Object and Background Coding by Different Neurons of Blowflies and the Benefit of Motion Adaptation

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Content

1. Summary

In a rich and complex world, it is a crucial task for animals, especially for fast moving ones, to detect objects in front of their background. Fast moving animals strongly rely on optic flow, i.e., the visual motion induced on their eyes during locomotion, to guide their behavior, such as to avoid obstacles, to estimate depth or distance to environmental objects, or to prepare for landing during flight. This thesis investigates with electrophysiological recording techniques the performance of different motion-sensitive neurons in representing objects and the spatial layout of the environment as well as how this representation is affected by adaptive processes. The analysis is done in the visual motion pathway of the blowfly, *Calliphora vicina*.

Only the translational component of the optic flow induced by an animal's self-motion contains spatial information, since the retinal images of close objects move faster than distant ones only during translatory movements, whereas during rotation, the retinal velocities are independent from the distance between objects and observers. Like several other groups of animals, blowflies pursue an active saccadic flight and gaze strategy to separate by their behavior the rotational and translational component of optic flow and, thus, to facilitate the processing of spatial information. During largely translational motion between saccadic turns, the gaze is stabilized and the spatial layout of the environments can potentially be encoded by the visual system flies.

How this may be accomplished is investigated for three types of motion-sensitive neurons, the horizontal system (HS), centrifugal horizontal (CH) and figure-detection (FD) cells in the third neuropil of the fly's visual system. Among the different types

of neurons, HSE/HSS (HS equatorial, southern), VCH (ventral CH) and FD1 (one subtype of FD) cells constitute major elements of a neural circuit which is assumed to be involved in object detection and distance estimation. CH cells receive retinotopic visual input from large parts of the ipsilateral visual field indirectly via dendro-dendritic electrical synapses from the large-field HS cells and transfer a GABAergic inhibitory signal to the FD1 cell and, thus, mediate its selectivity to small moving objects. In this thesis, neurons are confronted with semi-naturalistic optic flow as is seen by free-flying animals as well as targeted modifications of it. The results show that FD1 and HSE cells both respond strongly to nearby objects and are also affected by the distance to the background. The general performance of the FD1 cell not only to detect nearby objects, but also to represent spatial information is better than that of HSE.

The detectability of objects under given environmental conditions by motion sensitive neurons is not fixed but may be improved as a consequence of adaptive processes. Therefore, this thesis investigates the functional significance of motion adaptation for providing spatial information under the complex stimulus conditions encountered in a three-dimensional world. This is done in electrophysiological experiments on HS cells of the blowfly visual system. With manipulations of semi-naturalistic optic flow, motion adaptation is shown to facilitate the detection of objects in a three-dimensional environment although the overall neuronal response amplitude decreases during prolonged motion stimulation.

Furthermore, it was tested how motion adaptation is affected by different dynamic properties of the optic flow. In particular, this thesis assessed to what extent neuronal responses to an object located close to the flight trajectory depend on the dynamical characteristics of the optic flow before the object appears in the receptive field of the HS-cell. Object-induced responses were stronger in the adapted compared than the

non-adapted state. This effect holds for all types of adapting optic flow that have been used in the experiments. Adaptation with optic flow that lacked typical dynamical features resulting from natural flight dynamics, and even pure rotation at a constant angular velocity, was effective to enhance object-induced responses. The enhancement was slightly direction-selective, since preferred direction rotation was a more efficient adaptor than null direction rotation. These results provide evidence that the adaptive mechanisms are most likely distributed over different processing stages along the visual motion pathway and that the natural dynamics of optic flow is not a basic requirement to adapt neurons in a specific, presumably functionally beneficial way.

2. General introduction and discussion

Detecting objects embedded in a rich and complex surrounding world is a crucial requirement for animals to guide their behavior, such as identifying predators or a prey, to detect obstacles and avoid collisions with them, to estimate depth or distances to environmental objects or, in the case of flying animals, to prepare for landing. Depending on the type of animals, there are different possible cues to detect objects. An object can be discriminated from its background based on different texture properties such as color, shape, contrast and luminance. Even if all these features are shared by background and object, the object, at least if it is closer to the observer than the background, can still be detected solely on the basis of retinal motion cues. Motion cues, however, can only be employed for object detection, if the observer is moving in the environment. The continuous displacements of retinal images induced during self-motion of an observer are called optic flow. Self-motion is not sufficient as a basis for object detection. It rather has to contain a translatory component. Any movement of an animal can be decomposed into a translatory and a rotatory component, but only the translatory component contains spatial information. This is because only during translational motion the retinal images of a close object move faster than those of a more distant one. On the contrary, during pure rotation the retinal velocities are independent from the distance between objects and observers (e.g. Fig. 1).

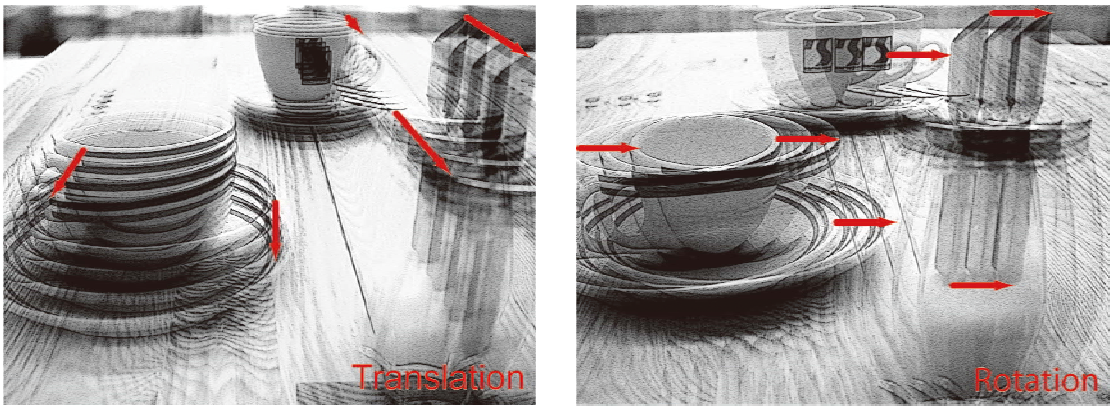

Figure 1: Schematic illustration of the consequence of translational (left diagram) or rotational (right diagram) self-motion for the resulting optic flow. Superimposed images were either generated by translating a camera forward or by rotating it around its vertical axis. Adapted from (Egelhaaf 2009).
(http://www.scholarpedia.org/article/Insect_motion_detection#Steps_of_visual_motion_computation)

Several groups of animals evolved active vision strategies to separate rotational and translational components of retinal image motion already by their characteristic behavior (e.g. Kral 2009; van Hateren and Schilstra 1999; Kern et al. 2006; Boeddeker et al. 2010; Boeddeker and Hemmi 2010; Eckmeier et al. 2008; Troje and Frost 2000). For instance, blowflies apply a saccadic flight and gaze strategy during their fast and acrobatic maneuvers (Schilstra and van Hateren 1999; van Hateren and Schilstra 1999). Their flight can be divided into two sets of episodes: 'saccades', when angular velocities of the head and body reach up to a few thousand degrees per second; and 'intersaccadic intervals', when the orientation of the head is well stabilized (Fig. 2). During intersaccadic intervals, the angular velocities of the head are generally lower than 100-200 degrees per second for any angular degree of freedom (*yaw, pitch* and *roll*: rotations around the vertical, the transverse or longitudinal axis of the animal, respectively). With high-speed cameras Boeddeker and Hemmi (2010) have found that honeybees visually stabilize their heads against rotation while performing fast lateral movements that are caused by periodic roll movements of the thorax. During such thorax roll movements, the head is held close

to horizontal, thereby minimizing rotational optic flow. Moreover, it could be shown for honeybees that they also employ a saccadic gaze strategy with respect to the yaw axis of the animal (Boeddeker et al. 2010). A similar gaze strategy has been observed also in avian species, the Zebra Finch *Taeniopygia guttata* (Eckmeier et al. 2008). The authors demonstrate that the birds separate rotational and translational optic flow by an alternation of fast rotational head shifts and intersaccadic periods where head rotations are relatively small and the translational optic flow component dominates. Although it is not yet clear, whether birds use this information source, the latter type of optic flow component could be used to gain information about the three-dimensional structure of the visual environment and to guide the animal's behavior. Another type of behavior where rapid and slow movements alternate is the so-called head-bobbing of several bird species, i.e., back and forth head movements with respect to the body, as has been extensively investigated in pigeons (Frost 1978; Davies and Green 1988, 1991; Troje and Frost 2000). During pigeons' walking, the head movement consists of two alternating phases: a thrust phase and a hold phase. Whereas in the thrust phase the head is quickly displaced forward, in the hold phase the head remains in a relatively fixed position in space (Troje and Frost 2000).

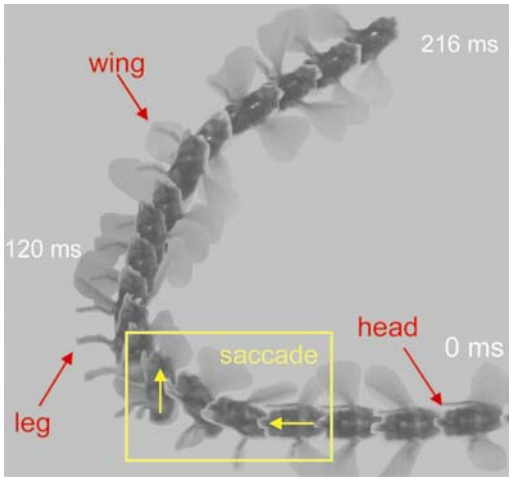

Figure 2: An example of saccadic flight of blowfly Calliphora. The pictures were taken from above and overlaid together and shown here at 12 ms time intervals. The rapid saccadic turn of about 90° (yellow frame) is executed within less than 50ms. Adapted from (Egelhaaf 2009). (http://www.scholarpedia.org/article/Insect_motion_detection#Steps_of_visual_motion_computation)

So far, it has been introduced that several animal groups employ gaze strategies

during locomotion behavior that largely separate the rotational from the translational optic flow components. Is this gaze strategy really used to segregate objects from their background on the basis of discontinuities in the optic flow field? The ability to detect such discontinuities in the retinal image flow has been studied in a broad range of animals (Kral 2003). For instance, bees were trained to select an object at a specific height above a structured ground from among several objects at various heights (Srinivasan et al. 1990). The bees were able to select the correct object despite variations in their size, shape and position, indicating that they are able to monitor the apparent motion of the object relative to the ground (Lehrer 1994; Wehner 1994). Also free-flying (Kimmerle et al. 1996) as well as tethered flies flying in a flight simulator (Reichardt et al. 1983; Egelhaaf 1985a; Kimmerle and Egelhaaf 2000b; Kimmerle et al. 2000) were shown to be able to use relative motion cues to discriminate objects from their background. Experiments on the empusid mantid *Empusa fascista* indicate that, when climbing among the branches of shrubs and jumping from one branch to another, the insects use relative motion cues from back and forth movements to estimate the distance to the nearest and most readily grasped object or landing target (Rossel 1996; Kral and Devetak 1999). Similar evidence has been found in birds such as pigeons. When flying, pigeons exhibit head-bobbing (Frost 1978) during the landing approach (Davies and Green 1990). In a study of hooded rats, Legg and Lambert (1990) investigated the significance for distance estimation of retinal motion cues arising from vertical translational head movements executed immediately before a jump to a platform. In psychophysical studies on the human visual system, relative motion in random dot patterns yields a vivid perception of surface boundaries and objects (Julesz 1971; Baker and Braddick 1982).

Taken together, we can conclude from these studies that translational optic flow facilitates for fast moving animals to gain relevant spatial information, such as approaching objects and distance of environments, for their visual navigation.

2.1 Why is adaptation interesting to study?

Our familiar experience, such as during and even an hour after a rock concert or a look into momentarily blinding sunlight, underlines the importance of sensory adaptation. The luminance level of light is just one of many features to which sensory systems adapt. For example, motion-sensitive neurons responding to a more complex feature than just brightness, such as motion, adapt to the preceding sequence of retinal image displacements ('motion adaptation'). Two aspects are important in this context. First, the changes in neuronal response properties with adaptation occur on a range of timescales from tens of milliseconds to many seconds (Kohn 2007). The rapid adaptation effects may contribute to instantaneous sensory processing. Since sensory neurons have limited operating ranges and are afflicted with noise (Vogels et al. 1989; Levine et al. 1988; Berry et al. 1997; Warzecha and Egelhaaf 1999), they cannot generate a unique response to any stimulus value. To solve this problem, neurons adapt to the prevailing conditions, so the same limited set of output values can be reassigned to different stimuli in different contexts. Secondly, adaptation emerges at different stages of sensory systems. For instance, visual adaptation is already found at the most peripheral level, the photoreceptors, but also at more downstream processing stages. Whereas, light adaptation has been intensively studied and now understood quite well (Dowling 1967; Autrum 1981; Laughlin 1989; Dunn and Rieke 2006), motion adaptation is still only partially understood. It is particularly unclear how the different stages of motion information processing along the visual pathway adjust to the current environment and how plasticity at one stage impacts responses at another.

2.2 The fly as a model system for the study of visual motion processing

The blowfly is used here as an experimental model system for analyzing visual

information processing. The reasons are: 1) several well identified motion-sensitive neurons in its visual pathway and its relatively easy accessibility for experiments (Hausen 1982a,b; Egelhaaf 1985b); 2) the possibility to associate neuronal response properties with their significance for behavior (Frye and Dickinson 2001; Borst and Haag 2002; Egelhaaf et al. 2002; Egelhaaf 2006, 2009; Maimon et al. 2010). Particularly, in the fly much is already known with respect to the main topics of this project, i.e., the neural mechanisms underlying object detection as well as the mechanisms and functional consequences of motion adaptation. This project, however, extends beyond the previous ones, because it concentrates on both object detection and motion adaptation under the complex stimulus conditions that come close to what a fly encounters during its normal flying behavior.

2.3 Naturalistic stimulus paradigms

Traditionally, studies on motion adaptation in vertebrates including humans as well as in invertebrates like flies apply relatively simple stimuli serving as the adapter, such as sinusoidal gratings moving at a constant velocity (e.g. Barlow and Hill 1963; Wallmann et al. 1982; Maddess and Laughlin 1985; Muller et al. 1999; Dragoi et al. 2002; Clifford and Ibbotson 2002). Other studies have applied a different approach: measuring the cells' tuning and responsiveness with dynamic stimuli, such as white noise velocity fluctuations (Brenner et al. 2000; Fairhall et al. 2001). However, these dynamic stimuli are characterized by a motion statistics that is exclusively determined by the experimenter and may deviate much from what an animal experiences during its behavior in the real world. There, sensory systems often face fluctuations of signals with specific characteristics in space and in time. For instance, the saccadic flight and gaze strategy of blowflies leads to visual input signals that fluctuate continually in a characteristic way. First of all, the optic flow of the flies is segregated into rotational (during saccades, large yaw velocity around 4000°/s) and translational

components (during intersaccadic intervals, low yaw velocity below 200°/s). The retinal input of flies that was reconstructed from such complex flights can nowadays applied for the experimental analysis. Additionally, we have a panoramic stimulation device, the so-called FliMax (Lindemann et al. 2003). This icosahedral display covers most of the visual field of the insects and extends the spatial reach of the conventional stimulation setups. The presentation of behaviorally generated naturalistic stimuli on such an instrument can most likely help us to further understand neural information processing and, in particular, its functional significance in a behavioral context. Since the stimuli are reconstructed from the trajectories and gaze direction of free-flying flies in a cubic arena with walls covered herbage pictures, they are still only an approximation to the natural optic flow induced from the self-motion of the flies in a cluttered environment. Moreover, we manipulated the optic flow stimuli for analytical purposes in targeted way. Therefore we call them in the following semi-natural optic flow or semi-natural stimuli.

2.4 Different neurons within a neural circuit underlying object detection and their functional significance

In the fly's third visual neuropil, the lobula plate, reside several large-field motion-sensitive neurons, the so-called tangential cells (TCs) (Hausen 1984). Most of these neurons have extended dendrites on which they spatially integrate the outputs of local motion sensitive elements. TCs thus respond in a direction-selective way to motion in large parts of the visual field. Among the TCs, a neural circuit constituted of three types of neurons is involved in object detection. These three types of neurons are the horizontal system (HS) cells (Hausen 1982a,b), the figure-detection (FD) cells (Egelhaaf 1985b) and the centrifugal horizontal (CH) cells (Eckert and Dvorak 1983; Egelhaaf et al. 1993; Gauck et al. 1997).

The HS family comprises three members that cover the northern (HSN), equatorial

(HSE) and southern (HSS) areas of the visual field. Their dendrites cover the dorsal, median, and ventral part of the lobula plate, respectively. They respond mainly to front-to-back motion in the ipsilateral visual filed and are inhibited by reverse motion. Moreover, both the HSE and HSN cells receive excitatory input from the contralateral eye during back-to-front motion via the H1 and H2 cells, although this input has a relatively impact on the HS responses (Fig. 3) (Horstmann et al. 2000; Krapp et al. 2001). The HS cells connect via descending neurons to thoracic ganglions (Hausen et al. 1980; Strausfeld and Bassemir 1985; Haag and Borst 2005), which ultimately control motor neurons for locomotion or head movments (Gronenberg and Strausfeld 1990; Gilbert et al. 1995). Thus, HS cells are traditionally thought to be involved in course control (Hausen 1981; Geiger and Nassel 1981; Hausen and Wehrhahn 1983; Wehrhahn 1985).

The second type, the CH cells, includes the dorsal (DCH) and ventral (VCH) cells, whose receptive fields also correspond to the location of their dendrites (Eckert and Dvorak 1983). They respond maximally to binocular rotation about the vertical axis of the animal in the dorsal and ventral part of the visual field, respectively (Egelhaaf et al. 1993). Similar to HS cells, CH cells get input from contralateral spiking neurons, the H1 and H2 cells (Eckert 1980; Hausen 1981, Egelhaaf et al. 1993; Gauck et al. 1997). CH cells are excited by ipsilateral front-to-back motion and also by contralateral back-to-front motion (Haag et al. 1999; Egelhaaf et al. 1993). Thus, CH cells respond not only to motion in front of the ipsilateral eye but also to motion in front of the contralateral eye (Krapp et al. 2001). Except for the contralateral excitatory input from H1 and H2 cells (Horstmann et al. 2000), the VCH cell gets additional contralateral inhibitory input from the Hu cell (Fig. 3) (Gauck et al. 1997; Haag and Borst 2001). In contrast to HS cells, CH cells do not receive ipsilateral visual input directly from columnar elements but indirectly via dendro-dendritic electrical synapses from the overlapping dendritic trees of HS cells (Fig. 3) (Haag and Borst 2002). This indicates that the ipsilateral retinotopic information from HS cells

is processed on via CH cells within a dendritic network of lobula plate tangential cells. Moreover, the VCH cell has input and output synapses, which are very close to each other (0.5-1.5 µm), located within its main dendritic arbor in the lobula plate (Gauck et al. 1997). Such close location of input and output synapses suggests that the spatial organization of its retinotopic synaptic input is more or less conserved in its inhibitory (Meyer et al. 1986) output pattern. By realistic compartmental modeling of dendritic electrical coupling between HS and VCH cells, Cuntz et al (2003) have showed that VCH cell dendrites serve as a kind of spatial low-pass filter, which produces a spatial blur of the motion image.

Within the group of FD cells, the FD1 cell is a well-examined example, which is specifically tuned to front-to-back motion of small objects (Egelhaaf 1985b). Motion of extended patterns elicits only small responses in the FD1 cell. The small-field tuning of the FD1 cell is based on the GABAergic inhibition from the VCH-cell (Fig. 3). The VCH-cell responds best to exactly that type of motion by which the activity of FD1-cell is reduced. By ablating the VCH-cell either pharmacologically or by photoinactivation, it has been evidenced that the VCH-cell inhibits the FD1-cell and thus medicates its selectivity to small moving objects (Warzecha et al. 1993).

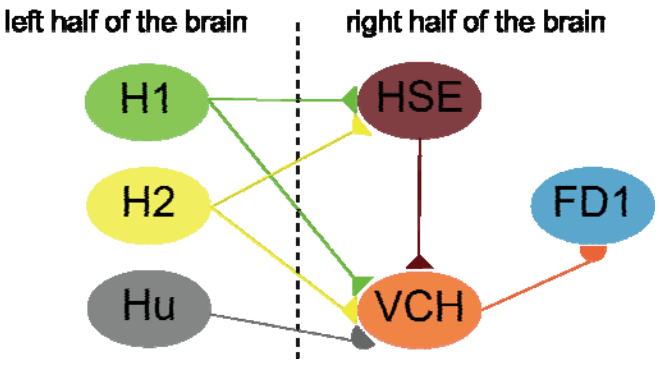

Figure 3: Relationship of the neuronal circuit. The cells are indicated by olive boxes. Excitatory and inhibitory synapses are indicated by triangles and half-circles, respectively.

2.5 Short summaries of the main projects of this thesis.

This dissertation addresses the following main issues. How do HS cells respond to object and background in the context of naturalistic stimulation and how their performance changes during prolonged stimulation? What is the possible functional significance of motion adaptation? Does the natural dynamics of the retinal image displacements contribute specially to the object induced response enhancement? The three types of motion-sensitive neurons (HSE, FD1 and VCH cells) are compared within the context of their functional significance, i.e., object detectability and distance coding, respectively. These issues are treated in the three projects of my thesis which are summarized below.

2.5.1 Functional relevance of motion adaptation in the context of naturalistic stimulation

Many response characteristics of neurons sensitive to visual motion depend on stimulus history and change during prolonged stimulation (e.g. Maddess and Laughlin 1985; Harris et al. 2000; Heitwerth et al. 2005). Although the changes are usually regarded as adaptive, their functional significance is still not fully understood. By using experimenter-defined stimuli, research on motion adaptation has mainly focussed, so far, on enhancing the detection of changes in the stimulus domain, on preventing output saturation and on energy efficient coding. This project will ground the functional significance of motion adaptation under the complex stimulus conditions encountered in the three-dimensional world. Motion adaptation is characterized in identified output neurons, HS cells, of the blowfly visual system. Neurons are confronted with reconstructed semi-naturalistic optic flow as is seen by free-flying animals. The optic flow sequence was modified by virtually inserting an object close to the flight pathway or changing the size of flight arena. Keeping the position of the object unchanged and increasing or decreasing the size of the flight

arena, the discontinuities induced by relative motion between the object and distant background are modified in a targeted way. With these stimulus manipulations, the neuronal responses to the motion induced by the sudden turning up of a nearby object and by the corresponding background when no object appears within the receptive field were analyzed. Under all tested conditions motion adaptation is shown to facilitate the detectability of objects in a three-dimensional environment although the overall neuronal response amplitude decreases during prolonged motion stimulation (details see Chapter 3).

2.5.2 Enhancement of object responses by visual motion adaptation and its dependence on the temporal characteristics of optic flow

Since motion adaptation is conventionally investigated with purely experimenter defined stimuli, it is still unclear how sensory systems efficiently encode signals with dynamical properties as experienced by animals in the real world and what role adaptation plays during normal behavior. This project addresses the performance of visual motion sensitive neurons of blowflies, the horizontal system (HS) neurons, with optic flow that is reconstructed from the head trajectories of semi-free-flying flies. To test how motion adaptation is affected by different dynamic properties of optic flow, the semi-natural optic flow was manipulated in different ways. The resulting stimuli comprised a broad range of dynamics covering naturalistic dynamics, just the rotational component of naturalistic dynamics without superimposed translational movements as well as simple rotations with constant velocities in the preferred and null direction of HS cells. Similar to the first project, the stimulus sequences were reconstructed from the optic flow experienced by the fly with an object virtually inserted close to the flight trajectory. As a functionally relevant effect of motion adaptation we assessed to what extent neuronal responses to an object located close to the flight trajectory depend on adaptation. Object-induced responses

were stronger in the adapted compared to the non-adapted state. This effect of motion adaptation holds for all types of adapting optic flow we used. Adaptation with optic flow that lacked the typical dynamic features of natural behaviorally generated optic flow and even pure rotation at a constant angular velocity was effective to enhance object-induced responses. The enhancement was direction-selective to some extent, since preferred direction rotation was a more efficient adaptor than null direction rotation. These results provide evidence that the cellular sites of motion adaptation are likely to be distributed along the visual motion pathway and indicate that the natural dynamics of optic flow is not a basic requirement to adapt neurons in a specific, presumably functionally beneficial way (details see Chapter 4).

2.5.3 Object responses and distance encoding in three dimensional environments by visual neurons of the blowfly

As mentioned in the general introduction, the three types of neurons, FD1, VCH and HSE/HSS cells, constitute, together with input neurons originating in the contralateral half of the visual system, the neural circuit assumed to detect objects and to encode distance information. Two sets of experiments to test object detectability and distance coding were carried out. In the first one, the behaviorally generated optic flow was modified by virtually inserting two objects close to the flight trajectory and by changing the size of the flight arena in order to analyze and compare the different neurons' performance in environments with different spatial characteristics. The second set of stimuli was reconstructed from ten different flight trajectories and the flight arena was virtually set to a wide range of sizes. The results show that FD1 and HSE cells both respond strongly to nearby objects and to close background. However, the general performance of the FD1 cell to detect nearby objects is better than that of HSE, particularly in the large environments. Distance information about the three dimensional environments is represented by the neural responses of HS cells and,

especially, of the FD1 cell, although many other aspects inherent in the complex behavioral optic flow influence the responses of the motion-sensitive visual neurons (details see Chapter 5).

2.6 General discussion

2.6.1 General functional benefits of adaptation

A proposed benefit of adaptation in the neural pathways of several sensory modalities is improvement of the detectability or discriminability of novel or rare stimuli (Kohn 2007). More precisely, novelty detection is thought to be accomplished by suppressing responses to frequent or persistent stimuli, thus leading to an enhancement of the relative strength of responses to novel stimuli. Improved novelty detection by adaptation has been proposed to be effective in the nervous system of some vertebrate species (Dragoi et al. 2002; Ulanovsky et al. 2003; Benda et al. 2005; Hosoya et al. 2005; Sharpee et al. 2006; Reches and Gutfreund 2008; Gill et al. 2008) as well as in insects (Maddess and Laughlin 1985; Kurtz et al. 2009; Ronacher and Hennig 2004). Adaptation can be viewed as reducing the transmission of redundant information by the sensory system, which optimizes the use of the limited dynamic range of the neural pathway for the coding of relevant stimuli (Attneave 1954). By reducing the redundancy in the responses of individual sensory neurons, the transmission of novel information about the stimulus is optimized (Clifford and Langley 1996). The functional considerations about redundancy reduction suggest that vision should be viewed as a dynamic process, with adaptive mechanisms continually operating to match the coding employed to the statistical properties of visual stimulation (Clifford 2002). For instance, Brenner et al. (2000) analyzed motion adaptation in flies and could provide evidence that the speed tuning of the H1 neuron adjusts to match the range of speeds in a stimulus ensemble. When H1 is

probed with a sequence of stimuli chosen from a low variance distribution of velocities (i.e., a narrow range of speeds), tuning is steep; when exposed to a high variance velocity distribution, tuning is substantially shallower. Adaptation can thus stretch or compress the range of stimuli over which the cell's responsiveness is modulated (i.e., it can change the slope of tuning curves) (Bair and Movshon 2004; Dean et al. 2005; Nagel and Doupe 2006). Moreover, Sharpee et al. (2006) could provide evidence that neural filters in cat cortical area V1 differ during and after exposure to a dynamic sequence of natural scenes or to filtered white-noise stimuli. Spatial frequencies that are common in a particular stimulus sequence become less effective in eliciting neural responses than rare spatial frequencies. Thus, during a period of adaptation, the input-output relationship varies according to changes of the statistical properties of the stimulus.

2.6.2 Comparison of neurons sensitive to small objects

Apart from the FD1 cell there exists in blowflies another type of identified visual neuron that is tuned to small objects. This neuron is called Male Lobula Giant 1 (MLG1) because it exists only in males (Hausen and Strausfeld 1980; Gilbert and Strausfeld 1991; Strausfeld 1991; Trischler et al. 2007). This neuron is presumably one major element of the pursuit system, which enables male blowflies to chase conspecifics by fixating their position in the dorso-frontal part of the visual field (Boeddeker et al. 2003; Trischler et al. 2010). Small-field selectivity of neurons was observed also in the moth and hoverfly. The moth 'target tracking' neurons respond only to discrete moving features, such as black or white spots, bars or edges, in a direction-selective manner, but they do not respond to large-field stimuli (Collett 1971). Recently, more and more small-target motion detector (STMD) neurons in the lobula complex of the male hoverfly *Eristalis* have been identified and characterized. They are sharply tuned to small moving targets and some STMDs are inhibited by

large objects (Nordström et al. 2006; Barnett et al. 2007; Geurten et al. 2007; Bolzon et al. 2009). Neurons tuned to small objects, similar to the MLG1 neuron of blowflies and STMD neurons of hoverflies, have been found also in the visual system of some vertebrate species. For instance, directionally selective neurons in the tectofugal system of pigeons respond strongly to small target motion and are inhibited by large-field motion as may occur during self-induced motion (e.g. Frost et al. 1990). Large-field inhibition also tunes object motion sensitive (OMS) cells in the retina of rabbits and salamanders (Ölveczky 2003). From the above mentioned examples from insects and vertebrates we can conclude that the selective responses of neurons to small objects are presumably due to inhibition by simultaneous background motion (e.g. Egelhaaf 1985b, 1988; Kimmerle and Egelhaaf 2000; Ölveczky 2003).

2.6.3 Neural mechanisms underlying object detection

It has been suggested that the small-field tuning of FD1 cells of blowflies is based on inhibition during large-field background motion (Egelhaaf 1985c; Egelhaaf and Borst 1993). The inhibitory large-field motion sensitive elements are the GABAergic VCH cells, which are supposed to form a large number of spatially distributed synapses with FD1 (Warzecha et al., 1993; Gauck et al. 1997; Hennig et al. 2008). The VCH cell receives not only its ipsilateral input from HS cells (Haag and Borst, 2002), but also its contralateral excitatory input from both H1 and H2 cells (Horstmann et al. 2000) and inhibitory input from the Hu cell (Gauck et al., 1997; Haag and Borst, 2001). As a consequence of dendro-dendritic electrical synapses between HS and VCH cells, VCH cell dendrites serve as a kind of spatial low-pass filter, which produces a spatial blur of the motion image (Cuntz et al., 2003). This property might well be functionally relevant in the context of object detection, because small motion patterns might be affected more by spatial low-pass filtering than larger motion patterns. In this way, inhibition of FD1 via VCH could be more pronounced for large

than for small patterns (Hennig et al. 2008).

In dragonfly, Bolzon et al (2009) have found that STMD not only get local lateral inhibition from early visual processing (Srinivasan et al. 1982), but also interocular inhibition from their contralateral counterpart. A similar way to tune cells to small objects has been proposed in the mammalian visual cortex (Hubel and Wiesel 1962). For instance, cortical hypercomplex cells of cats respond optimally to small moving targets, and are inhibited by motion of extended bars (Bishop et al. 1980). Hubel and Wiesel (1962) proposed that the lateral inhibition is probably the way to shape cortical responses and to tune cells to small objects. Recently, Anderson et al. (2001) suggested that this response inhibition is not only from lateral spatially discrete 'end-zones' within the receptive field, but also from decreased excitation from pre-synaptic cortical cells that are themselves target tuned (e.g. Fig. 4 in Nordström and O'Carroll 2009). Thus, the inhibition to tune the small-field cells can happen at different levels of the visual information processing pathway.

In conclusion, insect motion-sensitive neurons, like their mammalian counterparts, might as well employ multiple levels of inhibitory interaction to produce a specific sensitivity to small objects.

2.6.4 Behaviorally generated stimuli

The presentation of the naturalistic optic flow in electrophysiological experiments which was experienced by semi-free-flying flies extends the conventional methods to study the performance of visual motion-sensitive neurons. Nonetheless, the so-called replay experiments of the present and previous studies (Kern et al. 2005, 2006; Karmeier et al. 2006) differ much from the real flight situation. It is because that the neurons have been recorded in restrained flies although they have seen virtually the

same as in free flight. This might be a critical limitation for the study of sensory information processing. The reason is that the nervous system may be in a different state as compared to free locomotion. Rosner et al (2009) have found that the responses of blowfly TCs depend on the motor activity of flies, in particular, on halter movements. Halters are transformed hindwings of flies. When they oscillate the TCs responses are enhanced. Similarly, Maimon et al (2010) found that in fruitflies the gain of the response of VS cells (one type of TC) to wide-field stimuli is increased during tethered flight. Such behavioral state dependent performance of sensory neurons do not much limit the scope of the study described here, because only the response gain of the analyzed neurons increased during behavior, but not their stimulus tuning. Thus, the conclusion about the functional relevance of motion-sensitive neurons to detect small objects and to present distance information in a three dimensional environment as well as the enhancement of object-induced responses are most likely not affected by the limitations of our methods.

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3. Motion adaptation facilitates object detection in three-dimensional environment

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3.1 Introduction

When an animal moves, nearby objects are displaced on the retina faster than more distant ones in the background. Many animals including humans (Lappe et al., 1999; Warren et al., 2001) and other mammals (Legg and Lambert, 1990), birds (Wylie and Frost, 1999) as well as insects (Kral, 2003; Land and Collett, 1997; Kern et al., 1997; Kimmerle et al., 1996; Srinivasan et al., 1990) use the resulting motion discontinuities to segregate objects from their background and to estimate their distances. This segregation is possible only during translational self-motion, as during pure rotation the retinal velocities are independent of the distance between objects and observer and, thus, information on spatial discontinuities cannot be retrieved.

Several insect groups pursue active vision strategies to separate rotational and translational components of retinal image motion. They structure by their own behavior the optic flow on their eyes, thereby facilitating processing of spatial information by the nervous system (e.g. Collett and Zeil, 1996; Zeil, 1993a, b; Srinivasan and Zhang 2000). Blowflies shift their gaze by saccadic rotations of body and head, keeping their gaze virtually constant during translational locomotion between saccades (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999) (Fig. 1E). This gaze strategy appears to be utilised by a class of directionally selective motion sensitive output neurons, the Horizontal System-cells (HS-cell; Hausen, 1982a, b; Krapp et al., 2001). These cells were concluded to extract information about the spatial layout of the environment during the intersaccadic intervals (Kern et al., 2005; Karmeier et al., 2006; Boeddeker et al., 2005; Kern et al. 2006).

Motion sensitive cells of blowflies change their response characteristics during maintained motion stimulation. So far, research on motion adaptation has

concentrated mainly on enhancing the detection of velocity changes, on preventing output saturation and on energy efficient coding (e.g. Fairhall et al., 2001; Harris et al., 2000; Heitwerth et al., 2005; Maddess and Laughlin, 1985; Neri and Laughlin, 2005). From these studies with relatively simple experimenter-designed visual stimuli it is hard to infer the perceptual or behavioral significance of motion adaptation under the complex stimulus conditions encountered in the real world. Therefore, we analyze motion adaptation with semi-natural visual stimuli and address the following questions: Does the sensitivity of HS-cells for spatial discontinuities, i.e. for nearby objects, change with motion adaptation? Does object motion contribute to motion adaptation?

3.2 Material and methods

3.2.1 Stimulation

An almost circular section of a semi-free-flight trajectory was chosen from a large data set obtained from blowflies flying in a cubic arena (edge length 0.4m; walls covered with herbage photographs). This arena was placed in a Helmholtz coil; the position and orientation of the head were monitored by magnetic coils mounted on it (van Hateren and Schilstra, 1999). The semi-free-flight sequences recorded in this way do not differ in their saccadic structure from free-flight manoeuvers monitored with high-speed cameras under outdoor conditions (Boeddeker et al., 2005). The selected flight section was closed to a 717ms loop by interpolating the head position and gaze direction in a semi-natural way (Heitwerth et al., 2005). With gaze direction and the visual interior of the cage known, the visual stimulus could be reconstructed and presented in a panoramic display instrument, FliMax (Lindemann et al., 2003). Because of the looped trajectory, image sequences with repetitive structure (sequence

of loops) could be displayed continually to the blowfly. Ten of these loops made up one trial. To introduce spatial discontinuities, a homogeneously black vertical cylinder (diameter: 0.01m; height: 0.4m) was inserted into the virtual flight arena close to the flight trajectory, and the corresponding modified image sequence was reconstructed (Fig. 1A). To create spatial discontinuities of a different extent the edge length of the virtual flight arena was increased to 2.17m (large arena) or decreased to 0.16m (small arena). The wall pattern was scaled accordingly, but the distance between object and fly remained unchanged. Mirrored versions of the reconstructed image sequences were also presented. To assess the contributions of contrast and relative motion to the object responses, in control experiments an area on the original arena wall was blackened ('wall object'). This area corresponded in the analyzed intersaccadic interval to the azimuthally retinal size and position of the object in the other experiments. Different stimuli were presented in pseudo-random order. Between two stimuli, all light-emitting diodes of FliMax were set to the mean luminance for 20s to allow the fly's visual system to return to its pre-adaptation state.

3.2.2 Electrophysiological experiments

One- to three-day-old female blowflies (*Calliphora vicina*) were dissected as described by Dürr and Egelhaaf (1999). Temperatures during experiments amounted to 24-34°C. Responses were recorded intracellularly with glass electrodes from the axon of HS-cells in the right optic lobe. The resistance of the electrodes, filled with 1M KCl, was 20-50MΩ. Ringer solution (Kurtz et al., 2000) was used to prevent desiccation of the brain. Recordings were sampled at 4 kHz. The response of the left HS-cells was approximated by presenting a mirrored version of the reconstructed image sequences to HS-cells in the right half of the visual system.

3.2.3 Data analysis

Data analysis with Matlab 7.0.1 (The Math-Works, Natick, MA) is based on 14 HS-cells in the right half of the visual system (6 HSN, 7 HSE and 1 HSS). All cells tested with the mirrored image sequence simulating recordings from the left HS-cells will be termed 'left HS-cells'. Five of the HS-cells (4 HSN and 1 HSE) were tested, in addition with the original image sequence; they will be termed 'right HS-cells'. The data were averaged across different HS-cell types, because for any of them object detection and the functional consequences of motion adaptation did not differ in any obvious way. Responses to the control stimuli were recorded only from HSE-cells, because the wall object, due to its larger distance to the fly, had a smaller vertical angular extent than the nearby object and covered only the receptive field of HSE. The object was present in the receptive field of the left and the right HS-cells in different intersaccadic intervals. All response values represent a depolarization relative to the resting potential of the cell as determined before stimulation. The mean object and background response of the left HS-cells (Fig. 2A1-D1) were averaged during a 30ms time window in the respective intersaccadic intervals (indicated in Fig. 3C,D left grey areas). Shorter and longer (15 and 50ms) time windows led to qualitatively the same results. To check how much the object influences motion adaptation, the responses were averaged over 30ms in the subsequent intersaccadic interval while the object was no longer present in the cell's receptive field (Fig. 3C right grey area) or absent during the entire flight (Fig. 3D right grey area). The standard deviations (std) were calculated across all the cells' mean responses. Each cell was recorded 2 to10 trials.

The time constants τ with which the object and background responses decrease during adaptation were analyzed with DataFit Version 8.2.79 (Oakdale Engineering, Oakdale, PA) by fitting an exponential function of the form $y=a+b*\exp(-t/\tau)$ to the data.

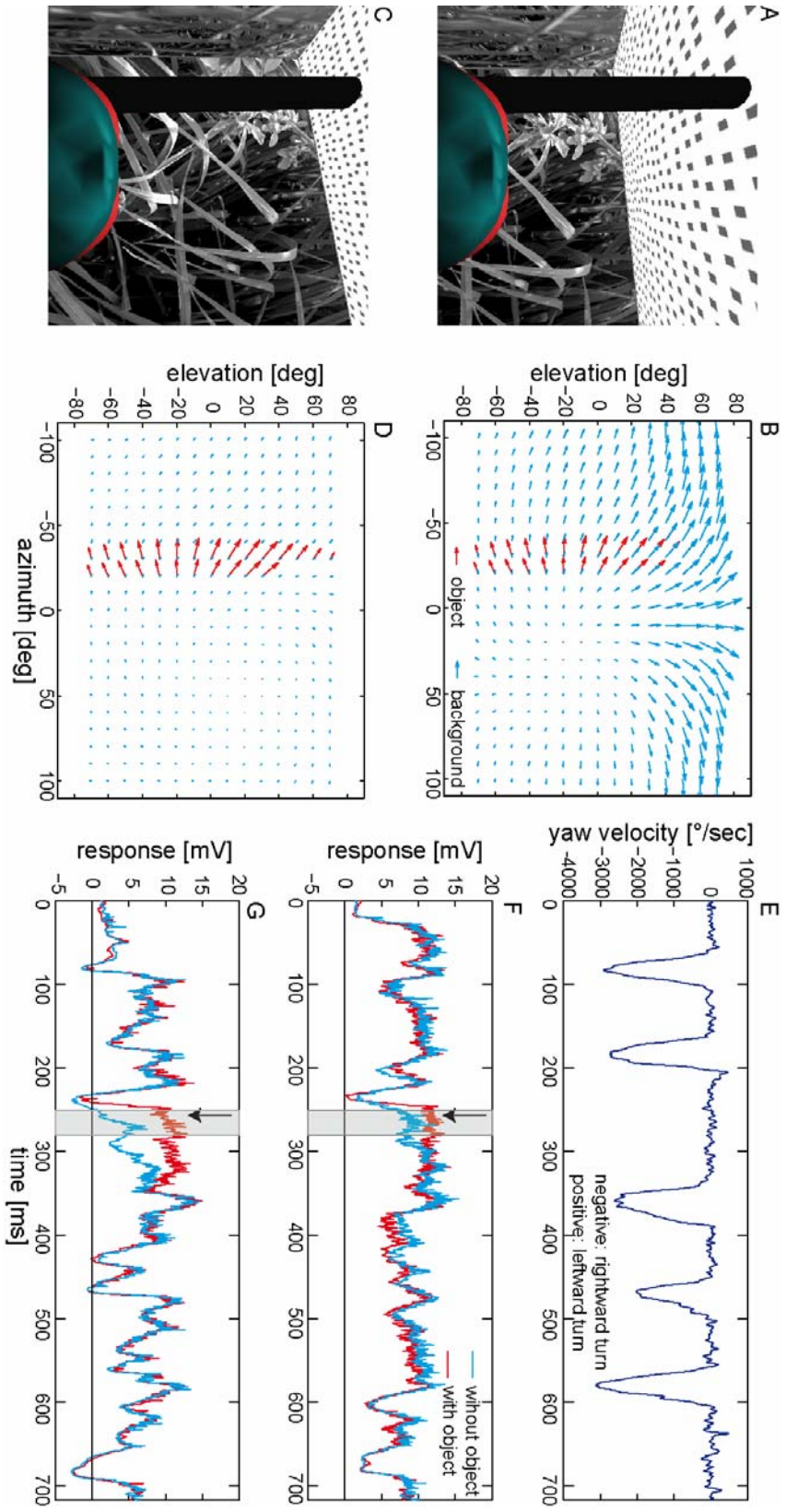
3.3 Results

3.3.1 Object-induced activity of HS-cells

The semi-natural flight trajectory consisted of five saccades which led to wide-field motion in the null direction of the right HS-cells and intervals of virtually rotation-free straight flight (Fig. 1E). In its mirrored version all saccades led to motion in the preferred direction. Inserting an object close to the flight trajectory allowed us to assess the impact of a spatial discontinuity on the responses of HS-cells. An example of the corresponding relative motion cues is shown in figure 1B for a moment of the flight in an experimental arena when the fly passes the cylindrical object (Fig. 1A). The retinal velocities induced by the nearby object are much larger than those induced by the background (Fig. 1B). Hence, the optic flow experienced when approaching an object is characterized by conspicuous discontinuities in the optic flow field, which are absent without object. Such discontinuities increase when the background is more distant (Fig. 1C,D) and decrease when it is closer (not shown). The time-dependent graded membrane potential fluctuations, averaged from 10 HS-cells reveal a stronger depolarization when an object is present in the cell's receptive field during the intersaccadic interval (Fig. 1F, red in grey area) than when it is absent (blue). On average, this increase was 52% of the response amplitude obtained without object (Fig.2B1 left). This increase in object-induced depolarization is not only visible on average, but in 98% of the individual responses (98 trials, 10 cells). The response increment is the larger the more distant the background and, thus, the larger the object-induced motion discontinuities (Fig. 1B,F vs. D,G; Fig. 2). The depolarization induced by the wall object was only 15% larger than the corresponding background response (Fig. 2D1). Hence, relative motion between object and background contributes considerably to the object-induced responses of HS-cells

during the intersaccadic interval. Accordingly, HS-cells provide information about the spatial layout of the environment.

Figure 1: Fly's view and neural responses. A The original-sized arena with a black object (part of the head from behind). B Reconstructed optic flow at the instant of time depicted in A. Blue and red arrows represent the velocity vectors at different points in visual space (shown as a cylindrical projection) induced by the background and the object, respectively. C, D same as A, B, but for the large arena. Since the head is not in the centre of the arena and slightly pitched upward, the fly's visual field does not cover the same area in A and C. E Time-varying yaw velocity during the flight. Between rightward saccades are intersaccadic intervals with near-zero yaw velocities. F, G Average responses of 10 left HS-cells during the flight in the original (F) and large arena (G), respectively. Red and blue curves indicate the responses to the behaviorally generated image displacements with and without object, respectively. Black arrows indicate the moment depicted in A, C. The grey areas in F, G correspond to the time interval (30ms) of strong HS-cells responses during the passage of the object through the receptive field.


A
C
B
elevation [deg]
object
background
D
elevation [deg]
azimuth [deg]
E
yaw velocity [°/sec]
negative: rightward turn
positive: leftward turn
F
response [mV]
without object
with object
G
response [mV]
time [ms]

3.3.2 Adaptation increases sensitivity of HS-cells for spatial discontinuities

On the basis of our looped flight a continuous sequence of semi-natural optic flow with a repetitive structure was generated and presented to the fly. As a consequence of adaptation of the left HS-cells by prolonged optic flow stimulation the intersaccadic background responses decreased much more (Fig. 2 blue data points) than the intersaccadic object responses (Fig. 2 red data points). Hence, the response increment induced by a nearby object increased with motion adaptation. Already after the third loop, 100% of individual object responses are larger than the corresponding background responses (98 trials, 10 cells). The object-induced response increment depends on the strength of the motion discontinuities, as tested by increasing or decreasing the distance between background and object while maintaining the position of the object relative to the flight trajectory. Without any relative motion, the intersaccadic responses decreased similarly with and without object (Fig. 2D1). In the large flight arena (Fig. 2C) the unadapted intersaccadic background response is already relatively close to the resting potential of the cell, since the background optic flow is very weak (Fig.1D). Nonetheless, the adaptation-induced decrease in the background response is larger than that of the corresponding object response (Fig. 2C1). 100% of the individual object responses are larger than the corresponding background responses irrespective of the adaptation level (98 trials, 10 cells). The strongest adaptation occurs in the small flight arena (Fig. 2A1), i.e. when the background is closest to fly and object. Then the object induces only very small motion discontinuities on the eyes. Nonetheless, an object-induced increment of depolarization is still visible (Fig. 2A1). The object-induced responses are now larger than the corresponding background responses in 79% of the trials (66 trials, 5 cells). Again, the object-induced response increment increases with motion adaptation, with

94% of individual object responses being larger than background responses after the fourth loop (66 trials, 5 cells). Similar results were obtained for the right HS-cells, although here all saccadic turns led to wide-field yaw rotation of the retinal image in the null-direction of the HS-cells (Fig. 2B2, C2). The larger the spatial discontinuities, the more pronounced is the object-induced intersaccadic response increment and the better the detectability of the object with motion adaptation.

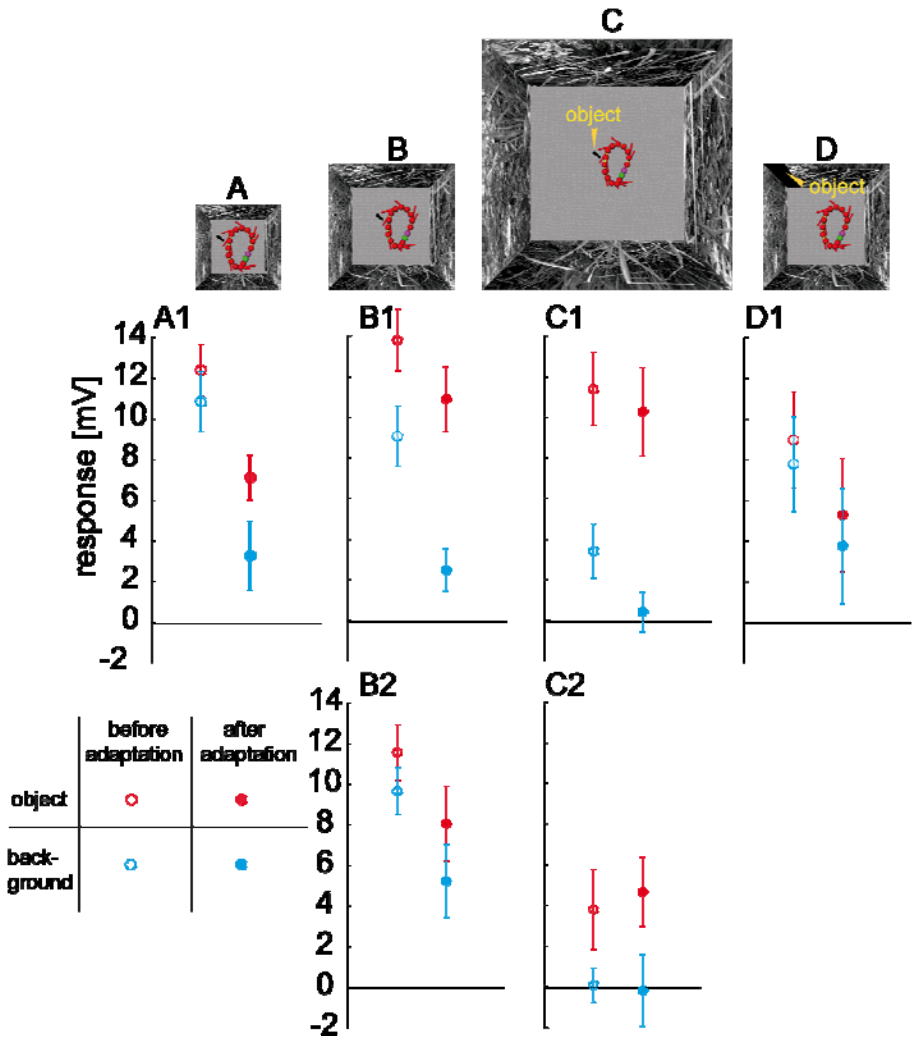


Figure 2: Mean responses (±std) to object and background before and after adaptation. A-D The same flight trajectory (top view) in small, original and large arena. The position of the fly's head and its orientation are shown every 45ms (red symbols). The location of the black object is given by the yellow markers. A1-D1 Responses within the 30ms intervals marked in Figs.1 F, G averaged of 5, 10, 10 left

HS-cells and 4 left HSE-cells, respectively. Data before and after motion adaptation are gathered during the first and the eighth loop. B2, C2 Mean responses of 5 right HS-cells, calculated in a 30ms time interval. The time interval corresponds to the presence of the object in the receptive field of the right HS-cells in the 'object' condition.

3.3.3 Time constants of motion adaptation

In accordance with previous studies on the time course of motion adaptation to constant velocity stimulation (Maddess and Laughlin, 1985), the intersaccadic background response amplitude decreases over time to a steady-state level (Fig. 3A blue line). The time constant of this decrease, as determined by an exponential fit to the average responses of each HS-cell during repetitive loops, amounts to, τ=1.1±0.3s, (10 cells) for the intersaccadic background responses and to τ=0.8±0.5s (10 cells) for the corresponding object responses (Fig. 3A red line). During motion adaptation the background response decreases by about 80%, while the object response declines by only about 25%.

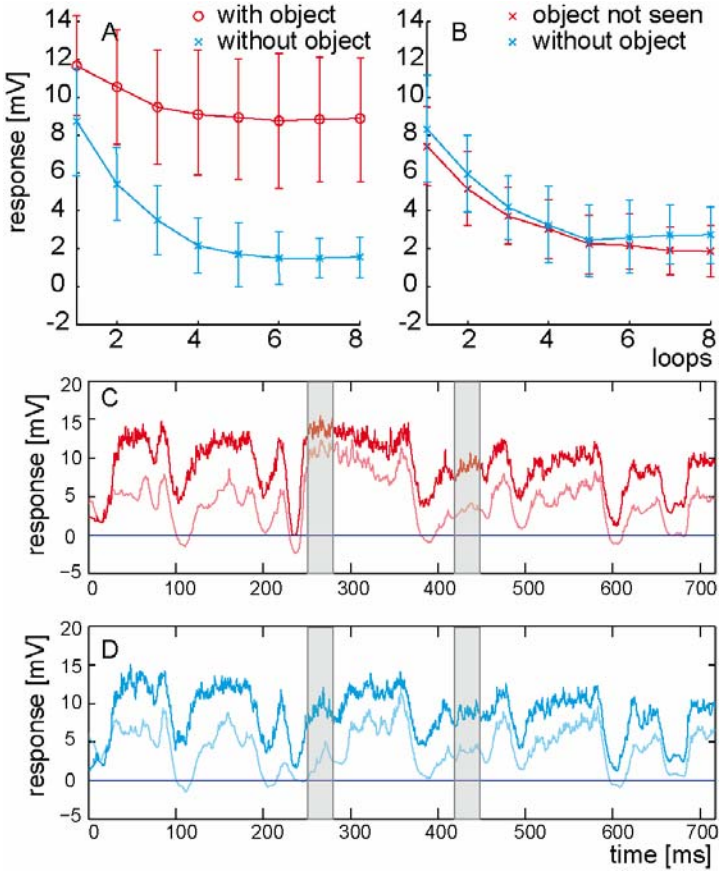


Figure 3: Time course of adaptation. A Mean responses (± std) in the intersaccadic interval with the object present (red; left grey area in C) and mean background responses (± std) in the corresponding time interval of the 'without object' condition (blue; left grey area in D). B Mean responses (± std) were determined either within the time interval where the object is not seen but is passed by the HS-cells' receptive field in the intersaccadic interval preceding the analyzed one (red; right grey area in C) or within the corresponding time interval of the 'without object' condition (blue; right grey area in D). C, D Time-dependent responses (averaged from 10 left HS-cells) in the original arena before (bold; first loop) and after (light; eighth loop) motion adaptation. C Responses to stimuli obtained in the arena with object. D Responses to stimuli obtained in the arena without object.

3.3.4 Contribution of object motion to motion adaptation

When the object is seen by the eye in the previous intersaccadic interval, but not visible to the HS-cell in the analyzed intersaccadic interval, the decrease in the background responses is very similar to that when there was no object in any of the

previous intersaccadic intervals (Fig. 3B). Hence, object motion is not the main source of motion adaptation. Only a minor contribution of object motion to motion adaptation is suggested, since all values obtained with the object in the previous intersaccadic interval are smaller by less than 0.9mV than those data points collected when there was no object at all. This conclusion is corroborated by another finding: When the object is absent during the initial eight loops and only inserted in the ninth loop, the object response (10.4±1.7mV) is very close to the level of the object response (11.6±1.8mV) when the object is present during the first loop (unadapted state), and insignificantly larger than the object response in the eighth loop (8.9±2.0mV, adapted state). Thereby, the object makes only a minor contribution to adaptation as compared with the impact of the background.

Comparing the time-dependent neuronal responses in the non-adapted and the adapted state (Fig. 3C,D) indicates that the reduction of the depolarization level during motion adaptation is primarily an overall shift of the responses to a more hyperpolarized level and only to a lesser extent a reduction in the modulation amplitude of the responses. Such a membrane potential shift has also been observed previously with simple experimenter-designed constant-velocity stimuli (Harris et al., 2000; Kurtz et al., 2000). However, a change in response gain that was also found by Harris et al. (2000) and concluded to be independent of the direction of motion is not obvious in our data.

3.4 Discussion

Discussion

The functional significance of motion adaptation in the blowfly visual motion pathway has been assessed in various previous studies (Maddess and Laughlin, 1985;

Fairhall et al., 2001; Brenner et al., 2000; Borst et al., 2005; Harris et al., 2000; Heitwerth et al., 2005). With experimenter-designed motion stimuli an increase in relative sensitivity to velocity increments or decrements superimposed on a constant velocity stimulus could be shown to accompany a decrease in absolute response amplitude (Maddess and Laughlin, 1985). In accordance with our results, the detectability of temporal discontinuities is improved as a consequence of motion adaptation, although the overall response amplitude decreased. Our results extend the adaptation benefit to three-dimensional complex environments.

Adaptation benefits are addressed in other studies as well. For instance, adaptive rescaling has been concluded to maximize the temporal information transmission by a fly motion sensitive neuron (Brenner et al., 2000) or to capture some of the statistical properties of a time-varying motion stimulus (Fairhall et al., 2001). Part of these results could be explained by modeling as being emergent properties of the motion detection system without any adaptive changes of system's parameters (Borst et al., 2005). It is not clear, so far, how these interpretations of the functional significance of motion adaptation based on experimenter-defined motion stimuli relate to our conclusion that adaptation enhances object detectability in a three-dimensional world.

Adaptation in motion sensitive neurons of vertebrates has been concluded to be beneficial in various ways. For instance, motion adaptation is proposed to re-centre tuning of motion sensitive neurons around the prevailing stimulus conditions in order to improve the discriminability of novel stimuli (Kohn, 2007). This can be accomplished by suppressing responses to frequent or persistent stimuli, leaving those to novel stimuli largely unchanged (Sharpee et al., 2006; Dragoi et al., 2002). Similar phenomena were observed in electrosensation of electric fish (Grau and Bastian, 1986; Reches and Gutfreund, 2008). This kind of novelty detection is similar to our finding that motion adaptation improves the detectability of an object suddenly

turning up, while the sustained background motion responses decrease. Novelty detection can be also viewed as an extension of a general predictive coding (Srinivasan et al., 1982) strategy of sensory systems, which improves efficiency by encoding the environment as differences of stimulus strengths in space or time (Barlow, 1961). Earlier studies interpreted motion adaptation mainly in terms of signal coding without recourse to its immediate perceptual (Dragoi et al., 2000; Maravall et al., 2007) or behavioral significance (Sharpee et al., 2006; Hosoya et al., 2005). Natural scenes have been already used in a recent study on motion adaptation (Sharpee et al., 2006). However, in contrast to our approach, where we reconstructed retinal image sequences as seen by semi-free-flying flies, the dynamics of the stimulus sequences used in the study of Sharpee et al. were obtained with a manually moved camera. These stimulus sequences presumably differ considerably from those image sequences experienced by behaving animals.

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4. Enhancement of object responses by visual motion adaptation and its dependence on the temporal characteristics of optic flow

The contents of this section have been submitted to *Journal of Neurophysiology*:

Pei Liang, Roland Kern, Rafael Kurtz, Martin Egelhaaf: Enhancement of object responses by visual motion adaptation and its dependence on the temporal characteristics of optic flow.

4.1 Introduction

The functional properties of sensory neurons may change during prolonged stimulation. Such changes are thought to be adaptive and have been extensively studied in a variety of systems ranging from receptor cells to high-level sensory neurons (Kohn 2007; Clifford and Ibbotson 2002). At the level of photoreceptors, adaptation to the mean light level adjusts their operating range to the huge variation of light intensities that may be encountered in a natural environment, maintaining sensitivity to fluctuations around this mean (Laughlin 1994; van Hateren 1997; Smirnakis et al. 1997; Fain et al. 2001). For the auditory system in the midbrain of cats and barn owls, it has been shown that adaptive processes increase neuronal responses to rare stimuli and decrease those to frequent stimuli (Ulanovsky et al. 2003; Reches and Gutfreund 2008). In a similar way, motion-sensitive neurons in the visual system of flies increase their sensitivity to sudden stimulus changes during prolonged motion stimulation, whereas the overall responses decrease (Maddess and Laughlin 1985; Liang et al. 2008; Kurtz et al. 2009b). Hence, adaptive processes adjust the operating range of neurons to the currently prevailing stimulus level not only at peripheral levels in sensory systems, but also at more downstream processing stages.

In most previous studies on adaptation in higher order sensory neurons, adaptation has been elicited by constant stimuli or stimuli with a simple temporal structure. In the real world, however, sensory systems often face highly variable fluctuations of signals with specific characteristics in space and in time. It is still not clear how sensory systems efficiently encode signals with real world statistics as experienced by animals during normal behavior and what role adaptation plays under such conditions (Rieke and Rudd 2009). Flies are widely used as a model system for the investigation of visual information processing for the following reasons: 1) the easy accessibility of their visual system and 2) the possible combination of electrophysiological data and

their significance for behavior (Frye and Dickinson 2001; Egelhaaf et al. 2002; Borst and Haag 2002; Egelhaaf 2006, 2009). Experiments on motion-sensitive neurons of the fly suggested that motion adaptation elicited by white noise velocity fluctuations rescales, on a wide range of timescales, the relationship between the motion input signals and the neural responses (Brenner et al. 2000; Fairhall et al. 2001). Although the motion stimuli used in these studies varied dramatically over time, the statistics of their dynamical properties deviates much from the dynamics of the retinal motion patterns experienced by the animal in behavioral situations. From responses to artificial stimuli, it is not easy to infer the functional significance of adaptation under natural operating conditions. To overcome this limitation, we use optic flow that is reconstructed from the head trajectories of virtually free-flying animals as well as targeted modifications of this optic flow. Such reconstructed motion sequences are as close as is currently possible to what the fly has seen during flight.

The dynamics of the retinal motion patterns of several insect groups is shaped by active vision strategies, which separate rotational and translational components of retinal image motion. These strategies facilitate the processing of spatial information by the nervous system (Zeil 1993; Collett and Zeil 1996; Srinivasan and Zhang 2000; Zeil et al. 2008; Boeddeker et al. 2010). For instance, blowflies shift their gaze by saccadic rotations of body and head, while keeping their gaze virtually constant during translational locomotion between the saccades (Schilstra and van Hateren 1999; van Hateren and Schilstra 1999; see also Fig. 1). During saccades the yaw velocity of their heads can reach up to 5000°/s. The corresponding retinal input is characterized by rapid rotational motion during saccadic turns separated by mainly translational optic flow during the intersaccadic intervals. Although the neural responses to these characteristic retinal motion patterns have been studied in some detail (Boeddeker et al. 2005; Kern et al. 2005, 2006; van Hateren et al. 2005; Karmeier et al. 2006), it is not yet known whether the dynamics of these patterns has a distinct impact on motion adaptation.

We performed our experiments on a specific class of directionally selective motion-sensitive output neurons of the visual system of blowflies, the horizontal system (HS) neurons (Hausen 1982a,b; Krapp et al. 2001). These neurons have been shown to encode information about the spatial layout of the environment during the intersaccadic intervals (Boeddeker et al. 2005; Kern et al. 2005, 2006; Karmeier et al. 2006), although they are conventionally thought to act as rotation sensors (e.g. Krapp et al. 2001; Farrow et al. 2006; Nordström et al. 2008). Additionally, it has been shown that motion adaptation enhances the responses of HS neurons during the intersaccadic intervals to suddenly appearing objects (Liang et al. 2008). Thus, HS neurons serve as a good model to study the consequences of motion adaptation and its stimulus dependence. Here we address the following questions: Does the intricate dynamics of natural optic flow play a crucial role in motion adaptation? Does, in particular, the temporal fine structure of optic flow, e.g. the frequency of changes between flows mainly caused by translation or fast rotation, influence the consequences of motion adaptation? If not, what else might be essential stimulus parameters contributing to motion adaptation?

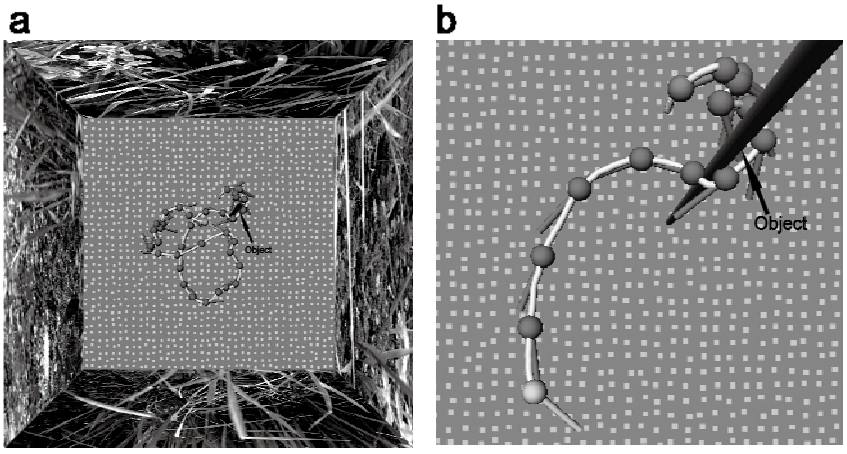


Figure 1: Top view of the flight trajectory of a blowfly in a cubic arena used for the generation of naturalistic optic flow. The track of the fly is indicated by the white line; the grey dots and short dashes indicate the position of the fly's head and its orientation, respectively; the slightly lighter grey dot indicates the start of the trajectory. a) Complete trajectory in the arena. b) Magnified part of the trajectory

constituting the reference/test phase; an inserted object (black cylinder) is located very close to the trajectory.

4.2 Materials and Methods

4.2.1 Stimulation

A flight trajectory (duration 3.45s) was chosen from a large data set obtained from blowflies flying in a cubic arena (edge length 0.4m; walls covered with photographs of herbage). This arena was placed in a Helmholtz coil; the position and orientation of the blowfly's head were monitored by means of magnetic coils which were mounted on it (van Hateren and Schilstra 1999). The semi-free-flight sequences recorded in this way do not differ in their saccadic structure from free-flight maneuvers monitored with high-speed cameras under outdoor conditions (Boeddeker et al. 2005). With known gaze direction and visual interior of the cage, the visual stimuli can be reconstructed and presented in a panoramic display instrument, the so-called FliMax (Lindemann et al. 2003). Our new system of FliMax with ultra-bright light-emitting diodes (WU-14-752GC, 525nm, 5mm diameter, Vossloh-Wustlich Opto, Kamp-Lintfort, Germany) has the following characteristics: (1) maximum luminance averaged over the whole array is more than 12,000 cd/m^2, which is about 30-fold relative to the old system used in our previous study (Liang et al. 2008); (2) it is able to display 190 different levels of light intensities; (3) it allows presentation of almost panoramic motion at a frame rate of 354 Hz, which is sufficiently high to account for the temporal resolution of the fly's visual system.

The time constant of the built-up of some components of motion adaptation has been shown in previous studies to be in the range two to four seconds (Maddess and Laughlin 1985; Harris et al. 2000; Fairhall et al. 2001; Wark et al. 2009; Liang et al. 2008). The chosen flight sequence (3.45s) was divided into two parts, the first part termed adaptation phase lasts 2.26s, and the second part, the test stimulus, 1.19s.

Presenting the second part in isolation delivered the reference neuronal responses. They were compared to test responses, i.e. responses to the identical stimulus following a motion adaptation stimulus. In order to create spatial discontinuities in the reference/test stimuli, a homogeneously black vertical cylinder (diameter: 0.01m; height: 0.8m) was inserted close to the flight trajectory (Fig. 1). The spatial discontinuities were enhanced, i.e. strong object responses relative to the background responses were induced, by doubling the edge length of the reconstructed virtual flight arena (0.8m) relative to the original arena. The wall pattern was scaled accordingly. In this way we could analyze the object and background responses before and after motion adaptation and take changes in the object-induced response increment as indicator of motion adaptation (Liang et al. 2008). A set of five different adaptation stimuli covering a broad range of dynamics were used to test the consequences of motion adaptation. Details of the various stimuli are described in Results. Different stimulus-pairs (the same dynamic stimuli with and without the object) were presented in pseudo-random order. Between two stimuli, all light-emitting diodes of FliMax were set to the mean luminance (about 4000 cd/m²) of the whole movie for 20s to allow the fly's visual system to return to its pre-adaptation state.

4.2.2 Electrophysiological experiments

One- to three-day-old female blowflies (*Calliphora vicina*) were dissected as described by Dürr and Egelhaaf (1999). Temperatures during experiments, measured close to the animal, amounted to 24-34°C. Voltage responses were recorded intracellularly with glass electrodes (GC100TF-10, Clark Electromedical Instruments, Pangbourne Reading, UK) from the axon of HS neurons (Hausen 1982a) in the right brain hemisphere. The responses of the left HS neurons were approximated by presenting a mirrored version of the reconstructed image sequences. The resistance of

the electrodes, filled with 1 M KCl, was 20-50 MΩ. Ringer solution (Kurtz et al. 2000) was used to prevent desiccation of the brain. Recordings were sampled at 8 kHz (DT 3001, Data Translation, Marlboro, MA).

4.2.3 Data analysis

Nine recordings of left HSE neurons were analyzed with Matlab 7.0.1 (The Math-Works, Natick, MA). The reason to record only the responses of the left HSE neuron is that the object appeared mostly in the receptive field of the left HSE, but not much in that of the right one. For each trial the responses were firstly offset by the resting potential (-40 to -60 mV), which was obtained by averaging the membrane potential over 500 ms before stimulation. Potentially as a consequence of recording quality and difference in cellular properties, some recordings contain action potentials of variable amplitude in addition to graded voltage changes. To focus on the graded potential signals, we used a low-pass filter (sigma = 3.7 ms) to smooth out the spikelets. Since the stimuli were displayed pair-wisely, i.e. the same stimuli with and without object were always displayed in direct, random succession, the part of the paired responses before the object moved into the neuron's receptive field should ideally be identical. Minimal differences were attributed to noise and compensated for by shifting the across-trial average paired response traces vertically to each other. This shift was half of the mean difference between the paired responses averaged over 330ms before the appearance of the object and over both stimulus conditions.

The object-induced response increment could be easily seen from the difference of the time dependent responses during the reference movie with and without object (Fig. 2 r6). The differences between object and background responses were analyzed in two groups of time windows. These two groups of windows were defined by the following steps. (1) Two windows were chosen from the pair response traces, where the object-induced response differences were clear visible. (2) Since the object might

not only induce depolarizations, but also hyperpolarisations when it moves in null direction, only the locations within the windows were chosen to include strong depolarizations of the membrane potential. As an objective criterion, for each cell only those locations of the windows were selected, where the increments were two times larger than the SD of the difference of the responses with and without object. (3) Once determined for the responses in the reference condition, these windows were used for all different stimulus conditions to compare object and background responses. As in Fig. 2 shown, the first group of time windows (pink area) starts about 330ms after the reference movie begins; the second group of windows (blue area) starts 670ms later and is located almost at the end of the movie.

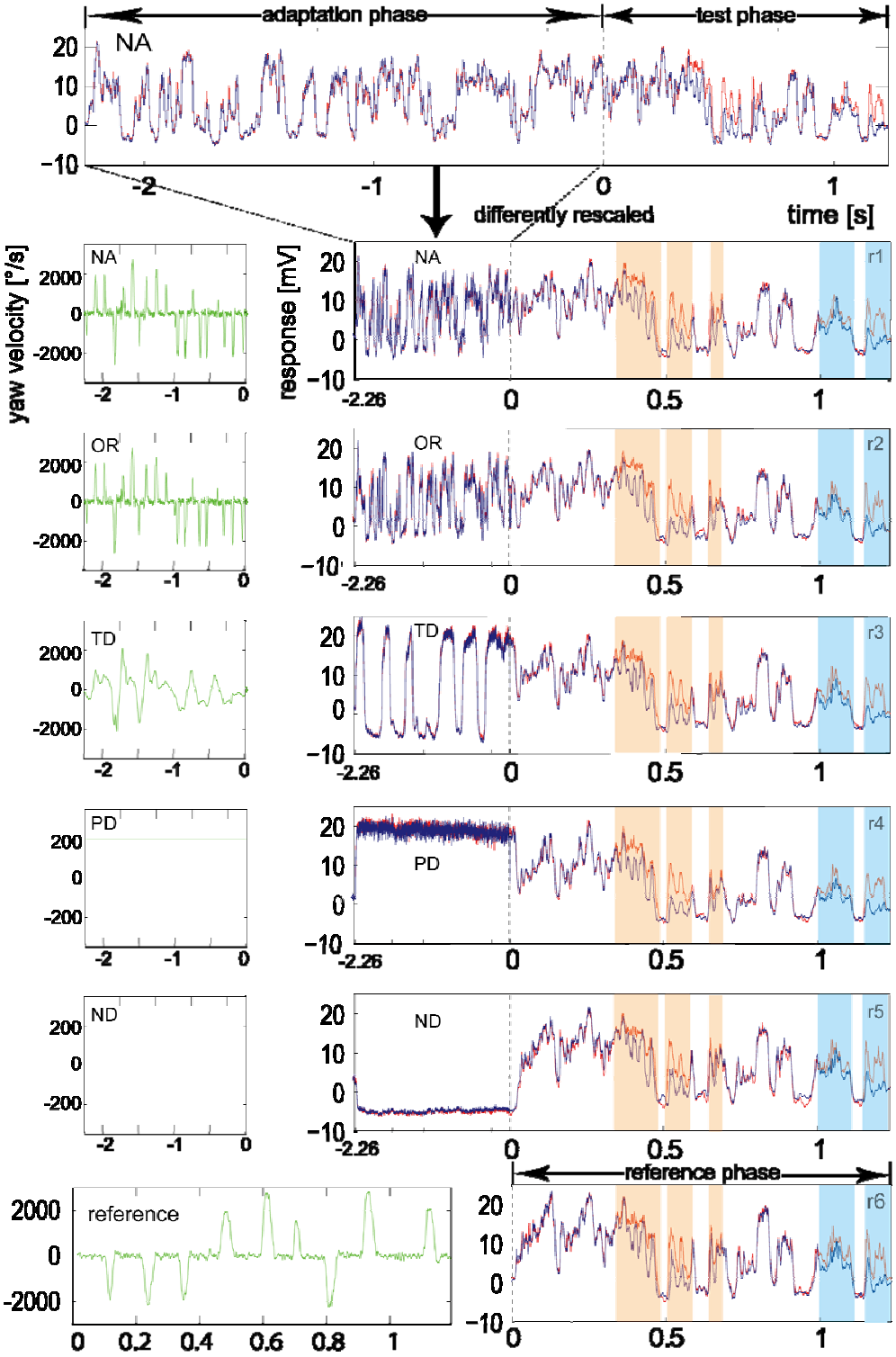
A pair-wise t-test was used to test the significance of differences in object-induced response increments.

4.3 Results

Natural optic flow of blowflies contains the succession of flight sections with virtually no rotations and brief sections dominated by fast rotations (Fig. 1; Fig. 2 left column, NA), which is characteristic of the animals' saccadic flight and gaze strategy. Does this dynamics of natural optic flow play a critical role in motion adaptation? To answer this question, five different adaptation stimuli, covering a broad range of dynamics, were used to test the consequences of motion adaptation (Fig. 2 left columns): (1) the motion sequence experienced on a semi-free-flight trajectory with its characteristic saccadic structure (naturalistic dynamics, NA); (2) the motion sequence that would have been seen by a fly while rotating with its semi-natural dynamics without translating at all in the intersaccadic intervals. To obtain basically the same trajectory of the eye, the intersaccadic translation of the original trajectories was added to the translation during saccades (only rotation, OR; Kern et al. 2005);

note that the resulting additional translational optic flow is negligible during saccades relative to the much larger rotational optic flow; (3) the motion sequence that would have been experienced by a fly with its gaze directed tangentially to the flight trajectory (track direction, TD); (4) motion sequences encountered during a yaw rotation in the HS neurons' preferred (PD) or (5) null direction (ND) at a constant velocity 200°/s. As a reference a stimulus without preceding motion adaptation was used (Fig. 2 left, reference). As indicators of motion adaptation, two response characteristics of HS neurons were used: (1) the decrement of the overall responses after prolonged motion stimulation (Maddess and Laughlin 1985; Harris et al. 2000; Kurtz et al. 2000; Reisenman et al. 2003); (2) the changes in the response increments that are elicited when an object passes the receptive field of the neuron during a translatory intersaccadic phase within a flight sequence (Liang et al. 2008).

Figure 2: Yaw velocities related to the stimuli employed and the corresponding responses of a single HSE-neuron (averaged from five to seven trials). The columns on the left side present the yaw velocities (green) during the respective motion adaptation stimulus phase: NA, OR, TD, PD and ND. The top diagram illustrates the responses to the semi-natural dynamic stimuli (NA) obtained in the with (red curve) and without (blue curve) object condition. The time intervals preceding and after 0 are defined as adaptation phase and test phase, respectively. The responses are differently rescaled and plotted underneath (r1). r2-r5 present responses to OR, TD, PD and ND stimuli, temporally rescaled in the same way. Starting from time point 0 (vertical dotted line) are the responses to the two test stimuli (with and without object), which are identical for all adaptation conditions. During reference phase the responses without preceding motion adaptation are shown in r6. The areas shaded pink and blue mark two groups of time windows in which the responses to the stimulus with object ("object responses") are considerably stronger than to the stimulus without object ("background responses") in the NA condition.


adaptation phase
test phase
NA
differently rescaled
time [s]
yaw velocity [°/s]
response [mV]
OR
TD
PD
ND
reference phase
reference
r1
r2
r3
r4
r5
r6

HS neurons respond to visual motion with prominent graded de- and hyperpolarisations of their axonal membrane potential, occasionally superimposed with action potentials of variable amplitude (which are inconspicuous in average traces) (Hausen 1982b). The responses to the different adaptation stimuli differ in most cases dramatically (time intervals preceding time zero in right columns of Fig. 2, named ‘adaptation phase’). Only the responses to NA and OR appear to be very similar (Fig. 2 r1 and r2, to the left of the black vertical broken line). It can be expected from previous studies that upon closer inspection these responses may differ during the intersaccadic intervals, because the intersaccadic translational optic flow is present in the naturalistic stimulus (NA) but absent in the OR stimulus variant (Kern et al. 2005). Irrespective of these fairly inconspicuous differences, the membrane potential shows pronounced fast fluctuations both during NA and OR (for details of the time course of HS responses to naturalistic motion stimuli, see Kern et al. 2005; Kern et al. 2006; van Hateren et al. 2005). In contrast, the responses to TD (Fig. 2 r3) are much smoother and vary on a much slower timescale. This difference in time course is the consequence of the much slower changes in the direction of the flight track compared with the much more rapid saccadic changes in head orientation and gaze direction (van Hateren et al. 2005). The responses to PD and ND are fundamentally different from those to the adaptation stimuli discussed so far. The neurons show either a constant depolarization (Fig. 2 r4) or hyperpolarisation (Fig. 2 r5) if stimulated with constant velocity motion in PD or ND, respectively. During motion in PD and ND the temporal modulations of the responses are both weak, as is characteristic when motion-sensitive neurons with large receptive fields are stimulated with panoramic constant motion.

For the reference as well as for the entire set of motion adaptation conditions we compared the responses to two types of stimuli, presented in the time interval following the adaptation phase (after time zero in right columns of Fig. 2). On the one

hand, we showed the original image sequence, which is close to what has been experienced by the semi-free-flying fly. On the other hand, an object (a vertical black cylinder) was inserted into the flight arena (object position shown in Fig. 1) before reconstructing the other presented image sequence. Even in the complex time dependent responses of the neuron, the object leads to a prominent depolarization of the neuron when it is displaced on the retina of the fly in the neuron's preferred direction (pink and blue areas in Fig. 2). These depolarizations become more evident when the responses during the condition *without object* ('background response'; Fig. 2 blue traces) are compared with responses during the condition *with object* ('object response'; Fig. 2 red traces). However, differences in these object-induced response increments between the various adaptation conditions are not immediately obvious. Therefore, the responses had to be further analyzed in more details.

To quantify the object-induced response increment, two groups of time windows were chosen to analyze the object and background responses (pink and blue areas in Fig. 2). The first group of windows starts about 330 ms after the reference movie begins; the second group of time windows starts 670 ms later. The reasons to choose these two groups of windows are: (1) the object should affect the response of the neuron, i.e. an object-induced response increment relative to the background condition should be clearly visible; (2) we aimed to assess whether the adaptation effect lasts over several hundreds of milliseconds by comparing the consequences of motion adaptation between the two groups of windows. The responses to the object and background were averaged for the two groups of windows respectively (Fig. 3a,b). In the first group of windows both the averaged object and background responses decrease after motion adaptation with NA, OR, TD and PD stimuli, but not with the ND stimulus (Fig. 3a). However, the decrement is stronger in the background responses than in the object responses. This discrepancy results in an enhancement of the object-induced response increment by motion adaptation. This effect of adaptation has already been

shown previously, although after a more sustained, repetitive sequence of semi-natural motion (Liang et al. 2008). Remarkably, differences in the strength of this adaptation effect between the various adaptation conditions are weak: the object-induced response increment does not only increase after an adaptation stimulus with naturalistic dynamics (NA), but also after all other tested adaptation stimuli (OR, TD, PD and ND; Fig. 3c, pair wise t-test, $P<0.05$). Interestingly, both the constant preferred and null direction rotations enhance the object-induced increments significantly, but the increment after PD motion is significantly stronger than that after ND motion. This discrepancy indicates two components of motion adaptation: one is independent of the direction of motion, the other is direction dependent. The object-induced response increments after motion adaptation with NA, OR and TD are in a very similar range, and their mean values lie between the increments after adaptation with PD (3.16±0.80mV) and ND (2.57±0.50mV). From the results presented so far we can conclude that (1) naturalistic dynamics of optic flow is not essential for the enhancement of object-induced responses by motion adaptation and that (2) stimulus dynamics, and thus the dynamics of voltage fluctuations, does not appear to influence motion adaptation in any conspicuous way with respect to the object-induced response increment.

In the second group of windows, we find a similar overall dependence on the different adaptation stimuli of the mean object and background responses (Fig. 3b) as well as the corresponding response increments (Fig. 3d). However, the adaptation dependent effects are considerably smaller than in the first group of time windows. The object-induced response increments after all adaptation stimuli are only slightly larger than that before motion adaptation (Fig. 3d; statistically not significant). Hence, the consequences of the different adaptation stimuli are much weaker or almost disappear until the second group of time windows. Moreover, differences between the various motion adaptation conditions might be attenuated because the adaptation state

of the neuron is already affected by the reference stimulus, which is the same for all conditions.

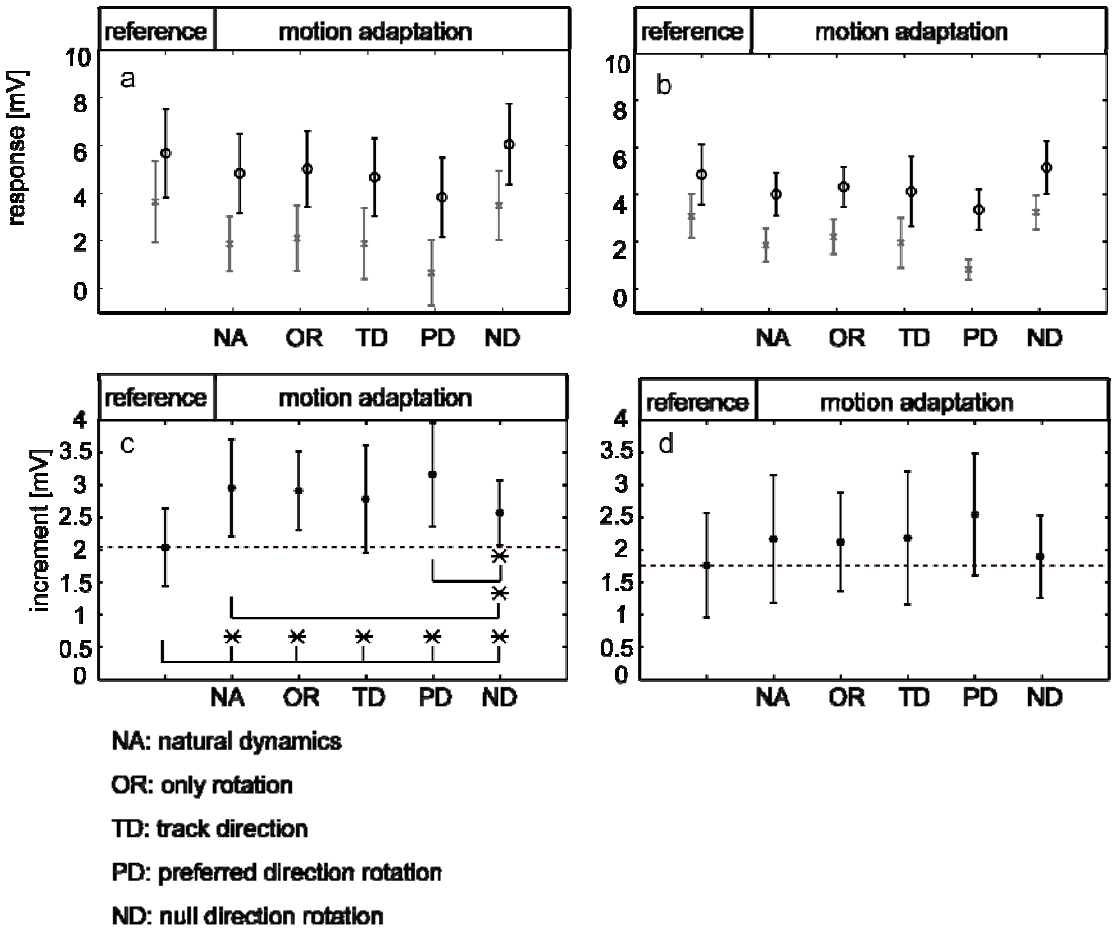


Figure 3: The averaged object and background responses with and without motion adaptation. a) The object (black circles) and background responses (grey asterisks) are averaged (nine cells; error bar: SD) from the first group of windows (pink area in Fig. 2). c) The object-induced response increments for the various stimulus conditions. Dotted line highlights the level reached in the reference condition. The object-induced response increments are significantly enhanced after motion adaptation with NA, OR, TD, PD and ND stimuli. The increment after PD motion is significant stronger than that after ND. The one after NA is also larger than that after ND. (indicates significant difference by the pair wise t-test, $p < 0.05$) b) and d) show analogous results analyzed from the second group of windows (blue area in Fig. 2). Increments in object-induced responses are not significantly different between the various adaptation conditions.*

While the dynamical properties of the adaptation stimuli do not seem to play a pronounced role in enhancing object-induced responses, what else might be the parameters that determine the strength of this effect of motion adaptation? The depolarization level of the neuron evoked by constant velocity stimulation has been suggested to influence the strength of motion adaptation (Kurtz et al. 2000, 2009a; Harris et al. 2000). We therefore investigated whether this finding generalizes across stimuli of various velocity profiles, such as those employed here for motion adaptation. We plotted the averaged object and background responses as a function of the time-averaged membrane potential during the adaptation phase (Fig. 4a,b). Despite considerable variability in the responses, there is a clear relationship between the responses and the averaged membrane potentials during the adaptation phase. Both the object and the background responses relative to the resting potential decrease when the neuron is more depolarized during adaptation (Fig. 4a,b). The averaged membrane potential during the adaptation phase with NA, OR and TD stimuli have almost the same level, and the corresponding object and background responses are, accordingly, very similar. Moreover, the object-induced response increment increases with an increasing positive average depolarization during the adaptation phase (Fig. 4c,d). Accordingly, the object-induced response increments are similar after NA, OR and TD motion stimulation. However, when the membrane potential gets negative relative to the resting potential, i.e. the neuron is hyperpolarized, the averaged object response increases very slightly and the background response remains almost at the same level (Fig. 4a left). As a consequence, the object-induced response increment gets larger even if the membrane potential is hyperpolarized. The latter finding cannot be explained on the basis of a direction selective mechanism of motion adaptation.

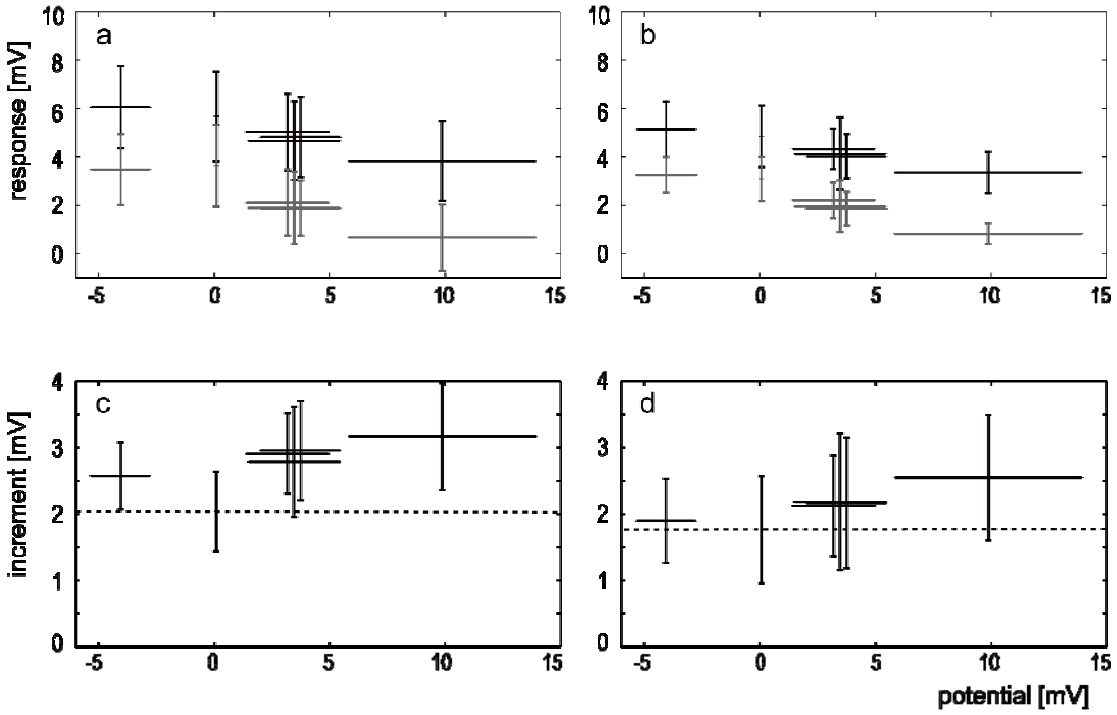


Figure 4: Average object and background responses plotted against average stimulus-induced membrane potential changes during the adaptation phase (nine cells; error bar: SD). a) The object (black) and background responses (grey), which are averaged from the first group of time windows (pink areas in Fig. 2), decrease when the averaged membrane potential increases. c) The object-induced response increment increases when the averaged membrane potential relative to the resting potential (set to 0 mV) gets positive and negative (the neurons are depolarized or hyperpolarized, respectively). b) and d) show analogous results from the second group of windows (blue areas in Fig. 2).

4.4 Discussion

We used naturalistic optic flow to adapt motion-sensitive HS neurons in the visual system of blowflies, and tested to what extent responses to an object suddenly entering the receptive field of an HS neuron are enhanced relative to the background responses. By modifying the dynamic characteristics of optic flow in various ways we were able to show that natural dynamics is not indispensable to generate this effect of

motion adaptation. The typical enhancement of object-induced responses with adaptation was preserved not only with adapting stimuli that led to a modified fine-structure of the neuronal response fluctuations, but even after pure constant-velocity rotation in the neurons' preferred or null direction, which led to a maintained de- or hyperpolarisation of the neuron, respectively. Moreover, null direction rotation differed in two ways from all other stimuli: it produced a net hyperpolarisation during the adaptation phase, and it left background responses after adaptation unattenuated. Nevertheless, similar to all other conditions object responses were enhanced after adaptation with null direction rotation.

Potential functional benefits from enhanced object responses after motion adaptation

Improved detectability of novel stimuli has been suggested as a major functional benefit of adaptation (Kohn 2007). Novelty detection is a crucial task for animals during natural behavior, especially for fast flying animals. It can be viewed as redundancy reduction by the sensory system, which improves the efficiency of encoding sudden changes in stimulus strength in space or time at the expense of a consistent encoding of absolute intensity levels (Attneave 1954; Barlow 1961). Novelty detection can be accomplished by suppressing responses to frequent or persistent stimuli, thus leading to an enhancement of the relative strength of responses to novel stimuli. Improved novelty detection by adaptation has been proposed to be effective in the nervous system of some vertebrate species (Dragoi et al. 2002; Ulanovsky et al. 2003; Benda et al. 2005; Reches and Gutfreund 2008; Gill et al. 2008) as well as in insects (Maddess and Laughlin 1985; Kurtz et al. 2009b; Ronacher and Hennig 2004). For instance, in the auditory and visual systems, the sensitivity to stimulus discontinuities increases with adaptation (Li et al. 1993; Gill et al. 2008; Maddess and Laughlin 1985; Kurtz et al. 2009b). These discontinuities in the stimulus could be sudden brief changes in one of the stimulus parameters, such as velocity, spatial contrast or orientation of a drifting visual grating or the frequency in

a sound. Consistent with this phenomenon, our previous paper (Liang et al. 2008) has indicated that motion adaptation enhances the response to an object suddenly turning up, whereas the sustained background motion response decreases. Compared with our previous study, which used a sustained optic flow sequence assembled from several shorter repetitive loop-like trajectories (Liang et al. 2008), our present experiments reproduced this phenomenon for a contiguous trajectory, and thus for optic flow stimuli that are closer to the situation during real flight. Although HS neurons have conventionally been regarded control elements for optomotor turning responses that compensate for deviations from an intended flight course, the enhancement of object responses with adaptation suggests that these neurons may also be functional in the context of object detection and collision avoidance. Consistent with this notion, it has been shown that HS neurons encode behaviorally relevant information about the spatial structure of the visual surround (Boeddeker et al. 2005; Kern et al. 2005; Karmeier et al. 2006). Nonetheless, the responses of HS neurons are depending on various stimulus parameters apart from retinal velocity (e.g. Hausen 1982a,b). Accordingly, from the activity of just a single HS-cell it is not possible, without additional information, to infer an object in its receptive field.

Role of statistical stimulus properties in motion adaptation

Using random velocity fluctuations and information theoretic approaches, it has been demonstrated how adaptive processes affect the input/output relation in fly visual motion detection (Brenner et al. 2000; Fairhall et al. 2001). Adaptation was shown to work on different timescales to match the neuronal response range to the dynamic range of the external environment and efficiently transfer information about the input signal. More precisely, the system stretches or compresses its tuning curve to match the range of the incoming modulations in motion velocity. However, our results show that the dynamics of optic flow experienced on a semi-natural flight trajectory do not conspicuously contribute to motion adaptation, namely the enhancement of

object-induced neural activity. Our results are not directly comparable with those of the previous studies (Brenner et al. 2000; Fairhall et al. 2001), in which white noise velocity fluctuations of a grating were used, and coding of a single stimulus parameter, velocity, was assessed. Moreover, our results do not exclude that the dynamics of a stimulus is relevant for adaptation, because under all conditions tested in the present study strong irregular modulations are expected to be present in the local inputs of the neurons recorded in our study. Only with spatial integration over many of these local inputs, a prominent feature of optic flow sensitive neurons, these modulations can be integrated into a fairly smooth response, as is the case during constant-velocity rotation (Egelhaaf et al. 1989; Single and Borst 1998).

Putative mechanisms underlying adaptation to naturalistic optic flow

In the present study two effects of motion adaptation were observed: (1) a decrease in the overall response level, which we termed background response; (2) an enhancement of response increments elicited by the appearance of an object in the receptive field. Whereas previous studies give hints on the location and cellular mechanism of the first effect, it is more difficult to find putative cellular origins of the second, more remarkable effect of adaptation to naturalistic optic flow. In the fly visual system as well as in the visual cortex of cats a component of adaptation exists, which is selectively elicited by motion in the preferred direction (Carandini & Ferster 1997; Harris et al. 2000). In fly HS neurons, this direction selective adaptation goes along with an increase in the conductance and becomes visible as a prominent after-hyperpolarisation following stimulus offset (Kurtz et al. 2000; Harris et al. 2000; Kurtz 2007). The attenuation of the background response found in the present study may be attributed, at least to some extent, to this form of adaptation. This assumption is plausible because, on the one hand, an after-hyperpolarisation can be experimentally evoked in HS neurons by membrane depolarization (Kurtz et al. 2009a) and, on the other hand, we found a correlation between the attenuation of the

background response and the average level of depolarization during the preceding adaptation stimulus (Fig. 4).

Although the enhancement of object-induced response increments is likely to be affected by direction-selective adaptation, it cannot result alone from this form of adaptation. This is because object responses are also enhanced by previous null direction rotation, a stimulus condition which generates net hyperpolarisation and, consequently, does not lead to an attenuation of background responses. This finding implies the components of adaptation that are independent from the direction of motion, contributing to the enhancement of object-induced response increments. Harris et al. (2000) described a prominent decrease in contrast gain of HS neurons, elicited by motion adaptation in any direction. An attenuation of contrast gain, which has also been reported for motion adaptation in cat visual cortex (Hietanen et al. 2007), could favour responses to an object if this is silhouetted from its background by contrasts that are in general higher than those of the textures in the background. Nevertheless, previous studies argue against the idea that adaptation of contrast gain alone can explain the enhancement of object-induced responses. When stimulating a fly optic flow sensitive neuron with a continually drifting grating, interrupted from time to time by brief changes in stimulus parameters, the responses to these discontinuities were enhanced in the course of adaptation. This simple adaptation protocol was effective to enhance the sensitivity for stimulus discontinuities consisting of changes in the velocity (Maddess and Laughlin 1985) as well as changes in other stimulus parameters, e.g. grating orientation, wavelength, and also contrast (Kurtz et al. 2009b). Thus the motion vision system might be equipped with similar adapting properties as the auditory system. Here adaptation is thought to be based on the specific attenuation of those elements within an ensemble of inputs which are strongly activated by the adapting stimulus. Inputs that are only weakly activated by the adapting stimulus thus remain responsive to the sudden appearance of a novel stimulus (Ulanovsky et al. 2003). Assuming that such a type of stimulus-specific

adaptation is also present in motion vision would imply that the enhancement of object-induced response increments found in the present study originates not from cellular processes in the optic flow sensitive neurons themselves but from adaptation at their input synapses or even more in the periphery. As already outlined above, this view is also consistent with the lack of effects of different dynamics of optic flow on this form of adaptation.

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5. Object detection and distance encoding in three dimensional environments by visual neurons of the blowfly

5.1 Introduction

Object detection is an important task for animals to guide their behavior in real environments, in particular when required to avoid obstacles or to prepare landing. In our rich and complex surrounding world, objects are embedded in visual scenes. Intuitively, an object can be discriminated from its background based on different texture properties such as color, shape, contrast and luminance. Even when all these features are shared by background and object, the object can still be detected by a moving observer just from the relative motion between a nearby object and its distant background. Thus, detection is possible also without elaborated stereoscopic vision which is characteristic of many insects. The ability to detect spatial discontinuities induced by nearby objects has been studied in a broad range of animals (Kral 2003), from humans (Regan and Beverly 1984; Lappe et al. 1999; Warren et al. 2001), monkeys (Miles and Kawano 1987), pigeons (Frost and Nakayama 1983; Wylie and Frost 1999), bees (Srinivasan et al. 1990) to flies (Virsik and Reichard 1976; Reichard et al. 1983; Egelhaaf 1985a; Kimmerle et al. 1996). Since fast flying animals strongly rely on motion information to segregate objects from background structures, the optic flow, i.e. the continuous displacements of the animals' retinal images during self-motion, seems to be the most relevant cue to guide their behavior. However, the ability to detect objects based on relative motion is limited to translational self-motion, since during pure rotation the retinal velocities are independent from the distance between objects and observers. Interestingly, several insect groups pursue active vision strategies to separate rotational and translational components of retinal image motion (Kral 2009). For instance, blowflies shift their gaze by saccadic rotations of body and head, keeping their gaze virtually constant during translational locomotion between saccades ('*intersaccadic intervals*', Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999).

It is largely unknown how natural visual information is being processed in the neuronal systems of animals. Relatively simple stimuli such as constant velocity stimuli or white-noise velocity fluctuations have been used in most of the studies on the performance of neurons in the visual pathway. Unfortunately, these approaches are not sufficient to assess how neurons represent complex natural input. Recently it became feasible to record from visual neurons during stimulation with behaviorally generated stimuli, which emulate the dynamic conditions encountered by freely behaving animals in a complex world. The fly is an excellent model to study visual information processing because of 1) the easy accessibility of its visual system; 2) the possible association of neuronal response properties with their significance for behavior (Frye and Dickinson 2001; Borst and Haag 2002; Egelhaaf et al. 2002; Egelhaaf 2006, 2009; Maimon et al. 2010); 3) the possibility to apply in the fruit fly *Drosophila* genetic techniques for targeted manipulation of the nervous system (Armstrong et al. 1995, Borst 2009).

In the fly's third visual neuropil, the lobula plate, exist several large-field, motion sensitive neurons, the so-called tangential cells (TCs) (Hausen 1984). Most of these neurons have extended dendrites on which they spatially integrate the outputs of local motion sensitive elements. TCs thus respond in a direction-selective way to motion in large parts of the visual field. Among the TCs, a neural circuit constituted of three types of neurons is involved in object detection. The figure-detection (FD) cells (Egelhaaf 1985b) possess, similar to other TCs, a large excitatory receptive field. Remarkably, they do not respond strongest when a motion stimulus extends entirely across this receptive field, but when a small moving object is presented anywhere in the excitatory receptive field. This small-field tuning of FD cells has been suggested to be accomplished by local processing of excitatory inputs with additional inhibitory inputs (Hennig et al. 2008). The inhibitory input is supplied by another TC, the ventral centrifugal horizontal (VCH) neuron, which provides information on large-field motion in the ipsi- and contralateral visual field (Warzecha et al. 1993).

Such sophisticated input organization enables FD cells to segregate a nearby object from a more distant background on the basis of relative motion (Kimmerle & Egelhaaf 2000). The third neuron type of the neural circuit, horizontal system (HS) cells, is involved in evaluation of optic flow during locomotion in the horizontal plane. HS cells lack the type of inhibition present in FD cells and are thus maximally excited during global horizontal motion as induced during turns of the fly around its vertical body axis or forward translation (Hausen, 1982 a,b, Krapp et al. 2001). HS cells provide major ipsilateral input to CH cells, to which they are coupled via extended dendro-dendritic electrical synapses (Haag & Borst 2002). Recently it has been shown that HS cells encode information about the spatial layout of the environment during the intersaccadic intervals (Boeddeker et al. 2005; Kern et al. 2005, 2006; Karmeier et al. 2006). Moreover, HS neurons were shown to depolarize during the intersaccadic intervals when an object suddenly moves in their preferred direction into their receptive fields (Liang et al. 2008). Therefore, FD and HS cells both are likely to play an essential role in visually guided orientation behavior (Hausen and Egelhaaf, 1989; Egelhaaf and Borst, 1993; Egelhaaf and Warzecha, 1999).

In the present study we use two approaches. First, for the object detection experiments the responses of one type of FD cells (FD1), VCH cells and two types of HS cells (HSE and HSS) to optic flow as experienced by a semi-free-flying fly in an arena are compared. These three types of neurons constitute the neural circuit assumed to encode distance information and to detect objects. The behaviorally generated optic flow was modified by inserting two objects close to the flight trajectory and by changing the size of the flight arena (Fig. 1) in order to analyze the different neurons' performance in environments with different spatial characteristics. Second, for distance encoding experiments the responses of HSE cells to stimuli which were reconstructed from ten different flight trajectories and the flight arena was virtually modified systematically in different sizes. Thus the following questions

are addressed: 1) how do representatives of different cell types respond to the nearby object and background in naturalistic dynamic conditions; 2) how is distance to environmental structures encoded by the neurons in the fly's visual pathway.

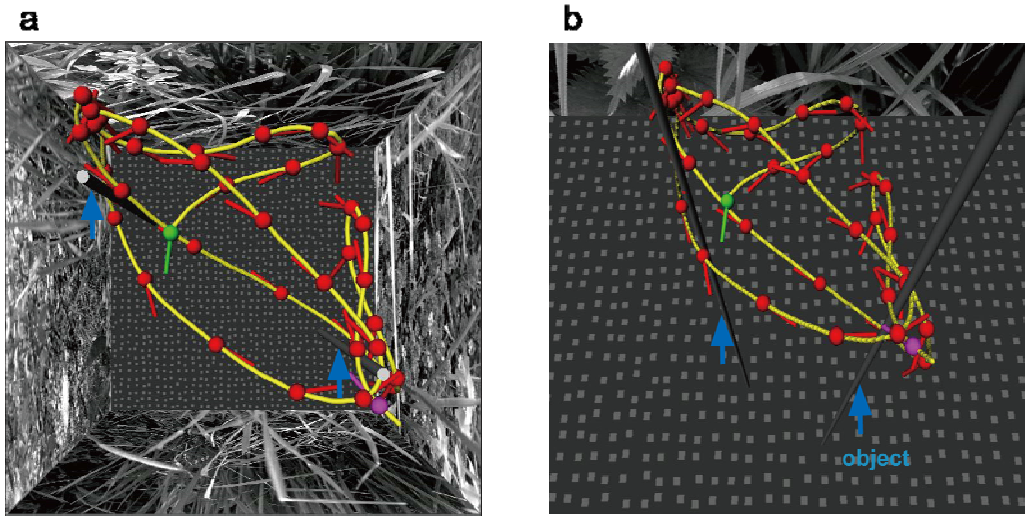


Figure 1: Top view of the flight trajectory of a blowfly in a cubic arena used for the reconstruction of optic flow. The track of the fly is indicated by the yellow line; the red dots and short dashes indicate the position of the fly's head and its orientation, respectively; the green and violet dots indicate the start and end of the trajectory. a) Complete trajectory in the small arena. b) The same trajectory in the large arena. In some of the stimulus sequences two virtual objects (homogeneous black cylinders, marked by blue arrows) are inserted at positions very close to the trajectory.

5.2 Material and methods

Generation of visual stimuli for object detection experiments

A flight trajectory (duration 3.45s) was chosen from a large data set provided by Dr. J.H. van Hateren (University of Groningen, NL). The data were obtained from blowflies flying in a cubic arena (edge length 0.4m; walls covered with photographs of herbage, see Fig. 1). This arena was placed in a Helmholtz coil; the position and orientation of the blowfly's head were monitored by means of magnetic coils, which were mounted on it (van Hateren and Schilstra 1999). The semi-free-flight sequences

recorded in this way do not differ in their saccadic structure from free-flight maneuvers monitored with high-speed cameras under outdoor conditions (Boeddeker et al. 2005). With known gaze direction and visual interior of the cage, the visual stimuli can be reconstructed and presented in a panoramic display instrument, the so-called FliMax (Lindemann et al. 2003). In order to introduce spatial discontinuities, two homogenous black vertical cylinders (diameter: 0.01m; height: 0.4m) were virtually inserted into the flight arena close to the already existing flight trajectory (which was recorded without object). The corresponding modified image sequence was reconstructed, similar as in our previous study (Fig. 1A in Liang et al. 2008). To create spatial discontinuities of a different extent the size of the flight arena was modified virtually, i.e. its edge length was increased to 2.17m (large arena). The wall pattern and the height of the objects were scaled accordingly, but the distance between objects and fly remained unchanged. To remove the background motion influence, we replaced the wall pattern of the arena by homogeneous grey colour while the positions of objects remained unchanged (*O-nB*). Altogether, the conditions described above add up to five different stimuli (see Results for details), which were presented in pseudo-random order. Between two stimuli, all light-emitting diodes of FliMax were set to the mean luminance of the just presented stimulus for 20s to allow the fly's visual system to return to an identical adaptation level in all stimulus runs.

Generation of visual stimuli for distance encoding experiments

Ten flight trajectories, each lasting 3.45s, were chosen from a large data set obtained from blowflies flying in a cubic arena (the same as described in the previous section). To analyze how the membrane potentials of HSE encode the distance between the fly and the walls of the flight arena, we changed the size of the virtual environment systematically. The edge length of the cubic arena was set to 0.41, 0.55, 1.05, 2.35, 7.35 m respectively, while each of flight trajectories remained unaltered. An approximation of the responses of the contralateral HSE during the same flight was

obtained by presenting a mirrored version of the reconstruction. In this way we obtained responses of HSE in both brain hemispheres by recording from one of them only.

Electrophysiological analysis

One- to three-day-old female blowflies (*Calliphora vicina*) were dissected as described by Dürr and Egelhaaf (1999). Temperature during experiments, measured close to the animal, amounted to 24-34°C. Voltage responses were recorded intracellularly with glass electrodes (GC100TF-10, Clark Electromedical Instruments, Pangbourne Reading, UK) from the axon of an HSE, HSS (Hausen 1982a) or a VCH cell (Eckert and Dvorak, 1983) in the right brain hemisphere. The resistance of the intracellular electrodes, filled with 1 M KCl, was 20-50 MΩ. Ringer solution (Kurtz et al. 2000) was used to prevent desiccation of the brain. Extracellular recordings were done with glass electrodes (G100TF-4, Warner Instruments, Connecticut, USA) pulled on a P97 Puller (Sutter Instruments, California, USA) and had resistances of 2 to 5 MΩ, with 1M KCl filled. Recording site was the input arborisation of the right FD1 cell in the right optic lobe. The amplified, band-pass filtered (LP=10 kHz; HP = 200 Hz) raw signals were sampled at 20 kHz (DT 3001, Data Translation, Marlboro, MA, USA) and stored on hard disk for offline analysis.

Analysis of data obtained in the object detection and distance encoding experiments

Three HSE, two HSS, and five FD1 cells were recorded for the object detection experiments and three to five HSE cells for the distance encoding experiments. The latter data of HSE have already been used in Kern et al. 2005. All the data were analyzed with Matlab 7.0.1 (The Math-works, Natick, MA). The spike activities of FD1 cells were transformed into peristimulus time histograms (PSTHs; temporal resolution 4 kHz). We subtracted the baseline spike activity (averaged over 500 ms from the beginning of the responses without object to the stimulus *O-nB*) from the

overall activity of analyzed FD1-cells and the resting potential (averaged over 500 ms before stimulation) from the intracellularly recorded membrane potential of all analyzed HS and VCH cells. To facilitate comparison of the responses of the different types of cells, we normalized for each individual cell all responses to the time-averaged responses in the small arena without object. Before normalization we rectified the hyperpolarisation (negative signal) part of the responses of HS and VCH cells, since the spike threshold of the FD1 cell produces a similar rectification. Hence, only the response components resulting from motion in the particular cell's preferred direction were used for the analysis.

To analyze the impact of an object on the cellular responses we quantified the responses in those intersaccadic intervals where an object passes the particular cell's receptive field ('object response'). These responses were compared with the responses in the same intersaccadic intervals in the flight situation without objects ('background response'). The intersaccadic intervals were selected by masking saccades (see methods in Kern et al. 2005). Briefly, saccades were detected by peaks in angular head velocity (>= 500 deg/s) and saccades that were close together were merged. To define the time windows when an object is present in the receptive field and moving in preferred direction, we used the FD1 responses to the reference stimulus where the dark objects are shown against a non-textured, homogeneously bright "grey" background. The windows for time intervals where object responses are evaluated had to satisfy two criteria. 1) The normalized time-dependent responses are larger than 0.6 (other thresholds ranging from 0.5 to 1 lead to similar results). 2) Only windows lying within intersaccadic intervals are considered, since we focused on the neural representation of spatial information, which can only be extracted from the translational optic flow during intersaccadic intervals. Within the windows determined in this way, the overall "object responses" and "background responses" of all cells (HSE, VCH and FD1) to all different stimuli were determined by

time-averaging across the windows (Fig. 3 marked in light green).

ROC analysis

To further specify and compare the detectability of an object on the basis of HSE and FD1 cell responses, we used the so-called Receiver Operating Characteristic (*ROC*) (Greiner et al. 2000). First, we define a threshold (0, 0.2 or 0.6) for the responses of FD1 cells in the stimulus condition when the object is shown against a non-textured background (Fig. 3 B1). Only if the response is above this threshold an object is assumed to be present in the receptive field and moving in preferred direction. In addition, we only analyzed the responses during intersaccadic intervals, i.e. during translational motion. Those points in time within intersaccadic intervals when response values exceed the respective threshold served as references when constructing the ROC and determining the percentage of correct and false detections of an object under the more demanding stimulus conditions, i.e. when the object is seen against a textured background. Under these conditions it is difficult to assess whether at a given instant of time the response is elicited by an object or by the background, because the responses of FD1 or HSE are affected by both object and background motion and strongly fluctuate (e.g. Fig. 3 A2, B2),. We define the object being detected correctly (‘correct detection’) if the response exceeds a given threshold and the object was indeed moving in preferred direction through the receptive field, as indicated by the reference. Correspondingly, a ‘false detection’ is obtained if the same threshold is exceeded without the object being in the receptive field of the cell and moving in preferred direction. By shifting the threshold from the largest attained response level to smaller values the percentage of correct detections increases, but also those of false detections. Useful object detection on the basis of the neuronal response profile requires the percentage of correct detections to initially increase more than the percentage of false detections when lowering the threshold. Otherwise correct and false detections increase, on average, in the same way. The

corresponding percentages of correct versus false detections for the different thresholds are plotted against each other in the ROC curve. The diagonal in the ROC curve indicates that the percentage of correct and false detections increases in the same way with decreasing threshold. The diagonal thus represents chance level and would imply that the object cannot be detected on the basis of the time-dependent neuronal response. The area under the ROC curve can be used to quantify object detectability. The closer the area is to 0.5, the closer it is to the diagonal and the less often the object can be detected. The closer the area is to 1.0, the better the object can be detected.

Nearness analysis

To analyze the relationship between the HSE, HSS and FD1 responses and the corresponding distance of the fly to the arena walls and the object, we averaged the responses of HSE and the corresponding nearnesses. This was done during selected intersaccadic intervals of all stimuli, where the optic flow was dominated by horizontal translational motion. The intersaccadic intervals were selected by three criteria: 1) movement in the horizontal plane is three times larger than along the vertical axis; 2) the duration of the intersaccadic interval is longer than 10ms; 3) the average pitch angle during the intersaccadic interval is smaller than 25°.

The nearness, i.e. the inverse of the distance between the fly and a point somewhere in the environment, is analyzed by the following steps. Within the receptive field of the HSE cell (Lindemann et al. 2005; Krapp et al. 2001; Hausen 1982b), we chose sample points equally spaced at 1° in azimuth from -45° to 101° and only one transect in elevation at -15°. Since the objects did not change in their vertical extent the coarse spacing along the vertical was found to be sufficient and saved computing time. The frontal equatorial direction is defined as 0°; the angular positions to right or left in azimuth are positive and negative, respectively. Elevations above the equator are

positive and below the equator negative.

From the known flight trajectory, the head orientation of the fly and the known geometry of the flight arena, we could calculate the distance from the head to the background for each selected point within the receptive field of the cell. The resulting distances were first converted to nearness (nearness = 1/distance), and then weighted by the sensitivity distribution of the cell.

$\Sigma\text{Nearness}(\psi, \theta) = \Sigma(\text{Nearness}(\psi, \theta) \times \text{Sensitivity}(\psi, \theta))$

with ψ and θ representing the position in azimuth and elevation, respectively.

The sensitivity distribution of HSE was the same as used in the model study by Lindemann et al. (2005) (Fig. 2 right). For HSS we only shifted the most sensitive position downwards to an elevation of -45° (Krapp et al. 2001; Hausen 1982b). For the FD1 cell we simulated the sensitivity distribution from the data of Egelhaaf (1985b) in azimuth and Warzecha et al. (1993) in elevation (Fig. 2 left). The most sensitive position is -30° in elevation. For all cells the azimuth range we took was the same as for HSE (-45° to 101°)

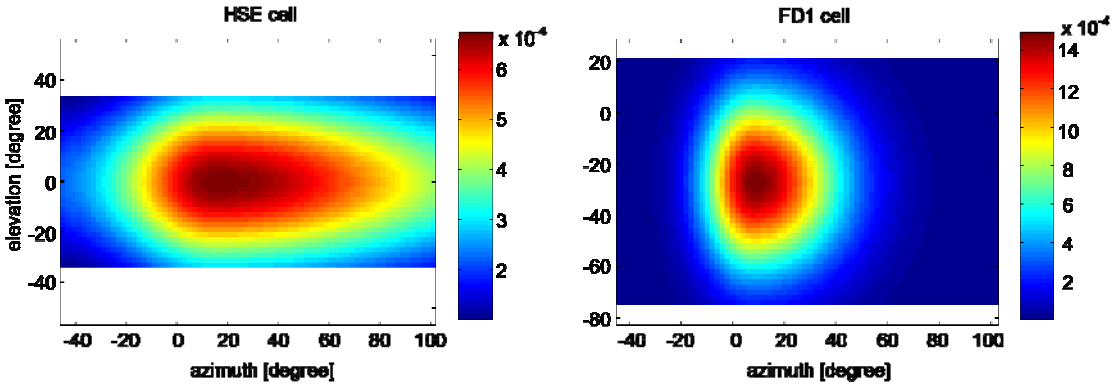


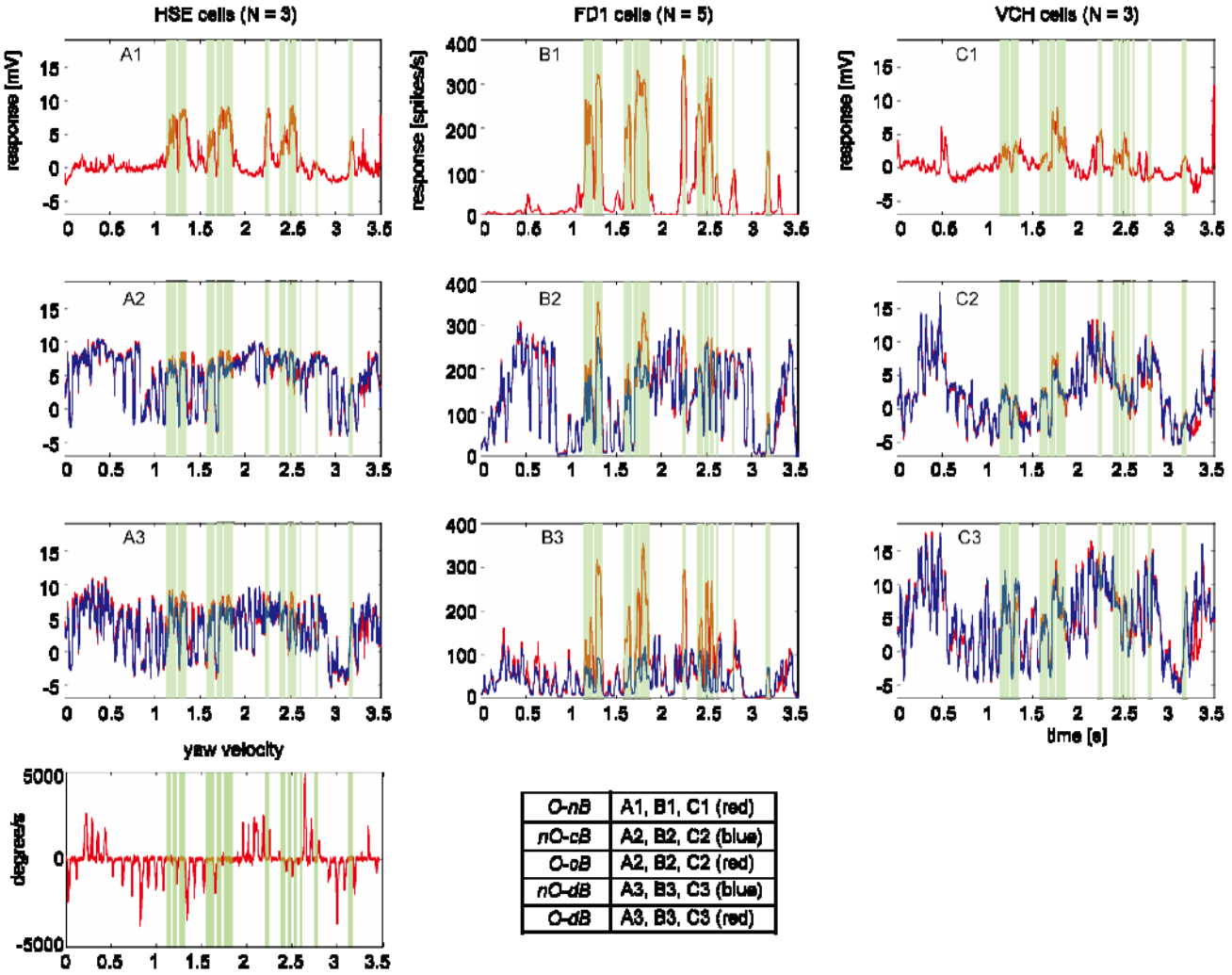
Figure 2: The modeled local sensitivity distributions of the HSE and FD1 cells of the right brain hemisphere, based on electrophysiological data for HSE from Hausen (1982b) and for FD1 from Egelhaaf (1985b) and Warzecha et al. (1993). The contours are plotted in cylindrical projection. Red areas indicate higher sensitivities (colorbar on the right side). The frontal equatorial viewing direction is at 0° azimuth and elevation. The most sensitive position in elevation for HSE is at -15°, for FD1 at -30°; in azimuth for HSE is at 15°, for FD1 at 10°.

5.3 Results

We analyzed the responses of individually identifiable cells in a neural circuit of the fly's visual system, which is assumed to play a role in providing spatial information and detecting objects. We asked how distinct cells encode spatial information during flight and, in particular, whether they may be able to detect a nearby object? To answer this question, five stimulus sequences were designed. The visual stimuli are based on the flight trajectory of a semi-free-flying fly and the time-dependent optic flow experienced by the fly on this trajectory. The optic flow sequence was replayed in its original version and in modified versions, generated by changing the size and texture of the virtual flight arena and by inserting two objects close to the flight trajectory. Five visual stimulus sequences were used in the experiments: 1) (*nO-cB*: no object, close background) the motion sequence experienced by the fly in the original small arena with photographs of herbage on the wall; 2) (*O-cB*: objects, close background) the motion sequence that would have been experienced on the same trajectory in the small arena with two objects inserted close to the flight path (Fig. 1a); 3) (*nO-dB*: no object, distant background) and 4) (*O-dB*: objects, distant background) the motion sequences from the same trajectory as in 1) and 2) but in a large flight arena with the locations of the objects in 4) remaining unchanged relative to the flight trajectory; the texture on the arena walls was scaled according to the increased arena size (Fig. 1b); 5) (*O-nB*: objects, no textured background) the reference stimulus that was reconstructed from the trajectory with the objects inserted at the same location as in 2) and 4) but with non-textured arena walls. In this condition displacements of the background did not lead to displacements of any contours and hence, did not induce any neural responses. The responses to the five stimulus sequences of three types of TCs in the lobula plate, HSE/HSS, VCH and FD1 cells, which are components of the neural circuit for object detection, were recorded. Due to technical limitations we

could not record these three cells simultaneously. HSE/HSS and VCH cells were recorded intracellularly. FD1 cells were recorded extracellularly, because FD cells have a smaller axon diameter (less than 5 μm, Egelhaaf 1985b) than HS and VCH cells, which makes it hard to record the cells with intracellular electrodes for a sufficiently long time. FD cells generate full-blown action potentials (Egelhaaf 1985b), whereas HS and VCH cells respond with pronounced graded axonal membrane potential shifts to motion. In the case of HS cells the graded potential shifts are superimposed by action potentials with variable amplitude (Hengstenberg 1977). The responses of HSE/HSS and FD1 cells to stimuli *nO-cB* and *nO-dB* were later used for distance encoding analysis as well (see section "Encoding of distances" below). Analysis of distance encoding was extended in the case of the HSE cell to recording with stimuli reconstructed from various trajectories flown in arenas of five different sizes.

Figure 3: The averaged time-dependent responses of HSE, FD1 and VCH cells to five different optic flow stimuli. A1, B1, C1 present the responses to the optic flow only induced by the objects, since the background is homogenously dark (O-nB). A2, B2, C2 show the responses to the motion sequence experienced by the fly in the small arena with (O-cB, red curves) and without objects (nO-cB, blue curves). A3, B3, C3 demonstrate the responses to O-dB and nO-dB, similar as A2, B2, C2, but in the large arena. In the bottom a plot of the yaw velocity during the flight (shown in Fig. 1) is shown. The light green columns in all diagrams mark the time windows within intersaccadic intervals when objects appeared in the receptive field of FD1 cells. The object induced response increments are visible in the responses of HSE and FD1 (compare the red and blue curves in A2, B2, A3, B3) and most pronounced for FD1 cells in the large arena.



O-nB	A1, B1, C1 (red)
nO-oB	A2, B2, C2 (blue)
O-oB	A2, B2, C2 (red)
nO-dB	A3, B3, C3 (blue)
O-dB	A3, B3, C3 (red)

Object detection

The display of the responses of HSE, FD1 and VCH cells (Fig. 3) shows that HSE and FD1 cells respond strongly to the reference stimulus (*O-nB*, Fig. 3 A1, B1). Both cell types generate large transient responses when an object moves in preferred direction within their receptive fields, whereas VCH cells respond with smaller fluctuation amplitudes (Fig. 3 C1). In the small arena with textured walls the responses of all cells fluctuate strongly (Fig. 3 A2, B2 and C2 blue curves). In the large arena the fluctuation amplitudes of the responses of FD1 cells are reduced dramatically while those of the HSE do not change much and those of VCH cells even increase in their overall amplitudes (Fig. 3 A3, B3 and C3 blue curves). When objects are inserted into the flight arena, both HSE and FD1 cells show object induced response increments, whereas VCH cells do not show obvious increments (Fig. 3 A2, B2 and C2 red curves). The increments in the FD1 responses are more pronounced than those in the HSE responses. Moreover, the increments are more obvious in the large arena, especially those of FD1 cells (Fig. 3 A3, B3 red curves).

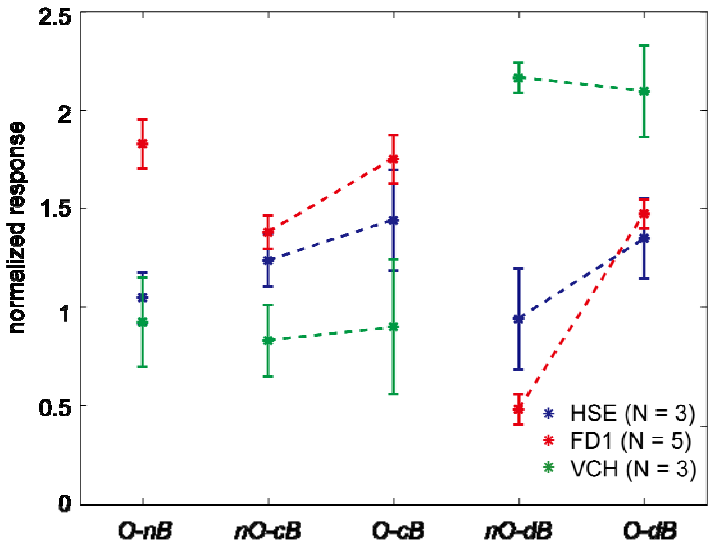


Figure 4: The normalized object and background responses of HSE (blue), FD1 (red) and VCH (green) cells under five different stimulus conditions are averaged from the

time windows marked in Fig. 3. Vertical lines are standard deviations across cells. Dashed lines connect the responses in the same flight arena with and without object.

To quantify the object and background responses of all three TCs (HSE, VCH and FD1), we determined the object and the corresponding background responses within those intersaccadic windows (Fig. 3 windows marked in green) where an object appeared within the receptive field of the particular cell (Fig. 4, details how time windows were defined given in Methods). The responses of FD1 cells, if there are only objects and no background motion, are larger than the HSE responses. In the small arena, both HSE and FD1 cells respond strongly to the background and object, and the object induced response increment (Fig. 4, compare the responses connected by dashed lines) of FD1 is slightly larger than that of HSE cells. In the large arena, the background responses of HSE decrease slightly relative to those obtained in the small arena, while the responses of FD1 cells decrease dramatically. On the other hand, the object induced response increment of FD1 cells in the large arena is much larger than that of HSE. So far, we can conclude that FD1 and HSE cells both respond strongly if the background or the object is close. The background responses of FD1 decrease much more than those of HSE when the distance to the background is increased. The object induced response increments of FD1 are generally larger than those of HSE, particularly in the large arena.

The intersaccadic responses of VCH in the large arena are about three times stronger than those obtained in the small arena (Fig. 4 green points). Moreover, the objects do not lead to a response increment with respect to the corresponding background response. Accordingly, the response amplitude is relatively small under the only object condition (*O-nB*). At first sight, these findings might be surprising, since VCH cell gets its main ipsilateral input from HSE/HSS cells (Haag & Borst 2002), which under the stimulus conditions of the present study respond in a markedly different

way. This difference is likely to be a consequence of the differences of VCH and HS with respect to the type and strength of the synaptic input originating from the contralateral visual field. VCH receives relatively strong excitatory contralateral synaptic input from two neurons, H1 and H2, and an inhibitory signal from the Hu cell (Hausen 1981; Eckert and Dvorak 1983; Haag and Borst 2001). Hu is excited by front-to-back motion in the contralateral visual field of VCH. The strong intersaccadic response of VCH in the large arena might thus be a consequence of a much smaller contralateral inhibitory input in the large arena as compared to the small arena, where the translational optic flow is larger and thus might stimulate the inhibitory Hu cell more than in the large arena.

To quantify how well an object might be detected on the basis of the responses of HSE or FD1 cells, we determined receiver operating characteristics (*ROCs*) for the detectability of the objects in the small and the large flight arena. The correct detection rate was plotted versus the false detection rate of the objects for the entire range of detection thresholds (details see Methods). Before we could construct the ROC curves, we had to define the time intervals within which an object was assumed to be within the receptive field of the cell and moving in preferred direction. This was done on the basis of the responses obtained under the object with non-textured background condition (*O-nB*) by setting an 'object defining' threshold, quite arbitrarily, to three values (0, 0.2 and 0.6; the black dashed lines in the right inset of Fig. 5). Small values indicate that an object is assumed to be present even at very small neural responses, although these may to some extent be the consequence of spontaneous activity fluctuations of the neuron. The detectability of objects was then determined on the basis of ROC curves for the more complex situation when also the background was textured and, thus, contributed to the time-dependent responses of HS and FD1 cells (Fig. 5). The object detectability based on FD1 responses is better in the large arena (Fig. 5 thick blue curve) than in the small arena (Fig. 5 thick red

curve). This is also true for the HSE cell responses (Fig. 5 thin dashed blue and red curves), although object detectability is considerably smaller for HS (thin dashed curves in Fig. 5) than for FD1 responses (thick curves in Fig. 5). The detectability of objects from the responses in the large and small arenas gets better when the object defining threshold gets larger, i.e. when the threshold is raised from 0 to 0.2 or 0.6. This difference can easily be explained by the fact that with a cutoff threshold at zero an object is assumed to have been almost all the time within the receptive field. The flight trajectory and body orientation (Fig. 1) shows that this was actually not the case.

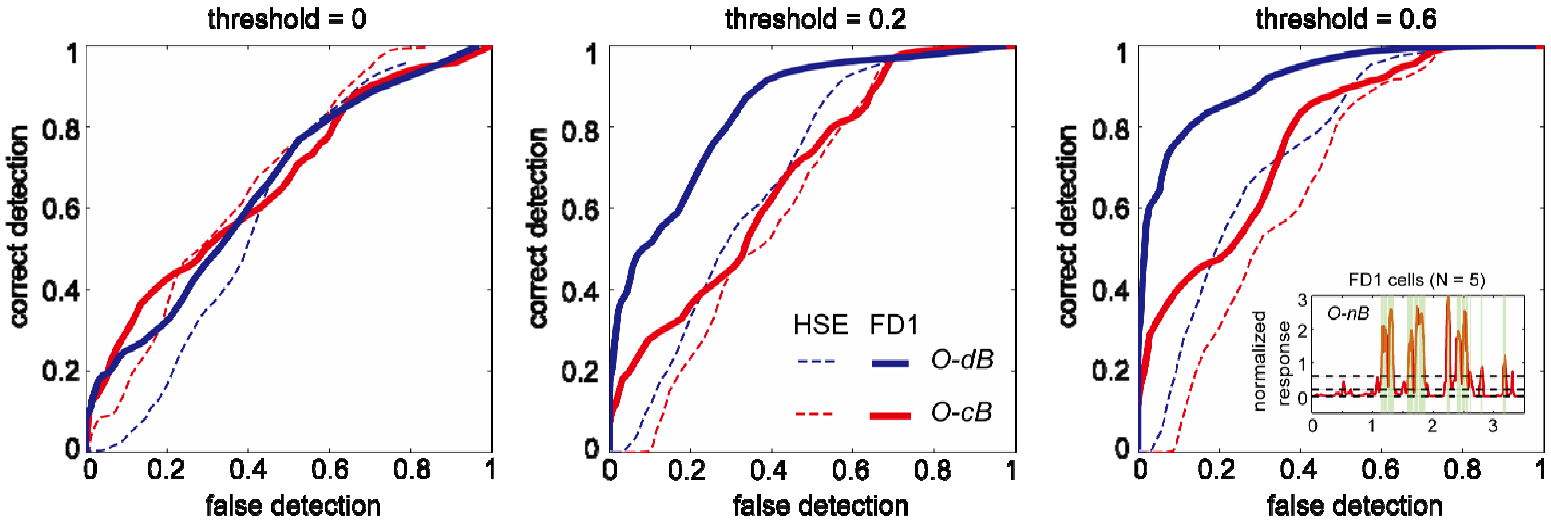


Figure 5: Diagrams from left to right side show ROC curves with three object defining thresholds (0, 0.2 and 0.6). The inset in the right shows normalized responses of FD1 to the "only object" stimulus (O-nB), plotted in red, with the thresholds marked by black horizontal dashed lines. In all the three diagrams red dashed and bold lines present the ROC curves of HSE and FD1 respectively in the small arena with objects inserted. Blue dashed and bold lines represent in the corresponding curves for the large arena. The larger the area below the ROC curve, the better is the detectability of the object.

Encoding of distances

It has been suggested that the responses of HSE cells during intersaccadic intervals reflect the spatial layout of the three dimensional environment (Kern et al. 2005; Karmeier et al. 2006). We address this important issue here in a complementary way for both HS and FD1 cells.

We first analyzed the responses of HSE to the stimuli which were reconstructed from ten different flight trajectories. The stimulus sequences were modified from the original flight arena in which the behavior had been filmed, to five 'virtual flight arenas' of different size (edge length: 0.41, 0.55, 1.05, 2.35, 7.35 m) in which the original flight trajectories were placed. We determined the dependence of the mean intersaccadic responses on the nearness of the cell's receptive field to the arena walls (details see Methods) in two ways. (1) Since the nearness of the eyes to the arena walls continually change during individual flights, the dependence of the intersaccadic responses on the corresponding nearness was determined for the differently sized flight arenas (246 intersaccadic intervals selected from ten flight trajectories in Fig. 6a-c; 64 intersaccadic intervals from three flight trajectories in Fig. 6d-e). (2) The average intersaccadic responses within a given flight arena were determined as a function of the corresponding average nearness for all differently sized arenas (Fig. 6f).

In the smaller flight arenas (Fig. 6a-c) where the nearness and, thus, the intersaccadic retinal velocities changed considerably during individual flights (compare the x-axes of the different diagrams in Fig. 6 a-e) there appears to be a systematic increase in the average response amplitude by almost a factor of 2 with increasing nearness, although the standard deviations of the responses are large. However, the response increments with increasing nearness may even completely vanish when the eye of the fly comes

too close to the arena walls. The large variability of the responses is likely to be a consequence of the fact that HS responses do not only depend on retinal velocity (which for a given flight speed depends on the nearness), but also on the direction of motion as well as the contrast and texture of the stimulus pattern (Hausen, 1982b; Egelhaaf and Borst, 1989). For the larger flight arenas where the nearness and the corresponding intersaccadic retinal velocities vary only slightly during a given flight, thus, the intersaccadic response amplitudes do not increase systematically with increasing nearness (Fig. 6d-e).

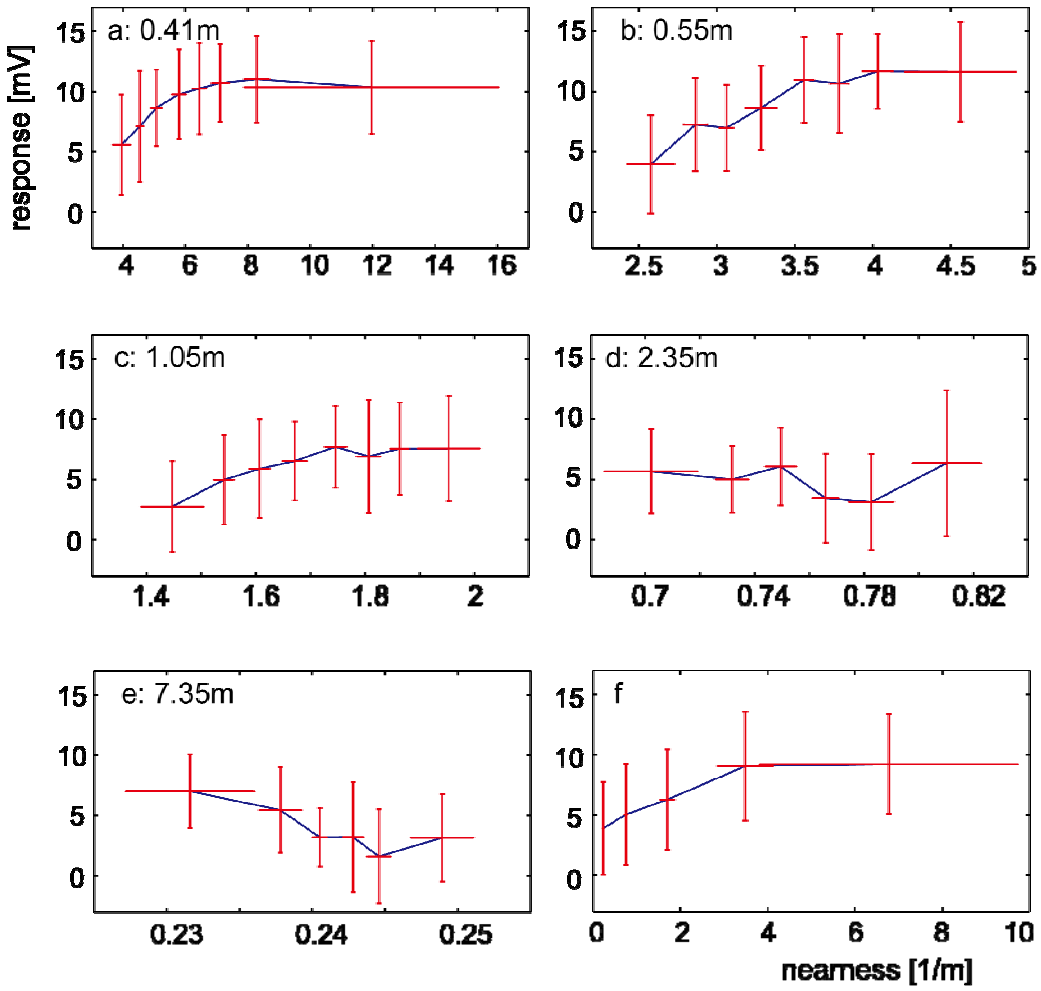


Figure 6: Averaged responses of HSE cells during the selected intersaccadic intervals (for details see Methods) are plotted against the corresponding nearness in virtual cubic arenas of five different sizes (a-c, 246 intersaccadic intervals; d-e, 64 intersaccadic intervals). All the responses are sorted by increasing nearness and then

divided into eight groups (a-c, 30 intersaccadic intervals per group, the remainder combined into the last group) or six groups (d-e, 10 intersaccadic intervals per group, the remainder is handled as before). The red vertical and horizontal lines show the standard deviations of responses and nearness, respectively, across the data values within one group. The HSE responses and nearness are averaged across all selected intersaccadic intervals within each arena (f).

A similar dependence of response amplitudes is obtained when we averaged the responses and nearness across all intersaccadic intervals for each arena (Fig. 6f). The average responses of HSE increase when the nearness increases, but levels off when the overall nearness gets too large. From these results we conclude here that the HSE responses could encode the distance to structures in its environment during translational motion.

To compare the performance in providing spatial information of HS cell with that of FD1 cells, we analyzed that part of the earlier data, which were used for object detection experiments, where no objects were presented. Because it is very hard to obtain sufficiently stable recordings from FD1 cells we employed only a single flight trajectory and two sizes of the flight arena (0.4 m and 2 m). To facilitate comparison of FD1 and HS responses, we used their normalized responses (see Methods). The relationship of intersaccadic responses and nearness for HSE, HSS and FD1 cells (Fig. 7) was analyzed in the same way as was done for the HSE cells under a larger variety of environmental conditions (cf. Fig. 6). The results demonstrate that HSS behaves similar to HSE: the responses seem to decrease slightly when the nearness decreases, but the decrement is not as strong as that shown in Figure 6f, where the average response in a large arena (2.35 m) is almost only 50% of that in a small arena (0.4 m). Such discrepancy is likely due to the much smaller amount of cells recorded from only a single flight condition. Nevertheless, the responses of FD1 cells decrease much stronger than HSE and HSS as the nearness reduces (Fig. 7).

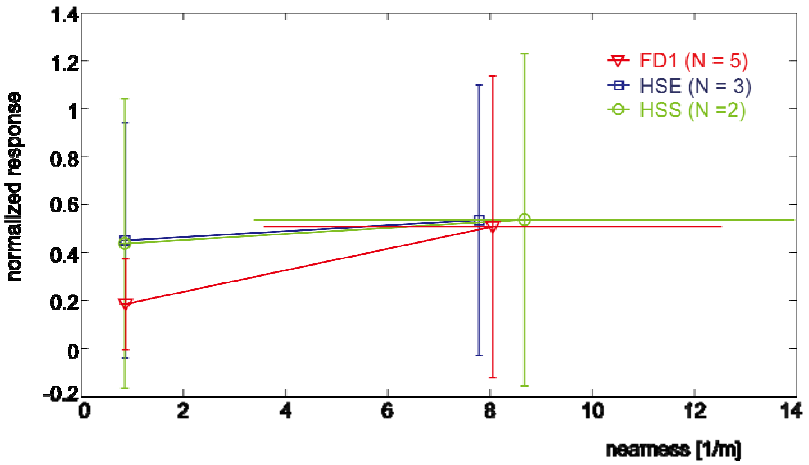


Figure 7: The responses and nearness of HSE, HSS and FD1 cells were averaged cross all the intersaccadic intervals in a small (0.4m) and large arena (2m). The original responses for HSE and FD1 have been shown as blue curves in Fig. 3 A2, B2, A3, and B3. The mean responses of HSE (N =3) and HSS (N =2) decreases very slightly when the nearness gets smaller, whereas FD1 responses decrease much more obvious in the large arena. Since the standard deviations of nearness in the large arena are about 0.09, they are almost invisible in the Figure.

Taken together, we conclude that FD1 and HSE cells both respond strongly to nearby objects and close background. When the distance to the background increases, the object detectability of both cells improves. The general performance of the FD1 cell to detect nearby objects is better than that of HSE, particularly in large environments. Despite many other factors during complex behavior which influence the responses of visual motion-sensitive neurons, distance information of three dimensional environments is present in the neuronal responses of HS cells and, in particular, of the FD1 cell.

5.4 Discussion

Motion-sensitive cells within a small neural circuit, which is presumably involved in object detection, were tested here with optic flow as experienced by a fly during its

flight in a three dimensional environment. By using different modifications of this behaviorally generated optic flow we show that two neurons in the circuit, FD1 and HSE, respond strongly to objects and background patterns when they get close. In a virtual environment with a large distance between objects and background, the object-specific response increments get larger in both cells, particularly in FD1. The object detectability as assessed by receiver-operator characteristics is better when based on the response of FD1 cells compared to HSE cells. With a large stimuli set consisting of virtual flight arenas of systematically different sizes, we found that HSE cells are able to encode the distance of the three dimensional environment during flight.

Object and background segregation

FD1 cell was first described by Egelhaaf (1985b) and has been shown to respond specifically to the motion of small objects, when either presented alone or as relative motion to a background moving at a different speed (Egelhaaf 1985b,c; Kimmerle & Egelhaaf 2000b). Kimmerle and Egelhaaf (2000a) showed that the activity of FD1 cells was almost exclusively determined by object motion and independent of background motion; FD1 cells respond only weakly during background motion alone. Our results reproduced the object specificity of FD1 cells with naturalistic stimuli (Fig. 3B red curves). However, in the present study FD1 cells were found to respond strongly to close background motion as well (Fig. 3 B2 blue curves). Moreover, the object induced response increments of FD1 cells get larger when the distance to the background increases. The reasons for the different effects of background motion in the two studies are likely the result of different stimulus conditions. Kimmerle & Egelhaaf (2000a) replayed optic flow experienced by a fly during tethered flight in a torque compensator. In this experiment, the fly fixated on a vertical stripe, visible by its relative motion in front of a simulated more distant background, consisting of a pattern with regularly spaced bars. In the present study, we reconstructed the optic

flow from real flight in an arena with walls covered with herbage pictures. Our stimuli are much more complex with respect to their temporal and spatial frequency content, the spatial orientation, and, in particular, the dynamic changes of direction of motion as a consequence of the saccadic flight and gaze strategy of blowflies. This gaze strategy largely separates rotational from translational motion components, which were superimposed in the stimuli used in the study by Kimmerle and Egelhaaf (2000a). Moreover, the translational velocity component Kimmerle & Egelhaaf (2000a) used for the background motion was constant at 15°/s along the azimuth within the right and left visual field, whereas in our stimuli the translational velocity varies continually up to round 200°/s, depending on the distance between the eye to the background and the heading direction. Moreover, the optic flow during forward translation in the semi-free-flight condition of the present study expands radially from a focus of expansion in the heading direction. Therefore the much larger changes in the temporal and spatial frequencies of background motion in the present study compared to Kimmerle & Egelhaaf (2000a) may lead to the higher sensitivity of FD1 to background motion, in particular when the background is close.

In contrast to FD1 cells, HS cells, which are thought to be major output cells of the neuronal network underlying optomotor course control (Hausen, 1981; Hausen and Wehrhahn, 1983; Wehrhahn, 1985) have been suggested not sufficient to account for figure-ground discrimination (Egelhaaf, 1985a). The present study supports this view, because HS cells respond strongly to background motion, even with a distant background. Nevertheless, as has been already observed in our previous study (Liang et al. 2008) object-induced response increments are clearly present also in HS and they increase with background distance.

Possible mechanisms underlying object specificity

It has been suggested that the small field tuning (i.e., the selectivity for small objects)

of FD1 cells is based on inhibition during large-field background motion (Egelhaaf 1985c; Egelhaaf and Borst 1993). The inhibitory large-field motion sensitive elements are GABAergic VCH cells, which are supposed to form large, distributed synapses with FD1 (Warzecha et al., 1993; Gauck et al. 1997; Hennig et al. 2008). The VCH cell receives its ipsilateral input from HS cells via dendro-dendritic electrical synapses (Haag and Borst, 2002) and its contralateral excitatory input from both H1 and H2 cells (Horstmann et al. 2000) and inhibitory input from the Hu cell (Gauck et al., 1997; Haag and Borst, 2001). As a consequence of its input via dendro-dendritic electrical synapses, VCH cell dendrites serve as a kind of low-pass filter, which produces a spatial blur of the motion image (Cuntz et al., 2003). This property might well be functionally relevant in the context of object detection, because small motion patterns might be affected more by spatial low-pass filtering than larger motion patterns. In this way, inhibition of FD1 via VCH could be more pronounced for large than for small patterns (see also the modeling approach in Hennig et al. 2008) The VCH cell prevents the FD1 cell from responding strongly to self-motion around the animal's vertical axis. Since the VCH cell does not respond much during forward translation (Egelhaaf et al., 1993), the FD1 cell is inhibited only weakly during this type of locomotion. In consistence with this we found that VCH responds stronger during intersaccadic intervals in the large than in the small virtual environment, where in general the translation component of optic flow may outweigh the rotation component. Accordingly, responses of FD1 to background motion are much weaker in the large than in the small arena.

Encoding distance of three dimensional environments

Recently Kern et al (2005) have shown that HSE cell encodes information about sideward translational optic flow, and thus, implicitly provide information about the spatial relation of the animal to its environment during intersaccadic intervals. We further analyzed the data in a complementary way by relating the distance between

the eye to the background walls in a three dimensional environment and the corresponding HSE responses during intersaccadic intervals (Fig. 6). We found that the responses of HSE generally increase with the nearness of the environment. Although tested only under a smaller number of conditions, a similar correlation appears to hold also for FD1, which is consistent with the lower object specificity of FD1 in a small compared to a large environment.

Taken together, given their specific properties, both FD1 and HSE are well suited to fulfill distinct roles in the guidance the fly's behavior in complex environments, FD1 cells are likely to be the key elements in figure-ground discrimination during flight and HSE cells appear to encode the distance of the flight environment and to provide important signals for optomotor course control as well.

5.5 Reference

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