„Why has not man a microscopic eye?

For this plain reason, man is not a fly.“

Alexander Pope, „An essay on man“ (1732)

# Representation of motion information in the visual system of the blowfly

Dissertation zur Erlangung des Grades
Doktor der Naturwissenschaften (Dr. rer. nat)
der Fakultät für Biologie der Universität Bielefeld

Vorgelegt von Christian Spalthoff

Betreut von Dr. Rafael Kurtz

Bielefeld, Dezember 2011

## Erklärung

Ich versichere, dass ich diese Arbeit selbständig und ohne unzulässige Hilfe verfasst habe, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und Zitate kenntlich gemacht habe.

Christian Spalthoff

# Contents

# Summary

In this study, I examined how spatiotemporal stimuli are represented at various levels of the visual system of the blowfly Calliphora. Flies, which possess remarkable manoeuvrability and a highly efficient visual system in spite of their miniature brains, are popular model organisms for the study of motion vision, also due to their easy accessibility with electrical recording techniques.

Despite the fact that several highly successful computational models for motion detection have been established in flies and extended to applications in other fields like computer vision, several key processing steps in the optic lobes of the fly are still unresolved, especially in regard to their neuronal implementation. This is partly due to the fact that these computations take place at the dendrites of cells or in densely packed neuropils that are difficult to access electrophysiologically.

I tried to shed light on these intermediate neuronal computations by examining how visual features like velocity, direction and orientation are represented at different stages of motion vision. For this, I used calcium imaging to label different classes of neurons and quantify their activity in vivo while the animal was stimulated with visual motion patterns.

The first step of the study looks at dendritic integration of motion signals by the large lobula plate tangential cells. These neurons integrate and process inputs from large parts of the visual field in a fashion that is characteristic for each of these individually identifiable cells, with specific preferences for global motion directions. I was able to show that the dendrites of this cells display a patterns of localized motion preferences that forms a dendritic map of the visual field, thus creating a selective filter for complex motion patterns. Also, I could show that distributed inputs from contralateral neurons act as additional influences on these retinotopic patterns, shaping their layout in a way that could not be predicted through axonal recordings alone.

The second part of the study focuses on the putative input elements of the tangential cells, the cells of the medulla. These neurons form a complex retinotopic mosaic of columns which are connected by lateral interactions, and are believed to be one

of the key neuropils for the extraction of image features in the fly visual system. Through a novel population staining method, I was able to introduce a calcium indicator into these cells and examine their responses to motion as well as various flicker stimuli. The stained neurons, which consisted of columnar elements as well as tangential processes, exhibited strong motion responses, but did not show a distinct preference for a single motion direction like the cells of the lobula plate. Instead, many of them showed symmetry in their motion responses, preferring one pattern orientation over the other but not differentiating between opposite motion directions. Also, when stimulated with either bright („on-“) or dark („off-“) edges, the neurons responded to both, with spatial composition of inputs depending on the dye injection site: Some cells simply integrated spatially on- and off- signals with similar receptive fields, while other populations displayed an integration over spatially separated channels. Both findings, orientation selectivity as well as integration of spatially separated on- and off-channels, exhibit homologies to known processing schemes in vertebrates, but have so far only been sparsely documented in insects.

In the third chapter, I review some of the techniques developed and used during the study in the context of other methods for examining and influencing cellular activity through the application of calcium indicators, caged calcium and phototoxic dyes, which allow monitoring and manipulation of cellular networks on a single cell basis.

# Introduction

## Visual sensory systems in the animal kingdom

To gather information about the surrounding by picking up and analysing light intensities is a highly successful strategy and has been an important driving force in the evolution of higher organisms. Light signals are fast, have long ranges in aquatic as well as terrestrial environments, and provide input not only in form of light intensity, but also in their spectral and polarized components, making them excellent sources of information.

It is no wonder that this information source has been tapped by most of the animal species known to us, both for vision as well as for more basic metabolic functions like the generation of circadian rhythms, reaching back to the early photosensitive structures that have been used as an energy source in organisms as primitive as archaebacteria.

Genetic studies indicate that the opsin family of proteins shares a common ancestor in early metazoans (Schwab, 2011), and the evolvement of light-sensitive organs arose in parallel in many species: Of 33 extant animal phyla, two-thirds possess light-sensitive organs, and while only 6 of these have evolved complex, image-forming eyes, these phyla make up about 96% of all species that currently exist (Land and Fernald, 1992).

While several components needed for the expression of an eye are believed to have commonly originated already in early bilaterian species (Fernald, 2006), image forming eyes have probably evolved later individually, with their structure often showing convergent development due to the functional constraints for creating a sharp, two-dimensional image. Eight major types of animal eyes with spatial resolution exist, which can be further subdivided into chambered eyes like those of vertebrates and cephalopods, and compound eyes like those of arthropods and crustacea, and also into additional subgroups based on whether they use reflection, refraction or shadows to form two-dimensional images.

All these approaches share a common challenge and limitation: To make sense of visual information, an animal needs to analyse and store stimulus patterns in a mea-

ningful way. Not all light-sensitive structures, like bacterial rhodopsins, are necessarily sensory in function, and while simple trophic responses to light might be regarded as the first and simple steps to a direct relationship of light perception and action, true „vision", as we know it, needs not only the formation of an eye structure with spatial resolution, but also the corresponding cellular structures to process the information from these inputs. Eyes without sophisticated neuronal structures exist, like in box jellyfish, where surprisingly sophisticated camera-type eyes provide input to only a very limited neural net (Joram and Zbynek, 2004), but in many animals, large parts of the brain are dedicated solely to the processing of visual information. This feature is shared in species of widely different size, habitat and phylogeny: In primates, for example, visual areas make up 50% of the structures in the neocortex (Barton, 1998). In flying insects, this proportion is even larger, and the optic lobes of the blowfly Musca do not only make up over half of total brain volume, but also possess a neuronal density which is more than twice as high as that of the average in all brain regions (Strausfeld, 1976). This, as well as the easy accessibility of their visual neuropils, makes these insects ideal model systems for the processing of visual information.

## Feature extraction from the visual panorama

With the multitude of information present in the visual surroundings, extraction of behaviourally relevant information is a highly complex task that can be theoretically approached from two directions: One the one hand, every subsequent computation needs a fundamental representation of the visual world to work with, in form of a reconstruction or model of the three-dimensional surroundings (Lennie, 1998). On the other hand, given the biological constraints to storage and processing capacity, some kind of reduction in complexity is needed, selectively extracting those stimuli that have a behavioural relevance to the animal.

Even though the different theoretical approaches to this topic have been subject to some controversy, the answer lies, as usually, somewhere in the middle: Early visual processing stages are probably dominated by largely retinotopic transmission of primary visual features like light intensity or colour, mainly enhancing basic image properties like local contrast adaptation, edge detection, colour constancy or signal-to-noise-ratio. Both in vertebrate as in invertebrate visual systems, the radial struc-

## Inset: Computational models for motion detection

A lot of different approaches have been made to create models for motion perception that consistent with the physiology in different animals and with human psychophysics. Several of these are based on global image matching (Lappin and Bell, 1972), but face problems when confronted with complex motion patterns or several stimuli moving at once. Also, these models are hard to realize with the limited neuronal complexity of insect brains.

A different approach models an array of local correlation units to gauge velocity and direction of small-field stimuli. This low-level computation of visual inputs is well suited for a rapid estimation of motion by elements relatively close to the periphery, which could then be used in further hierarchical processing steps for the detection of more complex motion patterns.

Two of these low-level motion detection models have proven very successful in the prediction of motion responses found in experimental data: The correlation detector described by Hassenstein and Reichardt (Hassenstein, 1961) and the motion-energy model described by Adelson and Bergen (Adelson and Bergen, 1985). The most striking feature of these two models is their high degree of similarity, and the output signals of both approaches are mathematically equivalent under certain filter conditions. The splitting of the multiplication operation into a physiologically more plausible 4-quadrant-operation like that in the Adelson-Bergen model has also been proposed for the Hassenstein-Reichard detector, a model that has, with the discovery of the segregation of on- and off-channels in the fly lamina, received renewed attention lately (Eichner et al., 2011).

Correlation-based motion detection has several characteristic limitations when used to measure image velocity: The output of a Hassenstein-Reichard-detector is strongly dependent on contrast as well as pattern frequency, and thus exhibits a strong texture-dependence. Also, it is susceptible to various optical illusions like reverse-phi-motion, where a pattern that reverses contrast while moving is perceived as motion in the opposite direction.

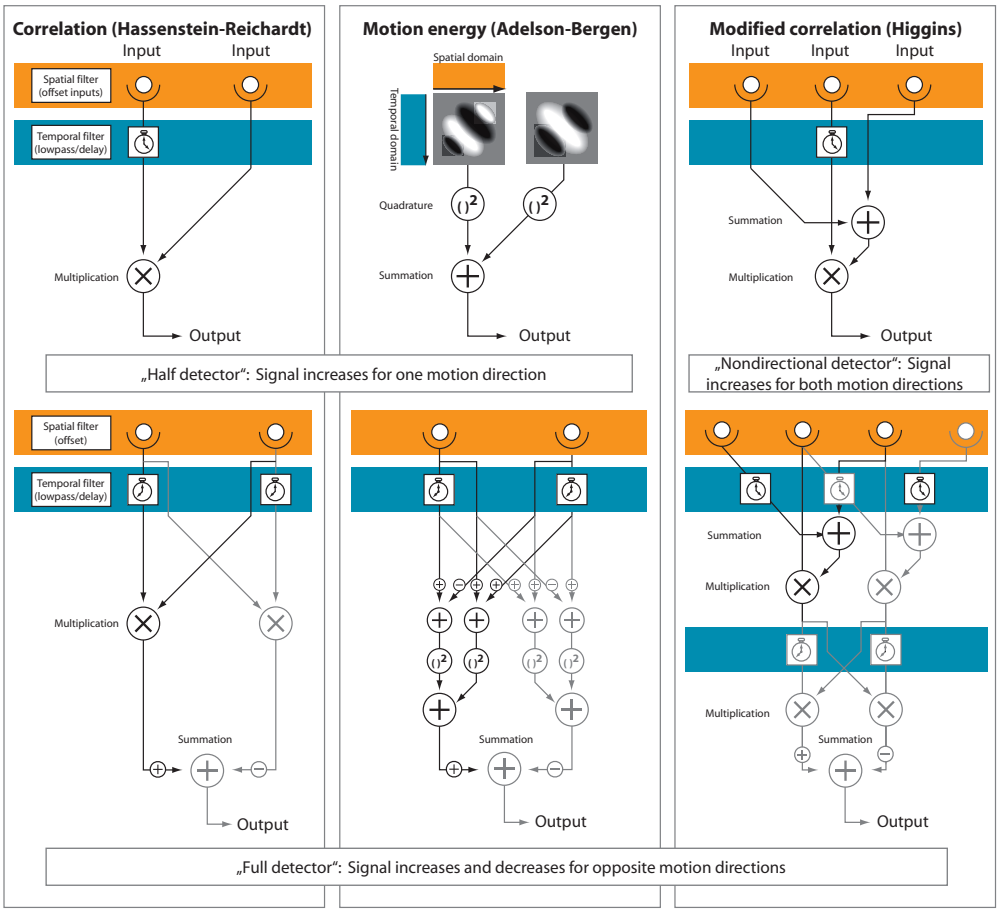


**Figure 2: Computational models for motion detection**
(A) Correlation type detection model. Top: Visual input passes through spatially offset filters, of which one is delayed by a low-pass operation. Output from both branches is multiplied. Bottom: Two branches with opposite directions are combined and the products from each branch subtracted from each other.
(B) Motion energy model. Top: Two linear filters whose responses are 90 degrees out of phase are squared and summed. Bottom: Combination of two of the above branches with opposite filter directions by summation and filtering.
(C) Modified two-step correlation detection model. Top: Symmetrical neighbouring visual inputs are added before delay and multiplication to give a nondirectional motion response. Bottom: Two nondirectional motion detectors form the input for the two branches of a Hassenstein-Reichardt detector. (A and B modified from Adelson 1985, C simplified from Higgins 2004)

These response characteristics have, however, also been found in neuronal and behavioural responses of flies, strongly supporting the fact that these animals use correlation-type motion detection.

ture of inputs is retained, but subsequently processed in layered structures which are specialized in enhancing and extracting salient local image properties (Sanes and Zipursky, 2010).

Later processing stages contain highly specialized individual neurons or neuronal populations that are selectively tuned to the extraction of behaviourally relevant stimuli from the combination of signals which arrive from the periphery, often sampling relatively large areas of the visual field. These „feature detectors" have been found in various animals, for example triggering behavioural responses for catching prey in dragonflies as well as in toads (O'Carroll, 1993; Schuerg-Pfeiffer et al., 1993), and act as late integrative stages that filter and combine certain subsets of image modalities to create selectiveness.

One of the most common visual feature that can be extracted through local as well as global processing is motion. Visual motion cues carry a strong behavioural relevance since they can provide information about self-motion, approaching prey or predators, or the three-dimensional layout of the environment. Motion cues are stimuli of high saliency to humans (Nothdurft, 1993), and basic motion sensitive behaviour like optomotor following responses can be observed in a large number of animals, from insects (Hassenstein, 1961; McCann and MacGinitie, 1965) to birds, reptiles, fish (Tauber and Atkin, 1968) and mammals (Abdeljalil et al., 2005). Thus, motion vision has played a prominent role in research on visual processing, and several computational models for motion detection have been successfully able to recreate key features of neuronal motion detection (See inset).

## Computation of image modalities in the visual neuropiles of the fly

Even though the structure and development of insect brains is widely different from that of vertebrates, a direct comparison of the layout of structures and the channels for information processing shows several functional similarities in their visual neuropils.

For an anatomical layout of the fly visual neuropils see inset. The most striking candidate neuropil in terms of early feature extraction is the medulla, which is marked by an organization which on the one hand consists of a retinotopic mosaic of co-

lumns, with homologous elements tiling the visual field, and on the other hand by a distinct stratification: Many of the columnar elements show lateral arborisations which are confined to distinct sublayers in a way that shows strong parallels to the organization of the mammalian retina and downstream LGN into layers and sublayers (Sanes and Zipursky, 2010). It is known that colour information is selectively processed in specialized layers of the medulla, since projections of the spectrally sensitive R7 and R8 photoreceptors of Drosophila form histaminergic synapses in confined strata (Gao et al., 2008), projecting on the dendrites of large tangential neurons which might mediate behaviours like phototaxis. It has been shown that motion detection and phototaxis work through systems independently from each other (Yamaguchi et al., 2010), and this segregation is most likely present at the organizational level of the medulla. Most of the remaining medulla layers are dominated by input from the monochromatic photoreceptors R1 to R6, which arrives indirectly via interneurons in the lamina. This information is then processed by a multitude of columnar interneurons which possess fine arborisations in one or several layers that provides finely structured lateral interaction between neighbouring columns (Fischbach and Dittrich, 1989). This organization makes involvement of these cells in spatiotemporal processing, like the computation of form, orientation or motion very plausible, but physiological information on most of these neurons is still lacking. Single cell recordings of individual neurons have shown, however, that orientation selective responses are present in two types of medulla cells, the T2 and T4 cells, in medulla amacrine cells which run tangentially along proximal medulla layers, as well as in subsequent wide-field neurons (Douglass and Strausfeld, 1996; Okamura and Strausfeld, 2007). Also, recordings from the T5 cell, which provides an output element from the medulla to the lobula plate, show that direction selectivity is already present in the medulla.

Output from the medulla is projected to several neuropils, the protocerebrum, the lobula and the lobula plate, of which especially the lobula plate has been well studied for its direction selectivity. The large tangential cells of this neuropil mark a transition from tiled, retinotopic elements to large integrative structures, and thus serve as collectors for precomputed motion information. The lobula plate is also segregated into layers, which are believed to group inputs specific to the cardinal motion directions (Buchner et al., 1984).

## Inset: Organisation of the visual system of the blowfly

The insect compound eye consists of a lattice of single eye units called ommatidia, which are mounted on a convex surface and cover large parts of the visual field. In flies, each ommatidium (about 3000 per eye in Calliphora) contains 8 photoreceptors, from which R1 to R6 are most sensitive to green light and provide achromatic input to the motion system, while R7 and R8 have blue and UV-sensitive pigments and provide colour vision (Yamaguchi et al., 2010). The lattice structure of the ommatidia is retained in the following two neuropils: Input from the six green-sensitive photoreceptors directed at one point in visual space is first bundled into a cartridge in the lamina in a wiring scheme known as „neural superposition".

The visual information is then, together with axons from R7 and R8 which bypass the cartridge structure of the lamina, projected through the first optic chiasm, which connects the frontal part of the lamina with the lateral part of the medulla and vice versa, and fed into a lattice of parallel medulla columns which continue this pattern of retinotopic organization. In the medulla, a complex network of lateral interactions between columns in a complex layered structure suggest wide-spread parallel processing of motion and colour information in this region. Neuronal densities in this neuropil are the highest in all of the optic lobes, with cell counts in Musca showing that more than half of the total neurons in the optic lobes are located in the medulla (Strausfeld, 1976). The medulla mosaic, which still retains the periodicity of the ommatidia, is then homotypically projected through the second optic chiasm to the surfaces of the lobula complex.

This structure consists of the third order neuropils, the lobula and lobula plate, and mark a transition from a widely retinotopic and repetitive wiring to a layered organization: While inputs to the lobula retain much of the columnar structure found in the medulla, many cells within the lobula are grouped together in bundles that process information from several ommatidia, often in a 1:3 periodicity (Strausfeld, 1976), which then send projections into the lobula plate.

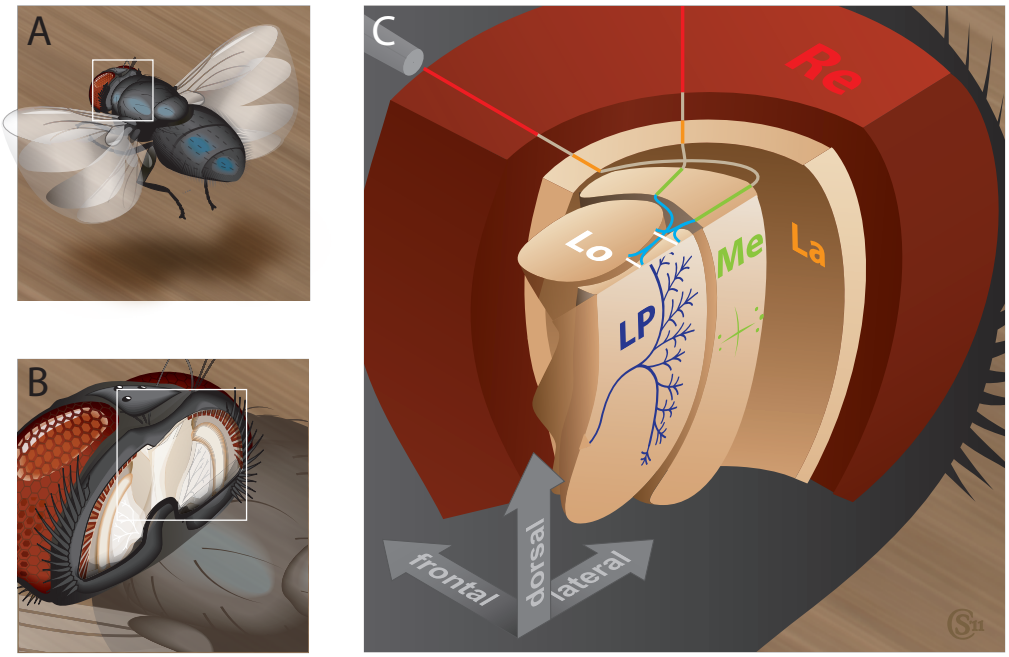


**Figure 1: The visual system of the blowfly** *Calliphora*
(A) Blowfly in flight
(B) Head capsule of the fly with the caudal cuticula removed, showing the superficially visible optic lobes (ommatidia not to scale).
(C) Schematic of a horizontal and frontal section through the right optic lobe, showing the layout of the retina (Re), lamina (La), medulla (Me), lobula (Lo) and lobula plate. The coloured lines indicate the path of visual information from two points of the visual field through these neuopils. The dark blue structure visible on the surface of the medulla and the green structure on the medulla indicate position and size of typical staining patterns obtained during the experiments in Chapter 3 and 4, respectively.

The lobula plate is home to a large population of individual cells with widespread tangential arborisations that spread out parallel to the surface of the brain. These lobula plate tangential cells (LPTCs) collect inputs from large parts of the visual field and compute directional motion signals in response to wide-field, or in some cases, specific small-field stimuli. Further processing steps are provided by connections between these cells in the form of dendro-dendritic and axonal synapses which can be electrical, chemical, or a mixture of both types (Gauck et al., 1997; Haag and Borst, 2002). Axonal projections from the lobula plate connect both brain hemispheres and provide input to the midbrain, glomerulus-like structures in the protocerebrum (Okamura and Strausfeld, 2007), or neurons descending to the ventral nerve cords.

The exact composition of the inputs that provide motion information to these cells, however, is still a matter of controversy. Several works suggest that directional selectivity is computed on the dendrite of these cells by the integration of excitatory (nACh-receptor mediated) and inhibitory (GABA-receptor mediated) elements (Single and Borst, 1998; Brotz et al., 2001), and suggest T4 and T5 cells for these inputs, but the question of whether these cells act directly or through connections from the lobula, and whether these inputs are orientation-selective, direction-selective, or a mixture of both, still remains unknown.

The exact compositon of the inputs that provide motion information to these cells, however, is still a matter of controversy. Several works suggest that directional selectivity is computed on the dendrite of these cells by the integration of exitatory (nACh-receptor mediated) and inhibitory (GABA-receptor mediated) elements (Single and Borst, 1998; Brotz et al., 2001), and suggest T4 and T5 cells for these inputs, but the question of whether these cells act directly or through connections from the lobula, and whether these inputs are orientation-selective, direction-selective, or a mixture of both, still remains unknown.

## Aims and goals of the study: From snapshot to response

When observing an animal in a typical situation, we can usually easily observe two immediate aspects: We see the layout of the environment that the animal is in and many of the stimuli it is exposed to, and we can observe the animals actions in response to these stimuli. How this interaction of action and perception works, and how the animal manages to generate these behavioural responses, however, is a far more complex question.

As mentioned above, neurobiological studies have classically approached the topic of neuronal feature extraction from two sides:

On the one hand, scientist have worked downwards from concepts like optomotoric following responses or control of self-motion that are present in many insects (Hassenstein, 1961; Krapp and Hengstenberg, 1996; Egelhaaf and Kern, 2002), or from problem-solving capabilities like pattern discrimination (Dyer et al., 2008) or navigation (Srinivasan, 2011), looking for mechanistic models to explain these behaviours and the corresponding neuronal substrates which might trigger them. These works have provided much insight especially into the comparatively simple but still complex sensory systems of invertebrates, not only in the form of pioneering models for motion detection as the Hassenstein-Reichardt correlation detector (Hassenstein, 1961), but also by finding corresponding neuronal systems for these tasks like the wide-field motion sensitive cells in the lobula plate of flies (Hausen, 1982; Hengstenberg, 1982) or the descending contralateral movement detector neurons in locusts, which trigger a direct behavioural response to approaching objects in the visual field (Rind and Simmons, 1997; Fotowat and Gabbiani, 2011).

On the other hand, the problem has been challenged from the other direction, looking at the visual input of animals in their natural surroundings (Geurten et al., 2010), examining which visual parameters like optic flow, contrast and pattern frequency occur (Wiederman et al., 2008) and how they are computed by the early stages visual system. These complementary approaches which work "upwards" from the input of the visual system to subsequent processing stages, have also proven fruitful and shed light on concepts like the adaptation and interaction properties of photoreceptors (Brinkworth et al., 2008), the coding range and properties of the synapses between photoreceptor and LMCs (Juusola et al., 1995) and the represen-

tation of emerging properties like the segregation of on- and off-signals and the subsequent interaction of these channels in the lamina (Rister et al., 2007; Reiff et al., 2010; Clark et al., 2011)

This year marks the fiftieth anniversary of the publication on the correlation-type movement detector by Hassenstein (Hassenstein, 1961), and while tremendous progress has been made in this field and countless theoretical models exist which try to integrate these information into a comprehensive picture of feature extraction in the insect visual system, many of the intermediate computational steps remain unknown. Still, little is known about which neurons form the basis of small-field motion detection or pattern recognition, how the input to the wide-field cells of the lobula plate is organized, and how and where the extraction and correlation of complex features from the unprocessed camera-like image that forms on the retina take place.

One of the reasons for this is that the candidate neuropils for this kind of computation, the medulla and lobula, have been hard to access physiologically: Their small and densely interwoven neurites and huge variety of cells do not lend themselves well to electrical recordings, while their complex network of interactions, the fact that they are partly located in deeper brain regions and the necessity for the conservation of a largely complete and intact visual pathway during experiments has hampered access with imaging methods.

This study aims to close this gap, trying to gain access to signals from these intermediate neurons, and shed light on the processing steps between large output elements of the lobula plate tangential cells and the early peripheral signals of the lamina.

## From local to global: Integration of direction sensitive inputs on the dendrite of lobula plate tangential cells

The first part of this work begins with the large wide-field-sensitive cells of the lobula plate. These cells represent a fairly late stage of visual processing, specializing in extracting information from wide-field motion patterns. These cells have extended dendritic trees that gather inputs from large parts of the visual field, and respond best to simultaneous global motion. The similarity of the input patterns to stimuli evoked by self-motion of the animal has led to the theory that these classes of cells

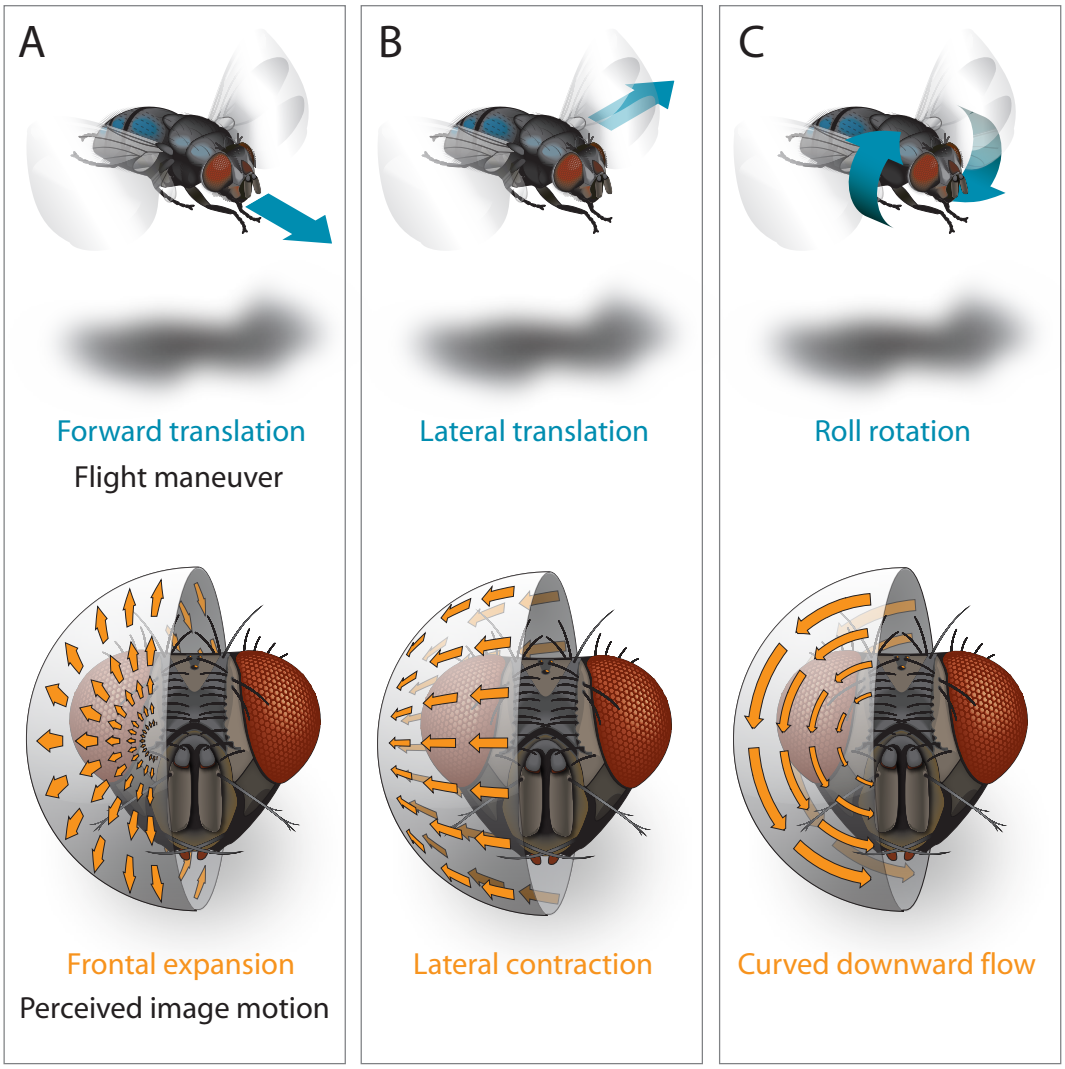


**Figure 3: Flight maneuvers lead to characteristic patterns of optic flow**
Top row: Sample flight maneuvers (A,B and C) of a fly in directions indicated by the blue arrows. Bottom row: Corresponding image motion (orange arrows) in the right visual hemisphere induced through movement of the visual panorama.

serves as feature extractors for the estimation of flight parameters like ground velocity, rotation speed (Krapp, 2000) or the proximity of nearby objects (Liang et al., 2008). Examples for these so called „optic flow field“ are pictured in Figure 3: Different types of flight manoeuvres lead to characteristic patterns of localized motion components, and conversely, extraction of information from these patterns could be used to gain information about flight parameters like direction (based on the focus of expansion) or velocity (based on perceived pattern velocity). Since localized optic

flow components can be the result of different kinds of self motion, for example the lateral optic flow in the dorsal visual field visible in Figure 2 B and C that results from both lateral translation and roll rotation, an extraction of movement components would require the integration of localized signals from large parts of the visual field. Neurons that selectively code for translational or rotational optic flow have been found in several animals, for example in the accessory optic system and vestibulocerebellum of pigeons (Wylie et al., 1998), or the extrastriate visual cortex of primates (Duffy, 1998).

Experiments that measure the responses of various LPTCs to small moving stimuli presented locally in different parts of the visual field show that the responses of these cells exhibit a strong dependence on motion direction, but that this local preferred direction is different in different parts of the visual field. For example, the „Vertical System" cell VS2 shows a preference for downward motion in the lateral visual field, but for progressive and regressive motion in the dorsal and ventral visual field, respectively (Krapp et al., 1998), a response field that strongly matches the optic flow created during roll manoeuvres (compare Fig. 3 C). This has led to the conclusion that the network of VS cells serves as matched filters for the estimation of roll velocities, with each cell's output encoding a slightly different axis of rotation along different azimuthal directions (Krapp, 2000; Karmeier et al., 2006)

Other LPTCs show similar responses in respect to the fact that they integrate inputs with different motion selectivity in their visual field, but this pattern of preferred directions does not always match optic flow fields created by typical components of self-motion. Also, it has been shown that „Horizontal System" (HS) cells are not purely matched filters for yaw rotation (as one might predict from their receptive field), but show a distinct response to objects in the vicinity that is enhanced by adaptation (Liang et al., 2008). Also, several LPTCs do not simply integrate retinotopic elements but form parts of large networks of cells which connect several classes of LPTCs from both hemispheres of the brain in a complex pattern of electrical as well as inhibitory and excitatory chemical synapses (Haag and Borst, 2003). These interactions lead to emergent properties like the tuning to small stimuli found in the so called „Figure Detection" (FD) cell that is dependent on the presence of dendro-dendritic inhibitory inputs from CH cells (Warzecha et al., 1993; Hennig et al., 2008).

Thus, dendritic processing plays a large role in the function of LPTCs, in the integration of local inputs as well as in interaction with the widespread neurites of other tangential cells. Also, the concept that fully direction selective responses arise at the dendrite itself by integration of non- or only weakly directional signals has been proposed for VS cells (Single and Borst, 1998), while the use of the dendritic morphological layout for spatial filtering is a well-known concept in vertebrate vision (Shapley et al., 2007). Previous studies in LPTCs have, however, mainly concentrated on electrophysiological recordings at the axon of these cells, only accessing the already integrated signal, and while some aspects of dendritic signalling like basic retinotopy have been shown through calcium imaging (Egelhaaf and Borst, 1995), the directionality of inputs has so far received little attention. In contrast to the vertebrate neurons mentioned above, the large size and superficial layout of the dendrites of LPTCs make them ideal model systems for the study of dendritic integration.

In the first part of my study, I examined how directionality is integrated on the dendritic tree of various LPTCs, focusing on three particular aspects:

First, I looked whether retinotopic directionality mapping exists on the dendritic tree. Due to the morphology of the optic lobes, which retains a columnar retinotopic structure through the first to third neuropil, one would expect to find a mapping of directional preference to the dendrite that matches those measured with small stimuli in the visual field (Krapp et al., 1998). However, it was unclear whether the differences in directionality of these signals would stay confined enough to observe a systematic segregation of directionality on the dendrites of a single cell. This was the case, as I found a clear change in directionality on the dendrites of VS cells that closely matched a map of the direction selectivities in the visual field. Other cells, namely HS, CH or the previously uncharacterized „Amacrine“ (Amx) cell exhibited similar dendritic directionality maps, with strong variations in local preferred directions. The fact that calcium signals in the Amx cell showed fully directional representation of stimuli in preferred and antipreferred direction with increases and decreases in calcium also allowed, in addition to the mapping of preferred directions, the conclusion that this cell is most probably a „second stage“ LPTCs which gets its input via electrical synapses from other LPTCs. This difference in dendritic calcium signals between cells with direct transmitter-driven input and cells with

precomputed input through electrical synapses had been previously demonstrated for HS and CH cells (Dürr et al., 2001).

Second, I examined the dendritic response fields of the vCH cells for deviations from a direct retinotopic mapping. While these cells are known to integrate signals from several other LPTCs both from ipsi- and contralateral brain hemispheres (Haag and Borst, 2003), the localization of these inputs on the dendritic tree of vCH has so far been unclear. Through individual stimulation of the visual hemifields I could show that these interactions clearly influence the dendritic directionality map of vCH, which leads to a complex non-retinotopic map that integrates excitatory and inhibitory inputs in form of localized synaptic projections from the contralateral LPTCs.

Third, I measured the fine-scale direction tuning of fine dendritic arbours with multiline two-photon-microscopy, a technique that allows the excitation and fluorescence signal registration from more localized structures than with conventional epifluorescence microscopy (Kurtz et al., 2006). While our measurements with wide-field-fluorescence and earlier electrophysiological recordings showed relatively smooth transitions in preferred direction, local direction selectivity could either arise from an a array of inputs with homogeneous preferred directions, or by averaging over several signals with heterogeneous preferences at the level of the LPTC dendrite: The hexagonal structure of the compound eye of the fly makes a segregation of motion detectors into cardinal directions which follow the axes of the ommatidial lattice plausible, and experiments with activity labelling in the optic lobes propose a confinement of the output structures from horizontally and vertically selective elements to different layers of the lobula plate (Buchner et al., 1984). My results, however, showed no distinct heterogeneities in the directional preference of localized dendritic arborisations of VS1 cells even at high spatial resolution, suggesting that inputs to LPTCs represent motion signals that have already been pooled at earlier stages of the visual pathway.

In conclusion, I showed that the analysis of dendritic signals allows deeper insights into the input organization of the motion sensitive neurons of the lobula plate than what had previously been deduced from axonal recordings, and how dendritic directional maps can contribute to feature extraction from large parts of the visual field. However, the exact composition of the motion-sensitive inputs to the lobula plate still remained elusive.

## From flicker to feature: Representation of spatiotemporal image components in the medulla

In the second part of my work, I went one step upstream in the visual system, to the second neuropil, the medulla. These cells in this region ultimately provide the retinotopic inputs to the lobula plate tangential cells I recorded from, but the exact nature of these connections, and whether they are direct or indirect via interneurons in the lobula, still remains unclear.

The medulla is the most densely packed of all visual neuropils in the fly (Strausfeld, 1976), and contains a multitude of different classes of neurons which can be distinguished by their morphological properties (Fischbach and Dittrich, 1989), organized into repetitive columnar structures that tile the visual field of the animal. However, very little is known about the functional properties of these cells, even though their dense interconnection and layered morphology suggests involvement in parallel processing, allowing feature extraction from visual stimuli (Sanes and Zipursky, 2010).

Both the small size of these cells and their diversity makes access through intracellular recordings difficult, and only a small subset of medulla neurons have so far been characterised electrophysiologically (Douglass and Strausfeld, 1995, 2003). Both of these limitations could, however, be circumvented by the introduction of calcium sensitive dyes via population staining. Several approaches to multi-cell-labelling in neuronal tissues exist in vertebrate systems (Garaschuk et al., 2006), and membrane permeable dyes have already been used in invertebrates, for example to stain fibre tracts in bees (Joerges et al., 1997), but work in the visual system has mainly relied on genetically expressed calcium indicators so far (Seelig et al., 2010). To achieve spatially confined staining of medulla columns, I used a novel technique that had been previously used to label neuronal structures in the silk moth (Fujiwara et al., 2009): A membrane impermeant calcium dye (Calcium Green-1 dextran 3000) was loaded into a glass micropipette and inserted into the medulla. Application of pulsed hyperpolarizing current of several μA then reversibly opens pores in nearby cell membranes through movement of polar membrane proteins (Chen et al., 2006), driving the dye out of the electrodes and into nearby cellular structures, selectively staining neuronal processes.

The labelling patterns achieved in this way contained cells from about 1-2 medulla columns, as well as tangential elements running perpendicular to the columnar elements in the injection site. Even though injection sites varied in terms of both position in the visual field (determined through position at different sections of the dorsal-ventral extent of the medulla) and processing layer (determined through the medial-distal position in the medulla), the calcium responses of the stained population showed several common characteristics when stimulated with moving patterns: The cells responded with strong increases in intracellular calcium when stimulated with moving gratings. Different than in LPTCs, all medulla signals were omnidirectional, and increased in response to motion in all directions with no singular preferred direction visible. However, several stainings showed orientation selectivity, responding stronger to a particular (usually vertical) pattern orientation. This was somewhat surprising: Even though single-cell recordings had previously showed the existence of orientation-selective cells in the medulla (Douglass and Strausfeld, 2003), the medulla has been proposed as the candidate neuropil for the processing of local motion detection, providing fully or at least partly (Single and Borst, 1998) direction selective signals that could provide inputs to the integrating dendrites LPTCs. Even though the population staining method did not rule out that single, direction selective cell responses were masked by the pooling of different signals from several cells, two findings spoke against that:

First, indiscriminate random pooling of cell responses should mask orientation selectivity as well as direction selectivity. But while significant orientation selectivity was found in several stainings, directional selectivity was never above chance level. Second, while epifluorescence imaging always results in blurring of the image and makes accurate discrimination of signals from individual neurites difficult, signals were localized enough to pinpoint them to localized regions, especially in the case of the tangential elements with their large and individually discernable structures, but I could not find inhomogeneities in local direction preference (like those found in LPTCs in the previous study) even in small and confined image regions.

The presence of an nondirectional motion selectivity as a hierarchical processing stage earlier than the computation of directionality in insects had previously been proposed to explain behavioural centring responses in bees (Srinivasan et al., 1993): A two-step model proposes an earlier centre-surround-like stage where signals from

symmetrically arranged inputs are pooled and then compared to the signal from a central input channel. This leads to a nondirectional signal with a broad temporal frequency optimum that is subsequently fed into an array of correlation detectors where additional low-pass filtering leads to the loss of response to high-frequency stimuli (Higgins et al., 2004). This hypothesis is supported by the temporal frequency tuning of the neurons I measured in the medulla, which lies between 10-20 Hz, while responses of LPTCs usually already drop off at about 5 Hz (Dürr et al., 2001; Jung et al., 2011). However, the two-step model also predicts a velocity tuning largely independent from the pattern frequency for low velocities, a relationship could not find when stimulating with different pattern frequencies.

Given the fact that several classes of large-field neurons exist which exhibit orientation-selective responses and have their input arborisations located in the medulla (Okamura and Strausfeld, 2007), the existence of a non-direction-selective processing step in the medulla that is either preliminary or parallel to the direction-selective pathway is very likely.

Another topic that has really received much attention is the presence of separate on- and off-channels in early steps of insect vision, which bears striking similarity to the on-off-segregation found in retina ganglion cells of vertebrates (Kuffler, 1953). While both the existence of lamina interneurons tuned to increases or decreases (Reiff et al., 2010), as well as direct connections between these systems (Joesch et al., 2010) have been shown, the functional significance this segregation has on later processing stages still remains largely unclear. Since the gratings used for stimulation contained both brightness increments and decrements, I also used single moving bright and dark edges to examine the responses of medulla cells to on- and off-stimuli.

All populations tested responded to bright as well as dark edges, with responses to dark edges usually slightly weaker, which supports the concept of pooling of the separate lamina channels at this stage, even though on-off selective responses could be found in some single somata. In addition to that, signals in tangential cells located in distal medulla layers exhibited a spatial separation of on- and off-responses, which would suggest the integration of separate channels from neighbouring ommatidia. A layout like this has been suggested in an earlier work after recordings in the first optic chiasm, which suggested a lateral off-on-off organization of inputs to the medulla (Arnett, 1972), but has received little attention in recent works.

Both the early computation of orientation selectivity and the integration of spatially separate neighbouring on- and off-channels found in the medulla both suggest a strong convergence of processing between invertebrate and vertebrate visual systems.

## From cell to camera: Accessing the fly neuronal system with electrical and fluorescent probes

One of the basic challenges in in-vivo neurophysiology has always been the development of methods that allow access to neuronal signals while in a way that disturbs the system as little as possible, while giving the experimenter the tools to record from and possibly alter cellular activity. Insects offer several practical advantages in the use with in vivo preparations, often allowing experimentation without the need for anaesthesia, body temperature control, artificial respiration or circulation, and facilitating work on neuronal tissues due to their small size and easy access through localized windows in the cuticula.

A large part of the studies in the insect visual system have been and are still performed with electrical recordings, with methods ranging from intracellular recordings with sharp glass micropipettes (e.g. Rien et al., 2011), patch clamp, extracellular recordings (Tang and Juusola, 2010), multielectrode arrays (Piironen et al., 2011) or neurons used as probes for local field potential (Weckström and Laughlin, 2010). While these approaches allow recordings of cellular signals, both spiking and sustained, with high specificity and temporal resolution, these methods also have several drawbacks: As neuronal structures become smaller, so does the probability of a successful and stable penetration needed for single-cell recordings. Because of this, especially neuropils with high neuronal densities have been hard to access in this way.

Also, recording from axons and cell bodies of large cells with extended dendritic trees always suffers from the „space clamp“ problem: Different compartments of the cells might exhibit different electrotonic characteristics, and the signal measured at distant neurites might not reflect the computation performed in other parts of the cell. This makes electrical recordings less suited for cells which exhibit a large degree of complex dendritic computation.

This chapter of my work concentrates on an overview of methods for the recording or manipulation of cellular signals with the help of calcium indicators, a group of substances that consist of the combination of a fluorophore group with a calcium-binding chelating domain. A multitude of calcium indicators have been developed recently, which differ in their fluorescence spectra, membrane permeability, calcium binding dynamics, two-photon cross section and the number of calcium ions bound (Adams, 2010). Optical recordings of calcium signals possess several advantages: fluctuations in calcium level stay locally confined, which allows the recording of dendritic integration processes as well as the pinpointing of interaction sites. Even with intracellular single-cell staining methods like those used for the filling of LPTCs, imaging of the labelled neuron is often possible for several hours, much longer than the typical intracellular recording duration. The recent development of multiphoton excitation techniques opens further advantages in calcium imaging: The confined excitation volume generated by fluorophore excitation through two-photon-processes greatly increases imaging resolution in the z-direction, allowing imaging of finer cellular structures, and the long wavelength of two-photon excitation light minimizes stimulation of fly photoreceptors, which makes this technique especially suited for application in the visual system.

In addition to the simple observation of neuronal activity by introduction of calcium indicators, related fluorescence-based techniques can also be used to manipulate cells. Photolysis of high-affinity calcium chelators, so called „caged calcium", allows artificial elevation of intracellular calcium levels through light exposition. Due to the involvement of calcium as a second messenger in a multitude of intracellular pathways, this method is especially potent to elucidate the effects of adaptation on a single-cell basis (Kurtz, 2007). Also, by exploiting the effect of phototoxicity, it is possible to introduce cell death in individual neurons labelled with fluorescing dyes, which makes it possible to explore the effect of network interactions in fine detail by selectively removing individual elements from the circuit.

In all of these applications, indication of calcium level, photochemically induced cell activation, and induced cell death, synthetic fluorescent probes find their counterpart in genetically expressed molecules (Luo et al., 2008): Chimerical proteins of, for example, GFP based fluorophores combined with calcium binding domains have seen much improvement over the last few years (Mehta and Zhang, 2011),

now offering high signal-to-noise ratios and a wide variety of excitation and emission spectra. The introduction of photosensitive ion channels, both depolarizing and hyperpolarizing, has opened wide opportunities and founded the new field of „optogenetics“ (for recent reviews, see Knöpfel et al., 2010; Fenno et al., 2011), and the genetic expression of neurotoxins is a well established tool for selective ablation of cell function (Evans, 1991). These methods have their own advantages and limitations, especially due to the ectopic protein expression through cell-specific driver lines (Elliott and Brand, 2008): While these approaches offer excellent specificity for certain cell lines, receptor or neurotransmitter expression, it is hard to achieve specific single-cell-resolution or conclusive staining of all cells in an anatomical tract or structure. Also, genetic cell labelling is limited to organisms in which the corresponding molecular tools for transfection and expression exist.

Thus, synthetic and genetically introduced indicators can serve complementary functions, and combination of the two methods should be considered to overcome the individual limitations of each individual approach.

## Concluding remarks

In my work I could show that the interaction between medulla and lobula plate, and thus in a way also the transition from retinotopic visual to emerging conceptual features, is a highly interesting field for the study of the processing of visual information. The complex interaction of inputs from the peripheral meshwork of medulla neurons as well as feedback from other higher-order-neurons makes the dendrites of LPTCs a central interface area that still leaves much opportunity for further research.

The results of the contralateral stimulation for CH cells and the moving on-off-edges in the medulla illustrated a fundamental challenge with system analysis approaches: You often can only find what you are looking for. Even though approaches to probing the visual system of flies include a wide range of approaches, from strictly geometrical gratings over white-noise-stimuli to naturalistic movies, it is hard to find a matched stimulation for a feature-detecting system if you don‘t know what it is coding for, and vice versa to determine the exact function of a cell without proper stimuli to drive it. Even though the thought of all neurons as perfect filters for distinct and singular features probably is simplified, expanding the repertoire of visual

stimuli to a broader but still manageable range of features is a challenging and probably necessary task to gain more insight into the segregation of visual features by insects.

In face of the multitude of converging signals, dendritig interaction and localized activity present in the fine denritic processes of neurons in the fly visual system, calcium imaging remains a powerful tool for the examination of visual processing. This method, however, has its drawbacks, mainly when calcium signals are used as a general indicator for membrane potential changes and thus, general cell activity, as has been also shown in other works which assessed the ambiguous dendritic calcium signals of LPTCs driven by inhibitory receptor activity. While there has been little work using voltage sensitive dyes in the fly, this method would be well suited for a complementary approach that makes use of the many of the advantages in application that calcium sensitive dyes have.

With all progress being made and a lot of detail that has been found out over the years, we are still far from a conclusive model for fly motion vision. With more and more computational models being proposed by different groups, there are always concepts which are overlooked, or theories and details which are left out in subsequent work without being challenged or implemented. In the recent light of parallel progress and surprising convergences regarding motion vision in so many different animal models, it is never wrong to look beyond the rim of our teacup.

## References

Abdeljalil, J., Hamid, M., Abdel-mouttalib, O., Stéphane, R., Raymond, R., Johan, A., José, S., Pierre, C., and Serge, P. (2005). The optomotor response: A robust first-line visual screening method for mice. Vision Research 45, 1439–1446.

Adams, S.R. (2010). How Calcium Indicators Work. Cold Spring Harbor Protocols 2010, pdb.top70.

Adelson, E.H., and Bergen, J.R. (1985). Spatiotemporal energy models for the perception of motion. Journal of the Optical Society of America A 2, 284.

Arnett, D.W. (1972). Spatial and temporal integration properties of units in first optic ganglion of dipterans. J. Neurophysiol. 35, 429–444.

Barton, R.A. (1998). Visual specialization and brain evolution in primates. Proceedings of the Royal Society of London. Series B: Biological Sciences 265, 1933–1937.

Brinkworth, R.S.A., Mah, E.-L., Gray, J.P., and O'Carroll, D.C. (2008). Photoreceptor processing improves salience facilitating small target detection in cluttered scenes. Journal of Vision 8,.

Brotz, T.M., Gundelfinger, E.D., and Borst, A. (2001). Cholinergic and GABAergic pathways in fly motion vision. BMC Neurosci 2, 1.

Buchner, E., Buchner, S., and Bülthoff, H. (1984). Identification of [3H]deoxyglucose-labelled interneurons in the fly from serial autoradiographs. Brain Res 305, 384–388.

Chen, C., Smye, S.W., Robinson, M.P., and Evans, J.A. (2006). Membrane electroporation theories: a review. Medical & Biological Engineering & Computing 44, 5–14.

Clark, D.A., Bursztyn, L., Horowitz, M.A., Schnitzer, M.J., and Clandinin, T.R. (2011). Defining the computational structure of the motion detector in Drosophila. Neuron 70, 1165–1177.

Douglass, J.K., and Strausfeld, N.J. (1995). Visual motion detection circuits in flies: peripheral motion computation by identified small-field retinotopic neurons. J. Neurosci 15, 5596–5611.

Douglass, J.K., and Strausfeld, N.J. (1996). Visual motion-detection circuits in flies: parallel direction- and non-direction-sensitive pathways between the medulla and

lobula plate. J. Neurosci. 16, 4551–4562.

Douglass, J.K., and Strausfeld, N.J. (2003). Retinotopic pathways providing motion-selective information to the lobula from peripheral elementary motion-detecting circuits. The Journal of Comparative Neurology 457, 326–344.

Duffy, C.J. (1998). MST Neurons Respond to Optic Flow and Translational Movement. Journal of Neurophysiology 80, 1816–1827.

Dürr, V., Kurtz, R., and Egelhaaf, M. (2001). Two classes of visual motion sensitive interneurons differ in direction and velocity dependency of in vivo calcium dynamics. J. Neurobiol. 46, 289–300.

Dyer, A.G., Rosa, M.G.P., and Reser, D.H. (2008). Honeybees can recognise images of complex natural scenes for use as potential landmarks. J. Exp. Biol. 211, 1180–1186.

Egelhaaf, M., and Borst, A. (1995). Calcium accumulation in visual interneurons of the fly: Stimulus dependence and relationship to membrane potential. J. Neurophysiol. 73, 2540–2552.

Egelhaaf, M., and Kern, R. (2002). Vision in flying insects. Curr. Opin. Neurobiol. 12, 699–706.

Eichner, H., Joesch, M., Schnell, B., Reiff, D.F., and Borst, A. (2011). Internal structure of the fly elementary motion detector. Neuron 70, 1155–1164.

Elliott, D.A., and Brand, A.H. (2008). The GAL4 system: a versatile system for the expression of genes. Methods Mol. Biol. 420, 79–95.

Evans, G.A. (1991). Toxigenics. Semin. Cell Biol. 2, 71–79.

Fenno, L., Yizhar, O., and Deisseroth, K. (2011). The development and application of optogenetics. Annu. Rev. Neurosci. 34, 389–412.

Fernald, R.D. (2006). Casting a genetic light on the evolution of eyes. Science 313, 1914–1918.

Fischbach, K.F., and Dittrich, A.P.M. (1989). The optic lobe of Drosophila melanogaster. I. A Golgi analysis of wild-type structure. Cell and Tissue Research 258, 441–475.

Fotowat, H., and Gabbiani, F. (2011). Collision Detection as a Model for Sensory-Motor Integration. Annual Review of Neuroscience 34, 1–19.

Fujiwara, T., Kazawa, T., Haupt, S.S., and Kanzaki, R. (2009). Ca2+ imaging of identifiable neurons labeled by electroporation in insect brains. Neuroreport 20, 1061–1065.

Gao, S., Takemura, S.-Y., Ting, C.-Y., Huang, S., Lu, Z., Luan, H., Rister, J., Thum, A.S., Yang, M., Hong, S.-T., et al. (2008). The neural substrate of spectral preference in Drosophila. Neuron 60, 328–342.

Garaschuk, O., Milos, R.-I., Grienberger, C., Marandi, N., Adelsberger, H., and Konnerth, A. (2006). Optical monitoring of brain function in vivo: from neurons to networks. Pflugers Arch. 453, 385–396.

Gauck, V., Egelhaaf, M., and Borst, A. (1997). Synapse distribution on VCH, an inhibitory, motion-sensitive interneuron in the fly visual system. J Comp Neurol 381, 489–499.

Geurten, B.R.H., Kern, R., Braun, E., and Egelhaaf, M. (2010). A syntax of hoverfly flight prototypes. Journal of Experimental Biology 213, 2461–2475.

Haag, J., and Borst, A. (2002). Dendro-dendritic interactions between motion-sensitive large-field neurons in the fly. J. Neurosci. 22, 3227–3233.

Haag, J., and Borst, A. (2003). Orientation tuning of motion-sensitive neurons shaped by vertical-horizontal network interactions. J. Comp. Physiol. A 189, 363–370.

Hassenstein, B. (1961). Wie sehen Insekten Bewegungen? Die Naturwissenschaften 48, 207–214.

Hausen, K. (1982). Motion sensitive interneurons in the optomotor system of the fly - 1.The horizontal cells: Structure and signals. Biol. Cybern. 45, 143–156.

Hengstenberg, R. (1982). Common visual response properties of giant vertical cells in the lobula plate of the blowflyCalliphora. J. Comp. Physiol. [A] 149, 179–193.

Hennig, P., Möller, R., and Egelhaaf, M. (2008). Distributed dendritic processing facilitates object detection: A computational analysis on the visual system of the fly. PLoS ONE 3, e3092.

Higgins, C.M., Douglass, J.K., and Strausfeld, N.J. (2004). The Computational Basis

of an Identified Neuronal Circuit for Elementary Motion Detection in Dipterous Insects. Visual Neuroscience 21, 567–586.

Joerges, J., Kuttner, A., Galizia, C.G., and Menzel, R. (1997). Representations of odours and odour mixtures visualized in the honeybee brain. Nature 387, 285–288.

Joesch, M., Schnell, B., Raghu, S.V., Reiff, D.F., and Borst, A. (2010). ON and OFF pathways in Drosophila motion vision. Nature 468, 300–304.

Joram, P., and Zbynek, K. (2004). Cubozoan jellyfish: an Evo/Devo model for eyes and other sensory systems. International journal of developmental biology 48, 719–729.

Jung, S.N., Borst, A., and Haag, J. (2011). Flight Activity Alters Velocity Tuning of Fly Motion-Sensitive Neurons. The Journal of Neuroscience 31, 9231–9237.

Juusola, M., Uusitalo, R.O., and Weckström, M. (1995). Transfer of graded potentials at the photoreceptor-interneuron synapse. J. Gen. Physiol. 105, 117–148.

Karmeier, K., van Hateren, J.H., Kern, R., and Egelhaaf, M. (2006). Encoding of naturalistic optic flow by a population of blowfly motion-sensitive neurons. J. Neurophysiol. 96, 1602–1614.

Knöpfel, T., Lin, M.Z., Levskaya, A., Tian, L., Lin, J.Y., and Boyden, E.S. (2010). Toward the second generation of optogenetic tools. J. Neurosci. 30, 14998–15004.

Krapp, H.G. (2000). Neuronal matched filters for optic flow processing in flying insects. Int. Rev. Neurobiol. 44, 93–120.

Krapp, H.G., and Hengstenberg, R. (1996). Estimation of self-motion by optic flow processing in single visual interneurons. Nature 384, 463–466.

Krapp, H.G., Hengstenberg, B., and Hengstenberg, R. (1998). Dendritic structure and receptive-field organization of optic flow processing interneurons in the fly. J. Neurophysiol. 79, 1902–1917.

Kuffler, S.W. (1953). Discharge patterns and functional organization of mammalian retina. Journal of Neurophysiology 16, 37–68.

Kurtz, R. (2007). Direction-selective adaptation in fly visual motion-sensitive neurons is generated by an intrinsic conductance-based mechanism. Neuroscience 146, 573–583.

Kurtz, R., Fricke, M., Kalb, J., Tinnefeld, P., and Sauer, M. (2006). Application of multiline two-photon microscopy to functional in vivo imaging. J. Neurosci. Met. 151, 276–286.

Land, M.F., and Fernald, R.D. (1992). The evolution of eyes. Annu. Rev. Neurosci. 15, 1–29.

Lappin, J.S., and Bell, H.H. (1972). Perceptual differentiation of sequential visual patterns. Perception & Psychophysics 12, 129–134.

Lennie, P. (1998). Single units and visual cortical organization. Perception 27, 889–935.

Liang, P., Kern, R., and Egelhaaf, M. (2008). Motion adaptation enhances object-induced neural activity in three-dimensional virtual environment. J. Neurosci. 28, 11328–11332.

Luo, L., Callaway, E.M., and Svoboda, K. (2008). Genetic dissection of neural circuits. Neuron 57, 634–660.

McCann, G.D., and MacGinitie, G.F. (1965). Optomotor Response Studies of Insect Vision. Proceedings of the Royal Society of London. Series B. Biological Sciences 163, 369–401.

Mehta, S., and Zhang, J. (2011). Reporting from the Field: Genetically Encoded Fluorescent Reporters Uncover Signaling Dynamics in Living Biological Systems. Annual Review of Biochemistry 80, 375–401.

Nothdurft, H.-C. (1993). The conspicuousness of orientation and motion contrast. Spatial Vision 7, 341–363.

O'Carroll, D. (1993). Feature-detecting neurons in dragonflies. Nature 362, 541–543.

Okamura, J., and Strausfeld, N.J. (2007). Visual system of calliphorid flies: Motion- and orientation-sensitive visual interneurons supplying dorsal optic glomeruli. The Journal of Comparative Neurology 500, 189–208.

Piironen, A., Weckström, M., and Vähäsöyrinki, M. (2011). Ultrasmall and customizable multichannel electrodes for extracellular recordings. J. Neurophysiol. 105, 1416–1421.

Reiff, D.F., Plett, J., Mank, M., Griesbeck, O., and Borst, A. (2010). Visualizing retinotopic half-wave rectified input to the motion detection circuitry of Drosophila. Nat. Neurosci. 13, 973–978.

Rien, D., Kern, R., and Kurtz, R. (2011). Synaptic transmission of graded membrane potential changes and spikes between identified visual interneurons. Eur. J. Neurosci. 34, 705–716.

Rind, F.C., and Simmons, P.J. (1997). Signaling of object approach by the DCMD neuron of the locust. J. Neurophysiol. 77, 1029–1033.

Rister, J., Pauls, D., Schnell, B., Ting, C.-Y., Lee, C.-H., Sinakevitch, I., Morante, J., Strausfeld, N.J., Ito, K., and Heisenberg, M. (2007). Dissection of the peripheral motion channel in the visual system of Drosophila melanogaster. Neuron 56, 155–170.

Sanes, J.R., and Zipursky, S.L. (2010). Design Principles of Insect and Vertebrate Visual Systems. Neuron 66, 15–36.

Schuerg-Pfeiffer, E., Spreckelsen, C., and Ewert, J.-P. (1993). Temporal discharge patterns of tectal and medullary neurons chronically recorded during snapping toward prey in toads Bufo bufo spinosus. Journal of Comparative Physiology A 173, 363–376.

Schwab, I.R. (2011). Evolution's Witness: How Eyes Evolved (Oxford University Press).

Seelig, J.D., Chiappe, M.E., Lott, G.K., Dutta, A., Osborne, J.E., Reiser, M.B., and Jayaraman, V. (2010). Two-photon calcium imaging from head-fixed Drosophila during optomotor walking behavior. Nat. Methods 7, 535–540.

Shapley, R., Hawken, M., and Xing, D. (2007). The dynamics of visual responses in the primary visual cortex. In Computational Neuroscience: Theoretical Insights into Brain Function, (Elsevier), pp. 21–32.

Single, S., and Borst, A. (1998). Dendritic integration and its role in computing image velocity. Science 281, 1848–1850.

Srinivasan, M.V. (2011). Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics. Physiol. Rev. 91, 413–460.

Srinivasan, M.V., Zhang, S.W., and Chandrashekara, K. (1993). Evidence for two

distinct movement-detecting mechanisms in insect vision. Naturwissenschaften 80, 38–41.

Strausfeld, N.J. (1976). Atlas of an insect brain (Berlin: Springer-Verlag).

Tang, S., and Juusola, M. (2010). Intrinsic activity in the fly brain gates visual information during behavioral choices. PLoS ONE 5, e14455.

Tauber, E.S., and Atkin, A. (1968). Optomotor Responses to Monocular Stimulation: Relation to Visual System Organization. Science 160, 1365–1367.

Warzecha, A.K., Egelhaaf, M., and Borst, A. (1993). Neural circuit tuning fly visual interneurons to motion of small objects. I. Dissection of the circuit by pharmacological and photoinactivation techniques. J. Neurophysiol. 69, 329–339.

Weckström, M., and Laughlin, S. (2010). Extracellular potentials modify the transfer of information at photoreceptor output synapses in the blowfly compound eye. J. Neurosci. 30, 9557–9566.

Wiederman, S.D., Shoemaker, P.A., and O'Carroll, D.C. (2008). A model for the detection of moving targets in visual clutter inspired by insect physiology. PLoS ONE 3, e2784.

Wylie, D.R.W., Bischof, W.F., and Frost, B.J. (1998). Common reference frame for neural coding of translational and rotational optic flow. Nature 392, 278–282.

Yamaguchi, S., Desplan, C., and Heisenberg, M. (2010). Contribution of photoreceptor subtypes to spectral wavelength preference in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 107, 5634–5639.

# Localized direction selective responses in the dendrites of visual interneurons of the fly

Christian Spalthoff1, Martin Egelhaaf1, Philip Tinnefeld2 and Rafael Kurtz1

**This chapter was published as: Christian Spalthoff, Martin Egelhaaf, Philip Tinnefeld and Rafael Kurtz, Localized direction selective responses in the dendrites of visual interneurons of the fly, BMC Biology, BMC Biol. 2010 Apr 12;8:36.**

1)Department of Neurobiology, Bielefeld University, Postbox 100131,

33501 Bielefeld / Germany

2)Angewandte Physik – Biophysik, Ludwig-Maximilians-Universität, Amalienstrasse 54, 80799 München / Germany

## Abstract

**Background**

The various tasks of visual systems, including course control, collision avoidance and the detection of small objects, require at the neuronal level the dendritic integration and subsequent processing of many spatially distributed visual motion inputs. While much is known about the pooled output in these systems, as in the medial superior temporal area of monkeys or in the lobula plate of the insect visual system, the motion tuning of the elements that provide the input has yet received little attention. In order to visualize the motion tuning of these inputs we examined the dendritic activation patterns of neurons that are selective for the characteristic patterns of wide-field motion in the lobula-plate tangential cells (LPTCs) of the blowfly. These neurons are known to sample direction-selective motion information from large parts of the visual field and combine these signals into axonal and dendro-dendritic outputs.

**Results**

Fluorescence imaging of intracellular calcium concentration allowed us to take a direct look at the local dendritic activity and the resulting local preferred directions in LPTC dendrites during activation by wide-field motion in different directions. These 'calcium response fields' resembled a retinotopic dendritic map of local preferred directions in the receptive field, the layout of which is a distinguishing feature of different LPTCs.

**Conclusions**

Our study reveals how neurons acquire selectivity for distinct visual motion patterns by dendritic integration of the local inputs with different preferred directions. With their spatial layout of directional responses, the dendrites of the LPTCs we investigated thus served as matched filters for wide-field motion patterns.

## Background

Fast flying insects such as flies need to integrate motion signals from their compound eyes for various tasks which include course control [1], collision avoidance and the detection of small objects. These tasks require that signals from individual ommatidia be combined into local motion signals, a process which is thought to take place in the medulla [2]. Local motion signals are then pooled and integrated on the dendrite of cells, which receive input from thousands of ommatidia. In the blowfly, this integration of retinotopic information occurs in the lobula plate, with a class of about 60 individually identifiable neurons showing direction selective responses to visual stimuli, the lobula-plate tangential cells (LPTCs). Among the best characterized of these cells are the neurons of the so called 'horizontal system' (HS), named after their strong response to progressive (front-to-back) motion, and those of the 'vertical system' (VS), which respond mainly to downward motion. However, similar to many large-field neurons in other species [3, 4], the motion direction that evokes maximal responses is not uniformly the same but varies in the different parts of the visual field. This led to the conclusion that several LPTCs are tuned to a particular type of optic flow: visual motion patterns like those encountered during specific flight manoeuvres [5-7]. It is known that many LPTCs receive input from local motion-detecting elements on their dendritic trees in a fundamentally retinotopic manner [8, 9]. Little is known, however, about the fine-scale structure of these input

signals. In particular, we are unsure about how the variations in local preferred directions across the large receptive fields are structurally represented.

Due to their small size, the local input elements of LPTCs do not lend themselves well to electrophysiological recording and there is little direct evidence for their responses to motion [10]. In order to circumvent this limitation, we measured localized calcium concentration changes at the dendrites of LPTCs. Ca2+ entry into the dendrite results from the activity of voltage-gated calcium channels [11, 12] and concentration changes of Ca2+ have, to a large extent, been shown to remain local [9, 13, 14]. Consequently, fluorescent calcium-sensitive dyes can be used as a general indicator of potential changes in the local dendritic membrane while simultaneously allowing the visualization of the dendritic fine structure.

In the present study we monitored the local direction tuning of dendritic calcium signals and compared it to that obtained by axonal voltage recordings in different types of LPTCs. The VS consists of 10 neurons per brain hemisphere, which respond predominantly to vertical motion within large, dorso-ventrally elongated sections of the visual field. It is a remarkable feature of VS neurons that their axonal voltage responses are made up of two components: Either graded changes in membrane potential, which consist of depolarization during motion in one direction (the 'preferred direction') and hyperpolarization during motion in the opposite direction (the 'antipreferred direction') or modulations in the rate of spikes. Rather than being all-or-none by nature these spikes may vary in their amplitude [15]. In contrast to the VS neurons, which show a mixture of spiking and graded responses, the second type of LPTC analysed in the present study - the 'ventral centrifugal horizontal' cell (vCH) - responds with purely graded voltage responses to visual motion. It receives input from the ipsilateral field of view via electrical synapses formed with the dendrites of HS cells [16] and is thus most sensitive to horizontal motion. We present data for a third type of LPTC which has so far not been physiologically characterized in a systematic way. Based on similarity with anatomical data presented in [17] we classified it as 'Amacrine cell' (called hereafter 'Amx').

In our study we aimed to investigate how the intricate layouts of the receptive fields of LPTCs, which distinguish themselves by highly specific patterns of local preferred directions, are formed by dendritic input integration. We find that dendritic activation patterns, as monitored by calcium imaging, reflect a retinotopic projection

of local inputs from the visual field onto the dendrite of individual neurons. This subcellular representation of inputs is similar to, for example, the retinotopy that is present on a multi-neuron level in the mammalian primary visual cortex. Thus, local variations of preferred directions, which are present across the receptive fields of LPTCs [6, 7], are also expressed across their dendrites. However, dendritic calcium activation patterns are further shaped by indirect non-retinotopic inputs, which modify direction specificity in subregions of the dendrite.

## Results and discussion

### Axonal direction tuning and extent of dendritic activation

As a mechanism underlying the specificity of individual LPTCs for particular patterns of optic flow, it has been presumed that LPTCs sample local inputs with different preferred direction across their dendrites [6, 7]. This conclusion is mainly based on axonal recordings which provide a limited insight into the actual process of dendritic information processing. In order to fill this gap, we monitored local activity levels and determined the local preferred directions in the dendritic trees of several types of LPTCs.

As a reference for our measurements of localized dendritic motion sensitivities, we first investigated the global spatially integrated direction selectivity in the output region of various LPTCs to our set of visual stimuli, which consisted of grating patterns moving in different directions in the frontal visual field. Figure 1 shows the setup, recording site and stimulation pattern (A) and gives an example of an intracellular recording from a VS2 or VS3 neuron during the presentation of a square wave grating (octagon with an extent of -40° to +40° in elevation and in azimuth) drifting upward (0°, Figure 1B, top left) or downward (180°, bottom left). Since VS2 and VS3 are difficult to differentiate on an anatomical basis and exhibit very similar response properties, we will refer to them together as VS2/3 from now on. The VS2/3 cell responds with a combination of spikes and graded membrane depolarizations to downward motion and with hyperpolarization to upward motion (Figure 1B). The global direction preference of the neuron was determined by measuring the responses to eight motion directions. The response amplitudes, plotted as vectors in Figure 1B (right), show a symmetrical, nearly sinusoidal, direction tuning with a resulting preferred direction at ~180° for the VS2/3 cell, consistent with what

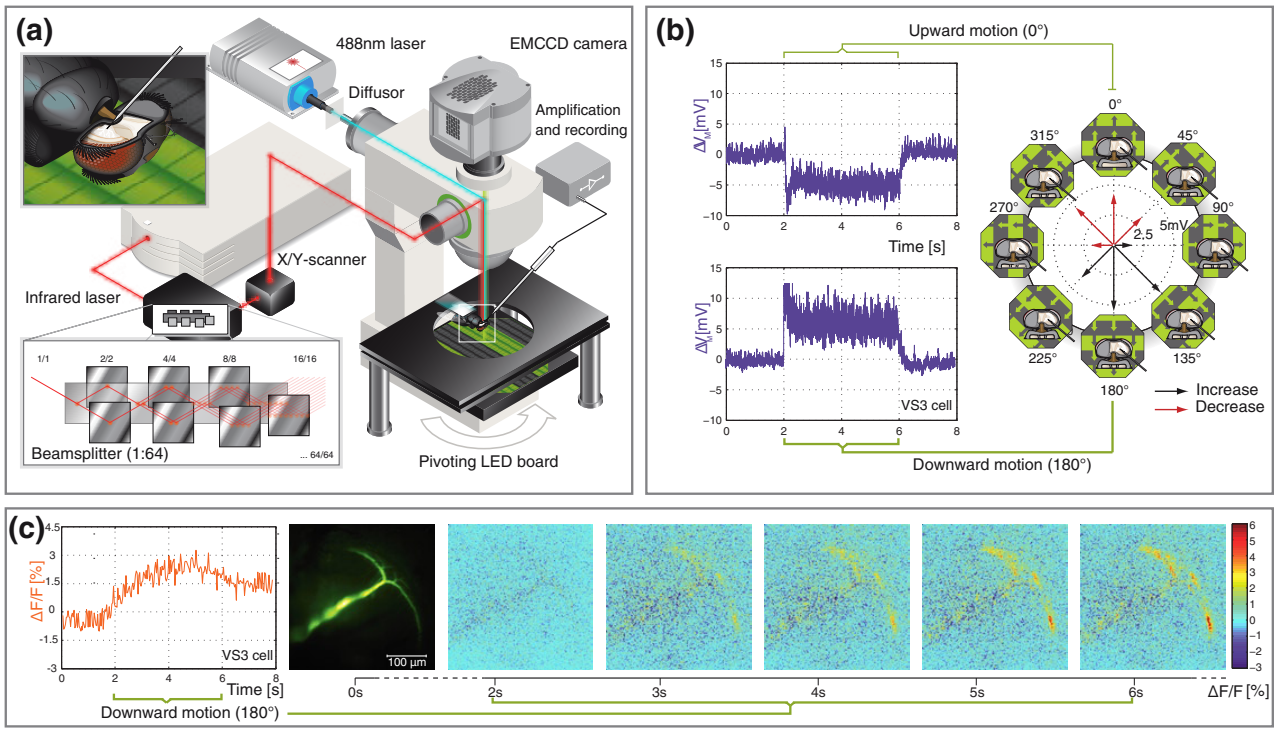


**Figure 1. Acquisition of voltage and calcium responses to visual motion.**
(A) Setup for simultaneous electrophysiology and calcium imaging by multifocal two-photon and conventional wide-field microscopy. Insets show the recording site (top left) and the multifocal beamsplitter for two-photon microscopy (bottom left). The motion stimulus consists of a drifting square wave grating generated by a light emitting diode board.
(B) Response of a vertical system (VS)2/3 neuron to a grating moving in preferred (bottom left) and antipreferred (top left) direction and directional tuning (right). Arrow directions indicate the direction of motion; arrow lengths represent response amplitudes averaged over an interval of 4s starting at motion onset minus the mean response during 2s before motion onset. Black arrows signal increases (depolarization); red arrows signal decreases (hyperpolarization).
(C) Calcium response to downward motion of the same VS cell stained with Oregon Green BAPTA-1. Time course of the calcium signal integrated over the whole dendrite (left) and series of colour-coded ΔF/F0 images showing local differences in fluorescence intensity for various time points (right). Resting fluorescence F0 was determined by averaging the last three frames before start of pattern motion. Images were taken at 10 Hz and 512x512 pixel resolution.

is known from earlier studies [6] (see also Figure 2a).

In order to visualize spatially resolved dendritic activity, the cells were filled with the fluorescent calcium-sensitive dye Oregon Green BAPTA 1 (see Methods). The calcium responses show that distinct areas of the dendrite were activated by a downward motion (Figure 1C). While the axon displays only weak calcium signals, the dorsal and ventral dendritic tips show strong increases in calcium. Our stimulus covered only part of the entire field of view of the fly's compound eye (-40° to 40° out of +90° to -75° in elevation and -5° to 40° out of 5° to >180° in azimuth for a single eye). Nonetheless, the calcium responses were expressed over almost the entire vertical extent of the dendrite, the tips of which nearly reach the dorsal and vertical rim of the lobula plate. Given the retinotopic layout of the lobula plate, this result implies that a disproportionately large amount of space is devoted to the processing of visual information from the frontal visual field, similar to the fovealization in the visual system of many vertebrates.

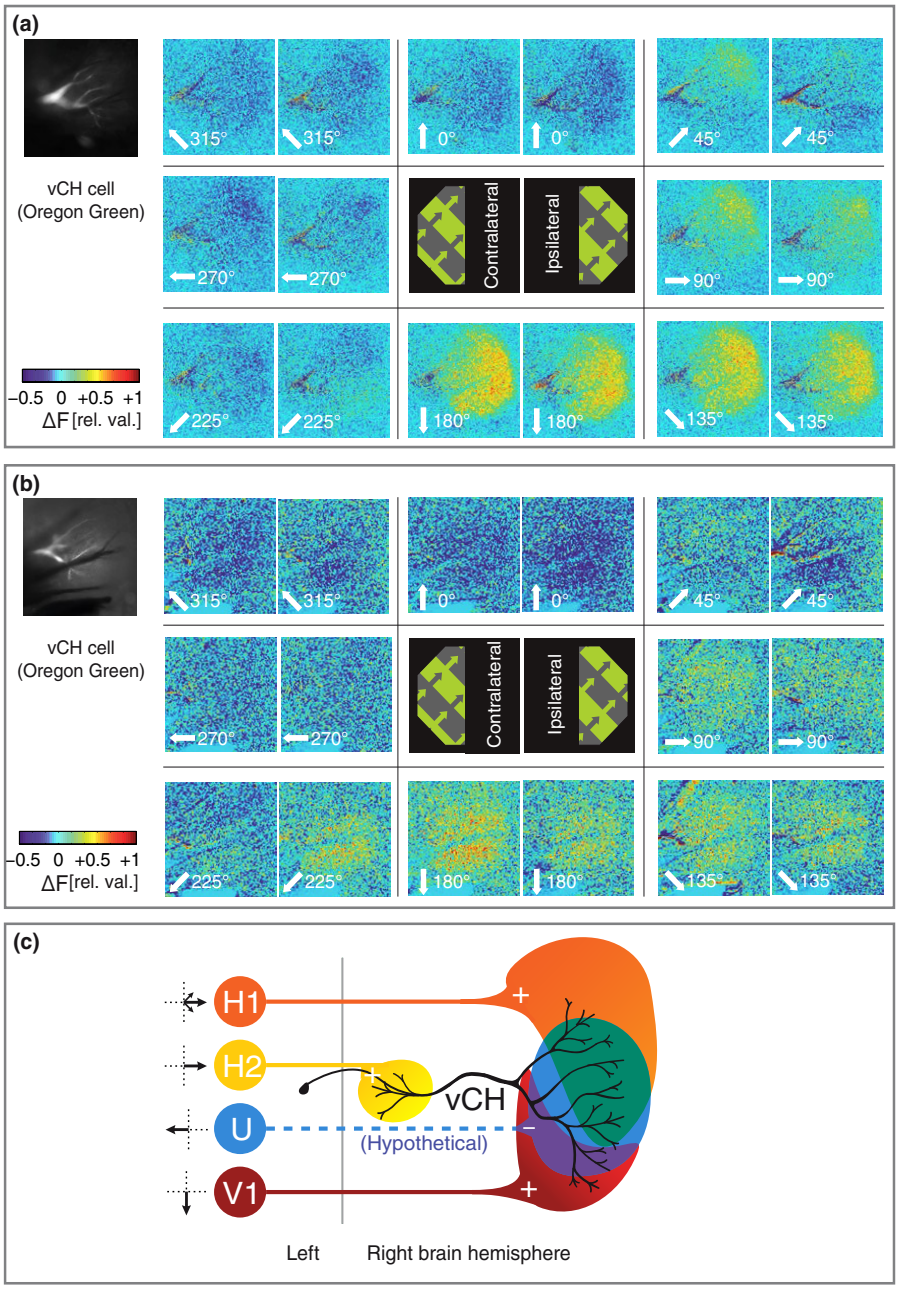


**Figure 2. Influences of ipsi- and contralateral inputs on ventral centrifugal horizontal (vCH) calcium signals.**
(A and B) Image pairs showing local differences in fluorescence intensity in a single vCH cell each during pattern motion in eight directions (white numbers), with only the contralateral (left panels) or ipsilateral (right panels) half of the stimulus pattern visible. Signals were averaged over the last 500 ms of stimulus motion. Images represent single recording traces and were taken at 10 Hz and 512x512 pixel resolution. (C) Wiring diagram of connections between contralateral lobula plate tangential cells and vCH. Black arrows next to the cells indicate the PD of visual motion in the contralateral receptive field of the cells. +: Excitatory input, -: inhibitory input. Wiring of H1, H2 and V1 after [23].

**Local dendritic directional preferences**

In order to investigate how variations in motion preference in the visual field are represented in the local activation patterns of the dendrite, we recorded calcium image series from different types of LPTCs while we presented motion in eight directions [Figure 3, first column (i)]. In order to visualize local direction preferences for motion in the different regions of the dendrite, the complete image series were divided into a grid of 16x16 square regions of interest (ROIs) comprising 32x32 pixels each. The relative fluorescence change in each ROI was then pooled and summed to a scalar value. This set of response amplitudes for each ROI is displayed as a set of eight arrows placed at the centre of each ROI, with length corresponding to amplitude (negative values are plotted in grey) and direction to stimulus direction, and the plots are overlaid on the raw fluorescence image of the cell [Figure 3, second column (ii)]. In order to calculate selectivity and preferred direction for each ROI, these eight vectors were added to a single resulting vector and plotted as arrows, with the arrow direction and length corresponding to the directional preference (see Methods) and the arrow brightness to the overall signal intensity [Figure 3, third and fourth columns (iii and iv)]. All cells examined show calcium signals over almost the whole extent of the dendritic tree but there are distinct variations in their local dendritic directional preferences.

In VS2/3 cells (examples shown in Figures 1C and 2A), the calcium signals mainly consist of increases, with no signal decreases in the dendritic branches. This effect was attributed to the fact that, during antipreferred direction stimulation, the calcium signal could consist not only of a concentration decrease due to closure of voltage-gated calcium channels but - at least close to synaptic input sites – also of an influx of calcium. The latter might result from a calcium influx through ACh receptors, which are thought to be Ca2+ permeable and slightly activated during motion in antipreferred direction [18]. Local preferred directions across the dendrite of VS2/3 reveal a curving pattern, with horizontal and downward/front-to-back selectivities predominant in the dorsal dendritic branch, purely downward selectivities near the major dendritic branching point and downward/back-to-front selectivities in the ventral branch. This pattern matches a retinotopic representation of the response field of VS2/3 measured by axonal voltage recordings during presentation of spatially confined stimuli [6]. In Figure 3A (iv) another example of a VS2/3 re-

cording is shown. The overall signal strength was weaker in this cell, which leads to increased background noise and weak signals in the dorsal dendritic branch. However, the ventral dendrite shows the same curving pattern of the direction selectivities as the first example. A similar response profile could be recorded in a third VS 2/3 cell (data not shown).

We also recorded calcium and voltage signals from two cells which we called Amx cells based on their anatomy. Similar to the Am1 cell [17], these cells have no prominent axon and their dendrites cover most of the lobula plate, showing a distinct double band of fine arborizations at and beyond the rim of the lobula plate [Figure 3B (i-iv)]. In voltage recordings, these cells displayed mainly graded responses, with a preference for back-to-front motion (data not shown). The direction selectivities of spatially resolved calcium signals of Amx cells differ considerably across the dendrite, with dorsal and ventral selectivity patterns smoothly changing from a 225° preference in the dorsal dendrite to a 270° preference in the medial and a 315° preference in the ventral dendrite. Motion in the local antipreferred direction led to strong decreases in the local calcium signals, but the relation of the amplitude of increases and decreases was inhomogeneous in different parts of the dendritic tree: calcium concentration decreases were particularly pronounced in medial parts of the dendrite. While the extent and response pattern of the field of view of this cell has not yet been characterized, the dendritic response field would suggest that the cell responds with strong hyperpolarization to an expanding flow field with a centre of expansion directly in front of the animal, a response characteristic which has not yet been found in other LPTCs.

Figure 3C shows two examples of a vCH, a cell that receives input from the ipsilateral field of view via electrical synapses formed with the dendrites of HS cells [16] . Unlike the VS neuron shown before, but similar to the Amx cell, vCH shows not only increases in calcium concentration during the preferred direction motion, but also decreases in response to motion in its antipreferred direction. This difference between the cell types has already been demonstrated by Dürr et al. [19] and might be attributed to the fact that vCH receives ipsilateral input via electrical synapses with HS neurons, so that influx via transmitter-driven channels (see above) has no effect on calcium concentration. HS neurons are primarily activated by a front-to-back motion in the ipsilateral field of view and by a back-to-front motion in the

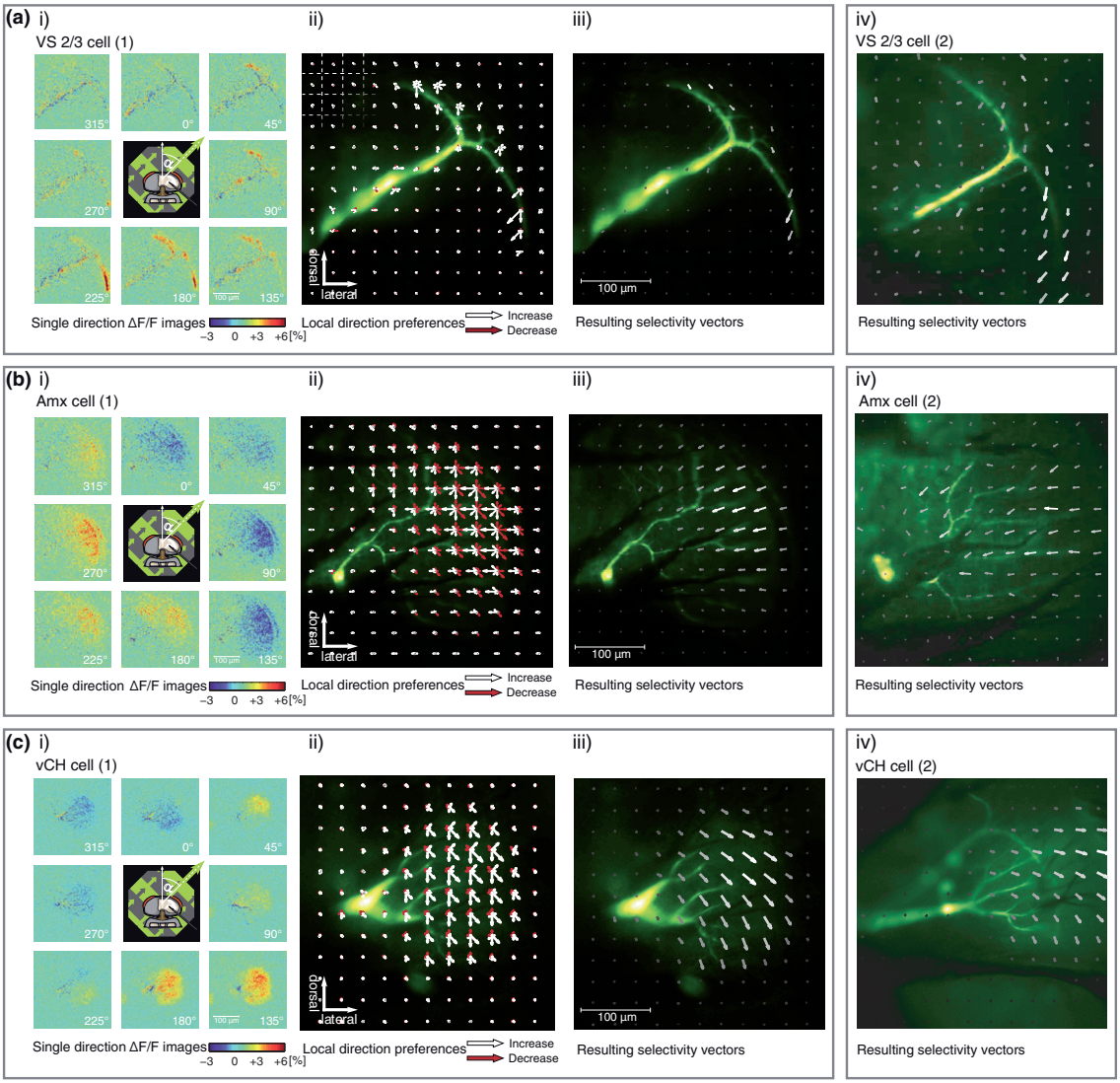


**Figure 3. Local dendritic directional preferences of various lobula plate tangential cells (LPTCs).** Calcium signals at the dendrites of three different LPTC classes: vertical system (VS)3 cells (A), neurons called Amacrine cell (B) and ventral centrifugal horizontal ventral centrifugal horizontal cells (C). (i) Local differences in fluorescence intensity recorded in a single cell during pattern motion in eight directions (α) in comparison to an average of the last three frames before onset of motion. Signals were averaged over the last 500 ms of stimulus motion. (ii) Integrated local calcium responses to pattern motion in eight directions - the same cell as in (i). The grid of region of interest (ROIs) is indicated in the upper left corner. White arrows show increases in calcium in the underlying ROI in response to motion in the direction of the arrow; red arrows show decreases. Arrow length (normalized to the arrow with maximum amplitude in the image) represents ΔF/F intensity. (iii): Resulting response vectors from vector summation of the individual response vectors to all stimulus directions (normalized as before) - the same cell as in (i). Arrow brightness represents overall response amplitude. (iv) Resulting response vectors of additional cells of each of the cell types recorded in different flies, calculated as in (iii). In C, right column, the profile faintly visible in the dorsal area is a VS cell which was accidentally stained during tissue penetration. The cell did not noticeably contribute to the calcium signals. Images represent single recording traces and were taken at 10 Hz and 512x512px resolution.

contralateral field of view [20, 21]. A corresponding horizontal motion sensitivity of the calcium response field of vCH would be expected if its dendritic responses were dominated by input from HS neurons. However, it is known that vCH also receives input from additional contralateral elements which may modulate the directional selectivity.

The dendritic calcium responses of vCH [Figure 3C(iii)] show a shift of the selectivity vectors from a front-to-back motion in the dorsal dendrite to a downward motion in the ventral dendrite, with similar results observed in two other vCH cells [one shown in Figure 3C (iv)]. This sensitivity to vertical motion was also found in electrical responses by Krapp et al. [22]. A plausible reason for this response is the input from the contralateral V1 cell, which shows a strong response to frontal downward motion stimuli and terminates in the ventral half of the lobula plate [22] (see Figure 2B). This cell has been shown to be coupled with vCH [23] and may contribute to the strong vertical sensitivity in the ventral part of the dendrite of vCH. This would add a non-retinotopic influence to the motion signals on this part of the dendrite of vCH.

**Influences of ipsi- and contralateral inputs on vCH calcium signals**

In order to test how strongly contralateral and ipsilateral inputs are represented in the dendritic calcium signal of the vCH cell, we covered one half of the stimulus area at a time, blocking most of the field of view of either the left or the right eye apart from the narrow region of binocular overlap, which reaches 5-10° into the respective contralateral visual field in female Calliphora [24]. Figure 2A shows the responses of two vCH cells to motion presented in either the ipsi- or contralateral visual field. The calcium signals show that responses to contralateral stimulation were about equally strong as to ipsilateral stimulation. This may partly be due to the fact that the receptive fields of most inputs to vCH cover the region of binocular overlap. However, as a consequence of retinotopy in its input from the ipsilateral eye, one would expect the responses to contralateral motion to be confined to the lateral part of the dendrite, which receives input from the frontal region of binocular overlap. Such a regional confinement is not present in our measurements. Thus, it is plausible that the strong responses to contralateral stimulation are to some extent caused by contralateral inputs converging on the dendritic tree.

Apart from the V1 mentioned above, sensitivity to contralateral motion might be mediated by the contralateral H1-neuron, which is known to be synaptically coupled with vCH [23]. This cell covers a large part of the dorsal dendrite of vCH with its terminal arborization (see scheme in Figure 2B), where responses are markedly stronger during contralateral than during ipsilateral stimulation. Another candidate mediating the decreases in calcium signal during contralateral stimulation, such as those observed in response to 270° and 315° motion, could be the so far unidentified neuron that provides inhibitory input to vCH during contralateral front-to-back motion called 'U' in Krapp et al. [22].

Overall, our results imply that, for vCH, contralateral elements not only play a role in the pooled axonal voltage response to wide-field motion as shown in [22], but that the extent and location of the arborizations of these cells can influence the dendritic representation of wide-field motion. For vCH, the layout of dendritic interactions is particularly relevant because the dendrite of vCH is not only an input region but forms a spatially distributed, inhibitory GABAergic synapse [25], which is part of a circuit for figure-ground discrimination [26, 27]. How this summation affects the dendritic response of vCH and, thus, the input to FD cells can only be addressed by further investigation with more localized stimuli.

**Fine-scale direction tuning of LPTC dendrites**

In order to investigate whether additional fine-scale inhomogeneities in direction tuning originating from individual input elements can be found, we examined several parts of the dendritic tree of a VS1 cell under higher magnification. For example, the pooling of motion signals originating from different pairs of neighbouring ommatidia might take place in locally distinct areas of the dendrite. In addition to the two-dimensional layout of the dendrite, this spatial separation of inputs might also be present in the z-direction: In the lobula plate, motion signals from local elements that are sensitive to vertical motion have been argued to terminate in more superficial tissue layers than those sensitive to horizontal motion [28]. In order to reduce cross talk between calcium signals originating from dendritic branches located in different depth layers of the tissue, we confined excitation to a single plane by using two-photon laser scanning microscopy (TPLSM). Our setup made use of a multifocal excitation scheme to enable the acquisition of entire images at a higher temporal rate than is possible with single focus scanning [29].

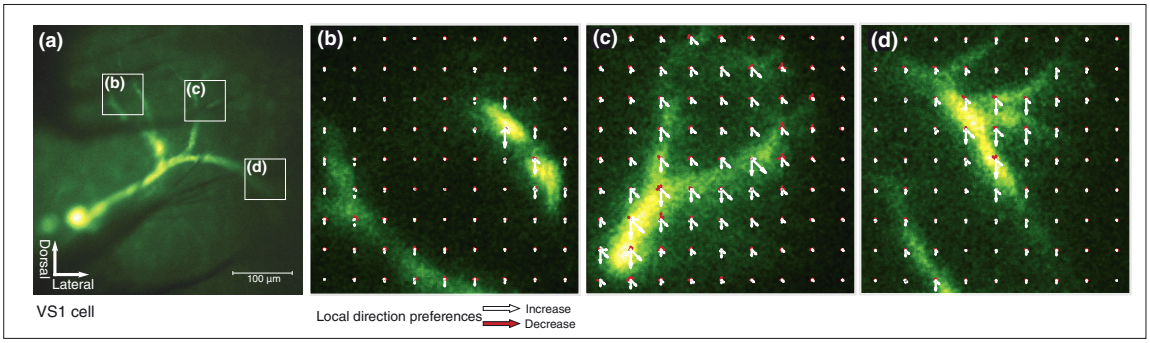


**Figure 4. Fine-scale direction tuning of vertical system (VS)1 cell dendrites.**
(A) VS1 cell stained with Oregon Green BAPTA-1 showing the positions of the recording areas B-D. (B) Calcium response field of the dorsomedial dendrite to a square grating moving in eight directions. Presentation as in Figure 3. (C) Response field of the dorsal and (D) of the lateral dendritic branch of the same VS cell. Images B-D were taken at 10 Hz and 256x256 pixel resolution with multifocal two-photon laser-scanning microscopy.

Figure 4 shows an image of a VS1 cell (A) and three calcium response fields of small dendritic areas examined with TPLSM (B-D). In all these areas direction tuning appears fairly homogeneous over the whole extent of the dendrites. Slight shifts in the preferred direction are present within the imaged regions, but abrupt changes from one branch location to the next are absent. In contrast, the preferred directions of single motion detectors are likely to correspond to the distinct axes of the ommatidial lattice [30]. The smoothness of shifts in direction tuning across dendritic branches therefore suggests that the measured local dendritic activity represents pooled signals from several motion detectors instead of single inputs. Alternatively, the smoothness of shifts might result from the fact that the ommatidial lattice orientations themselves shift across the eye, resulting in spatial differences in the alignment of the stimulus with the ommatidial lattice [30].

## Conclusions

By using calcium imaging to visualize dendritic activation during visual motion in various directions, we were able to obtain a direct view at the local variations in directional selectivity of the dendrites of individual fly LPTCs. These dendritic response patterns form a filter for the evaluation of complex motion patterns in large parts of the visual field of the animal as may be induced during different types of flight manoeuvres. Our experiments show that in the fly LPTCs differences in local preferred directions are maintained on the level of cytosolic calcium concentration changes across the dendrites. Thus, these neurons are capable of providing their postsynaptic targets with a matrix of spatially varying outputs via dendritic synapses or, alternatively, with integrated output via single axonal output synapses. However, rather than simply integrating inputs in a purely retinotopic way, some LPTCs also exhibit local variations in direction selectivity that are the result of indirect non-retinotopic inputs, which converge via extended synapses from the contralateral brain hemisphere. Even though individual motion detector inputs still remain to be uncovered, this direct insight into processes which take place on the dendrites of these cells is a first step to a more detailed understanding about how motion signals are integrated and processed by single neurons in order to generate behaviourally relevant output information.

## Methods

### Flies

Blowflies (Calliphora vicina) were raised in the department's stock at 25°C in a 12h light/12h dark cycle. Experiments were carried out on females collected <3 days after eclosion.

### Preparation

After affixing the fly's thorax in a horizontal position to a glass cover slide, legs, antennae, proboscis and the digestive tract were removed and the openings in the cuticle closed with beeswax. The head was pulled downwards and attached to the thorax to allow access to the head capsule. An opening was cut into the right half of the caudal head cuticle and a hole was cut into the dorsal thorax to insert the reference electrode. The exposed tissue was supplied with insect ringer solution [29] to prevent desiccation.

### Intracellular recording

Sharp borosilicate glass electrodes (G100TF-4, Warner Instruments, Conneticut, USA) were pulled on a Brown-Flaming P-2000 Puller (Sutter Instruments, CA, USA) in order to create resistances of 95 - 110 MΩ when filled with Oregon Green/ 1M KCl. A glass reference electrode was placed in the fly's thorax and connected to a supply of insect Ringer's solution, which also served as the medium for water immersion microscopy when needed. Recording was done in the right lobula plate. Electrode signals were amplified by a factor of 10 using a custom-built amplifier. Responses were sampled at 4000 Hz (DT2801A, Data Translation, MA, USA) and stored on hard disk for offline analysis.

### Calcium Imaging

For the visualization of calcium signals, we filled the tips of the recording electrodes with 15mM Oregon Green 488 (BAPTA)-1 hexapotassium salt (Molecular Probes, OR, USA) in 1.7mM KOH / 33mM (4-(2-hydroxyethyl)-1-peperazineethanesulphonic acid / 3.3mM KCl. The dye was injected by passing hyperpolarizing currents of 0.6 to 1 nA for 7–20 min (varying between experiments) during the determination of cell type by means of electrophysiological recordings. Relative cytosolic Ca2+ concentration changes were monitored by epifluorescence imaging of Ore-

gon Green emission using a dry Leica high-performance liquid chromatography Fluotar 10x and a water immersion Olympus LUMPlan FI/IR 40x objective at an upright fixed-stage microscope (Leica DMLFSA, filter set: excitation BP 470/40 nm, dicroic mirror 510 nm, emission LP 515 nm and BP 530/50 nm) equipped with an electron-multiplying charged-coupled device camera (Andor iXon DV887-BI, Andor Technology PLC, Belfast, Northern Ireland), operated at frame rates of 10 to 26 Hz and at a resolution of 512x512 pixels (pxs). A 488nM laser (FiberTEC, Blue Sky Research, CA, USA), diffused by a frosted glass, was used as an excitation light source for one-photon wide-field imaging. For multifocal two-photon imaging, we used a tuneable Mai-Tai infra-red laser (Spectra-Physics, CA, USA) at 800 nm and a multifocal beam splitter (TriM-Scope, LaVision BioTec, Bielefeld, Germany) set to eight excitation foci covering a scan area of 357x254 px/102x73 µm (for details see [29]). During the recordings, no noticeable bleaching could be found. Usually the reason for terminating a measurement series was a fairly abrupt deterioration of the visually induced calcium response.

**Visual stimulation**

A board of 22x45 green light-emitting diodes (LEDS; each measuring ~4.8 x 2.5 mm, emission maximum at ~570 nm, covered with a LP550 filter in order to reduce interference with fluorescence emission light) was used to simulate a moving high contrast square wave pattern (temporal frequency: 4 Hz, spatial frequency: 10°, mean luminance: ~30 cdm-2). The visible pattern consisted of an octagonal area centred in the frontal visual field with an angular extent of ~80x80°. The LED plate could be pivoted around the centre, allowing us to change the direction of motion in 45°-steps whilst leaving the visible area constant. Stimulus directions were changed pseudorandomly (sequence: 180°-0°-135°-315°-90°-270°-45°-315° or reverse). In some experiments half of the pattern was covered, leaving only the ipsilateral or contralateral part visible. In these series, which were recorded in consecutive blocks for technical reasons, the same stimulus sequence was used in ipsi- and contralateral stimulation to enhance comparability.

**Data analysis**

Camera control and image acquisition were performed using ImSpector 3.20 (LaVision Biotec). Routines written in C (Borland, CA, USA) were used to control visual

stimulation and electrophysiological data acquisition. Frame triggers from the camera were used to synchronize laser scanning, image acquisition, visual stimulation and voltage recording. Matlab (The Mathworks, MA, USA) was used for data analysis. Ca2+ concentration signals were evaluated as background-subtracted pixel-wise changes from baseline levels of the fluorescence of the Ca2+-sensitive dye divided by the baseline value (ΔF/F0). To compensate for global fluctuation in image brightness, mean intensity values of a reference area outside the dendrite were taken and subtracted from the signal. Baseline values F0 were calculated from the mean of the first three images in the series. For selectivity arrow plots, single direction response amplitudes were calculated by averaging ΔF during stimulus duration. For the calculation of the resulting vector, length and direction were calculated by vector addition of the eight vectors representing single directions, while brightness was calculated by scalar addition of the absolute values for the eight single direction responses.

## List of abbreviations

Amx = amacrine cell; BAPTA = 1,2-bis(o-aminophenoxy)ethane-N,N,N‘,N‘-tetraacetic acid; EMCCD = electron-multiplying charge-coupled device; HS = horizontal system; LED = light emitting diode; LPTC = lobula plate tangential cell; px = pixel; ROI = region of interest; TPLSM = two-photon laser-scanning microscopy; vCH = ventral centrifugal horizontal; VS = vertical system.

## Authors‘ contributions

CS performed the experiments, analysed the data and drafted the manuscript. RK conceived the concept of the study and contributed to interpretation of data and writing the manuscript. PT participated in the technical design of the experiments. ME was involved in interpretation of data and writing the manuscript. All authors have read and approved the final manuscript.

## Acknowledgements

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (KU-1520/3). We are grateful to Professor Dr Markus Sauer for allowing laboratory use and for his support of our experiments.

## References

1. Egelhaaf M, Kern R: Vision in flying insects. Curr Opin Neurobiol 2002, 12: 699-706.

2. Borst A, Egelhaaf M: Principles of visual motion detection. Trends Neurosci 1989, 12 :297-306.

3. Wylie DRW, Bischof WF, Frost BJ: Common reference frame for neural coding of translational and rotational optic flow. Nature 1998, 392: 278-282.

4. Britten KH: Mechanisms of self-motion perception. Annu Rev Neurosci 2008, 31: 389-410.

5. Krapp HG: Neuronal matched filters for optic flow processing in flying insects. Int Rev Neurobiol 2000, 44: 93-120.

6. Krapp HG, Hengstenberg B, Hengstenberg R: Dendritic structure and receptive-field organization of optic flow processing interneurons in the fly. J Neurophysiol 1998, 79: 1902-1917.

7. Karmeier K, van Hateren JH, Kern R, Egelhaaf M: Encoding of naturalistic optic flow by a population of blowfly motion-sensitive neurons. J Neurophysiol 2006, 96: 1602-1614.

8. Strausfeld NJ: Functional neuroanatomy of the blowfly's visual system. In Photoreception and Vision In Invertebrates. Ali MA, ed. New York: Plenum Press; 1984: 483-522.

9. Borst A, Egelhaaf M: In vivo imaging of calcium accumulation in fly interneurons as elicited by visual motion stimulation. Proc Natl Acad Sci USA 1992, 89: 4139-4143.

10. Douglass JK, Strausfeld NJ: Visual motion detection circuits in flies: peripheral motion computation by identified small-field retinotopic neurons. J Neurosci 1995, 15: 5596-5611.

11. Oertner TG, Brotz TM, Borst A: Mechanisms of dendritic calcium signaling in fly neurons. J Neurophysiol 2001, 85: 439-447.

12. Haag J, Borst A: Spatial distribution and characteristics of voltage-gated calcium signals within visual interneurons. J Neurophysiol 2000, 83: 1039-1051.

13. Borst A, Single S: Local current spread in electrically compact neurons of the fly. Neurosci Lett 2000, 285: 123-126.

14. Dürr V, Egelhaaf M: In vivo calcium accumulation in presynaptic and postsynaptic dendrites of visual interneurons. J Neurophysiol 1999, 82: 3327-3338.

15. Hengstenberg R: Spike responses of ‚non-spiking' visual interneurone. Nature 1977, 270: 338-340.

16. Haag J, Borst A: Dendro-dendritic interactions between motion-sensitive large-field neurons in the fly. J Neurosci 2002, 22: 3227-3233.

17. Hausen K, Egelhaaf M: Neural mechanisms of visual course control in insects. In Facets of Vision. Stavenga D, Hardie R, eds. Berlin: Springer-Verlag; 1989: 391–424.

18. Single S, Borst A: Different mechanisms of calcium entry within different dendritic compartments. J Neurophysiol 2002, 87: 1616-1624.

19. Dürr V, Kurtz R, Egelhaaf M: Two classes of visual motion sensitive interneurons differ in direction and velocity dependency of in vivo calcium dynamics. J Neurobiol 2001, 46: 289-300.

20. Hausen K: Motion sensitive interneurons in the optomotor system of the fly - 1.The horizontal cells: structure and signals. Biol Cybern 1982, 45: 143-156.

21. Hausen K: Motion sensitive interneurons in the optomotor system of the fly - 2. The horizontal cells: receptive field organization and response. Biol Cybern 1982, 46: 67-79.

22. Krapp HG, Hengstenberg R, Egelhaaf M: Binocular contributions to optic flow processing in the fly visual system. J Neurophysiol 2001, 85: 724-734.

23. Haag J, Borst A: Orientation tuning of motion-sensitive neurons shaped by vertical-horizontal network interactions. J Comp Physiol A 2003, 189: 363-370.

24. Beersma DGM, Stavenga DG, Kuiper JW: Retinal lattice, visual field and binocularities in flies. J Comp Physiol A 1977, 119 :207-220.

25. Gauck V, Egelhaaf M, Borst A: Synapse distribution on VCH, an inhibitory, motion-sensitive interneuron in the fly visual system. J Comp Neurol 1997, 381: 489-499.

26. Warzecha AK, Egelhaaf M, Borst A: Neural circuit tuning fly visual interneurons to motion of small objects. I. Dissection of the circuit by pharmacological and photoinactivation techniques. J Neurophysiol 1993, 69: 329-339.

27. Hennig P, Möller R, Egelhaaf M: Distributed dendritic processing facilitates object detection: a computational analysis on the visual system of the fly. PLoS ONE 2008, 3: e3092.

28. Buchner E, Buchner S, Bülthoff H: Identification of [3H]deoxyglucose-labelled interneurons in the fly from serial autoradiographs. Brain Res 1984, 305: 384-388.

29. Kurtz R, Fricke M, Kalb J, Tinnefeld P, Sauer M: Application of multiline two-photon microscopy to functional in vivo imaging. J Neurosci Meth 2006, 151: 276-286.

30. Petrowitz R, Dahmen H, Egelhaaf M, Krapp HG: Arrangement of optical axes and spatial resolution in the compound eye of the female blowfly Calliphora. J Comp Physiol A 2000, 186: 737-746.

# Representation of motion and orientation in the fly medulla

Christian Spalthoff*, Ralf Gerdes and Rafael Kurtz

This chapter is currently prepared for submission.

Department of Neurobiology, Bielefeld University, Postbox 100131,

33501 Bielefeld / Germany

*Corresponding Author: christian.spalthoff@uni-bielefeld.de, Tel: ++49-(0)521 106 5727,

Fax: ++49-(0)521 106 89034

## Summary:

In insects, the first segregation of motion and direction clues from local brightness modulations is thought to take place in the medulla, but the representation and localization of these responses in this neuropil remain widely unknown. In this study, we used optical imaging with calcium indicators in medulla neuron populations of the blowfly to study the responses to spatial and temporal brightness modulations. We find that these neurons show orientation, but not direction selectivity, a temporal frequency tuning with an optimum higher than that observed in lobula plate tangential cells, and a response both to on- and off-stimuli that is in some cases spatially separated. In this organization, medulla neurons show interesting similarities to some established principles of motion and edge detection in the visual system of vertebrates.

## Introduction

The integration of many individual local brightness signals into a meaningful image of structure and motion of the surroundings is a task that many animals face. Even

though the visual systems of insects and vertebrates differ in many structural aspects, they utilize many common design principles in their parallel processing of pathways that mediate information about color, form and motion [1]. In vertebrates, the neuronal representation of the mechanisms which are responsible for pattern discrimination and the intermediate processing steps necessary for these tasks are relatively well known. Prominent examples are the on- and off-channels formed in the retina and the integration of these channels into orientation-selective units in the visual cortex [2], [3].

In compound eye insects, these mechanisms are less well studied, with motion detection having been the main focus of classical studies in several species like beetles, dragonflies, flies, bees and locusts [4–8]. Recently, a series of studies demonstrated that, similar to the vertebrate visual system, photoreceptor signals are split into separate on and off channels [9], [10]. However, whether these channels enter the motion pathway separately or whether they interact is still controversial [11], [12]. While studies in bees have shown that insects are able to distinguish between clues like pattern orientation [7], [13], object size, or color [14], it is not yet clear where orientation clues are first represented in the visual system. Likewise, the preliminary steps leading to motion detection remain elusive, and it is unknown whether the extraction of stationary image features interacts with the computation of motion information.

In flies, the large cells in the downstream neuropiles of the lobula plate, in which motion information is integrated, are a well studied field [15]. The cells which are responsive for the computational steps in the two neuropils between these stages, however, have been elusive: A good candidate for the segregation of visual information into separate features is the medulla, which retains the columnar retinotopic layout found in the lamina, but also contains a higly complex network of tangential elements and interneurons crossing the boundaries between columns. In addition to the green-sensitive R1-R6 photoreceptors, which provide input to the motion detection pathway, the medulla also receives spectral input from the UV- and blue-sensitive R7 and R8 photoreceptors in flies [16]. Detailed accounts of the anatomical structure of the medulla exist for [17] and Musca [18], with estimates of 60-100 different cells involved in the processing of a single retinotopic column. Unfortunately, functional analysis of the neural substrates of motion or shape detection by electro-

physiological methods is hampered by the small size of neurons in the medulla.

Extracellular single-unit recordings in the medulla have yielded a first documentation of neurons which respond selectively to the orientation of moving or stationary gratings, termed "Class I units" [19]. Later intracellular recordings combined with staining of single neurons led to the conclusion that three different cells, a transmedullary cell, a T2 neuron and a Y-cell (Y19), display different responses to visual stimuli, namely non-specific responses to flicker, selectivity for motion orientation and selectivity for motion direction, respectively [20]. These experiments, however, were only successful in brief recordings from a single neuron of each type, and were able to access only a small subpopulation of the many different neuronal classes in the medulla, still leaving the question open whether orientation selectivity is ubiquitously represented in the medulla and whether an anatomical segregation of different stimulus modalities can be found in the different compartments of this neuropil.

The concept of nondirectional orientation tuning like that found in one of the medulla neurons plays an important role in feature extraction across widely different animal species. Orientation selectivity has been found in neurons of vertebrates like cats, monkeys, rats or pigeons, as well as in advanced processing stages in insects: Orientation selective cells with large receptive fields exist in the brains of dragonflies [5] and bees [21], and in the protocerebrum of flies [22], but their direct input elements remain unknown. Also, the role of nondirectional [23] or only weakly directional [24] motion as a preliminary step in motion detection has been discussed in flies, although these models have so far only been deduced from observations in the later processing stages in the lobula plate.

We have addressed these issues by examining the responses of medulla neurons through population staining with calcium selective dyes, circumventing the need for intracellular recordings or genetically induced labeling methods. One major finding is that neuronal selectivity for different orientations is present early in the invertebrate visual system, similar to what was shown to be the case for the separation of visual input into on and off channels. Our result point to the fact that, once more, surprisingly similar functional design principles are realized in vertebrate and invertebrate visual systems.

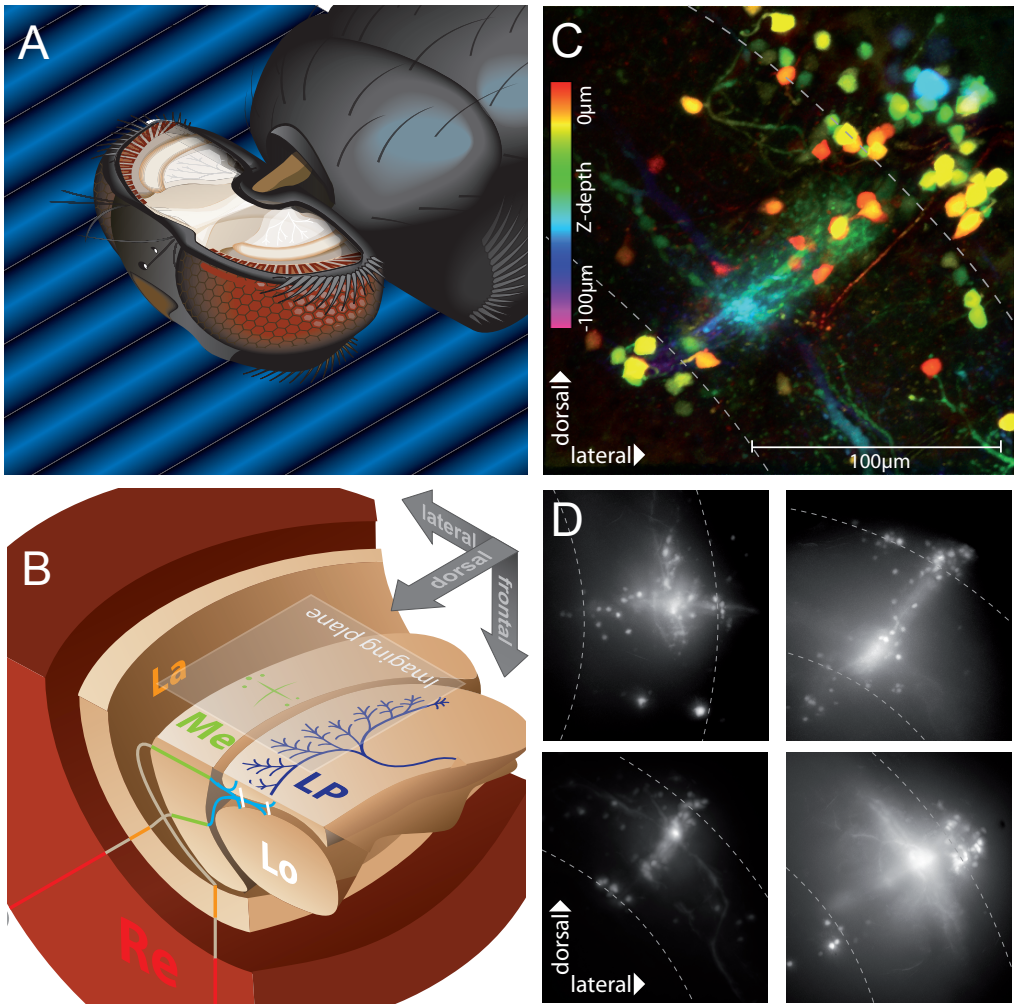


**Figure 1: Morphology of stained neurons in the medulla**
A: Positioning and preparation of the fly. The head of the animal is tilted forward and fixed with beeswax to look down at a screen presenting the visual stimulus. The caudal head capsule is cut open to access the optic lobes. B: Schematic of visual neuropiles of the right hemisphere, same alignment as in A, showing retina (Re), lamina (La), medulla (Me), lobula (Lo) and lobula plate (LP). Two planes represent a horizontal (lower left) and frontal (upper quadrant) cut through the brain. A large cell of the lobula plate (dark blue) and an exemplarystaining pattern in the medulla (green) are drawn in for clarity. The two lines represent the propagation of retinotopic information through the system, with inputs from the frontal and lateral visual fields crossing at the first optic chiasm between lamina and medulla and at the sccond chiasm between medulla, lobula and lobula plate. The imaging plane of the camera is aligned with the caudal surface of the brain, so that dorsal is on top. C: Collapsed confocal image stack of an exemplary Calcium Green dextran population staining in the medulla, showing a columnar bundle of axons, perpendicular tangential dendrites, and two groups of somata located superficially near the proximal and distal boundaries of the medulla. Color coding of the structures indicates depth. Medulla boundaries are indicated by the dashed lines. D: Collapsed images stack obtained with widefield microscopy of four examples of population staining in different parts of the medulla. Stainings usually show two groups of somata and tangential as well as columnar elements, with the crossing point corresponding to the injection site in different layers of the medulla.

## Results

### In vivo local electroporation stains columnar and tangential structures in the medulla

We accessed the brain from the back side of the head capsule, so that our plane of imaging corresponded to the caudal brain surface and the plane in which the stimuli were presented (Figure 1 A and B). Loading of neurons with Calcium Green dextran via electroporation to injection sites near the surface of the medulla led to stereotypic „cross-shaped“ staining patterns (Figure 1C). Visible structures consisted of somata mostly located superficially above the distal and proximal boundaries of the medulla, bundles of straight axonal projections crossing the medulla in a radial direction, and tangential elements arranged orthogonally to these bundles. The point of intersection of these columnar and tangential structures was centered on the injection site and in different stainings was located in different medulla layers. Based on the number of somata visible, the total number of cells stained in one injection was usually between 20 and 40.

Columnar elements usually showed a high number of axons located in the medulla, diffuse arborizations in proximal and distal layers, and relatively small cell bodies situated above the distal rim of the medulla which were connected by long thin protrusions, a structure which matches the anatomy of Mi („Medulla intrinsic“) neurons described in Drosophila (##REF Fischbach 1989). A smaller number of cells had axonal protrusions which extended across the proximal rim of the medulla and dived down into the gap between medulla and lobula plate, a structure described for TM („trans-medulla“) neurons, which possess distally located somata, as well as T2 cells [17], [20]which have their somata located near the surface of the proximal medulla.

Tangential elements had comparatively thick processes, which in cells located more superficially were mostly smooth, but in deeper structures often studded with distinct varicosities. These tangential elements were located in different layers, depending on the injection site. Estimating from the rough layout of the structures, these processes were most commonly located in in the distal part (layers 1-2), the proximal part (layers 9-10) or just distally from the serpentine layer (layer 5-6).

The arborizations were mainly confined to a single depth level, running parallel to the caudal surface of the medulla.

No stained structures extending into the lamina were visible, which would correspond to L1-L5 type lamina interneurons or R7 or R8 photoreceptors, but a co-staining of these cells cannot be ruled out because the curvature of the first optic chiasm obstructs the view to these cells from a caudal observation point.

**Neuronal activity is tuned to grating orientation, but not motion direction**

To examine the responses of the medulla cells to time-varying stimuli, we stimulated the fly with drifting sine wave gratings in the frontal visual field (stimulus size: ca. +/- 40 degrees elevation, +/- 50 degrees azimuth). These stimuli evoked robust and wide-spread increases in cytosolic calcium level in tangential as well as columnar structures (Figure 2B), with small superimposed ripples corresponding to the temporal frequency (4Hz) of the motion stimulus. To avoid saturation effects, the temporal frequency of the pattern was chosen below the optimum contrast freqency for the cells recorded (see below). We compared ΔF/F responses in a given region of interest for four different pattern orientations and two directions each, giving eight stimulus directions at 45°-intervals. The data shown here had the regions of interest centered on the crossing between tangential and columnar elements, since signals usually were strongest in that area. While other regions of the cells often showed faster or slower time courses of the signal, we could not find prominent differences in the direction tuning of different regions in a single staining (Data not shown). If single somata were visble, their calcium signals were usually too weak to discern from background fluorescence changes (but see Figure 5C for an exception).

Even though the medulla has a repetitive structure and staining patterns in different animals were similar, the anatomical orientation, receptive field, and direction tuning was different for each injection site. For this reason we could not simply average individual ΔF/F traces between different animals. To quantify the selectivity to stimulus orientation and direction selectivity for each staining, we plotted each single recording as a as a data point in a polar plot, with response amplitude averaged over the entire duration of stimulus presentation given as the distance of the data point from the center in the direction of stimulus motion (Figure 2C). To these points, we least-mean-square fitted a standard ellipse [25]. This allowed us to take

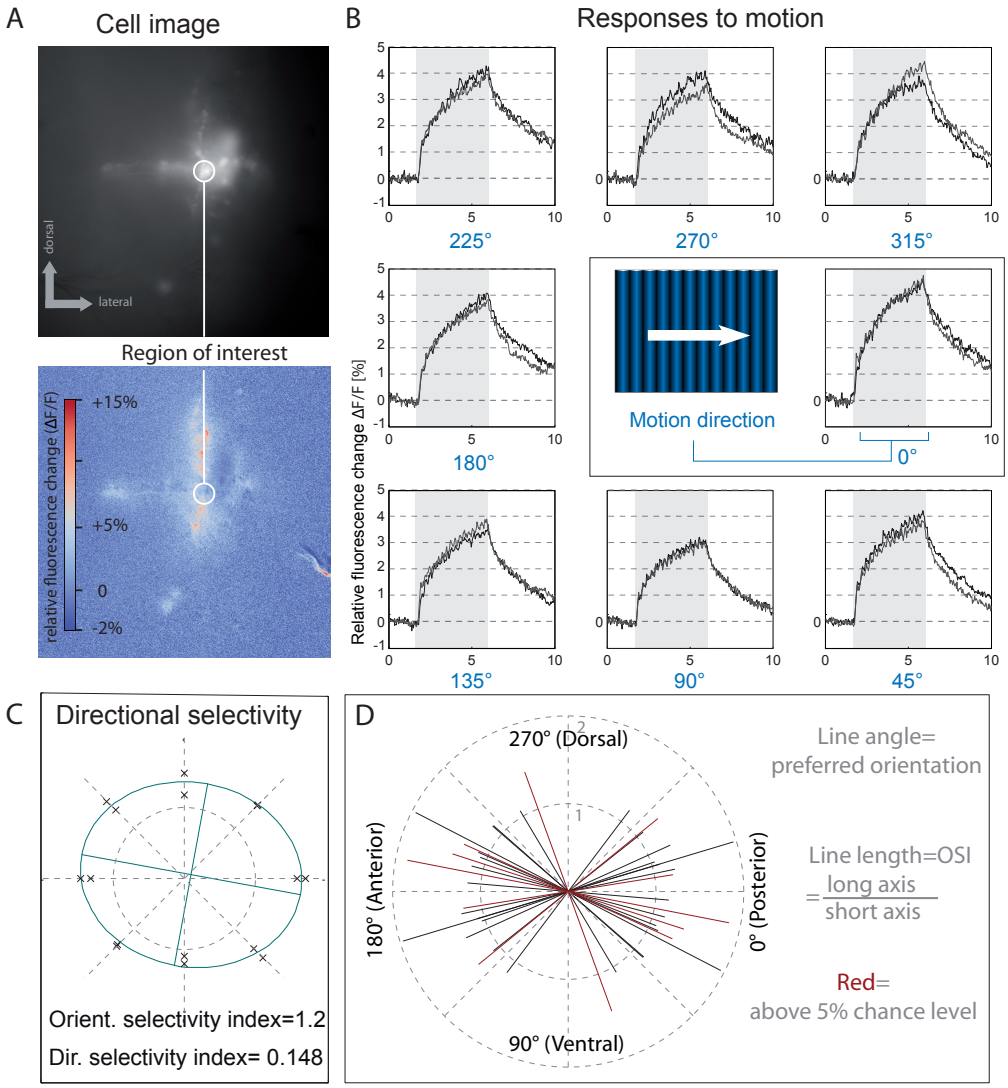


**Figure 2: Optical imaging of medulla neurons during presentation of moving gratings**
A: Top: 10-frame average image of a population of medulla neurons filled with Calcium Green dextran. Bottom: 10-frame average image showing relative differences in local fluorescence intensity during the last 300 ms of stimulation compared to baseline fluorescence. The circle outlines a typical region of interest centered on the injection site. The branch-like structure visible in the bottom right is an artifact caused by movement of a superficial trachea. B: Relative fluorescence changes (ΔF/F) in the region of interest indicated in A in response to a grating drifting in eight directions. Grey rectangles indicate duration of stimulus movement. C: Standard ellipse fitted to the data presented in C. Each data point represents a single recording, with distance from the center corresponding to mean response amplitude during stimulation and vector direction corresponding to stimulus direction. The ellipse plotted in green represents a least-mean-square fit to the data, with the ratio of minor to major radius giving the orientation selectivity index (OSI) and the ratio of center displacement to major axis the direction selectivity index.
D: Preferred orientations and orientation ratios of medulla cells, each line (N=19) represents averaged signals from one population staining. Lines colored in red show significant orientation specificity ($P<0.05$).

the major axis orientation θ as the preferred orientation, and the ratio between long and short axis as a measure for orientation selectivity. This ratio, called orientation selectivity index (OSI) in the following, has a value of 1 for recordings that are not orientation selective and would, theoretically, rise to infinity when responses are obtained only for a single orientation of the grating. The strength of direction selectivity was quantified by taking the displacement of the center of the ellipse from the center of the coordinate system and dividing this value by the length of the longest radius to normalize for differences in ellipse size. Thus, the direction selectivity index (DSI) can vary between 0, indicating no direction selectivity and 1 indicating maximum direction selectivity.

To test for statistical significance, we used a Monte-Carlo-approach to estimate the distribution of chance level orientation or direction selectivity indices obtained for the data set of a given recording. As a basic principle of this approach, the recorded data traces were randomly assigned to the different stimulus conditions. Standard ellipses were fitted to each of 10,000 control datasets generated by this random shuffling procedure As a measure of error probability we then determined how many of the fits to these random datasets produced orientation or direction selectivity indices higher or equal to the one measured.

Results for 21 stainings are shown in figure 2D. Preferred orientations are mainly grouped around the horizontal axis, corresponding to movement in the anterior-posterior or posterior-anterior direction, with 19 of 21 measurements falling into a range of +/-45° around the horizontal. Six of the stainings showed an orientation selectivity above 5% chance level, with orientation selectivity indices generally reaching values between 1 and 2.

Direction selectivity indices were between 0.02 and 0.20, and never showed statistical significance above chance level. For comparison, dendritic calcium signals in optic flow processing lobula plate tangential cells obtained in an earlier experiment gave direction selectivity indices between 0.9 and 0.6, depending on cell type (data from [26]).

Selectivity for grating orientation and motion direction was also tested using an LED board, which allowed us to present stimuli at a higher refresh rate than with the TFT display (4 kHz vs. 60Hz). Four out of nine stainings in this series of experiments

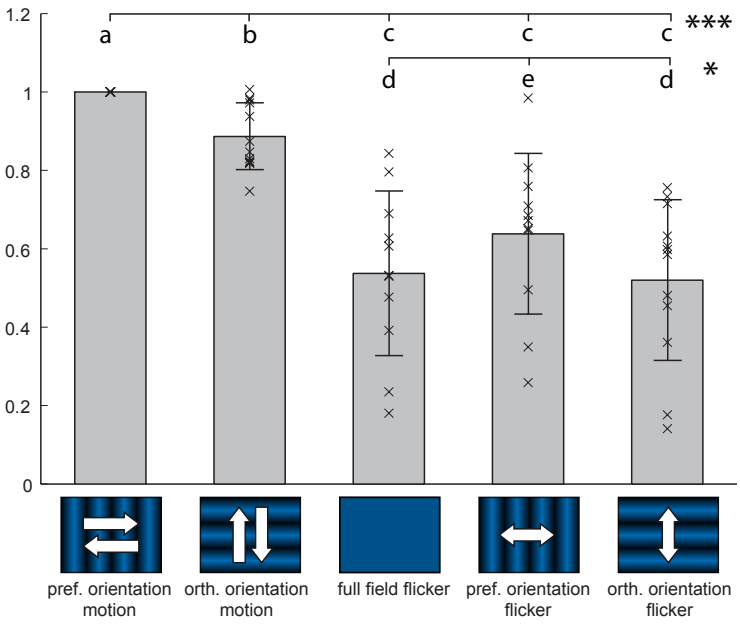


**Figure 3: Flicker stimuli elicit weaker responses than motion**
Response of 12 medulla cell populations during stimulation with two orthogonal orientations of motion, full-field-flicker and two corresponding orientations of counterphase-flicker. Each data point represents average responses from one staining and 2-3 trials, from a circluar Region of Interest centered on the injection site. Data has been normalized to preferred orientation motion response. Grey bars represent the mean ± standard deviation. Different letters denote significant difference (Wilcoxon signed-rank test) at P<0.001 for letters a-c or P<0.05 for letters d-e .

showed significant orientation selectivity as tested by the Monte-Carlo-approach ($p < 0.05$, data not shown). One out of the four orientation-selective stainings also showed direction selectivity ($p < 0.05$).

These results suggest that directional selectivity is not yet or only sporadically present on the processing stage of the medulla columns, but that the orientation of moving stimuli is represented in the signals of medulla neurons. However, since our recordings always consist of a population-average of the stained medulla cells, we cannot rule out that individual signals with stronger selectivity for orientation or direction are pooled into an average with a broader tuning.

### Responses to flicker are weaker than to motion

Motion stimuli always induce local modulations of brightness, which might also elicit responses in cells which are not selective for motion. We tested whether medulla elements respond stronger to motion than to brightness modulations that lack the motion-defining spatio-temporal correlations. For this, we stimulated the cells with several versions of flicker with spatial components similar to the motion stimuli shown in Figure 2B:

Two orientations of a sine wave grating, which smoothly inverted their phase with a 4-Hz-frequency (counterphase flicker), were tested. Additionally, a 4Hz sinusoidal untextured brightness change which lacks pattern orientation information (full-field

flicker) was used A direct comparison of stationary counterphase flicker stimuli with moving gratings is problematic because, apart from the minima and the maxima of the sinusoidalpattern, counterphase flicker induces a lower local brightness modulation than moving gratings as well as full-field flicker. For neurons with receptive fields much smaller than the pattern wavelength, this difference in local brightness modulation between counterphase flicker and motion can be compensated by shifting the flicker grating to align one of its minima or maxima with the receptive field. Therefore, we varied the position of the flicker grating in four steps, each equal to ⅛ of the pattern period.

We found that responses to counterphase flicker, at any of the tested phase positions, as well as full-field flicker were signifcantly lower than to motion stimuli of the same temporal frequency (Figure 3). Orientation preference for counterphase-flicker was the same as for motion. While the lowered contrast of the counterphase stimuli in comparison with the moving grating makes a quantitative comparison of the responses difficult, the attenuated response to full-field flicker in comparison to motion corroborates the presence of spatial filtering, resulting in orientation preference, on the level of the medulla.

**Medulla neurons are tuned to temporal frequency.**

Recordings from lobula-plate tangential cells, direction-selective neurons of the fly, usually show responses that depend not only the velocity of a stimulus, but also on its spatial structure [27]. When drifting gratings with different wavelengths are used as stimuli, the optimum velocity is shifted, such that the temporal frequency remains at a fixed value. As fixed tuning to temporal frequency (rather than velocity) is one of the key predictions of a correlation-type motion detector, the Hassenstein-Reichardt detector, this property of lobula-plate tangential cells has been taken as evidence that is type of motion detector is implemented in the input. Temporal frequency optima of lobula plate tangential cells in Calliphora have been estimated at 2-5 Hz during steady state and 10-20 Hz briefly after stimulus onset [27], [28], In Lucilia temporal frequency optima were shown to rise from a fairly narrow tuning function with a maximum at 2Hz at 2 Hz in resting flies to a broad peak of 2-7 Hz during tethered flight [29].

To measure the velocity tuning of medulla cells, we used sine wave gratings drifting

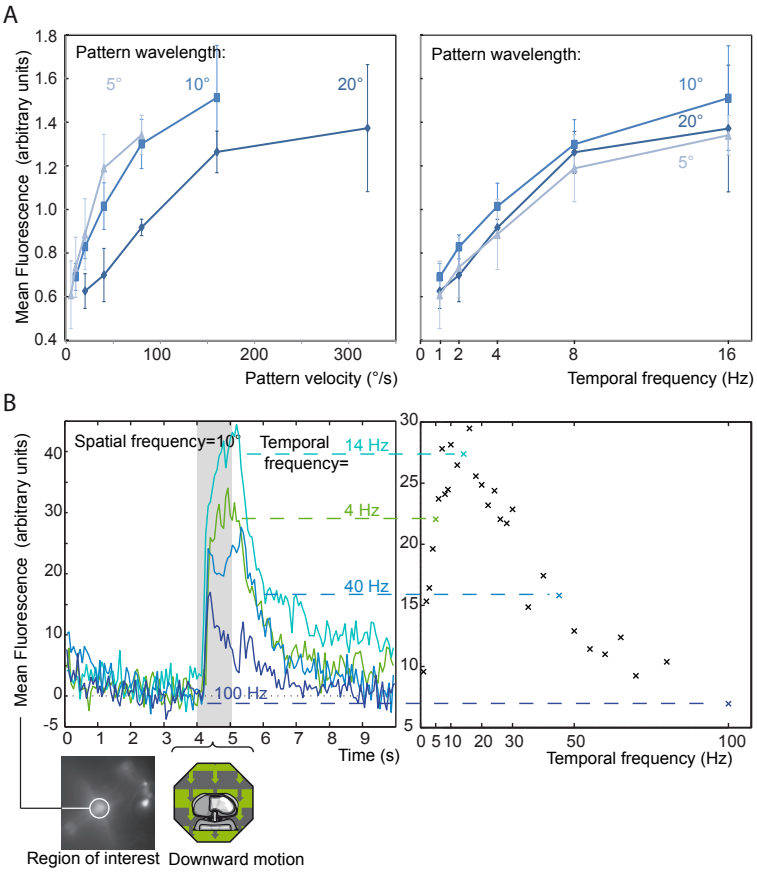


**Figure 4: Tuning to different temporal and spatial frequencies**
A: Average response of medulla population stainings to 4 seconds motion of a drifting sine-wave grating with preferred orientation at different velocities (left) or temporal frequencies (right) and pattern wavelengths. Each data point represents normalized average response from 6 stainings and 19 single recordings, each from a circular ROI each centered on the injection site, +/- SEM. Stimuli were presented on a 60Hz TFT screen.
B: Response of a single population staining (bottom left) to 1 second of downward motion with varying temporal frequency. Each data point represents a single trial response, from a circular ROI centered on the injection site, with four corresponding example ΔF traces shown to the left. Stimuli were presented on a high-speed LED array.

in a direction that elicits a strong response. Pattern wavelength and temporal frequency of the stimulus were varied to give 15 combinations between 5 and 20° and 1-16 Hz (Figure 4A, Data from 6 stainings and 19 individual series. Each series has been normalized to the average response to the set of all 15 stimuli). All individual stainings show response peaks at temporal frequencies equal to or or greater than 8 Hz (right panel), with no difference in response peak visible for the different spatial frequencies. In contrast, when plotting response amplitude versus image velocity, different response maxima for each pattern wavelength are obtained (left panel, same data as in the right). Thus, medulla cells also show the same strong dependence on spatial frequency in their response properties as lobula-plate tangential cells, but are tuned to higher frequencies.

Our data suggest that the peak response of medulla neurons might lie even beyond our highest measured stimulus frequency (16 Hz). Since the 60 Hz frame rate of the TFT screen makes presentation of stimuli with higher temporal frequency proble-

matic, we used an LED array to present a 10° square wave grating drifting at temporal frequencies of 1-100 Hz (Figure 4B). The peak response under these conditions lies at about 15 Hz, which is consistent with the results from the TFT stimulus. The response amplitudes show a marked decrease at temporal frequencies above 20 Hz. This differs from the dynamic properties of lamina monopolar cells, the neurons forming the major input pathways to the medulla, which respond with high gain to brightness fluctuations up to more than 100 Hz. This comparison suggests that the signals from the lamina are subject to temporal processing in the medulla, such as low-pass filtering, which attenuates the responses to high temporal frequencies.

At high temporal frequencies, the time course of the calcium signal shows a second rise after stimulus motion has stopped (Figure 4B, left panel, 40 and 100 Hz traces). A likely explanation of this effect is as follows: temporal frequencies higher than the temporal resolution of the cells might be perceived as an intermediate "grey" blur. Thus, the dark and bright bars of the stationary pattern are effectively seen to change their brightness to an intermediate level at the onset of pattern motion and back to dark and bright when motion stops.. We also tested the temporal frequency tuning using counterphase flicker presented on the LED array (data recorded in another staining, not shown). Similar to the optimum during motion stimulation, the largest responses to flicker were obtained at about 30 Hz and only weak responses were elicited by frequencies above 100 Hz.

**Tangential elements show retinotopic dendritic input and respond to on- and off-edges**

Recently, the possibility of the existence of seperate on- and off-channels in the visual pathway of the fly, a common feature in vertebrate vision, has received much attention: Selective genetic blockage of lamina neurons suggest that the responses of lobula plate tangential cells to on- and off- stimuli are dependent on the input from distinct types of lamina neurons [9].

To examine the representation of on- and off-stimuli in the medulla, and to characterise the visual field of the stained neurons, we stimulated the fly with bright and dark edges moving across the screen (Figure 5). We tested 5 different stainings, 2 with the injection site located in the distal part of the medulla and 3 with the injection site located more proximately in the medulla. All stainings responded to bright

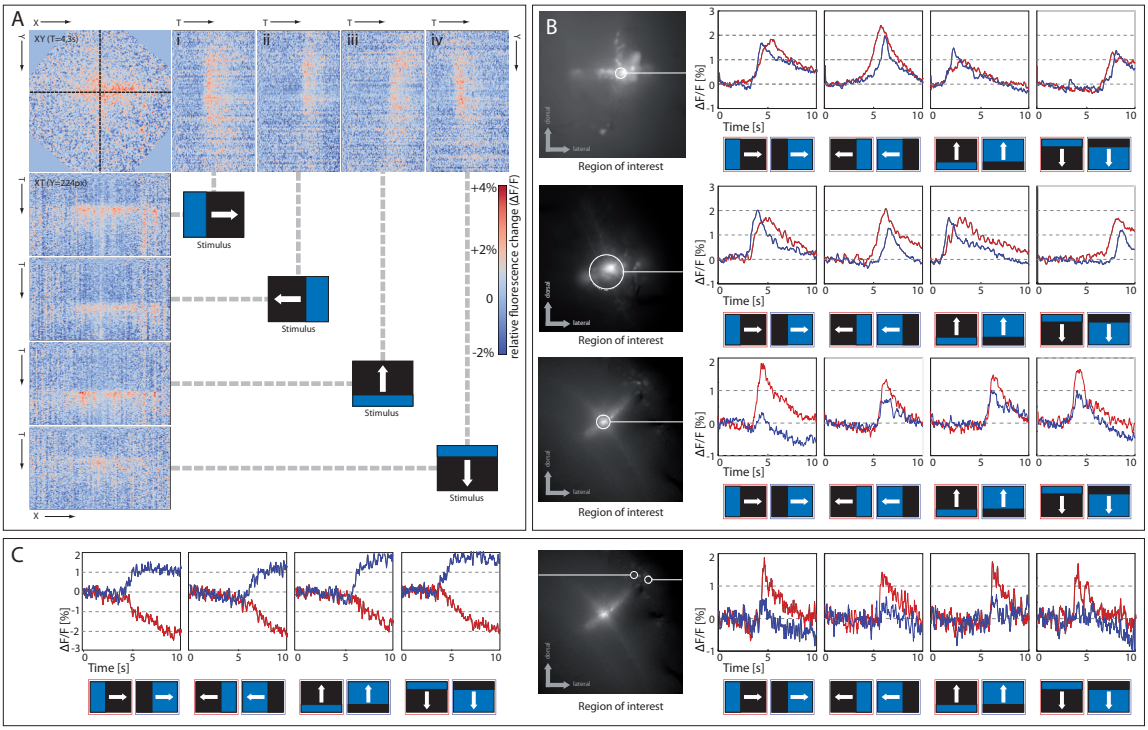


**Figure 5: Responses to moving bright and dark edges**
A: Orthogonal views of a x-y-t-stack of relative fluorescence changes (ΔF/F) in a staining of the distal medulla (same cell as in C, image rotated by 20°). The top left plot shows the spatial activation pattern of the cell population at a point in time, while the plots at the top (i-iv) and to the left show the temporal activation pattern of a single row or column in response to a bright moving bar stimulus. Similar results were obtained in five further stainings.
B and C: Relative fluorescence changes (ΔF/F) in the region of interest indicated in response to a bright edge (red) and dark edge (blue) drifting in four directions. Regions of interest in C are centered on two somata near the distal rim of the medulla.
Stimuli were presented on a 60 Hz TFT screen.

as well as dark edges and to motion in all four directions with transient increases in calcium level that were located in tangential as well as the columnar structures (exemplary response to an on-stimulus shown in Figure 5A). From the time during which the calcium signal increased, we estimate the response of this cell population to cover about 1 second of stimulus travel time. This corresponds to a receptive field of about 8°, which is considerably larger than the typical interommatidial distance of about 1.5° in Calliphora. Interactions among neighboring retinotopic columns are a prerequisite for the computation of direction (as well as orientation) selectivity, and interactions spanning as much as four ommatidia in a row have been shown to be influential in fly local motion computation [30].

In response to edges moving in vertical directions, all stainings showed a consecutive localized activation of the tangential structures, which corresponded to the motion of the stimulus through the visual field: With the stimulus moving upward, the ventral part of the dendrite was activated first while downward led to activation in the dorsal parts of the dendrite. This can be seen in the pattern of activation being tilted rightward in the yt-plot for upward motion (Fig. 5A(iii)), and leftward for downward motion (Fig. 5A(iv)). For edges moving horizontally, we could observe no such localized activation.

In the timing of the on- and off-responses, we could see a difference in the recordings with distal tangential elements stained, but not in the recordings of proximal tangential elements: In the examples for the distal layers shown in Figure 5B, top and middle, off-responses showed a clear displacement in peak timing relative to the on-responses, with off-responses leading during progressive and upward motion, and on-responses leading during regressive and downward motion. This suggests input from spatially separated on- and off-channels to the dendrite of the tangential elements located in the distal medulla. In the stainings where stratifications in the proximal layers of the medulla were stained (Example in Figure 5B, bottom), such direction-specific differences in response timing were not visible.

Typically, we could observe little separated localization of signals associated with on- and off-stimuli. The same areas within the stained cell population usually responded to all stimuli tested, although in some cases on and off responses appeared to differ in strength (see Figure 5B, bottom). In some stainings, however, some of the somata located at the distal rim of the medulla showed responses that differed from the typical population signal (Figure 5C): One soma showed a strong selectivity for stimulus polarity, responding with sustained increases in calcium to off-stimuli and with sustained decreases to on-stimuli, while a different soma displayed transient signals and a strong preference for on-stimuli. An on/off segregation was previously shown to be present in the fly visual pathway, because blocking one of the major types of output neurons from the lamina to the medulla, L1 or L2, led to selective loss of on and off responses, respectively [9]. However, since synaptic terminals of L1 and L2 in the medulla were recently found to respond to on as well as off stimuli [11], it was until now unclear whether on/off segregation takes place in a postsynap-

tic stage of the medulla or later in the lobula complex. The heterogeneity in response characteristics of medulla neurons demonstrated in the present study (Figure 5C) supports the notion that the segregation into separate channels for on and off stimuli takes place in the medulla.

## Discussion

The medulla neuropil of insects is characterized by processing of visual information from the retinotopic elements of lamina and retina and their segregation into parallel pathways by populations of highly ordered, stratified cells. These cells confine their processes into distinct sublayers of the neuropil in a similar fashion as cells of the plexiform layers in the vertebrate retina, suggesting a functional similarity of both systems [1] in the extraction of visual features from the purely retinotopic image represented on the retina. With its large number of densely interwoven neurites, the difficulty of intracellular recordings and the multitude of sensory modalities possibly represented, still relatively little is known about the representation of stimuli in the insect medulla.

In this study, our method allowed us to gain access to signals from medulla cell populations with a large number of previously inaccesible cell types and examine their responses to various spatial and temporal stimulus patterns. Similar to staining with membrane-permeant calcium dyes widely used in vertebrate cortical tissue, identification of individual types of neurons is hampered by the fairly large number of neurons stained in each experiment when using local electroporation. Nevertheless, it is possible to characterize which functional neuronal features exist in general in a given tissue following these staining procedures.

While studies exist about the processing of colour information [31] in the medulla, it has been unclear how motion information is processed and represented in this neuropil, and whether orientation selectivity plays a role as a separate or preliminary computation step to the full directionality emerging at later stages of the visual pathway [32].

Our findings show that orientation selective responses are a widespread feature in a large number of medulla cells of different layers. Due to the fact that we mainly measured population, not single cell responses, the orientation selectivity values we found provide the lower estimate of the orientation selectivity actually present, with

single cell orientation selectivity possibly higher than the values measured. Especially for tangential elements encompassing signals from several columns, an intrinsic broadening of orientation tuning due to combination of several input elements is plausible: While recordings from a single T2 cell have shown sharp orientation tuning [20], recordings from orientation selective wide-field cells from the lobula and lobula plate by Okamura and Strausfeld [22] exhibited a broader orientation selectivity, which was comparable to our recordings, possibly due to the pooling of input elements with diverging orientation selectivity. These findings also suggest that information about edge orientation plays an important role in the late processing stages of the insect visual system.

The possibility of nondirectional motion computation as a prerequisite or parallel pathway to the directional motion computation performed by the Hassenstein-Reichard-detector has also been proposed earlier as an explanation for certain response characteristics of bees in behavioural experiments [7]. These nondirectional responses show a broad frequency tuning with strong responses also visible to temporal frequencies above 50 Hz, while directional responses of fly lobula plate tangential cells exhibit a much lower temporal frequency tuning that already drops off at 10 Hz. These differences led to the conclusion that nondirectional motion computation would have to happen at earlier stages of visual processing [23], with some of this high-frequency information being lost at the later computational stages. This theory would be supported by our finding that medulla cells exhibit responses to a broader spectrum of higher temporal frequencies than lobula plate tangential cells, hinting at a subsequent low-pass-filtering of signals in the computation of directional motion signals. However, while modelling of cell responses with a nondirectional motion detection stage by Higgins [23] predicts a more robust velocity tuning that is largely insensitive to pattern frequency at low velocities, we still observed a generally strong pattern dependence of motion responses in the medulla.

Several earlier works exist which use flicker to assess the combination of spatial and temporal filtering that forms the basis of motion detection [33]. Experiments with directionally selective neurons in the lobula plate have shown that the responses to counterphase flicker stimuli show the same relationship that we found in the medulla: With counterphase flicker, the preferred pattern orientation was orthogonal to the preferred motion direction, i.e. vertical stripes elicited the largest responses

in a H1 cell that responded selectively to horizontal motion [34], while the temporal frequency optimum of these recordings also show the same low values as that for motion.

This orientation selectivity during flicker stimulation was attributed to the input from an array of sampling units with an excitatory center and two horizontally aligned inhibitory regions, which were called „sustaining units" in recordings from the chiasma between medulla and lamina of the flesh fly Lucillia by Arnett [35]. In these recordings, the sampling units comprise several interommatidial angles, reaching 6°-8° in lateral displacement [18].The spatial separation of units responding to on- and off-signals in the units found by Arnett bears a striking resemblance to the spatial displacement between on- and off-signals we found in the tangential processes in the distal medulla, with the difference that the medulla responses are not symmetric, but unidirectional, with responses to on-edges leading in one stimulus direction and off-edges leading in the opposite direction. While the analysis of the significance of lamina interneurons L1 and L2 to convey information about on- and off-stimuli to the later processing stages has recently made large progress due to the application of genetic tools in Drosophila, [10], [11], [36] it is still not clear how this information is further processed and how lateral interaction between optical cartridges are involved in motion and pattern detection.

The retionotopic activation of the tangential structures we found in the medulla shows that the arborizations of these cells are not simply output regions that distribute signals from one column to its neighbours, but serve as input structures that integrate signals from a larger population. A correlation of orientation tuning to dendritic morphology has been shown to exist for the small columnar T2 neurons in the medulla [20], and the same principle could also account for the spatial filtering that leads to orientation selectivity in larger medulla tangential cells. As noted earlier [34] extraction of specific orientation information prior to motion computation might be functionally beneficial to reduce noise introduced by motion which does not originate from coherent structures of a certain size in the environment. A similar design principle appears to exist in vertebrate visual cortex, in which motion-processing areas receive input from areas such as V1, in which one of the major neuronal characteristics is orientation selectivity. [2]

Both the correlation of spatially separated on- and off channels and the use of dendritic morphological layout for spatial filtering are established concepts in vertebrate vision [37], [38]. In the general approach to develop models for visual feature extraction, further investigation of the similarities and differences between taxa will remain a challenging and rewarding field of study.

## Experimental Procedures

### Flies

Blowflies (Calliphora vicina) were raised in the department's stock at 25°C in a 12h light/12h dark cycle. Experiments were carried out on females collected <3 days after eclosion.

### Preparation

After affixing the fly's thorax in a horizontal position to a glass coverslide, legs, antennae, proboscis and the digestive tract were removed and the openings in the cuticle closed with beeswax. The head was pulled downwards and attached to the thorax so that the caudal surface of the head was aligned horizontally parallel to the glass slide. An opening was cut into the right half of the caudal head cuticle and the dorsal thorax was opened to insert the reference electrode. We superfused the exposed tissue with insect ringer solution [39] to prevent desiccation during the staining, and later to provide an immersion medium for microscopy.

### Calcium dye loading

We pulled micropipettes (Warner G150TF-4 glass tubing, Warner Instruments, Hamden, CT, USA) pulled with a Sutter p-97 puller (Sutter Instruments, San Rafael, CA, USA) to have resistances of 8 to 10 MΩ and filled them with 5% Calcium Green-1 dextran (3000MW, Molecular Probes, Eugene, OR, USA) in 50 mM HEPES/5 mM KCl in the tip and 1M KCl in the back end. Under visual control through a stereo microscope, we inserted the microelectrode superficially into the medulla and applied negative current pulses (8 μA amplitude, 30 ms pulses with 270 ms intervals) for 45 s to label the neurons by electroporation (method modified after [40]). In some animals, a second and third injection site was stained in the same manner. Flies were left to incubate for 1-3h at room temperature.

### Morphological identification and calcium imaging

We recorded relative cytosolic Ca2+ concentration changes by epifluorescence imaging of Oregon Green dextran emission using a water immersion 40x (Olympus LUMPlan FI/IR) or 25x (Leica HCX IRAPO L 25x/0.9 W) objective at an upright fixed-stage microscope (Leica DMLFSA) equipped with an electron-multiplying charged-coupled device (EMCCD) camera (Andor iXon DV887-BI, Andor Technology, Belfast, Northern Ireland). Image resolution was 512x512 pixels at frame rates of 15 to 30 Hz. 470 nm excitation light was provided by a Leica Fluo LED4000 light source (filter set: excitation BP 470/40 nm, dicroic mirror 510 nm, emission LP 515 nm and BP 530/50 nm).

### Visual stimulus

We used two different stimulus devices to account for the shortcomings of each method: A high-brightness 10.4" TFT display (F510EK005, Reikotronic, Cologne, Germany) with a frame rate of 60 Hz to present sine-wave gratings, full-field flicker or counterphase flicker (temporal frequency: 1 to 16 Hz, spatial frequency: 5, 10 or 20°, mean white luminance:  1000 cdm-2). Stimuli were designed using OpenGL / Vision Egg [41] and presented using the blue channel through a dichroic glass filter (Blueberry 8, 515 nm short pass, Lee Filters, Hampshire, GB) which prevented bleed-through of the stimulus light to the fluorescence signal. Resulting from the high temporal resolution of the fly's visual system, the use of stimulus devices with a refresh rate below 150 Hz might cause coupling of neuronal activity to the refresh rate. Although these issues are not likely to be critical for the topic of the present study, we validated our major conclusions by the use of an LED-based stimulus device.

For these experiments we used a board of 22x45 green LEDs (each measuring ~4.8 x 2.5 mm/, emission maximum at ~570 nm, covered with a LP550 filter to reduce interference with fluorescence emission light) to simulate a moving high contrast square wave pattern (temporal frequency: up to 200 Hz, spatial frequency: 10°, mean luminance through filter: ~30 cdm-2). The LED board was updated at a rate of 4 kHz using an analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA, USA) and program routines written in C (Borland, Scotts Valley, CA, USA). The visible pattern consisted of an octagonal area centred in the frontal visual field

with an angular extent of ~80x80°. The LED board could be pivoted around the centre, allowing changes of motion direction in 45°-steps while leaving the visible area constant The stimulus consisted of 2 seconds presentation of the stationary pattern, followed by 4 seconds of motion or flicker (1 second for data presented in Fig. 3B), and 4 seconds stationary pattern again. Stimulus presentation order was pseudorandom, and stimulus presentation was paused for at least 10 seconds before starting the next recording.

**Data analysis**

Camera control and image acquisition were performed using ImSpector 3.20 (La-Vision Biotec, Bielefeld, Germany). We used Matlab (The Mathworks, Natick, MA, USA) and ImageJ (U. S. National Institutes of Health, Bethesda, MD, USA ) for data analysis. Ca2+ concentration signals were evaluated as background-subtracted pixel-wise changes from baseline levels of the fluorescence of the Ca2+-sensitive dye divided by the baseline value ($\Delta F/F0$). For the baseline values (F0) we used the average of the images during the first 1.5 seconds in the series. For presentation as mean $\Delta F$ false-color-plots, the image stacks were filtered with a xy-gaussian blur and a 2-frame running average in t-direction.

## Acknowledgements

We thank Jens Lindemann for generating the motion stimuli programs and helping with image processing routines.

References

[1] J. R. Sanes and S. L. Zipursky, "Design Principles of Insect and Vertebrate Visual Systems," Neuron, vol. 66, no. 1, pp. 15-36, Apr. 2010.

[2] D. H. Hubel and T. N. Wiesel, "Receptive fields, binocular interaction and functional architecture in the cat's visual cortex," The Journal of Physiology, vol. 160, pp. 106-154, Jan. 1962.

[3] D. Ferster and K. D. Miller, "Neural mechanisms of orientation selectivity in the visual cortex," Annual Review of Neuroscience, vol. 23, pp. 441-471, 2000.

[4] B. Hassenstein, "Wie sehen Insekten Bewegungen?," Die Naturwissenschaften, vol. 48, pp. 207-214, 1961.

[5] D. O'Carroll, "Feature-detecting neurons in dragonflies," Nature, vol. 362, no. 6420, pp. 541-543, Apr. 1993.

[6] A. Borst and J. Haag, "Neural networks in the cockpit of the fly," J. Comp. Physiol. [A], vol. 188, no. 6, pp. 419-437, Jul. 2002.

[7] M. V. Srinivasan, S. W. Zhang, and K. Chandrashekara, "Evidence for two distinct movement-detecting mechanisms in insect vision," Naturwissenschaften, vol. 80, pp. 38-41, Jan. 1993.

[8] A. C. James and D. Osorio, "Characterisation of columnar neurons and visual signal processing in the medulla of the locust optic lobe by system identification techniques," Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology, vol. 178, no. 2, pp. 183-199, Feb. 1996.

[9] M. Joesch, B. Schnell, S. V. Raghu, D. F. Reiff, and A. Borst, "ON and OFF pathways in Drosophila motion vision," Nature, vol. 468, no. 7321, pp. 300-304, Nov. 2010.

[10] D. F. Reiff, J. Plett, M. Mank, O. Griesbeck, and A. Borst, "Visualizing retinotopic half-wave rectified input to the motion detection circuitry of Drosophila," Nature Neuroscience, vol. 13, no. 8, pp. 973-978, Aug. 2010.

[11] D. A. Clark, L. Bursztyn, M. A. Horowitz, M. J. Schnitzer, and T. R. Clandinin, "Defining the computational structure of the motion detector in Drosophila," Neuron, vol. 70, no. 6, pp. 1165-1177, Jun. 2011.

[12] H. Eichner, M. Joesch, B. Schnell, D. F. Reiff, and A. Borst, "Internal structure of the fly elementary motion detector," Neuron, vol. 70, no. 6, pp. 1155-1164, Jun. 2011.

[13] S. Zhang, S. Schwarz, M. Pahl, H. Zhu, and J. Tautz, "Honeybee memory: A honeybee knows what to do and when," The Journal of Experimental Biology, vol. 209, no. 22, pp. 4420-4428, Nov. 2006.

[14] M. Giurfa, "Conditioning procedure and color discrimination in the honeybee Apis mellifera," Die Naturwissenschaften, vol. 91, no. 5, pp. 228-231, May 2004.

[15] A. Borst, J. Haag, and D. F. Reiff, "Fly motion vision," Annual Review of Neuroscience, vol. 33, pp. 49-70, 2010.

[16] S. Yamaguchi, C. Desplan, and M. Heisenberg, "Contribution of photoreceptor subtypes to spectral wavelength preference in Drosophila," Proceedings of the National Academy of Sciences of the United States of America, vol. 107, no. 12, pp. 5634-5639, Mar. 2010.

[17] K. F. Fischbach and A. P. M. Dittrich, "The optic lobe of Drosophila melanogaster. I. A Golgi analysis of wild-type structure," Cell and Tissue Research, vol. 258, no. 3, pp. 441-475, 1989.

[18] N. J. Strausfeld, Atlas of an insect brain. Berlin: Springer-Verlag, 1976.

[19] L. G. Bishop, D. G. Keehn, and G. D. McCann, "Motion detection by interneurons of optic lobes and brain of the flies Calliphora phaenicia and Musca domestica.," J. Neurophysiol., vol. 31, no. 4, p. 509, 1968.

[20] J. K. Douglass and N. J. Strausfeld, "Retinotopic pathways providing motion selective information to the lobula from peripheral elementary motion detecting circuits," The Journal of Comparative Neurology, vol. 457, no. 4, pp. 326-344, Mar. 2003.

[21] E.-C. Yang and T. Maddess, "Orientation-sensitive Neurons in the Brain of the Honey Bee (Apis mellifera)," Journal of Insect Physiology, vol. 43, no. 4, pp. 329-336, Apr. 1997.

[22] J. Okamura and N. J. Strausfeld, "Visual system of calliphorid flies: Motion- and orientation-sensitive visual interneurons supplying dorsal optic glomeruli," The

Journal of Comparative Neurology, vol. 500, no. 1, pp. 189-208, Jan. 2007.

[23] C. M. Higgins, J. K. Douglass, and N. J. Strausfeld, "The Computational Basis of an Identified Neuronal Circuit for Elementary Motion Detection in Dipterous Insects," Visual Neuroscience, vol. 21, no. 4, pp. 567-586, 2004.

[24] S. Single and A. Borst, "Different mechanisms of calcium entry within different dendritic compartments," J.Neurophysiol., vol. 87, no. 3, pp. 1616-1624, Mar. 2002.

[25] Batschelet, Circular Statistics in Biology, vol. 24. Academic Press, 1981.

[26] C. Spalthoff, M. Egelhaaf, P. Tinnefeld, and R. Kurtz, "Localized direction selective responses in the dendrites of visual interneurons of the fly," BMC Biology, vol. 8, p. 36, 2010.

[27] K. Hausen, "Motion sensitive interneurons in the optomotor system of the fly - 2. The horizontal cells: Receptive field organization and response," Biological Cybernetics, vol. 46, no. 1, pp. 67-79, Dec. 1982.

[28] R. Hengstenberg, "Common visual response properties of giant vertical cells in the lobula plate of the blowflyCalliphora," J. Comp. Physiol. [A], vol. 149, pp. 179-193, 1982.

[29] S. N. Jung, A. Borst, and J. Haag, "Flight Activity Alters Velocity Tuning of Fly Motion-Sensitive Neurons," The Journal of Neuroscience, vol. 31, no. 25, pp. 9231 -9237, Jun. 2011.

[30] F. H. Schuling, H. A. K. Mastebroek, R. Bult, and B. P. M. Lenting, "Properties of elementary movement detectors in the fly Calliphora erythrocephala," Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, vol. 165, no. 2, pp. 179-192, Mar. 1989.

[31] J. Morante and C. Desplan, "The color-vision circuit in the medulla of Drosophila," Current Biology: CB, vol. 18, no. 8, pp. 553-565, Apr. 2008.

[32] J. P. Dyhr and C. M. Higgins, "Non-directional motion detectors can be used to mimic optic flow dependent behaviors," Biol. Cybernetics, vol. 103, pp. 433-446, Dec. 2010.

[33] A. Borst and M. Egelhaaf, "Principles of visual motion detection," Trends

Neurosci., vol. 12, no. 8, pp. 297-306, Aug. 1989.

[34] D. Dvorak, M. V. Srinivasan, and A. S. French, "The contrast sensitivity of fly movement-detecting neurons," Vision Research, vol. 20, no. 5, pp. 397-407, 1980.

[35] D. W. Arnett, "Spatial and temporal integration properties of units in first optic ganglion of dipterans," J. Neurophysiol., vol. 35, no. 4, pp. 429-444, Jul. 1972.

[36] J. Rister et al., "Dissection of the peripheral motion channel in the visual system of Drosophila melanogaster," Neuron, vol. 56, no. 1, pp. 155-170, Oct. 2007.

[37] H. Jia, N. L. Rochefort, X. Chen, and A. Konnerth, "Dendritic organization of sensory input to cortical neurons in vivo," Nature, vol. 464, no. 7293, pp. 1307-1312, Apr. 2010.

[38] R. Shapley, M. Hawken, and D. Xing, "The dynamics of visual responses in the primary visual cortex," in Computational Neuroscience: Theoretical Insights into Brain Function, vol. 165, Elsevier, 2007, pp. 21-32.

[39] R. Kurtz, M. Fricke, J. Kalb, P. Tinnefeld, and M. Sauer, "Application of multiline two-photon microscopy to functional in vivo imaging," Journal of Neuroscience Methods, vol. 151, no. 2, pp. 276-286, Mar. 2006.

[40] T. Fujiwara, T. Kazawa, S. S. Haupt, and R. Kanzaki, "Ca2+ imaging of identifiable neurons labeled by electroporation in insect brains," Neuroreport, vol. 20, no. 12, pp. 1061-1065, Aug. 2009.

[41] A. D. Straw, "Vision egg: an open-source library for realtime visual stimulus generation," Frontiers in Neuroinformatics, vol. 2, p. 4, 2008.

# Examination of fly motion vision by functional fluorescence techniques

Rafael Kurtz, Julia Kalb and Christian Spalthoff

**This chapter was published as: Rafael Kurtz, Julia Kalb, Christian Spalthoff, Examination of fly motion vision by functional fluorescence techniques, Frontiers in Biosciences, 2008 Jan 1; 13:3009-21.**

Neurobiologie, Universitat Bielefeld, Postfach 100131, D-33501 Bielefeld, Germany

## Abstract

Over the past years, classical electrophysiological approaches to elucidate the functioning of nerve cells have been complemented by functional optical methods, in particular fluorescence imaging. This review illustrates how optical methods have proved helpful in the analysis of the neuronal principles underlying visual motion processing in the fly, a model system which allows physiological investigation under in vivo conditions. Many aspects of dendritic processing in large-field motion-sensitive neurons of Calliphora have been investigated by Ca2+ imaging. In addition, the function of Ca2+ can be addressed directly by manipulating its concentration via UV photolysis of caged Ca2+. The extraction of specific motion information from visual stimuli depends on interactions between individual neurons. A powerful technique to dissect the motion-vision circuit is the photoablation of single neurons. By selective photoablation the role of individual neurons within synaptic networks has been clarified. Further advances in the disclosure of visual motion processing may in the future be achieved by imaging the activity of single neurons during the processing of natural inputs. Moreover, the combination of genetic tools with functional fluorescence approaches will help elucidate the role of classes of neurons in the visual motion pathway of the blowfly's smaller companion, the fruitfly Drosophila.

## Introduction

When trying to understand how a neural system manages a specific task, the experimenter needs a technical approach that provides information on the activity of nerve cells or a method for manipulating neuronal activity. In this review we will illustrate how optical methods have been utilized to this end in a particular model system - visual motion-processing in the fly brain. This model system demonstrates in an exceptional way the usefulness of various fluorescence-based and optical techniques for studying the processes taking place in the intact brain of a living animal. Several excellent reviews have been written about optical techniques that we will address in this review, namely Ca2+ imaging, UV photolysis of ‘caged Ca2+‘ and laser ablation (1-4). However, we will focus on aspects of how these techniques have been applied to resolve specific questions in the context of fly motion vision.

All optical methods used so far to study fly motion vision have been built on the feasibility of introducing fluorescent substances or caged compounds selectively into single neurons or into small numbers of neurons. Such a specific targeting of cells requires a combination of optical techniques either with electrophysiological methods or with genetic tools. Before describing these optical techniques in detail, this review will briefly outline the basic principle of electrical recording of fly visual motion-sensitive neurons. The genetic approaches that are increasingly becoming tools of choice for the study of fly motion vision in combination with fluorescence techniques are addressed later in this review.

### Optic-flow processing neurons in the fly brain

During flight a fly is confronted with a rapid succession of images on its eyes, so-called optic flow. It is the task of the brain to make sense of this optic flow and to employ it for the control of a multitude of locomotor behaviours, such as targeted changes in flight direction, collision avoidance and the accurate initiation of landing manoeuvres. The velocity of flight movements – during sudden turns the blowfly Calliphora can reach more than 3000 degrees per second (5) – gives an impression of how challenging it is to use optic flow for the rapid control of locomotion (6). Calliphora has proven to be an outstanding model system for tracing activities in the brain that serve to process optic flow from the eyes (7-10), since experimental analyses can be conducted here employing a broad spectrum of methods.

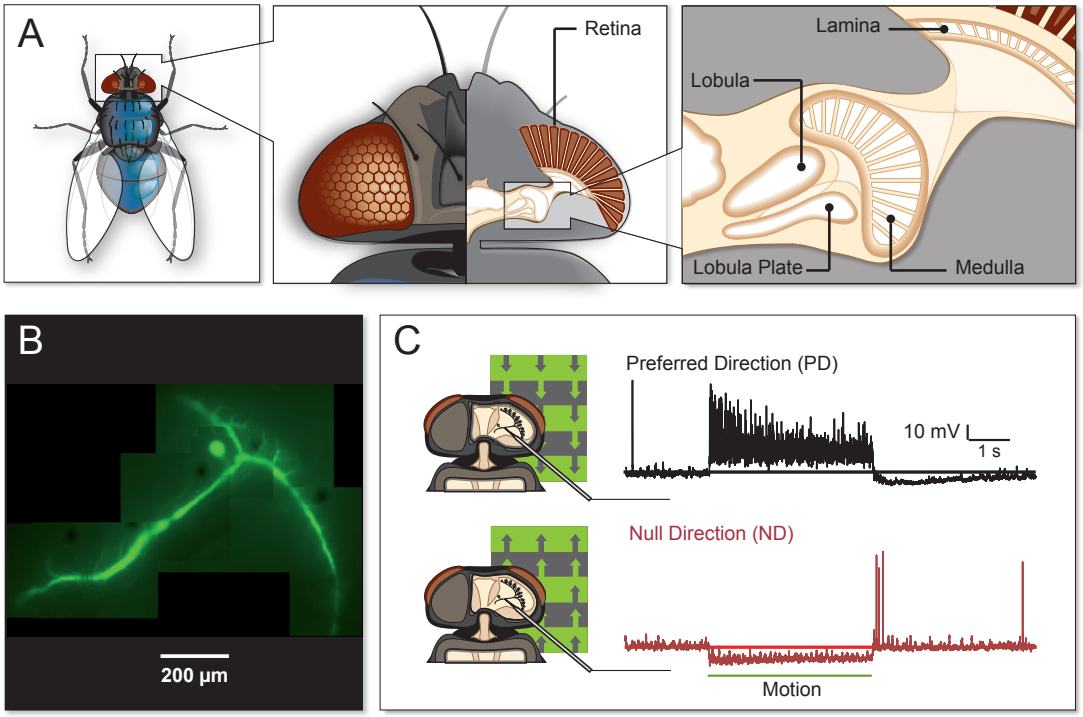


**Figure 1. Neuronal processing of visual motion in the fly brain.**
A, Schematic horizontal cross-section through the fly head. Photoreceptors in the retina send their axons to the lamina. Both the lamina and the next processing stage, the medulla, are organized into columns that represent positions in the visual space in a retinotopic way. This means that in lamina and in medulla the neighbourhood relationships between image points are conserved. Neurons in the lobula complex, consisting of two structures, the lobula and the lobula plate, integrate the outputs of medulla neurons in a specific way. About 60 lobula plate tangential cells (LPTCs) are known and can be individually identified. These neurons respond in a directionally selective way to visual motion within large receptive fields, comprising in some cases almost the whole visual hemisphere. B, Image of a Vertical-System LPTC (VS-neuron). The cell was penetrated with a sharp glass electrode and filled with a fluorescent dye. C, Intracellular recording of changes in the axonal membrane potential of a VS-neuron during presentation of a moving pattern. During downward motion, the PD, a graded depolarisation is observed (top). Fast depolarizing transients of variable amplitude ('spikelets') are superimposed on the graded depolarization and are occasionally present during rest. The response to PD motion is followed by a long-lasting after-hyperpolarisation (AHP). Upward motion, the ND, causes a graded hyperpolarisation in the axon (bottom). Cessation of motion elicits a brief burst of spikelets.

Steering an animal's locomotor behaviour based on optic flow information is a complex task. It is therefore surprising that there appears to be only a small number of neurons that integrate visual motion information over a large part of the visual field. These neurons, the tangential cells in the fly's lobula plate (LPTCs), form a set of about 60 individually identifiable cells in each brain hemisphere (Figure 1A,B) (7, 10, 11). The LPTCs integrate a large number of inputs and owe their sophisticated motion-computation capacity to complex pre-processing of visual stimuli in upstream processing layers. In the first stage of the visual system, the retina, photoreceptor cells only register the brightness in a certain part of the visual field (Figure 1A). Motion information is not explicitly given by the photoreceptor activity but has to be computed in subsequent processing stages from the pattern of brightness changes as sensed by the array of photoreceptors (12). Motion detection is thought to be implemented on a local basis by neuronal circuits in the medulla (13). LPTCs pool these local motion signals on large, retinotopically organized dendrites (14, 15). By integrating motion signals over large parts of the visual field LPTCs can extract relevant information from optic flow. This comprises information about the fly's self-motion and about the three-dimensional layout of its environment (16-18).

**Recording electrical activity of fly visual motion-sensitive neurons**

One crucial advantage of the fly as a model system for the study of motion vision is its accessibility to a large spectrum of physiological techniques. The position of LPTCs near the surface of the fly brain and their comparatively large size makes it possible to apply electrophysiological as well as optical methods in a nearly intact preparation. This allows an examination of the processes occurring in the motion-vision centre of the brain while the animal perceives its natural sensory inputs (9, 10). In the classic electrophysiological approach cell activity is recorded by measuring cell membrane potential with a sharp glass electrode inserted into the neuron (intracellular recording) or by placing an electrode close enough to the neuron to record its action potentials (extracellular recording).

Figure 1C illustrates the response of a VS-neuron ('Vertical System') to visual motion, registered by an intracellular electrode inserted into its axon. VS-neurons form one of the major output elements of the fly's motion-vision system (19, 20). In the lobula plate of Calliphora, there are ten VS-neurons per brain hemisphere. They respond predominantly to vertical motion within dorso-ventrally elongated sections

of the visual field (21). The sensitivity of different VS-neurons is shifted along the azimuth, so that the entire VS ensemble covers nearly the entire visual hemifield. A prominent depolarisation is registered during downward motion in the receptive field of the VS-neuron, the cell's preferred direction (PD). In contrast, motion in the so-called null direction (ND) leads to membrane hyperpolarisation. These graded axonal voltage responses are superimposed by fast depolarising transients, in particular during PD motion and after the cessation of ND motion (22). Unlike ordinary action potentials these transients are variable in amplitude and have therefore been termed 'spikelets' by some researchers.

## Monitoring activity in the fly motion system by calcium imaging

### Investigation of dendritic processing by Ca2+ imaging

Electrophysiological recordings of neuronal activity generally provide information on neuronal output signals. Unfortunately, monitoring of output activity is often insufficient to understand how neurons process incoming signals. LPTCs possess large dendrites, and these form major sites of information processing (15, 23). Electrical recordings from small dendritic branches are hardly feasible, but fluorescence microscopy provides a means to examine dendritic computation. In particular Ca2+ imaging can help making local dendritic activity visible.

In LPTCs, as in many other neurons, Ca2+ is a good marker of activity because its cytosolic concentration is largely regulated by the activity of depolarisation-activated Ca2+ channels (24). Different parts of the neurons may differ in the level of their depolarisation during ongoing signal processing, depending on the sites of local synaptic inputs and on the activation of voltage-dependent ion channels. Hence, imaging of cytosolic Ca2+ concentration changes presents a means to visualize the neuronal activation pattern (4). In order to monitor the changes in the cytosolic Ca2+ concentration of an individual cell, a fluorescent Ca2+ indicator dye has to be loaded into the neuron. In the case of LPTCs, this is accomplished by iontophoretic injection of charged dye molecules into the neuron from an intracellular electrode. Ca2+ dyes change their fluorescence properties upon binding of Ca2+. As a consequence, the intensity of the fluorescence emitted from a dye-filled neuron upon excitation with a specific wavelength is dependent on the concentration ratio

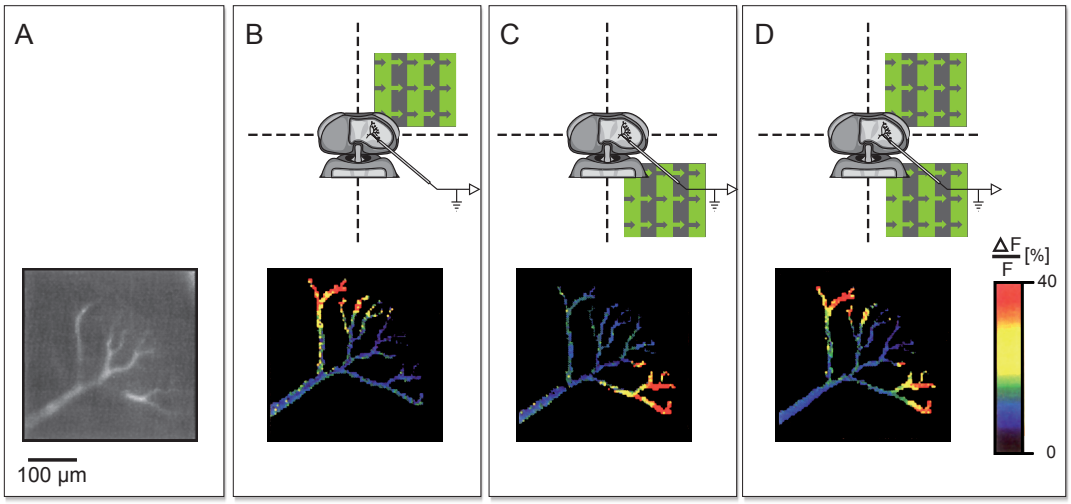


**Figure 2. Retinotopic dendritic organisation of LPTCs as demonstrated by Ca2+ imaging.** An HSE-cell ('Horizontal System Equatorial') was filled with a Ca2+-sensitive dye (Ca2+-Green 1) via the electrode during intracellular recording. Changes in fluorescence intensity during specific excitation of the dye were monitored by a charged-coupled device (CCD-camera) mounted on the microscope. An increase in the calculated fluorescence signal (deltaF/F) indicates a rise in the cytosolic Ca2+ concentration relative to its resting level. The color-coded signal is only shown in regions with high staining intensity, i.e. the major dendritic branches and the axon (see raw fluorescence image in A), in order to decrease noise in the calculation of deltaF/F-values. B, During presentation of PD motion (front-to-back) in the upper visual field Ca2+ rises predominantly in dorsal dendritic branches (color-coded image taken 7.5 s after motion onset). C, PD motion in the lower visual field leads to Ca2+ accumulation in the ventral dendrite. D, Simultaneous motion in both parts of the visual field induces Ca2+ accumulation in both ventral and dorsal dendritic branches. Experimental data from (75).

of Ca2+-free versus Ca2+-bound dye molecules, and can thus be used to estimate the intracellular Ca2+ concentration. Two different classes of Ca2+ dyes have been used in LPTCs: 1) Fura-2 and some of its analogues, which combine a stilbene fluorophore with a Ca2+-binding group (25), and 2) Calcium Green or Oregon Green 488 BAPTA, which are derivatives of fluorescein (26). Whereas the first group of dyes requires excitation in the near-UV range, the second group can be excited with visible light. In LPTCs, the first Ca2+ imaging experiments demonstrated that the dendrites possess a retinotopic input structure (14). This was concluded from the fact that dendritic Ca2+ accumulation is restricted to a certain section of the dendrite when motion is presented only in a sub area of the receptive field (Figure 2).

Axonal membrane potential responses of LPTCs have been recorded during the presentation of motion in restricted sections of the receptive field (18, 21, 27). These experiments have shown that motion sensitivity and local direction selectivity vary

across the visual field in a highly ordered way. These motion sensitivity patterns, the so-called 'response fields', are specific for each of the LPTCs and were assumed to be matched to the motion patterns that are generated on the eyes during certain types of self motion. Variations in motion sensitivity and direction selectivity across the dendrite can also be monitored directly by Ca2+ imaging. However, the resolution of conventional wide-field microscopy is often insufficient to resolve potential differences in Ca2+ dynamics between small neighbouring neurites. Two-photon laser-scanning microscopy provides a higher spatial resolution than wide-field microscopy (3, 28). In wide-field microscopy image blur is caused by emission from out of focus planes within the sample. The superior spatial resolution of two-photon microscopy relative to wide-field microscopy is a consequence of the fact that the simultaneous absorption of two photons is required to bring a fluorophore to the excited state (Figure 3A). Thus, only in the focal plane of the laser, which is used to scan the sample, is excitation intensity sufficiently high to produce fluorescence emission. One drawback of laser-scanning microscopy is its relatively slow rate of image acquisition. Frame rates are usually not higher than in wide-field microscopy, since the scan speed of the laser is limited by the inertia of moving parts. This constraint is removed, to a certain extent, by simultaneous scanning with several laser beams. In one implementation of the multifocal principle, the laser beam is split into an array of up to 64 unitary beams which can be used to create multiple excitation foci in the sample (29, 30). This excitation principle has been used to monitor Ca2+ signals at small dendritic branches of fly LPTCs during motion in different directions (Figure 3C).

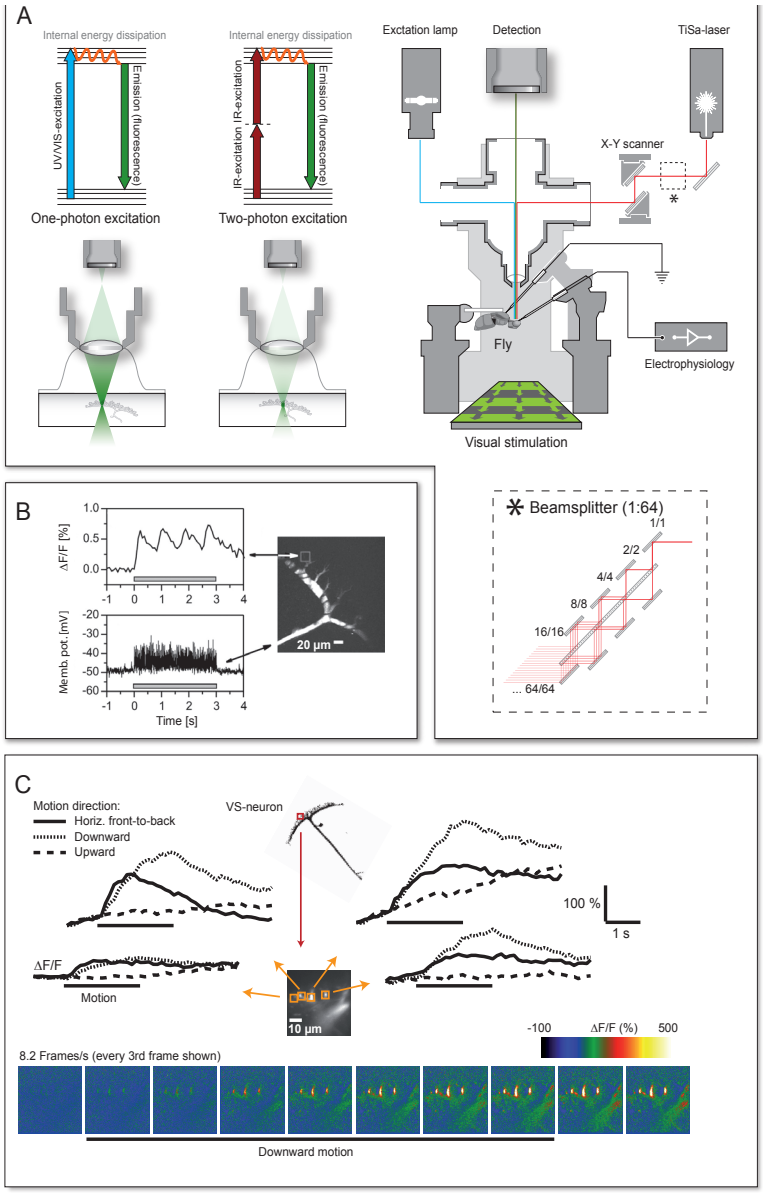


**Figure 3.** A The principles of one-photon (left) and two-photon excitation (middle) illustrated in Jablonski diagrams (top) and sketches of the resulting spatial distributions of signal generation in one- and two-photon microscopy (bottom). Visible or UV light is used for excitation in one-photon microscopy, whereas near-infrared light is used in two-photon microscopy. A quadratic dependency of excitatition probability on laser intensity arises from the nonlinear nature of two-photon excitation. Excitation probability has a quadratic dependency on laser intensity in two-photon microscopy, since nearly simultaneous absorption of two photons is required. This confines the occurrence of excitation events to the vicinity of the laser focus and thus reduces image blur caused by out-of-focus fluorescence. Additionally, photodamage is reduced by the confinement of excitation to the focus. A schematic of a microscope set up (right) illustrates the use of two-photon microscopy in the study of fly motion vision. A fly is immobilized with bees wax, mounted on a microscope stage and stimulated with moving visual patterns. Micromanipulators holding recording and reference electrodes are installed to perform simultaneous Ca2+ imaging and electrophysiological recording. Widefield fluorescence excitation with a Hg-lamp is available in addition to two-photon excitation by a pulsed Titanium-sapphire laser. In contrast to conventional two-photon microscopes, in multifocal two-photon microscopy a single laser beam is split up into several beamlets. One way to multiply the number of laser foci in the sample is the use of a mirror-optical beamsplitter (see inset indicated by asterisk), which generates an array of laser foci by repeated separation of the laser beams at a 50%-beamsplitter mirror and reflection at high-reflectivity mirrors. Multifocal laser scanning accelerates image acquisition, but necessitates the use of a camera instead of a photomultiplier tube for the detection of emission light. See (29) for a critical evaluation of multifocal two-photon microscopy and its application to Ca2+ imaging in fly LPTCs. B, Example of two-photon Ca2+ imaging during simultaneous intracellular recording of the axonal membrane potential. The fluorescence signal within a small area of the dendrite of a VS-neuron filled with Ca2+-Green 1 shows characteristic modulations corresponding to the temporal frequency of a sinewave grating moving at constant velocity. No modulation is visible in the membrane potential recording because local dendritic signals, which are phase-shifted with respect to each other, are integrated into an axonal response. C, Example of Ca2+ imaging with multifocal two-photon microscopy at a VS-cell dendrite. A color-coded image sequence indicating changes of cytosolic Ca2+ concentration in a small region of the dendrite of a VS neuron is shown for downward motion (bottom). Ca2+ concentration signals obtained by excitation of Oregon-Green 1 were quantified for four different branches during motion in three different directions. The corresponding response traces hint at differences in direction selectivity between branches and differences in Ca2+ signal timecourse between motion directions. B adapted from (31).

**Dendritic Ca2+ dynamics provide hints on the type of visual motion detection**

Due to the retinotopic input structure of LPTC dendrites, local dendritic Ca2+ signals show characteristic temporal modulations which reflect properties of the spatial pattern (15). These local modulations are phase shifted along the direction of motion (and thus along one axis of the retinotopically organized dendrite). As a consequence, they cancel out when local dendritic activity is spatially integrated into an axonal voltage change. The dependence of the local dendritic Ca2+ modulations on the properties of the visual stimuli has been used to elucidate the computational principle of the local motion detectors that deliver input to LPTCs (31). If the dendrites of LPTCs receive input from correlation-type local motion detectors, local modulations synchronous with the temporal pattern frequency are expected. Implementation of an alternative processing scheme, the gradient detector, would not produce such modulations. It was suggested that the visual system might be able to shift from a correlation-type motion detection scheme at low ambient contrast to a gradient scheme at high contrast. In principle, such a shift might help the system to benefit from the specific advantages of both detection schemes. Unlike the correlation-type detector, the gradient detector provides a signal that is proportional to image velocity. On the other hand, the advantage of the correlation-type detector over the gradient detector is its lower susceptibility to noise, in particular at low contrast. Although modulations in local dendritic Ca2+ signals have previously been demonstrated (15), a potential contrast-dependent switch between detector schemes could not be excluded. This is due to the fact that conventional wide-field fluorescence microscopy was used, which suffers from a low effective contrast of the stimulation pattern, because the excitation light causes spurious excitation of the photoreceptors. Therefore, two-photon laser-scanning microscopy was used to resolve the question of whether different motion detection scheme are implemented at different contrast conditions. Two-photon excitation of Ca2+ dyes is generated by infrared light, to which the fly's photoreceptors are insensitive. Thus, spurious stimulation of photoreceptors was eliminated and stimulation of the fly during Ca2+ imaging with movement of a high-contrast grating was possible. In these experiments modulations of local dendritic Ca2+ signals could be demonstrated over a wide range of contrast and luminance conditions (Figure 3B).

This result suggests that fly motion vision is based on a correlation-detector scheme irrespective of the signal-to-noise ratio of the visual input.

**Analysis of neuronal connectivity by Ca2+ imaging**

Ca2+ imaging can be applied to establish synaptic connectivity and to assess its functional properties. In this approach one neuron is intracellularly recorded and another neuron is filled with a Ca2+ dye. The two cells are connected by an excitatory synapse if depolarizing current injection into the first cell generates a Ca2+ signal in the second cell. In this way several interactions between LPTCs have been unravelled. In particular, it has been shown that bidirectional transmission by electrical synapses exists and that some specific computations are performed at dendro-dendritic synapses (32). One feature of dendro-dendritic connectivity in the fly visual motion system is the maintenance of the retinotopic organization principle. Model simulations led to the assumption that interaction by inhibitory dendritic synapses can lead to enhancement of motion contrast. This property resembles lateral inhibition in the vertebrate retina and might constitute a crucial step in figure-ground discrimination based on relative motion (33).

The use of Ca2+ imaging in combination with intracellular recordings of potentially coupled neurons has one advantage over dual intracellular recordings: it is not only possible to demonstrate the existence of a synaptic connection, but also to localize the site of interaction. In this way, it has been shown that VS neurons with neighboring receptive fields are synaptically coupled in their axon terminal region (34). Injection of depolarizing current into one VS neuron leads to an increase in the concentration of Ca2+ in an adjacent VS neuron. Unlike the injected neuron, which shows a Ca2+ increase both in its dendrite and in its axonal region, the increase in the adjacent neuron was strongest in the axon terminal region (Figure 4A).

## Analysis of synaptic connectivity by single cell photoablation

In the fly visual system it is feasible to fill a single neuron with a fluorescent dye that is toxic upon intense illumination, e.g. Carboxy-fluorescein. It is then possible to selectively ablate the dye-filled neuron by directing a laser beam on the preparation. All other neurons that are not filled with the dye are left intact. Single-cell photoablation, also termed the 'fill-and-kill technique', is an elegant technique to assess the function of a neuron by recording neurons at postsynaptic processing stages before and after the ablation (35). Due to its specificity, laser ablation is superior to any other lesion technique. Microsurgical brain lesions, for instance, usually affect fibre tracts containing axon projections of several neurons (36). Lesions of neuronal precursors in larval flies can also be performed by laser ablation. Unlike single-cell laser ablation with phototoxic dyes in adult animals, this technique results in elimination of classes of neurons or entire brain regions (37, 38). Certain classes of neurons can also be targeted by the expression of neurotoxins in transgenic Drosophila (39, 40) (see also section 6). However, single-neuron specificity has not yet been reached in genetics-based lesion approaches.

**Dendritic and axonal interactions elucidated by photoablation**

In the first study to use single-cell photoablation in the fly brain, it was established that a particular LPTC - a so-called Figure-Detection neuron (FD1) - is inhibited by a GABAergic Ventral Centrifugal Horizontal neuron (VCH) (41). This inhibition leads to an important feature of the FD1 neuron, so-called 'small-field tuning': FD1 responds only weakly when a motion stimulus covers its entire receptive field. In contrast, when pattern motion is restricted to a small part of the receptive field FD1 shows a strong response (42). This 'small-field tuning', which is thought to mediate visual-motion based figure-ground discrimination, was abolished after ablation of VCH. This circuit was further analyzed by photoablation of neurons that are presynaptic to the CH-cells. The dorsal and ventral CH-cells (DCH and VCH) receive their main ipsilateral input not from retinotopic local motion-detecting elements, as is the case for other LPTCs, but via dendro-dendritic synapses from HS cells. The response of CH-cells to ipsilateral motion was abolished after ablation of HS neurons (43).

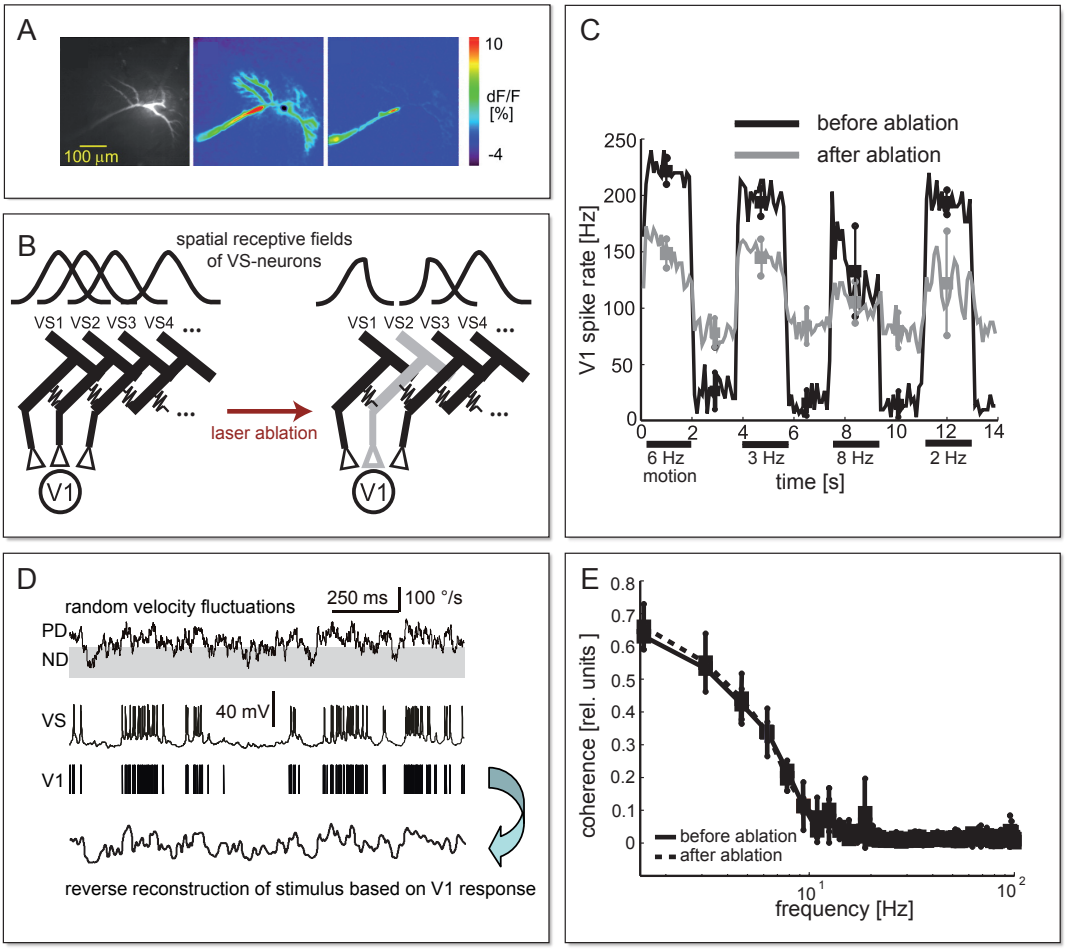


**Figure 4. Examination of the VS-V1 network in the fly brain by Ca2+ imaging and single-cell laser ablation.** A, VS neurons are coupled at their axon terminal region: fluorescence Ca2+ concentration signals were monitored in a VS1-neuron (left, raw fluorescence image) during depolarizing current injection into the neuron itself (middle) and into the neighboring VS2-neuron (right). The response during current injection into the neighboring cell was strongest in the axon terminal region, suggesting this region to be the site of synaptic contacts. B, Scheme illustrating network connections and receptive fields. VS neurons are thought to be connected by electrical synapses with their neighbours in a chain-like manner (45, 76). The V1-neuron receives strong synaptic input from VS1 – VS3. VS-neurons possess dorsoventrally elongated stripe-like spatial receptive fields. In the chain of VS neurons the centres of maximum sensitivity shift from frontal to lateral, with considerable spatial overlap in the receptive fields of neighbouring VS-neurons. Selective laser ablation of one VS-neuron leads to narrowing of the spatial receptive fields of its neighbours on the side of the ablated neuron. See (44) for details. C, In the postsynaptic V1-neuron the ablation of one of the presynaptic VS-neurons leads to a decrease in motion-induced responses and to an increase in baseline firing rate. The averaged timecourse of V1's spike responses to a square-wave grating moving with different temporal frequencies is shown before (black) and after VS-ablation (grey). D, Example responses of VS and V1 to dynamic motion stimulation. The coherence is a measure of how well the stimulus can be reconstructed from the response (in this case the response of V1) by convolution with a linear filter (reconstructed stimulus trace shown schematically). See (49) for details. E, The coherence between V1 and the dynamic motion stimulus is plotted for different frequencies of velocity fluctuations. The coherence when V1 receives input from the intact VS-cell ensemble is not different from the state in which one VS-neuron is ablated. A adapted from (34). Experimental data in C and E from (50).

Interactions in the ensemble of LPTCs sensitive to vertical motion have also been unravelled by single-cell photoablation. The ten VS-neurons form one major output element of the lobula plate (see sections 3,4). Since the dendrites of VS-cells have been shown to receive retinotopically arranged input, one would expect that their receptive fields correspond to the spread of their dendrites in the lobula plate. This is not the case however, since the VS-neurons respond to vertical motion in a much larger section of the visual space than estimated from the extent of their dendrites (20). This discrepancy was resolved by a photoablation study: the receptive field of a VS-neuron was compared before and after photoablation of a neighbouring VS-neuron (44). Photoablation led to narrowing of the receptive field of the recorded VS-neuron on the side of the lesioned neighbouring cell (Figure 4B). The form of synaptic interaction between neighbouring VS-neurons was resolved by current injections during dual intracellular recordings: VS-cells are electrically coupled, having stronger connections to their immediate neighbours than to the VS-cells with more distant receptive fields (45). This suggests a chain-like coupling of the VS-neurons. As described above (see section 3.3 and Figure 4A), by Ca2+ imaging the axonal regions were identified as the sites of electrical coupling (34). Interestingly, a very similar role of electrical synapses in enlarging receptive fields was recently demonstrated in vertebrate retina. The receptive fields of individual horizontal cells in the mouse retina were found to extend beyond their particular dendritic arbors. However, deletion of connexin57 (Cx57), a constituent of vertebrate gap junctions, significantly reduced horizontal cell receptive field size (46).

**Investigation of synaptic integration by photoablation of inputs**

One neuron that reads out the ensemble activity of VS-neurons is the V1-neuron (47). V1 receives strong input from VS-cells with frontal to slightly lateral receptive fields (VS1 – VS3) and transforms the resulting postsynaptic potential into spike activity (Figure 4B). This integrated motion signal is conveyed to the contralateral lobula plate, where V1 forms a large output arborisation. One postsynaptic target of V1 is the VCH-neuron, which gains sensitivity to contralateral vertical motion by this interaction (48).

During flight, a fly is rarely confronted with visual motion of uniform velocity. As a somewhat more naturalistic stimulus, the response of fly LPTCs was investigated using moving patterns that continually modulate their velocity. This approach tested

how well the graded-potential VS-neurons and the spiking V1-neuron can encode different motion velocities. Both the presynaptic VS-neurons and the postsynaptic V1-neuron were shown to encode PD velocity fluctuations linearly and reliably over a frequency range of up to about 10 Hz (49). The overall synaptic transfer between VS and V1 was shown to operate almost linearly over a range of presynaptic voltage fluctuations that covers this frequency range. But what is the contribution of individual VS-neurons to motion velocity coding in V1? Is the convergence of several VS-neurons onto a single V1-neuron essential to guarantee reliable transfer of velocity information? To address these questions the visual motion response of V1 before and after eliminating individual VS-neurons by laser ablation was registered (50). Ablation of single VS-neurons had two effects on the postsynaptic V1-neuron. First, motion-induced responses of V1 decreased and baseline spike rate increased after lesioning a VS-neuron (Figure 4C). The most parsimonious explanation of the latter effect is an increase in leak conductance in VS-neurons that are nearest neighbours of the lesioned VS-cell. Such effects have been reported to accompany the ablation of electrically coupled counterparts in neuronal networks (51). Increased leak conductance in VS would lead to depolarization and, consequently, to increased graded synaptic input to the V1-neuron. The second effect of ablation of single VS-neurons on the postsynaptic V1-neuron was that coding of fluctuations in motion velocity by V1 was quantified by information measures before and after ablation of a VS-neuron. Surprisingly, in most cases there was no pronounced effect on motion velocity coding by V1 when a presynaptic VS-neuron was ablated (Figure 4D,E). This indicates the presence of redundancies in the synaptic integration of motion information by the V1-neuron. Otherwise, elimination of a presynaptic element would strongly degrade V1's capacity to code motion velocity. Redundancies in synaptic wiring patterns might help establish robust representations of neuronal information, particularly when sensory information is sparse or fragmentary.

## Manipulation of calcium signalling in single neurons by UV photolysis of caged calcium

As detailed in section 3, Ca2+ signals in neuronal dendrites present a good marker of local activity. But what is the functional role of Ca2+ in the dendrite? Prompted by correlations between Ca2+ accumulation in the dendrites of LPTCs and membrane after-hyperpolarisation (AHP) following motion in PD (Figure 1C), a Ca2+-dependent inhibitory conductance, such as Ca2+-activated K+-channels, has been proposed as a physiological basis of direction-selective adaptation (52). The AHP following PD motion is an interesting effect for two reasons. First, it modulates neuronal sensitivity in a specific way. Second, it can explain a peculiar behavioural after-effect observed in Drosophila: after the cessation of rotatory visual motion deviations in the resumption of a straight walking course were observed. This after-effect is reminiscent of the well-known 'waterfall illusion' in human psychophysics, in which the observer experiences the impression of movement in opposite direction after viewing a pattern moving in one direction for several seconds (53-55). In fly LPTCs, Ca2+ appeared to be a plausible mediator of direction-selective adaptation for two reasons: first, as outlined in section 3.1, Ca2+ accumulation stays local within the dendritic tree (14, 56). Likewise, adaptation is stronger if adapting motion and test motion are presented at the same location within the receptive field of the LPTC (57, 58). Second, the time courses of the build up and the recovery from adaptation resemble a temporally low-pass-filtered version of the membrane potential (52). These adaptation time courses are similar to the dynamics of cytosolic Ca2+ signals (52, 56, 59). In this sense, Ca2+ could act as a kind of memory signal for stimulus history.

Although Ca2+ imaging is helpful to assess correlations between the cytosolic Ca2+ dynamics and neuronal adaptation, this correlation alone does not necessarily imply a causal relationship. To test the involvement of Ca2+ in the control of adaptation in LPTCs, the cytosolic Ca2+ concentration has to be manipulated in the absence of visual stimulation (60). Through this approach, the effect of Ca2+ can be dissociated from other activity-dependent mechanisms. One way to quickly and selectively manipulate the cytosolic Ca2+ concentration in a single neuron is through the release of Ca2+ from a UV-sensitive caged compound (1). NP-EGTA is a chelator of diva-

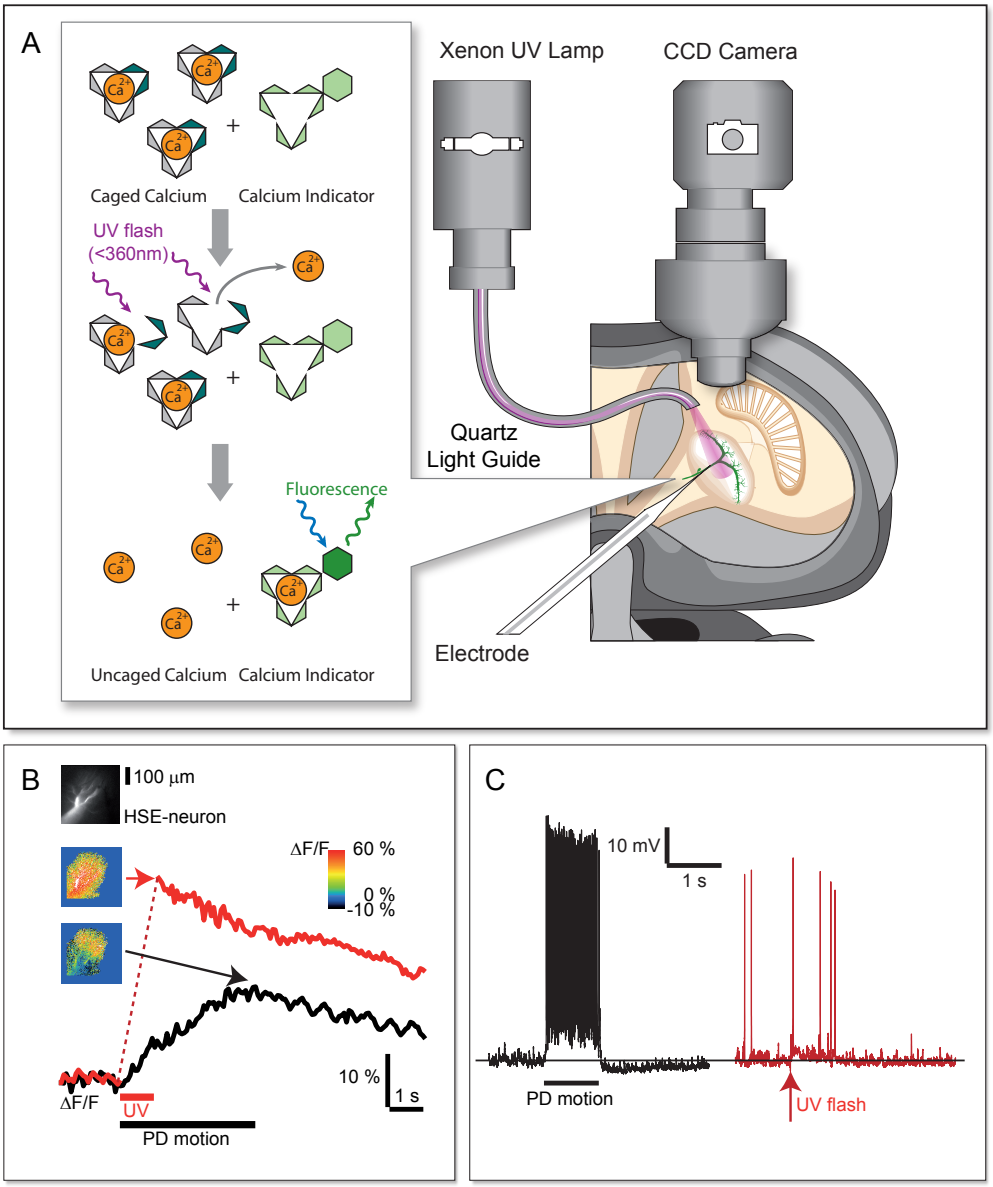


**Figure 5. UV photolysis of caged Ca2+ in single LPTCs.**
A, A LPTC in the fly brain is filled via a recording electrode with Ca2+ indicator dye and NP-EGTA, so-called 'caged Ca2+', which releases Ca2+ upon exposure to UV light. Filtered UV light, either from a Xenon flash lamp or from a continuous UV light source, is delivered to the sample by a quartz light guide to induce release of Ca2+ into the cytosol during membrane potential recording and fluorescence Ca2+ imaging. B, Example traces of dendritic Ca2+ elevation in an HSE-neuron by UV photolysis of caged Ca2+ (red) and during visual stimulation with PD motion (black). The color-coded images show the fluorescence signals at the end of UV illumination and of visual stimulation, respectively. C, Membrane potential traces of an HSE-neuron during visual stimulation with PD motion (black) and during UV photolysis of caged Ca2+ (red). Whereas an AHP follows PD motion (see also Figure 1C), no such response is present after artificial elevation of cytosolic Ca2+ by UV photolysis of caged Ca2+. In contrast, a slight depolarisation, presumably caused by excitation of photoreceptors by the UV flash, is observed. This speaks against an induction of AHP by Ca2+, and thus against Ca2+-dependent regulation of adaptation in LPTCs (see main text for details). Experimental data from (60).

lent ions, which exhibits a high selectivity and affinity for Ca2+ (61). UV illumination causes cleavage of NP-EGTA, leading to a several thousand fold decrease in its affinity for Ca2+. Thus, NP-EGTA, which can be selectively introduced into a cell by a iontophoretic current through a recording electrode, can be used to switch single neurons rapidly from a low- Ca2+ to a high- Ca2+ state (Figure 5A). Surprisingly, release of Ca2+ by UV photolysis of NP-EGTA in LPTCs neither elicited an AHP nor a rise in conductance (Figure 5B,C). Both effects were expected if Ca2+ would activate an inhibitory conductance. Nevertheless, a rise in conductance was present after adaptation with motion in PD. It is therefore plausible that direction-selective adaptation is caused by an activity-regulated inhibitory ion conductance that is independent of Ca2+ (60). One potential candidate is a Na+-regulated conductance, which appears to exist in fly LPTCs (62). Alternatively, direction-selective adaptation might be generated by a voltage-gated inhibitory ion conductance with very slow activation and deactivation characteristics, such as members of the KCNQ family of K+-channels, which have been shown to exist in Drosophila (63). In vertebrates, activation of KCNQ-type channels generates an AHP, suggesting a role in neuronal adaptation (64-66).

## Combination of optical methods with genetic approaches

Penetrations with intracellular electrodes are used to fill single neurons with fluorescent substances, e.g. calcium-sensitive probes or phototoxic dyes. However, intracellular recordings from LPTCs in the fly brain are a difficult and time-consuming procedure. By using genetic approaches in Drosophila it is, in principle, possible to circumvent this problem by expressing proteins in particular groups of neurons. For example, it is already possible to address the function of classes of neurons in the fly visual system by the targeted expression of toxins that block synaptic transmission (39, 40). These methods have not yet reached specificity for single neurons. Nonetheless, the ability to test large numbers of flies with identical defects will make these genetic approaches good complements to existing methods with single-cell specificity such as photoablation. A prerequisite to draw parallels between Drosophila and Calliphora is, of course, the close anatomical similarity of their visual systems. Importantly, both fly species possess the same classes of lobula plate visual motion-sensitive neurons, LPTCs, although the number of neurons within a class and cell shape of individual neurons may differ (67, 68). One major drawback of Drosophila

in functional neurophysiological studies is its tiny body size. Whereas behavioural tests, such as monitoring of optomotor responses, are well applicable in Drosophila, it is still difficult to assess neuronal responses directly. Therefore, Calliphora still presents the best model both for electrophysiological recording and for functional imaging. With respect to the latter, approaches to express genetically encoded functional fluorescence dyes in classes of neurons in Drosophila are promising, given the enhanced sensitivity of recently developed genetically encoded Ca2+ dyes (69, 70). By the MARCM method (71), i.e. by mosaic analysis with a repressible cell marker, it has recently become possible to express specific proteins in small numbers of LPTCs derived from a common progenitor or even in single LPTCs (72). It is, however, not feasible with this method to generate large numbers of individuals carrying exactly the same cellular expression pattern, which is the prerequisite to perform a systematic functional analysis of individual neurons.

## Perspective

Fluorescence imaging in living animals offers a wide range of possibilities to investigate neuronal activity in real time and at increasing spatial resolution, complementing classical electrophysiological methods. Technical advances in the field of fluorescent indicators and caged compounds provide not only the possibility to monitor various ionic concentrations in vivo, but also to influence these concentrations and other cellular processes on fast timescales.

The high spatial resolution of confocal and two-photon-imaging opens up a new experimental approach to small animals like Drosophila, whose small neurons previously made in vivo approaches difficult. The introduction of transgenic fluorescent probes and functional compounds, especially in Drosophila, might some day solve the problem of selectively marking single cells which are hard to access with intracellular dye injection techniques. Until then the exquisite amenability of larger flies, in particular Calliphora, to the simultaneous application of functional optical and electrophysiological techniques will help elucidating how the brain manages sensori-motor control tasks by specific wiring and synaptic processing in small neuronal circuits. With Calliphora it is possible to monitor by sophisticated techniques body movements during flight in order to reconstruct the visual stimuli seen in a natural situation for replay when recording neuronal activity (17, 73, 74). Such approaches

will help clarify how behaviourally relevant visual information is processed in the insect brain.

## List of abbreviations:

AHP: after-hyperpolarisation, CCD: charged-coupled device, CH: centrifugal horizontal, DCH: dorsal centrifugal horizontal, FD: figure detection, HSE: horizontal system equatorial, ND: null direction, PD: preferred direction, LPTC: lobula plate tangential cell, UV: ultraviolet, VCH: ventral centrifugal horizontal, VS: vertical system

## Acknowledgements

Martin Egelhaaf, Matthias Fricke, Markus Sauer and Philip Tinnefeld were involved in some of the projects summarized in this review. The work in the author's laboratory is supported by the Deutsche Forschungsgemeinschaft (DFG). We are grateful to Emily Baird for helpful comments on the manuscript.

## References

1. Ellis-Davies G.C.: Caged compounds: photorelease technology for control of cellular chemistry and physiology. Nat Methods 4, 619-628 (2007)

2. Helmchen F. & W. Denk: Deep tissue two-photon microscopy. Nat Methods 2, 932-940 (2005)

3. Svoboda K. & R. Yasuda: Principles of two-photon excitation microscopy and its applications to neuroscience. Neuron 50, 823-839 (2006)

4. Takahashi A., P. Camacho, J.D. Lechleiter & B. Herman: Measurement of intracellular calcium. Physiol Rev 79, 1089-1125 (1999)

5. Hateren J.H. & C. Schilstra: Blowfly flight and optic flow. II. Head movements during flight. J Exp Biol 202 (Pt 11), 1491-1500 (1999)

6. Egelhaaf M. & R. Kern: Vision in flying insects. Curr Opin Neurobiol 12, 699-706 (2002)

7. Borst A. & J. Haag: Neural networks in the cockpit of the fly. J Comp Physiol [A] 188, 419-437 (2002)

8. Egelhaaf M. & A. Borst: A look into the cockpit of the fly: visual orientation, algorithms, and identified neurons. J Neurosci 13, 4563-4574 (1993)

9. Egelhaaf M., R. Kern, H.G. Krapp, J. Kretzberg, R. Kurtz & A.-K. Warzecha: Neural encoding of behaviourally relevant visual-motion information in the fly. Trends Neurosci 25, 96-102 (2002)

10. Egelhaaf, M., J. Grewe, K. Karmeier, R. Kern, R. Kurtz, and A. K. Warzecha, Novel approaches to visual information processing in insects: case studies on neuronal computations in the blowfly., in Methods in insect sensory neuroscience, edited by T. A. Christensen, pp. 185-212, CRC Press, Boca Raton, 2005.

11. Hausen, K., The lobula-complex of the fly: structure, function and significance in visual behaviour., in Photoreception and vision in invertebrates, edited by M. A. Ali, pp. 523-559, Plenum Press, New York, London, 1984.

12. Borst A. & M. Egelhaaf: Principles of visual motion detection. Trends Neurosci 12, 297-306 (1989)

13. Douglass J.K. & N.J. Strausfeld: Visual motion detection circuits in flies: peripheral motion computation by identified small-field retinotopic neurons. J Neurosci 15, 5596-5611 (1995)

14. Borst A. & M. Egelhaaf: In vivo imaging of calcium accumulation in fly interneurons as elicited by visual motion stimulation. Proc Natl Acad Sci U S A 89, 4139-4143 (1992)

15. Single S. & A. Borst: Dendritic integration and its role in computing image velocity. Science 281, 1848-1850 (1998)

16. Karmeier K., H.G. Krapp & M. Egelhaaf: Population coding of self-motion: applying bayesian analysis to a population of visual interneurons in the fly. J Neurophysiol 94, 2182-2194 (2005)

17. Kern R., J.H. van Hateren, C. Michaelis, J.P. Lindemann & M. Egelhaaf: Function of a Fly Motion-Sensitive Neuron Matches Eye Movements during Free Flight. PLoS Biol 3, e171 (2005)

18. Krapp H.G. & R. Hengstenberg: Estimation of self-motion by optic flow processing in single visual interneurons. Nature 384, 463-466 (1996)

19. Hengstenberg R.: Common visual response properties of giant vertical cells in the lobula plate of the blowfly Calliphora. J Comp Physiol [A] 149, 179-193 (1982)

20. Hengstenberg R., K. Hausen & B. Hengstenberg: The number and structure of giant vertical cells (VS) in the lobula plate of the blowfly Calliphora erythrocephala. J Comp Physiol [A] 149, 163-177 (1982)

21. Krapp H.G., B. Hengstenberg & R. Hengstenberg: Dendritic structure and receptive-field organization of optic flow processing interneurons in the fly. J Neurophysiol 79, 1902-1917 (1998)

22. Hengstenberg R.: Spike responses of ‚non-spiking' visual interneurone. Nature 270, 338-340 (1977)

23. Haag J., M. Egelhaaf & A. Borst: Dendritic integration of motion information in visual interneurons of the blowfly. Neurosci Lett 140, 173-176 (1992)

24. Haag J. & A. Borst: Spatial distribution and characteristics of voltage-gated

calcium signals within visual interneurons. J Neurophysiol 83, 1039-1051 (2000)

25. Grynkiewicz G., M. Poenie & R.Y. Tsien: A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 260, 3440-3450 (1985)

26. Eberhard M. & P. Erne: Calcium binding to fluorescent calcium indicators: calcium green, calcium orange and calcium crimson. Biochem Biophys Res Commun 180, 209-215 (1991)

27. Krapp H.G., R. Hengstenberg & M. Egelhaaf: Binocular contributions to optic flow processing in the fly visual system. J Neurophysiol 85, 724-734 (2001)

28. Denk W., J.H. Strickler & W.W. Webb: Two-photon laser scanning fluorescence microscopy. Science 248, 73-76 (1990)

29. Kurtz R., M. Fricke, J. Kalb, P. Tinnefeld & M. Sauer: Application of multiline two-photon microscopy to functional in vivo imaging. J Neurosci Methods 151, 276-286 (2006)

30. Nielsen T., M. Fricke, D. Hellweg & P. Andresen: High efficiency beam splitter for multifocal multiphoton microscopy. J Microsc 201, 368-376 (2001)

31. Haag J., W. Denk & A. Borst: Fly motion vision is based on Reichardt detectors regardless of the signal-to-noise ratio. Proc Natl Acad Sci U S A 101, 16333-16338 (2004)

32. Haag J. & A. Borst: Dendro-dendritic interactions between motion-sensitive large-field neurons in the fly. J Neurosci 22, 3227-3233 (2002)

33. Cuntz H., J. Haag & A. Borst: Neural image processing by dendritic networks. Proc Natl Acad Sci U S A 100, 11082-11085 (2003)

34. Cuntz H., J. Haag, F. Forstner, I. Segev & A. Borst: Robust coding of flow-field parameters by axo-axonal gap junctions between fly visual interneurons. Proc Natl Acad Sci U S A 104, 10229-10233 (2007)

35. Miller J.P. & A. Selverston: Rapid killing of single neurons by irradiation of intracellularly injected dye. Science 206, 702-704 (1979)

36. Hausen K. & C. Wehrhahn: Neural circuits mediating visual flight control in flies. II. Separation of two control systems by microsurgical brain lesions. J Neurosci

10, 351-360 (1990)

37. Nassel D.R., G. Geiger & H.S. Seyan: Differentiation of fly visual interneurons after laser ablation of their central targets early in development. J Comp Neurol 216, 421-428 (1983)

38. Geiger G. & D.R. Nassel: Visual orientation behaviour of flies after selective laser beam ablation of interneurones. Nature 293, 398-399 (1981)

39. Keller A., S.T. Sweeney, T. Zars, C.J. O'Kane & M. Heisenberg: Targeted expression of tetanus neurotoxin interferes with behavioral responses to sensory input in Drosophila. J Neurobiol 50, 221-233 (2002)

40. Rister J. & M. Heisenberg: Distinct functions of neuronal synaptobrevin in developing and mature fly photoreceptors. J Neurobiol 66, 1271-1284 (2006)

41. Warzecha A.K., M. Egelhaaf & A. Borst: Neural circuit tuning fly visual interneurons to motion of small objects. I. Dissection of the circuit by pharmacological and photoinactivation techniques. J Neurophysiol 69, 329-339 (1993)

42. Egelhaaf M.: On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly: II. Figure-detection cells, a new class of visual interneurons. Biol Cybern 52, 195-209 (1985)

43. Farrow K., J. Haag & A. Borst: Input organization of multifunctional motion-sensitive neurons in the blowfly. J Neurosci 23, 9805-9811 (2003)

44. Farrow K., A. Borst & J. Haag: Sharing receptive fields with your neighbors: tuning the vertical system cells to wide field motion. J Neurosci 25, 3985-3993 (2005)

45. Haag J. & A. Borst: Neural mechanism underlying complex receptive field properties of motion-sensitive interneurons. Nat Neurosci 7, 628-634 (2004)

46. Shelley J., K. Dedek, T. Schubert, A. Feigenspan, K. Schultz, S. Hombach, K. Willecke & R. Weiler: Horizontal cell receptive fields are reduced in connexin57-deficient mice. Eur J Neurosci 23, 3176-3186 (2006)

47. Kurtz R., A.K. Warzecha & M. Egelhaaf: Transfer of visual motion information via graded synapses operates linearly in the natural activity range. J Neurosci 21, 6957-6966 (2001)

48. Haag J. & A. Borst: Orientation tuning of motion-sensitive neurons shaped by vertical-horizontal network interactions. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 189, 363-370 (2003)

49. Warzecha A.K., R. Kurtz & M. Egelhaaf: Synaptic transfer of dynamic motion information between identified neurons in the visual system of the blowfly. Neuroscience 119, 1103-1112 (2003)

50. Kalb J., M. Egelhaaf & R. Kurtz: Robust Integration of Motion Information in the Fly Visual System Revealed by Single Cell Photoablation. J Neurosci 26, 7898-7906 (2006)

51. Szczupak L. & W.B. Kristan, Jr.: Widespread mechanosensory activation of the serotonergic system of the medicinal leech. J Neurophysiol 74, 2614-2624 (1995)

52. Kurtz R., V. Dürr & M. Egelhaaf: Dendritic calcium accumulation associated with direction-selective adaptation in visual motion-sensitive neurons in vivo. J Neurophysiol 84, 1914-1923 (2000)

53. Götz K.G. & H. Wenking: Visual control of locomotion in walking fruitfly Drosophila. J Comp Physiol 85, 235-266 (1973)

54. Sekuler R.: Aftereffect of seen motion with a stabilized retinal image. Science 139, 419-420 (1963)

55. Srinivasan M.V. & D.R. Dvorak: The waterfall illusion in an insect visual system. Vision Res 19, 1435-1437 (1979)

56. Dürr V. & M. Egelhaaf: In vivo calcium accumulation in presynaptic and postsynaptic dendrites of visual interneurons. J Neurophysiol 82, 3327-3338 (1999)

57. Maddess T. & S.B. Laughlin: Adaptation of the motion-sensitive neuron H1 is generated locally and governed by contrast frequency. Proc R Soc Lond B Biol Sci 228, 251-275 (1985)

58. Neri P. & S.B. Laughlin: Global versus local adaptation in fly motion-sensitive neurons. Proc Biol Sci 272, 2243-2249 (2005)

59. Kurtz R.: Ca2+ clearance in visual motion-sensitive neurons of the fly studied in vivo by sensory stimulation and UV photolysis of caged Ca2+. J Neurophy-

siol 92, 458-467 (2004)

60. Kurtz R.: Direction-selective adaptation in fly visual motion-sensitive neurons is generated by an intrinsic conductance-based mechanism. Neuroscience 146, 573-583 (2007)

61. Ellis-Davies G.C. & J.H. Kaplan: Nitrophenyl-EGTA, a photolabile chelator that selectively binds Ca2+ with high affinity and releases it rapidly upon photolysis. Proc Natl Acad Sci U S A 91, 187-191 (1994)

62. Haag J., F. Theunissen & A. Borst: The intrinsic electrophysiological characteristics of fly lobula plate tangential cells: II. Active membrane properties. J Comput Neurosci 4, 349-369 (1997)

63. Wen H., T.M. Weiger, T.S. Ferguson, M. Shahidullah, S.S. Scott & I.B. Levitan: A Drosophila KCNQ channel essential for early embryonic development. J Neurosci 25, 10147-10156 (2005)

64. Gu N., K. Vervaeke, H. Hu & J.F. Storm: Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. J Physiol 566, 689-715 (2005)

65. Lawrence J.J., F. Saraga, J.F. Churchill, J.M. Statland, K.E. Travis, F.K. Skinner & C.J. McBain: Somatodendritic Kv7/KCNQ/M channels control interspike interval in hippocampal interneurons. J Neurosci 26, 12325-12338 (2006)

66. Yue C. & Y. Yaari: KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. J Neurosci 24, 4614-4624 (2004)

67. Rajashekhar K.P. & V.R. Shamprasad: Golgi analysis of tangential neurons in the lobula plate of Drosophila melanogaster. J Biosci 29, 93-104 (2004)

68. Scott E.K., T. Raabe & L. Luo: Structure of the vertical and horizontal system neurons of the lobula plate in Drosophila. J Comp Neurol 454, 470-481 (2002)

69. Mank M., D.F. Reiff, N. Heim, M.W. Friedrich, A. Borst & O. Griesbeck: A FRET-based calcium biosensor with fast signal kinetics and high fluorescence change. Biophys J 90, 1790-1796 (2006)

70. Reiff D.F., A. Ihring, G. Guerrero, E.Y. Isacoff, M. Joesch, J. Nakai & A. Borst:

In vivo Performance of Genetically Encoded Indicators of Neural Activity in Flies. J Neurosci 25, 4766-4778 (2005)

71. Lee T. & L. Luo: Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci 24, 251-254 (2001)

72. Raghu S.V., M. Joesch, A. Borst & D.F. Reiff: Synaptic organization of lobula plate tangential cells in Drosophila: gamma-Aminobutyric acid receptors and chemical release sites. J Comp Neurol 502, 598-610 (2007)

73. Boeddeker N., J.P. Lindemann, M. Egelhaaf & J. Zeil: Responses of blowfly motion-sensitive neurons to reconstructed optic flow along outdoor flight paths. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191, 1143-1155 (2005)

74. Karmeier K., J.H. van Hateren, R. Kern & M. Egelhaaf: Encoding of naturalistic optic flow by a population of blowfly motion-sensitive neurons. J Neurophysiol 96, 1602-1614 (2006)

75. Egelhaaf M. & A. Borst: Calcium accumulation in visual interneurons of the fly: stimulus dependence and relationship to membrane potential. J Neurophysiol 73, 2540-2552 (1995)

76. Haag J. & A. Borst: Dye-coupling visualizes networks of large-field motion-sensitive neurons in the fly. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191, 445-454 (2005)

# Acknowledgments

First, I'd like to thank my supervisor, Rafael Kurtz, who invested countless hours of teaching methods, explaining concepts, providing funding, proofreading manuscripts, working kinks out of malfunctioning equipment, and taking part in valuable discussions. Thanks for lending me a hand whenever I needed it, leaving me all the freedom to pursue my own ideas, and beeing a nice person to have around on top of it.

I also want to thank Martin Egelhaaf for his guidance, scientific input, and all the effort he invested to make the neurobiology group the nice and friendly place to work in it has been for me for the last five years.

Special thanks go to Jens Lindemann, for his support in all computer issues. Even though it meant him working with dreaded microsoft systems, his programming skills and helping hand proved invaluable for my work.

To all my other colleagues, PhD-students, post-docs and master/diploma students, many thanks for being around, providing me wih chocolate, company in the ups and downs of electrophysiology work, and uncounted evenings of pub crawls.

A special mention goes to Bart Geurten, who not only provided me with encouragement, feedback, and invaluable help in all PhD related issues, but also put up with me after work as a flatmate and fried.

For all the support in the background, and all the little help in everyday labwork, thanks to Heike Sieb, Gerd Wiebusch, Grit Schwertfeger and Tim Siesenop.

And, last but not least, I'd like to thank my family, for providing me with never ending support and encouragement, as well as my friends, for coming all this way with me.