velocity is still encoded very well even though motion noise reduces the spike rate to about 50%. The expected coherence is somewhat lower for strong motion noise than under the other two stimulus conditions suggesting that the noise in the responses increases when the strong motion noise is added to the stimulus.

The information transmitted by spike responses can be estimated from the coherence spectrum under certain conditions. For additive Gaussian noise, the so-called coherence rate is related to the Shannon information (van Hateren and Snippe, 2001) and is given in bits/s. Even if these requirements are not met, the coherence rate can still be treated as an approximation of the Shannon information. Figure 5 (rightmost plots) shows the coherence rates and the expected coherence rates for the three conditions. For this sample recording the coherence and expected coherence rates are about the same for the conditions with and without weak motion noise (31.6 and 30.4 bits/s coherence rates and 41.3 and 43.5 bits/s expected coherence rates), but are reduced to a coherence rate of 26.3 bits/s and an expected coherence rate of 33.7 bits/s when strong motion noise is added. Again, the coherence rate in the presence of strong motion noise still amounts to 86.5% of the coherence rate obtained under the no motion noise condition, although the spike rate dropped to only 43.6% (49.5 ±3.25 and 21.6 ±2.5 spikes, for no noise and strong motion noise, respectively). Thus, the contribution of a single spike to the coherence rate is higher when strong motion noise is added to the dynamic motion stimulus than without added noise. Figure 6 shows the coherence rate per spike for the two conditions (black symbols) as an
average over 5 cells. We find the coherence rate per spike almost doubled (0.55 ±0.06 bit/spike to 1.07 ±0.22 bit/spike under the no-noise condition and strong motion noise condition, respectively). Similar results were obtained using a different measure (Brenner et al. 2000) which estimates the information transmitted per spike on the basis of the time dependent spike rate (0.912 ±0.10 bit/spike and 1.22 ±0.26 bit/spike for the two conditions, grey symbols in Figure 6). Despite the increased information per spike the total transmitted information decreases. The dashed lines in Figure 6 indicate the coherence rate (information) per spike that would be needed to fully counterbalance the decreased spike rate when strong noise is added to the dynamic motion stimulus. A similar change in the information per spike was found in the context of motion adaptation: the loss of spikes was largely balanced by an increased information per spike (Heitwerth et al. 2005).

To conclude, H1 is well able to encode the time course of pattern velocity, even if a dynamically moving pattern is combined with strong motion noise that reduces the response amplitude quite drastically. The loss of spikes is partly compensated by the increased information carried by individual spikes.

Discussion

We addressed how different types of synaptic inputs interact on dendritic trees under in vivo conditions. By manipulating the natural sensory input of fly tangential cells (TCs), we find that visually induced responses and the input resistance of the cells are strongly affected by motion noise that was designed to be balanced and not to drive the cell on its own. Although the response amplitudes to coherent visual motion are strongly reduced in the presence of motion noise, functionally important properties like the direction tuning and the ability to encode time-varying motion are robust against motion noise. From these results we hypothesize that the integration of functionally different synaptic inputs is a mechanism that immanently equalizes the ensemble’s sensitivity irrespective of the specific stimulus conditions (see below).

Integration of functionally different synaptic inputs
Inputs to neurons in the primate lateral geniculate nucleus were classified as drivers or modulators depending on their origin (Sherman and Guillery, 1998). For rat cortical neurons a classification was suggested based on the context in which the inputs are activated rather than on
their origin (Chance et al. 2002). Those excitatory and inhibitory inputs which are active at
different times drive the neuron, while those active simultaneously cancel each other and
constitute the modulating input. Simultaneous activation of excitatory and inhibitory inputs
affects the membrane potential or the firing rate only little, but increases the membrane
conductance and therefore represents a shunting input. In our stimulus design the impact of a dot
of the motion noise moving in one direction is counteracted by another dot moving in the
opposite direction. Thus, the motion noise as a whole constitutes a modulating input and does not
drive the cell on its own. In contrast, those inputs which are activated by coherent motion drive
the neuron by either depolarizing or hyperpolarizing it, depending on the direction of motion.
Motion noise was found reduce the cell’s sensitivity to simultaneously presented coherent motion
by reducing the cell’s input resistance and can therefore be considered as a shunting input to the
cell. Even though this classification of the synaptic inputs into drivers and modulators is artificial
in the case of TCs, especially since both components are embedded in the same visual input, it is
useful for the system analysis presented here. In the real in vivo situation the actual role of an
input signal depends on the context in which it is active, as was put forward for cortical neurons
(Chance et al. 2002).

The specific consequences of a shunting input on dendritic computation of driving inputs is still
controversially discussed. On the level of the postsynaptic potential, a decreased input resistance
acts divisively on driving inputs (e.g. Holt and Koch, 1997). The slope of the input output
characteristic is reduced as the membrane conductance increases. Things appear differently when
the postsynaptic potential is converted into spikes. Several in vitro and modeling studies
concluded that a tonic shunt then shifts the input-output characteristic along the input axis
(Berman et al. 1992; Connors et al. 1988; Holt and Koch, 1997). This is often described as a
subtractive effect since it resembles the effect of a hyperpolarizing input although the underlying
postsynaptic potential is in fact divisively modulated (e.g. Holt and Koch, 1997). Recent analyses
mimicking the natural synaptic input by the dynamic clamp technique concluded that shunting
can modulate a neuron’s gain resulting in slope changes of the input-output characteristic
(Chance et al. 2002; Fellous et al. 2003; Mitchell and Silver, 2003). Prerequisite is an increased
membrane potential noise induced as a by-product of the shunting input as is characteristic of
synaptic signals. Consequently, driving stimuli, which on their own would not suffice to induce
spiking, are enabled to cross spike threshold, an effect reminiscent of stochastic resonance (e.g. Wiesenfeld and Moss, 1995).

In our experiments done under *in vivo* conditions, the shunting effect and the increased membrane potential fluctuations induced by the modulating input (Figure 1) lead, at least in principle, to conditions for a divisive change of the spike response. However, the changes observed in the input-output characteristics (Figures 2 and 3) do not allow us to infer a shift or a slope change as exclusive mechanism. On the other hand, subtractive and divisive mechanisms affect tuning curves of spiking neurons very differently: in case of a subtractive shift, non-optimal directions fail to induce spiking and the tuning curve is sharpened, the so called iceberg effect (e.g. Anderson et al., 2000). As a consequence of a divisive mechanism the overall spike rate is scaled but the width of the tuning curve stays the same. Thus, the observed reduction in directional gain with an almost unaffected tuning width (Figure 4) suggests a divisive in favor of a subtractive effect on the H1 spike responses. As mentioned above, this might be the consequence of the increased membrane potential fluctuations at higher motion noise levels which allow occasional action potential generation even at suboptimal directions, i.e. when the average postsynaptic potential is below spike threshold. This is in line with conclusions drawn by (Anderson et al. 2000) who showed that membrane potential noise is the key for contrast invariant tuning curves in cat complex and simple cells.

**Alternative explanations of gain control**

Similar changes of the input-output characteristics as described above are also found in simple cells of the macaque visual cortex. For instance, the contrast dependence of the response to an optimally oriented drifting grating is shifted when simultaneously presented with an orthogonal grating which has no own driving impact. Likewise, superimposed visual noise reduces the response to the drifting grating (Carandini et al. 1997; Carandini and Heeger, 1994). These gain changes were interpreted by shunting input to the considered neuron mediated by another cortical neuron pooling many local retinotopic inputs (Carandini et al. 1997; Carandini and Heeger, 1994). This scheme is reminiscent of a model originally proposed to account for gain control of fly TCs which also relies on a shunting pool cell (Reichardt et al. 1983). Since in later studies this gain control could be explained in a more parsimonious way by realizing, in line with our present findings, that the driving input does not only drive the cell, but also leads to pronounced shunting.
(Borst et al. 1995), a similar influence of the direct peripheral input of cortical cells on the cell’s input resistance may also play a role in controlling the gain of these neurons.

**Robustness of temporal encoding properties**
Both effects of motion noise, the reduced gain and the increased membrane potential noise could impair the coding performance of TCs. Surprisingly, the stimulus time course can be recovered from the neuronal responses almost as well in the presence of motion noise as in its absence, although the corresponding spike rate decreased to 50%. This result seems to contradict findings that visual flicker impairs the ability of monkey area MT/MST neurons to discriminate between motion in different directions (Churan and Ilg, 2002). This discrepancy may be a consequence of the method used to discriminate between neuronal responses. Churan and Ilg, (2002) employed a measure derived from signal detection theory to discriminate between two alternative directions of motion on the basis of the mean response to constant velocity stimuli combined with flicker. In contrast, we tested how well the time course of the dynamic stimulus can be recovered from the neuronal response.

**Functional implications**
The fly’s visual system offers the opportunity to link neuronal performance to the animal’s behaviour (reviews: Egelhaaf et al. 2002; Egelhaaf and Borst, 1993; Hausen, 1984). TCs are involved in the evaluation of optic flow and have been shown to encode information about the three-dimensional layout of the environment (Kern et al. 2005) as well as certain self-motions like body roll (Karmeier et al. 2005; Krapp et al. 1998; Krapp et al. 2001). Some TCs project directly to motoneurons that control compensatory head movements (Gilbert et al. 1995; Gronenberg et al. 1995; Milde et al. 1995; Strausfeld et al. 1987).

During aerobatic flight maneuvers the fly induces optic flow, which is characterized by peculiar patterns of motion vectors depending on the type of self-motion and the environmental layout. While some maneuvers induce motion vectors optimally driving a certain TC, they are ineffective for other TCs (Karmeier et al. 2006). Irrespective of the efficiency of driving a specific combination of TCs at a given time, the input resistance and thus the gain of all TCs is decreased regardless of the actual stimulus situation. Hence, under natural conditions the gains of all TCs can be expected to be about the same level at any time. This level may be low when the animal is flying rapidly and thus confronted mainly with rapid retinal motion, somewhat higher when the
animal is walking and thus experiencing mainly slower retinal motion and at its highest level when the animal is just sitting and thus experiencing virtually no motion at all. Hence, all TCs are proposed to operate with a similar gain and thus a similar sensitivity to motion, irrespective of the overall strength of the optic flow and thereby the behavioral context. Thus, the continuous bombardment with motion stimuli, though not necessarily driving all TCs, can equalize the network’s gain, leaving it well able to code for different parameters as we could show for the direction tuning of constant motion or a dynamically varying stimulus.
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Legends

Figure 1:
Measurement of the TC input resistance. A, Average response of one VS-cell (5 trials) to coherent null direction motion and to motion noise (first and second shaded area). The strongest motion noise was chosen for this measurement. In half of the trials (black trace) 1nA hyperpolarising current pulses of 200ms duration were injected into the cell. The input resistance was calculated from the difference between the “current responses” and the “no-current” responses (black and grey traces, which were recorded alternately). B, Input resistance on single trial basis (black dots) and on average across the 5 trials on this condition (asterisks) of the same cell as shown in a). Input resistances were measured at rest, during null-direction motion and motion noise. C, Pooled results from 4 VS-cells recorded in 4 different animals, each dot is the input resistance estimated in a single trial. The input resistances are normalized to the respective input resistances at rest. The different symbols denote results from the different cells.

Figure 2:
Influence of motion noise on VS sensitivity to preferred direction motion. A, Individual VS1 responses to coherent preferred direction motion, motion noise and the combination of coherent motion and motion noise recorded close to the axon terminal. These responses were evoked by the strongest motion stimulus (64 dots moving coherently) and the strongest motion noise (maximal brightness of the 112 motion noise dots). VS-cells show graded shifts of the membrane potential superimposed with spikes. B, Input-output characteristics of the graded membrane potential shift on different motion noise intensities. Motion strength was varied by increasing the number of dots moving coherently while the intensity of the motion noise was altered by varying the brightness of the dots constituting the motion noise (solid line, dashed line with open circles, dotted line with open squares, and dash-dotted line with open diamonds represent no, weak, medium and strong motion noise, respectively). Response amplitudes are normalized to the response to the strongest coherent motion (64 dots moving in preferred direction). C, Same as B but for the spike response of this cell.

Figure 3:
Influence of motion noise on H1 sensitivity to preferred direction motion. A, Averaged responses of a single H1-cell shown as PSTHs (peri stimulus time histograms, bin width 20 ms) to coherent preferred direction motion, motion noise, and the combination of coherent motion and motion noise (columns from left to right) for three different motion noise intensities (weak, medium, and strong motion noise in the upper, middle, and bottom row). B, Input-output characteristics averaged over four cells recorded in four different animals at different motion noise intensities (black solid, dashed, dotted, and dash-dotted lines represent no, weak, medium, strong motion noise, respectively; the grey solid line is the spontaneous activity). Errorbars are standard errors.

Figure 4:
H1 Direction tuning when combined with motion noise. A, H1 responses as a function of the coherent motion direction (64 dots) without motion noise (solid line) and combined with medium and strong motion noise (dashed and dash-dotted lines, respectively). 180° corresponds to the right H1’s preferred direction. Plots are averages across 6 H1 recordings done in 6 different animals. Errorbars represent the standard error. The right inset sketches the ommatidial lattice of the fly compound eye. The dashed lines indicate the three different connections responding to horizontal motion from left to right. B, Same as A but with 16 dots moving coherently.

Figure 5:
Representation of pattern velocity in the H1 responses. Left column: stimulus time-course. Positive and negative velocities denote movement in the preferred and null-direction, respectively. The coherent motion following this velocity profile was presented without motion noise and combined with weak and strong motion noise. The middle column shows the averaged responses of a single H1-cell (80 stimulus presentations per condition) as PSTHs and the response power spectrums. Right column presents the coherence (grey solid line) and expected coherence (black solid line) spectra and expected coherence rates (black) and the coherence rates (grey) obtained under the three conditions (no, weak, and strong motion noise, top to bottom).

Figure 6:
Information content of individual spikes. The information transmitted by each single spike is shown for two conditions (no motion noise and combined with strong motion noise). The information was estimated from the coherence analysis (black symbols) and according to Brenner
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