Genetic intervention in sensory systems of a fly

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1. Introduction

1.1. Structure-function correlation

A variety of behaviors including learning and memory, circadian behavioral rhythms, olfactory and visually guided orientation in walking and flight, and complex courtship behaviors have been described for *Drosophila melanogaster*. These behaviors are generated by a peripheral nervous system, a thoracic ganglion and a brain that consists of only 200.000 to 300.000 neurons. This low number of neurons (compared to the 10^8 to 10^{11} neurons in mammalian brains) that generate complex behaviors make the *Drosophila* brain an ideal model to study information processing.

A useful approach to understand functional brain architecture is the correlation of brain structures with functions. In mammals this is mainly done on the level of brain areas, but the small size of the *Drosophila* brain allows to assign behavioral functions to single neurons and subsequently to neuronal networks that mediate the behavior under study (for example: Waddell et al., 2000).

One way to assign a function to a neuron is to measure the activity of the neuron when the animal performs a behavioral task or processes a sensory input. This can be done by recording voltage changes in a neuron or in extracellular fluids. Although intracellular recordings at the larval neuromuscular junction and extracellular recordings at sensory structures are routinely performed (reviewed in Keshishian, 1996), electrophysiology in the central nervous system of *Drosophila* is notoriously difficult. However, in larger *Dipterans* like *Musca* and *Calliphora* intracellular recordings are possible and contributed to the understanding of the functional architecture of the fly brain. An alternative method to identify active neurons in the brain is deoxyglucose labeling (Buchner et al., 1979). In *Drosophila*, this method has a resolution to the cellular level (Bausenwein et al., 1990). However, the temporal resolution of this method is rather poor. Recently, Ca^{2+} imaging was applied to the *Drosophila* brain (Wang et al, 2001). The signals could not yet be attributed to individual neurons, but patterns of neural activity were analyzed. Electrophysiology, deoxyglucose labeling and calcium imaging tell the experimenter to what sensory inputs the neurons under study are sensitive. It is not possible from this kind of experiments to decide if the neurons under study are necessary and/or sufficient for a certain behavioral task. To obtain this information, strategies involving behavioral experiments are needed. To test if a neuron

is necessary for a behavior, flies in which the neuron is missing, non-functional or modified need to be generated and then tested for the behavior under study. Methods such as microsurgery, cooling of brain regions, and application of pharmacological reagents have been used to interfere with the function of specific neurons or brain regions in larger *Dipterans*. In *Drosophila*, mutants have been instrumental in mapping certain behavioral tasks to specific neurons in the brain. Flies lacking the giant fibers of the lobula plate are strongly impaired in their optomotor responses (Heisenberg et al., 1978; Brunner et al., 1992) and flies with aberrantly developed giant fibers of the cervical connective lack the escape reflex known as the jump response (Thomas and Wyman, 1984). Olfactory learning (Heisenberg et al., 1985), control of spontaneous walking activity (Martin et al., 1998), and context generalization in visual learning (Liu et al., 1999) all depend on the correct functioning of the mushroom bodies and, finally, the central complex is involved in the initiation, regulation and fine tuning of motor programs (Strauss et al., 1992; Strauss and Heisenberg, 1993; Ilius et al., 1994; Martin et al., 1999).

The use of mutants for structure-function correlation is not without drawbacks. Expressivity of the structural defects in most central brain mutants is variable and, secondly, nearly all such mutations show pleiotropic effects. Moreover, expression of the phenotype may depend on genetic background (deBelle and Heisenberg, 1996) which makes comparison with control lines difficult. For these reasons alternative methods for studying structure-function relationships are highly welcome. With the UAS/GAL4 technique (Figure 1), any transgene can be expressed in a spatially and temporally restricted pattern. For this purpose a construct carrying the gene of the yeast transcription factor GAL4 is inserted in the *Drosophila* genome. Depending on where the construct is inserted, GAL4 expression is driven in a spatially and temporally controlled pattern. On a second construct the GAL4-binding site (UAS) and a downstream gene are encoded. The gene is expressed in a GAL4-dependent manner, i.e. only in the cells and at the time at which GAL4 is expressed.

Figure 1

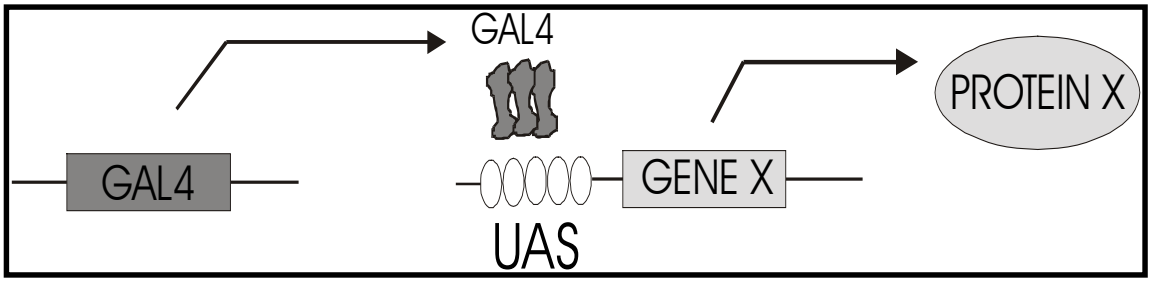

Figure 1: The UAS/GAL4 technique allows to express any gene X in a temporally and spatially restricted fashion. The yeast transcription factor GAL4 is expressed in a manner determined by the regulatory genomic elements close to the insertion site of the GAL4 enhancer trap construct. On a second transgenic construct the gene of interest (X) is cloned downstream of the GAL4 binding site (UAS). The gene of interest (X) is expressed in a GAL4 dependent manner, i.e. with the same temporal and spatial restrictions.

There is a growing number of GAL4 lines expressing in different structures, making it possible to express any transgene in the structure of interest. The UAS/GAL4 technique can be used to identify neurons necessary for certain behaviors by expressing a gene, which codes for a protein that kills the neuron or alters its function. Not only necessary neurons but also sets of neurons sufficient for a certain narrowly defined behavioral function could be identified using the UAS/GAL4 technique. This could be done by rescuing a gene, necessary for neuronal function, in a GAL4 driven manner in an otherwise mutant brain. So far, no such experiments have been reported, but the strategy has been used successfully to show in which structures synaptic plasticity is sufficient to perform in learning tasks (Zars et al., 2000a; Zars et al., 2000b).

1.2. Use of the UAS/GAL4 system to study structure-function correlation

With the UAS/GAL4 technique any gene can be expressed in a temporally and spatially restricted manner. Several effectors that kill cells or alter the function of neurons have been constructed (Table 1). They will be discussed here, with respect to how useful they are for structure-function correlation in the *Drosophila* nervous system.

Table 1

diphtheria toxin A chain	inhibits protein synthesis	Kunes and Steller, 1991
ricin toxin A chain	inhibits protein synthesis	Hidalgo et al., 1995
***reaper* (*rpr*)**	induces apoptosis	Zhou et al., 1997
***head involution defective* (*hid*)**	induces apoptosis	Zhou et al., 1997
human inwardly rectifying potassium channel (KIR)	suppresses initiation of potentials	Baines et al., 2001
tetanus neurotoxin (TNT)	blocks transmitter release	Sweeney et al., 1995
***shibire* (*shi^{ts1}*)**	blocks transmitter release	Kitamoto, 2001
activated stimulatory GTP-binding protein α subunit (*Gαs)**	interferes with the cAMP cascade	Connolly et al., 1996
***rutabaga* (*rut*)**	rescues *rut* function in the cAMP cascade	Zars et al., 2000a
***transformer* (*tra*)**	feminizes cells	Ferveur et al., 1995
neuropeptide processing enzyme (PHM)	rescues PHM function in neuropeptide processing	Taghert, Washington, pers. comm.

Table 1: Eleven effectors that can be used to kill neurons or alter their function are listed together with their mode of action and the respective reference. Diphtheria toxin A chain, ricin toxin A chain, *reaper* and *head involution defective* kill cells, whereas the human inwardly rectifying potassium channel, tetanus neurotoxin and *shibire* block signal transmission. The activated stimulatory GTP-binding protein α subunit, *rutabaga*, *transformer* and the neuropeptide processing enzyme alter more specific neuronal functions.

Two different strategies have been used to kill cells. One possibility is to use a heterologous gene the product of which specifically inhibits protein synthesis. For this purpose the genes for diphtheria toxin A chain and the ricin A toxin chain have been used (reviewed by O'Kane and Moffat, 1992). Diphtheria toxin A chain inhibits protein synthesis through ADP-ribosylation of elongation factor 2 (Chung and Collier, 1977), whereas ricin toxin A chain inactivates eukaryotic ribosomes by a specific depurination event in 28S rRNA, thus also inhibiting protein synthesis (Endo and Tsurugi, 1988).

Diphtheria toxin A chain as well as ricin A chain have two major disadvantages: both affect neuronal as well as non-neuronal cells and, after the necrotic cell death, the cellular debris as well as the toxin are released into the extracellular space. To my knowledge, no behavioral experiments with flies expressing a heterologous protein synthesis toxin have been reported so far. A second way to kill cells is to induce programmed cell death (apoptosis). Apoptosis induction in *Drosophila* is controlled among others by the genes *grim*, *head involution defective* (*hid*) and *reaper* (*rpr*) (reviewed in McCall and Steller, 1997). *rpr* and *hid* have been used in combination with the UAS/GAL4 technique to kill cells by inducing apoptosis. Flies carrying a *GMR-rpr* insertion that drives *rpr* expression in the eye show a dosage dependent ablation of the eyes (White et al., 1996). When *rpr* is used in combination with the UAS/GAL4 system to ablate eclosion hormone cells, this produces discrete deficits in eclosion behavior (McNabb et al., 1997). However, not all cells are accessible to apoptosis induction by *rpr* or *hid*. Resistance to apoptosis induction by one of the two proteins alone is described for embryonic central nervous system midline cells. In this case expression of *rpr* and *hid* in combination is necessary to induce apoptosis (Zhou et al., 1997). The disadvantage of the apoptosis inducers is, that, like the protein synthesis inhibitors, they affect neuronal as well as non-neuronal cells. To circumvent this, genes that do not kill but alter specific functions of neurons have been employed.

One possibility to alter neuronal function is the ectopic expression of a human inwardly rectifying potassium channel (KIR). Expression of KIR in motorneurons results in an almost total absence of excitatory junctional currents in its target muscle (Baines et al., 2001). This is consistent with a block of the evoked release of neurotransmitter by a mechanism that suppresses initiation of potentials.

Other effectors do not block action potential initiation but transmitter release at chemical synapses. This can be done either by ectopically expressing the light chain of the tetanus neurotoxin (TNT) (Sweeney et al., 1995) or by overexpressing a temperature-sensitive allele of *shibire* (shi^{ts1}) (Kitamoto, 2001). TNT specifically cleaves *neuronal-synaptobrevin* (*n-syb*). This is a synapse-specific protein proposed to target the synaptic vesicles to the presynaptic membrane (Schiavo et al., 1992b; review of synaptic vesicle cycle, see Jahn and Südhof, 1994). Cleavage of *n-syb* eliminates evoked, but not spontaneous, synaptic vesicle release (Sweeney et al., 1995). TNT has been used in numerous behavioral experiments ranging from regulation of activity to ethanol tolerance and learning and memory (reviewed in Martin et al., 2001). The

shibire (*shi*) gene product is essential for synaptic vesicle recycling, and shi^{ts1} is a semidominant mutant allele of the gene, therefore a temperature shift leads to fast and reversible effects on synaptic transmission of shi^{ts1} expressing neurons. shi^{ts1} was used successfully in behavioral studies (Dubnau et al., 2001; McGuire et al., 2001; Waddell et al., 2000).

Several other effectors have been constructed that alter more specific aspects of neuronal function. Synaptic plasticity of neurons has been altered by interfering with the cAMP cascade. Expression of a constitutively activated stimulatory heterotrimeric guanosine triphosphate-binding protein α subunit (*Gαs**) targeted to the mushroom bodies abolishes olfactory learning, suggesting that regulated *Gαs* signaling in *Drosophila* mushroom bodies is necessary for learning (Connolly et al., 1996). By expressing the *rutabaga* adenylat cylclase (*rut*) in a rut^- background (Zars et al., 2000a; Zars et al., 2000b) the structures in which synaptic plasticity is sufficient were determined for two learning paradigms (Zars et al., 2000a; Zars et al., 2000b). Both strategies can be used to identify structural correlates of behaviors involving cAMP-dependent neuronal plasticity.

To study sex specific functions of brain structures, the *transformer* gene (*tra*) that feminizes cells in male flies can be used. Experiments with male flies with regionally feminized brains showed that the antennal lobes and the mushroom bodies may function in the recognition of sex-specific pheromones, in the control of sex-specific behaviors, or both (Ferveur et al., 1995). Not only structures that are involved in courtship and mating can be mapped with this method, but any behavior that shows sexual dimorphism. The area controlling the sexually dimorphic locomotor activity was mapped to a small cluster of neurons in the pars intercerebralis (Gatti et al., 2000).

Recently, the neuropeptide processing enzyme, PHM, that is required for peptide amidation has been used as an effector (Taghert, Washington, pers. comm.). When used in a PHM negative background, this can be useful for identifying structures that mediate behaviors depending on amidated neuropeptides.

1.3. GAL4 driven tetanus neurotoxin expression

The most widely used gene for structure-function correlation in the *Drosophila* nervous system is the light chain of the tetanus neurotoxin (TNT) (reviewed in Martin et al., 2001). TNT is the causative agent of tetanus. It is produced by the gram positive anaerobe, *Clostridium tetani*, and belongs to the family of clostridial neurotoxins which

normally are found as a dichain holotoxin consisting of a heavy chain and a light chain. The light chain of the neurotoxin contains the intracellularly toxic moiety which is responsible for blocking synaptic exocytosis when delivered into the cytoplasm of a neuronal cell. The proteolytic substrate of TNT is *neuronal-synaptobrevin* (*n-syb*) (Schiavo et al., 1992b; Link et al., 1992). *Synaptobrevin* is a small C-terminally anchored membrane protein which was first identified as a component of synaptic vesicles in *Torpedo* electric organ (Trimble et al., 1988) and later cloned from a variety of species from yeast to human including two from *Drosophila* (Südhof et al., 1989; Chin et al., 1993; DiAntonio et al., 1993). *Synaptobrevin* has been found to interact with a number of presynaptic proteins of importance to the process of regulated exocytosis (for a recent detailed review, see Schiavo et al., 2000). However, a central importance of *synaptobrevin* to the process of neurotransmitter release is evidenced by the observation that cleavage of *synaptobrevin* by TNT elicits a complete blockade in evoked exocytosis (Schiavo et al., 1992b, Link et al., 1992). The identification of *synaptobrevin* as the substrate for the light chain of tetanus neurotoxin and five of the botulinal neurotoxins (O'Kane et al., 1999) indicates a core role for *synaptobrevin* in the process of synaptic vesicle exocytosis. *Synaptobrevin* is found in a tight complex with two other synaptic proteins, *syntaxin* and *SNAP-25*, both of which are also targets for three clostridial neurotoxins and loss of either, again, results in failure of evoked synaptic exocytosis. Two forms of *synaptobrevin* have been found in *Drosophila*, a ubiquitously expressed, TNT insensitive form, *dsyb* (Südhof et al., 1989; Chin et al., 1993; Sweeney et al., 1995), and a neuronally expressed, TNT sensitive form, *dn-syb* (DiAntonio et al., 1993; Sweeney et al., 1995). These facts together with the apparent specificity and cell autonomy of TNT have made the latter an attractive tool to study exocytosis in a number of preparations including *Drosophila*. Linking these properties to the UAS/GAl4 system made TNT a particularly useful tool for the dissection of behaviors in *Drosophila*. TNT blocks only chemical synapses, which is problematic, because there is evidence that electrical synapses are not uncommon in insect brains. The gap junction forming *innexins* are expressed in large parts of the brain during development (Watanabe and Kankel, 1992) and cobalt coupling, which indicates electric coupling, is widespread in the adult nervous system (for example, Milde and Strausfeld, 1986).

1.4. Inducible and conditional systems

Compared to vertebrates surprisingly few strategies to induce expression have been tested in *Drosophila*. This is, because in poikilothermic *Drosophila* heat dependent promoters can be used to induce expression.

Alternative strategies to induce expression include doxycycline dependent expression systems (reviewed in: Gossen et al., 1993). In the “tet-off” system, the GAL4 line drives expression of a doxycycline dependent transcriptional activator (tTA). The tTA only binds to its binding site (tetO) in the absence of doxycycline. Any gene that is cloned behind the tetO will only be expressed in the absence of doxycycline (Bello et al., 1998) . In the “tet-on” system, the GAL4 line drives expression of a doxycycline dependent reverse transcriptional activator (rtTA) (Figure 2) (Stebbins et al., 2001). The rtTA only binds to its binding site (tetO) in the presence of doxycycline. Any gene that is cloned behind the tetO will only be expressed in the presence of doxycycline (Bieschke et al., 1998). The doxycycline dependent system is reversible and it allows to control expression levels. The level of expression is linear to the doxycycline concentration over a wide range. The only effector useful for structure-function correlation available as a "tet-on" construct is the apoptosis inducer *reaper* that is used in this study to explore the properties of the "tet-on" system.

Figure 2

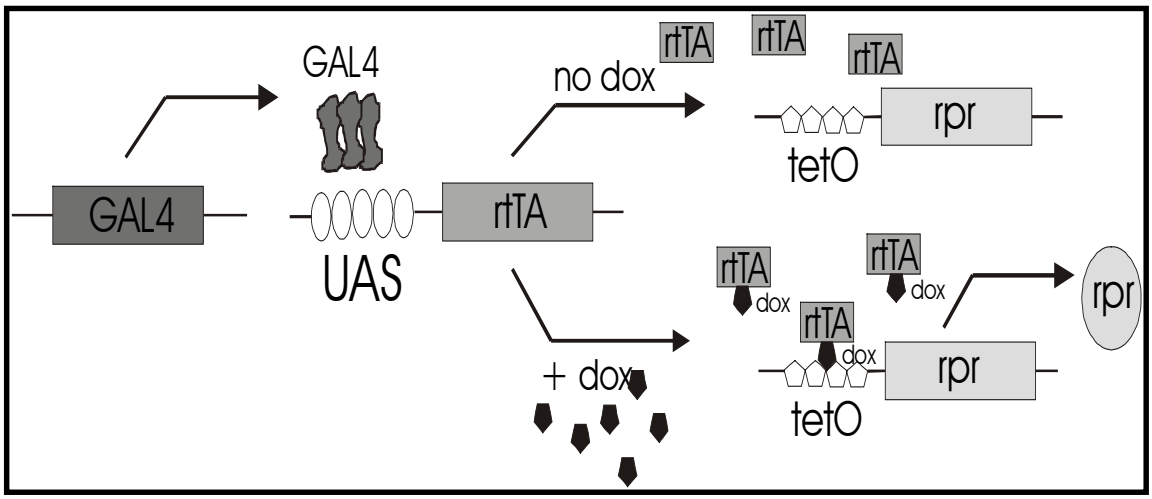

Figure 2: To achieve doxycycline inducible *rpr* expression, the UAS/GAL4 technique is used to drive expression of the reverse doxycycline-dependent transactivator (rtTA). The rtTA only binds to its binding site (tetO) in the presence of doxycycline. When the tetO is cloned upstream of the *rpr* gene, *rpr* is expressed in a doxycycline dependent way.

Another way to induce expression, although in a non-reversible and non-quantitative way, is to combine the UAS/GAL4 system with the flp/FRT system (Figure 3). The flp/FRT system is a two component recombination system in which the yeast recombinase flp is encoded under control of a heat shock promoter. After being heat shocked, flp is expressed and can excise any DNA that is flanked by two of its recognition targets (FRTs). Three transgenic constructs need to be integrated in the *Drosophila* genome to create an inducible expression system. The first construct encodes the effector gene separated from a UAS by the *white* gene and transcriptional termination signals that are flanked by FRTs. As long as the termination sites are present, the effector is not expressed. To excise the *white* gene and the transcriptional termination signals the recombinase activity of the flp recombinase is needed. The flp recombinase is encoded on the second construct under control of a heat shock promoter. When the flies are exposed to a heat shock, flp is expressed, the *white* gene and the transcriptional termination signals are removed and, from that moment on, the effector is expressed in that cell and all its progeny. The third construct is any GAL4 line that determines the spatial restriction of expression. In this study tetanus neurotoxin was used as an effector.

Figure 3

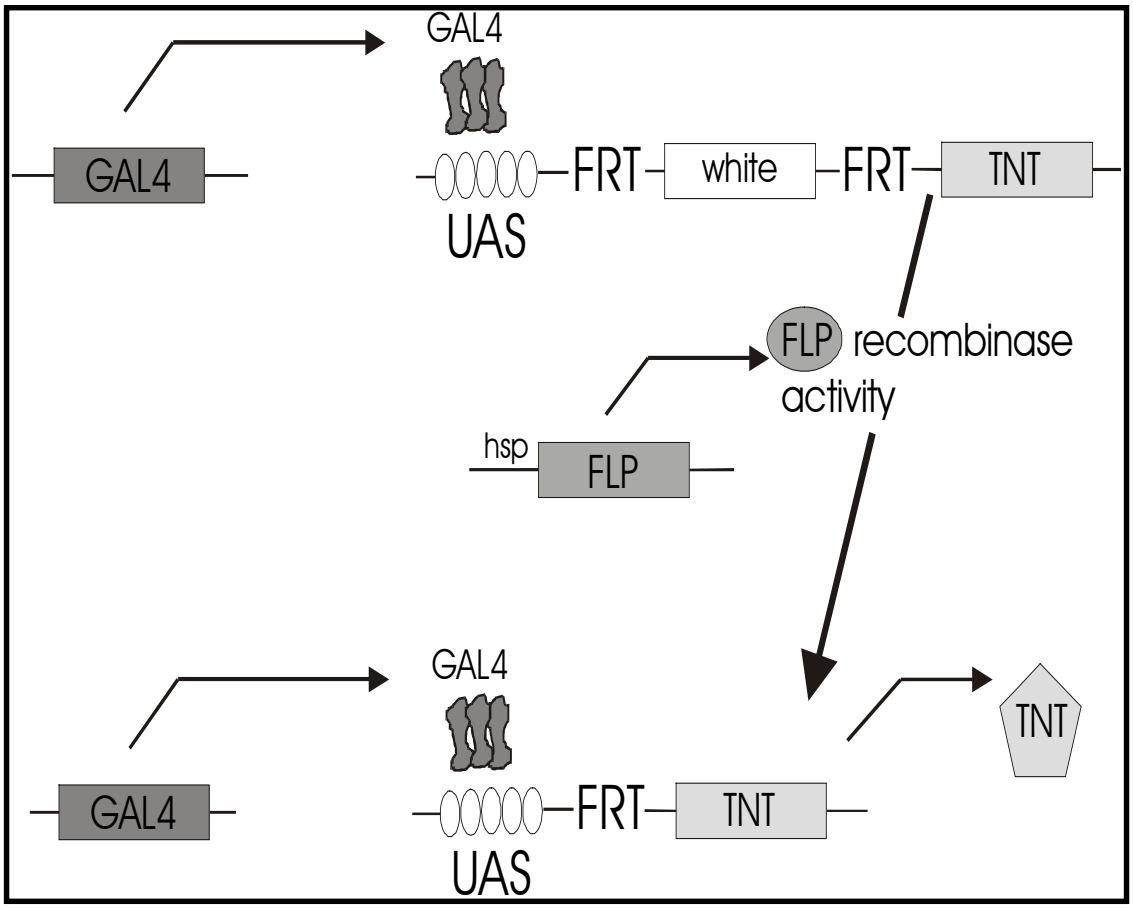

Figure 3: Three transgenic constructs are needed for an inducible TNT expression system, using the flp/FRT technique. The first construct encodes the TNT gene that is separated from a UAS by the *white* gene and transcriptional termination signals that are flanked by FRTs. As long as the termination signals are present, TNT is not expressed. The *white* gene and the transcriptional termination signals are excised by the activity of the flp recombinase. The second construct encodes the flp recombinase under control of a heat shock promoter. When the flies are exposed to a heat shock, flp is expressed, the *white* gene and the transcriptional termination signals are removed, and TNT is expressed in that cell. The third construct needed is a GAL4 line that determines the spatial restriction of TNT expression.

Inducing expression of an effector is not the only way to conditionally alter neuronal function. Since *Drosophila* is poikilothermic, temperature sensitive proteins can be used to generate neuronal mosaics with temperature dependent functionality. For the two protein synthesis inhibitors ricin A chain and Diphtheria toxin A chain there are also temperature sensitive mutants available. These are easy to screen for in yeast, since they are toxic for any eukaryotic cell. This is different for the other effectors (TNT, KIR, *tra*, *Gαs*, *rpr*, *hid*, PHM), none of which exists in a temperature sensitive form. The temperature dependent protein synthesis inhibitors are non-specific, have a high

background and affect neuronal as well as non-neuronal cells. A more specific temperature dependent block of vesicle recycling at chemical synapses can be achieved by using a temperature sensitive semidominant allele of the *shibire* gene (shi^{ts1}). shi^{ts1} was used in several behavioral studies to conditionally block synaptic transmission (Dubnau et al., 2001; McGuire et al., 2001; Waddell et al., 2000). The effect of shi^{ts1} depends on the relation of the *shibire* protein and its temperature sensitive form in the cells under study. This relation depends on the endogenous *shibire* level of the neuron as well as on the GAL4 dependent level of the mutant form.

A way to circumvent this dosage dependence is to generate a mosaic of temperature sensitive neurons by mutating a gene necessary for neuronal function at higher temperatures. For example the synaptic protein *cystein string protein* (*csp*) that causes temperature dependent neuronal dysfunction when mutated can be rescued with a construct in which the *csp* gene is flanked by two FRT sites. Using a GAL4 line and a UAS-flp construct, the GAL4 expressing tissue can be altered in such a way that it is dysfunctional at higher temperatures in an otherwise normal nervous system (Natascha Reisch, Würzburg, pers. comm.). This system is completely independent of levels of endogenous and ectopically expressed proteins. Induction only requires a single recombination event per neuron that can be detected immunohistochemically.

1.5. Sensory modalities in *Drosophila*

Sensory modalities are of principal importance to guide animals in their environment and to inform them about their appropriate behavior for survival and reproduction. Mechanoreception, chemoreception and photoreception are major modalities in *Drosophila* currently under study to understand how an animal captures and responds to environmental cues.

The stimuli relayed by the mechanosensory system include touch, relative body-part position (proprioreception), and sound. These stimuli are mediated by neurons with single dendrites (type I mechanoreceptors) in sensory bristles, chordotonal organs, campaniform sensilla, and multidendritic type-II mechanoreceptors (Jan and Jan, 1993). Chordotonal organs and type-II mechanoreceptors are internal sensory organs, whereas sensory bristles and campaniform sensilla use external sensory structures that detect external mechanical signals. Sensory bristles are numerous and distributed over the entire body surface of *Drosophila*. Campaniform sensilla, in contrast, are restricted to the wings, the basal and middle segment of the halteres, and the trochanter and femur of

the legs (Bryant, 1978). Bristles, campaniform sensilla and chordotonal organs are found in *Drosophila* legs. The mechanoreception-defective mutants *unc* and *uncl* can neither stand nor walk (Kernan et al. 1994). Which of the three types of mechanoreceptors contribute to coordinated walking is unknown. Bristles are contact sensors. Chordotonal organs measure joint angles (proprioreception), whereas Campaniform sensilla sense the deformation of the exoskeleton rather than the joint angles (reviewed in: Zill and Seyfarth, 1996). Campaniform sensilla are also found in the wing and the halteres. The campaniform sensilla in the halteres are equilibrium organs sensing Coriolis forces resulting from rotations of the body and mediating corrective reflexes (Nalbach and Hengstenberg, 1994). Previous studies (Trimarchi and Murphey, 1997) have shown that the synapses made between the haltere afferents and a flight motorneuron have both an electrical and a chemical component. In insects, *innexins* are thought to be the counterparts of the electrical synapses-forming *connexins* (Phelan et al., 1998). Mutations in one of the *Drosophila innexin* genes were shown to disrupt the electrical synapses between the haltere afferents and a flight motorneuron. The cholinergic component of these synapses remained unaltered in the mutants (Trimarchi and Murphey, 1997). The importance and functional significance of the two components of this synapse are unknown.

In adult *Drosophila*, olfactory and gustatory information is processed at different successive levels. Chemosensory neurons are located on the third antennal segment, the maxillary palps, the labellum, the pharynx, the tarsi, the wings, and the female genitalia (for a review of chemoreception, see Stocker, 1994). The olfactory receptors of the maxillary palps and the third antennal segment (plus certain pharyngeal sensilla) project to the antennal lobe. The antennal lobe is a conspicuous brain structure composed of 43 anatomical subunits, referred to as glomeruli (Laissue et al., 1999). Interestingly, individual afferent chemoreceptor fibers are invariably glomerulus-specific (Stocker et al., 1983). The anatomical organization of the antennal lobes led to the speculation that individual glomeruli are functionally specialized. This was later supported by the findings, that different odors excite specific subsets of glomeruli (Rodrigues, 1988) and that antennal chemoreceptors expressing a given receptor gene project to specific glomeruli within the antennal lobe (Vosshall et al., 2000). Taken together these findings indicate that the quality of an odor may be represented at the level of the antennal lobe by the level of excitation of the glomeruli.

Photoreception in flies is mediated by the two compound eyes, the three dorsal ocelli**,** extraretinal photoreceptors at the compound eye's posterior margin (Hofbauer and Buchner, 1989), and by brain neurons containing a deep brain photoreceptor (Emery et al., 2000). The information gathered by the compound eyes is processed in the optic lobes, the most prominent structures of the adult brain (for a detailed description of optic lobe neuroanatomy, see Fischbach and Dittrich, 1989; for Musca, see Strausfeld, 1976).

1.6. Processing of visual information in the lamina

The optic lobes of *Drosophila* and other flies consist of four neurocrystalline neuropils (lamina, medulla, lobula and lobula plate) through which information from single visual sampling units is processed in columns. In the *Drosophila* eye the units sampling the visual surround are not the ommatidia but visual elements consisting of eight photoreceptors from seven adjacent ommatidia which are directed toward the same point in the visual surround. All through the optic lobe there is crosstalk between neighboring columns. In *Drosophila*, in the first optic neuropil, the lamina, information is processed in 12 types of columnar neurons in each column (Meinertzhagen and O'Neil, 1991). The 11 types of small field neurons per column include receptor neurons (R1-6, R7, R8) as well as five types of lamina monopolar cells (L1-5), one type of second order interneuron (T1(beta)), and two types of medulla cells, the centrifugal neurons C2 and C3. In addition to the 11 columnar small field neuron types the large field amacrine cell alpha is found in each column. All of the connections between the 12 types of neurons in the lamina column are believed to be identified anatomically (Meinertzhagen and O'Neil, 1991) (Figure 4). This knowledge comprises a primary resource to study information processing unmatched in most other neural systems.

Figure 4

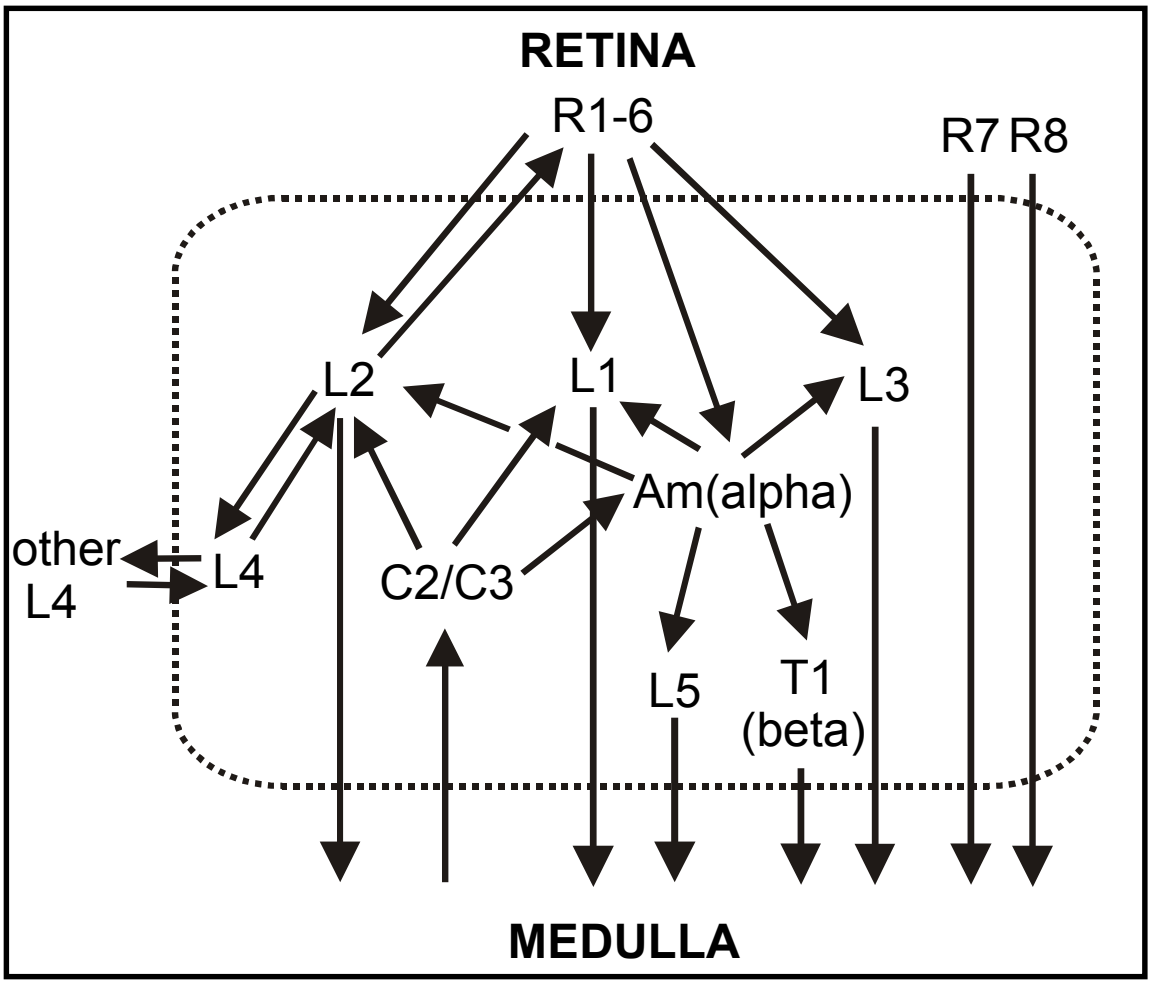

Figure 4: The twelve types of columnar neurons in the lamina (R1-6; R7; R8; L1; L2; L3; L4; L5; C2; C3; Am(alpha); T1(beta)) and their synaptic connectivity in *Drosophila* are shown according to Meinertzhagen and O'Neil (1991). The large field amacrine cell alpha is the only large field neuron. L1, L2, L3 and alpha receive input by common tetrad synapses from the retina, whereas C2 and C3 receive input from the medulla. L1, L2, L3, L5 and T1 project to the medulla. The only neuron providing feedback to the retina is L2.

Despite the detailed knowledge of lamina anatomy and connectivity, little is known about the function of specific lamina neurons. The fact that the R1-6 photoreceptors give identical input to three types of lamina monopolar cells (L1-L3) and the large field amacrine cell alpha, illustrates that at this very early stage in visual processing, information from one point in visual space is processed in parallel pathways within one column. Whether this parallel information processing reflects a functional specification is unknown. So far the methods to investigate information processing in the lamina have been limited. Electrophysiology and other ways to record neuronal activity are not useful to study the functional specification of neurons that receive identical input. To investigate for which behaviors the two large lamina monopolar cells L1 and L2 are necessary, the *Drosophila* mutant *vacuolar medulla* (*vam*) in which L1 and L2 degenerate was tested behaviorally (Coombe 1986; Coombe and Heisenberg, 1986; Coombe et al., 1989). However, the interpretation of these experiments with respect to the role of L1 and L2 in motion detection remained non-conclusive (Coombe 1986; Coombe et al., 1989).

The lamina monopolar cells L1-3 and the large field amacrine cell alpha receive direct input from R1-6 photoreceptors. L1 and L2 neurons are evolutionarily conserved across taxa, they have large axons and share inputs from R1-6 photoreceptors, the large field amacrine cell alpha as well as C2 and C3. L2 differs from L1 in being pre- and postsynaptic to L4. L4 cells receive input exclusively from L2 and build a matrix of crossconnections between columns, therefore L2 provides exclusive input to a whole field connection at this very early level. Another remarkable feature of L2 is that it provides feedback to photoreceptors by being presynaptic to R1-6. The two other pathways (L3 and amacrine cell alpha) are interconnected. The large field amacrine cell alpha is presynaptic to L3. No neurons in the lamina are postsynaptic to L3 and its target in the medulla is unknown.

Since in the second optic neuropil, the medulla, directionally sensitive small field elements have been found in large flies (DeVoe and Ochleford, 1976; DeVoe, 1980; Gilbert et al., 1991) and deoxyglucose mapping in *Drosophila* identified patterns in the medulla specifically activated by visual motion (Buchner et al., 1984; Bausenwein et al., 1992), elementary movement detection might take place in the lamina. Signal transfer between neighboring columns is a prerequisite for motion

detection. L4 and the large field amacrine cell alpha are the only two types of neurons that provide connections between neighboring columns upstream of the medulla.

1.7. Optic lobe output neurons

In flies, much of the midbrain volume is taken up by an extensive system of tracts containing axons of neurons from the medulla and lobula complex (Figure 5; reviewed in Strausfeld and Nässel, 1981). Some of them project to optic foci on the ipsi- or contralateral side of the central brain, whereas others, such as the posterior optic tract, link the left and right medullae or the lobula plates. Both, small field and large field output neurons of the optic lobes that project to characteristic targets have been found. Many of them have been described anatomically in detail (Strausfeld, 1976).

Output neurons of the lobula and lobula plate have attracted special attention since Bishop and Kheen (1966) showed directional motion-sensitivity in these neurons. Since then, numerous recordings from all kinds of optic lobe output neurons in a wide variety of species have been reported. These electrophysiological studies did provide very detailed information about the responses of identified types of optic lobe output neurons to different stimuli. In *Calliphora*, about 50 types of large tangential cells have been found to be sensitive to motion (Hausen and Egelhaff, 1989). Of these, the H1 cell and the HS and VS cells have been studied most extensively in different fly species (reviewed in: Hausen and Egelhaff, 1989; Strausfeld, 1989; Franceschini et al., 1989).

Figure 5

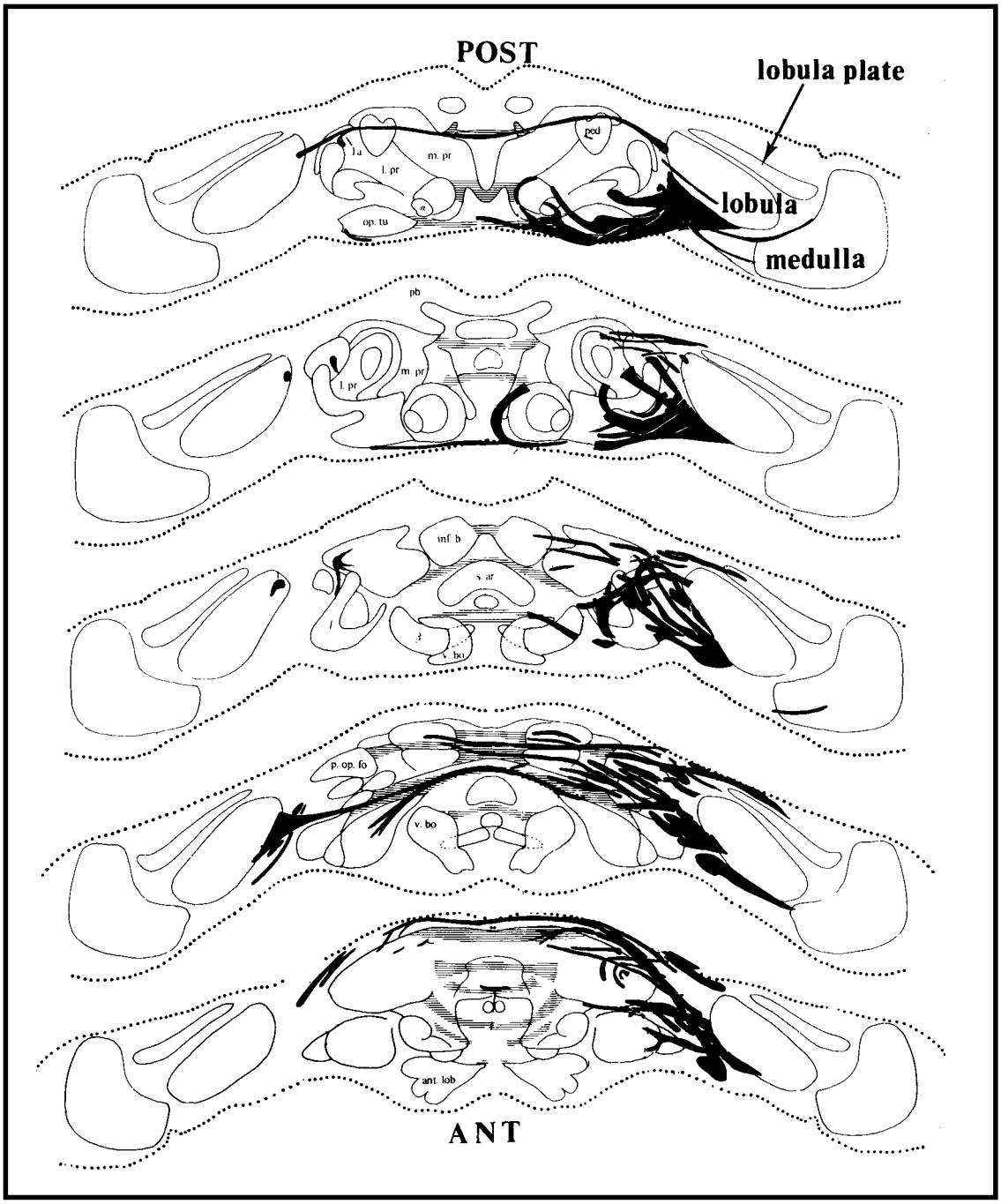

Figure 5: Projections of axon bundles from the optic lobes into the midbrain of *Musca*. The figure is taken from Strausfeld and Nässel, 1981 (adapted from Strausfeld, 1976). Axon bundles traced from the optic lobes into the midbrain through selected serial sections are illustrated. Each population of neurons (similarly shaped neurons invading a characteristic layer of a neuropil) projects to a characteristic target either in the central brain or in the contralateral optic lobe.

The electrophysiological properties of HS/VS cells indicate a role in the processing of visual motion signals. HS cells are sensitive to horizontal motion and VS cells to vertical motion. Considering the variety of motion-sensitive tangential cells, and the fact that various other tangential cells show similar responses to a moving stimulus as VS and HS cells (Hausen, 1984), the question remains, if VS and HS cells are essential for behaviors induced by motion stimuli. The only way to address this question is, to generate flies in which VS and HS cells are absent or non-functional. Two strategies to mechanically manipulate the cells have been employed. After laser ablation of the HS/VS precursor cell (Geiger and Nässel, 1981) the response to regressive stimuli is reduced whereas the response to progressive stimuli is only slightly affected (Geiger and Nässel, 1981). Microsurgical lesion of the axonal trunks of all three HS cells of the right lobula as well as the contralateral FD and H2 cells eliminates the response to clockwise horizontal binocular stimulation (Hausen and Wehrhahn, 1983). A *Drosophila* mutant in which VS and HS cells are absent emerged from a genetic screen for mutants defective in optomotor turning behavior (Heisenberg and Götz, 1975). In this mutant, called omb^{H31}, the large field response was found to be impaired while the object response is still functional (Bausenwein et al., 1986). As a consequence, optomotor responses in walking (Heisenberg et al., 1978) and yaw, roll and pitch optomotor responses during flight are impaired (Blondeau and Heisenberg, 1982). Additionally, these flies did show no head roll and pitch and a head yaw response that is reduced to 25 % of the wildtype value (Roland Hengstenberg, Tübingen, pers. comm.). Fixation of one black stripe is absent in omb^{H31} flies. omb^{H31} is also defective in Benzer's fast phototaxis paradigm (H. Weikert and M. Heisenberg, unpublished), and in a courtship paradigm omb^{H31} males are less successful than wildtype males in light but not in the dark (Tompkins et al., 1982). Another visual paradigm in which omb^{H31} was found to differ from wildtype is a forced-choice fixation Y-maze in which flies have to decide at each decision point to turn towards a small black dot, covering the receptive field of about one ommatidium. Wildtype flies turn towards the small black dot, whereas omb^{H31} flies avoid it (Bülthoff et al., 1982).

In addition to omb^{H31}, four mutations of the *omb* locus that cause the loss of HS and VS cells in allelic combination with omb^{H31} have been characterized (Brunner et al., 1992). In these deficiencies smaller parts of the 3'-regulatory region are removed. The

OLR1 and OLR2 regions that are removed in omb^{H31} are unaffected in these alleles (Figure 6).

Figure 6

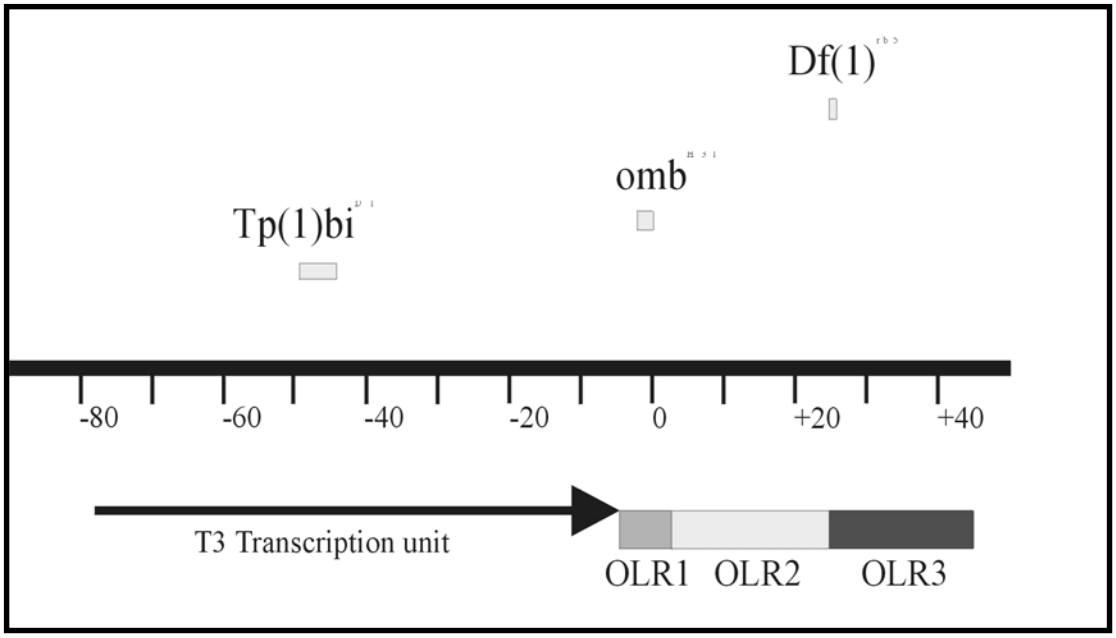

Figure 6: The downstream regulatory region of the *omb* gene locus. The breakpoints of the chromosomal aberrations of the *omb* alleles used in this study are shown. The gray boxes give the confines to which the breakpoints have been mapped. The scale is in kb with the zero point at the location of the inversion breakpoint of omb^{H31}. All three aberrations in combination with omb^{H31} do remove the HS and VS cells, but $Df(1)^{rb5}$, which removes only the OLR3 region, in combination with omb^{H31} shows less severe impairments of the optomotor behaviors. The black arrow represents the length and orientation of the *omb* transcription unit T3. Many transcription units have been described in the *omb* locus (Pflugfelder et al., 1990). Only the T3 transcription unit is shown here. The extents of the optic lobe regulatory regions (OLRs) are indicated by differently shadowed bars. (modified after Brunner et al., 1992)

Surprisingly, in the allelic combinations, the optomotor phenotype is much less pronounced than in omb^{H31}. Such mutants show a wildtype response to progressive stimuli during flight and 25 % of the wildtype response to regressive stimuli. The original omb^{H31} mutant shows a 50 % response to progressive and no response to regressive stimuli in the same experiment. This finding challenged the assumption that in omb^{H31} the loss of the HS and VS cells is causally related to the alteration in responses to moving stimuli. omb^{H31} shows additional anatomical defects that may as well be responsible for the behavioral defects (reviewed in Pflugfelder and Heisenberg, 1995). In omb^{H31} the two M-fibers are missing, the volume of the lobula plate is reduced by 30 %, the fiber number in the anterior optic tract is reduced from 1300 to 1000, the

order of the inner optic chiasm (IOC) is disturbed, very rarely a preimaginal orientation of the medulla is observed in adult flies and a set of lamina tangential cells labeled by a monoclonal antibody (nb169) lack their normally extensive arborizations in the lamina in omb^{H31}. Three of these anatomical defects found in omb^{H31} have been studied in the allelic combinations. The reduction in number of fibers in the anterior optic tract and the lack of arborizations in the lamina tangential cells labeled by nb169 is observed in both mutants (Brunner et al., 1992 and Hofbauer, Regensburg, pers. com.).The disruption of order in the IOC observed in omb^{H31} does not occur in the allelic combinations (Brunner et al., 1992), but, since there is no correlation between the optomotor defect and the severity of the disruption of the IOC, this can not explain the behavioral differences. However, anatomical defects, that were not studied in both genotypes, may also be less severe in the allelic combinations.

HS and VS cells are prominent tangential cells of the fourth optic neuropil, the lobula plate, but there are also numerous tracts connecting the lobula and the medulla with the central brain or the contralateral optic lobe (reviewed in Strausfeld and Nässel, 1981). These cells are less well studied electrophysiologically since their axon tracts are more central and therefore less accessible to electrophysiology. In genetically modified *Drosophila*, these optic lobe output neurons can be studied using the UAS/GAL4 system. A variety of GAL4 lines labeling specific types of tangential cells are available and additional lines are emerging from an ongoing screen (Kei Ito, Okazaki, pers. comm.). Some optic lobe output neurons are known to mediate functions other then optomotor course control. For example, in locust (Gabbiani et al., 1999) or *Manduca* (Wicklein and Strausfeld, 2000) neurons sensitive for expanding stimuli have been found. And, of course, all other aspects of the visual environment that induce a change in behavior have to be propagated from the visual system to the central brain, too.

2. Materials and Methods

2.1. Behavior

Unless stated otherwise, experimental flies are raised on standard *Drosophila* medium (cornmeal, agar, molasses, yeast, nipagin) under constant light-dark cycle (16L/8D) at 25°C and 60 % relative humidity. Fly stocks are kept on 18°C and 60% relative humidity. Unless indicated otherwise, flies are kept as homozygotes and females are tested in the behavioral paradigms. Flies are anaesthetized by cooling to 4° C. In cases were wings are cut off, this is done at least three hours prior to the experiment. Most tests are performed using three or four day old flies.

GENERAL BEHAVIOUR

Courtship latency*:* Flies tested for courtship latency are collected 1-7 h post-eclosion and stored individually before being tested. Four day old virgins are placed together with four day old males in a 0,4 cm^3 plastic observation chamber. Time till copulation is measured. If no copulation occurs the experiment is terminated after 15 minutes.

Activity: For the analysis of time spent walking flies with clipped wings walk freely on an illuminated circular disc (85 mm diameter) surrounded by a water-filled moat for 5 or 15 minutes. Their tracks in a visually unstructured environment are recorded by a video scanning device at a sampling rate of 5 Hz and the time spent walking is calculated as described in Strauss et al. (1992).

LOCOMOTOR BEHAVIOUR

Walking speed: For the analysis of average walking speed flies with clipped wings walk freely on an illuminated circular disc surrounded by a water filled moat for 5 or 15 minutes. Their tracks in a visually unstructured environment are recorded by a video scanning device at a sampling rate of 5 Hz and the average walking speed of the flies is calculated as described in Strauss et al. (1992).

Ability to walk on vertical surface: For the analyses of the ability to walk on vertical surfaces, a countercurrent apparatus (Benzer, 1967) is used. The experiment is performed under red light. For each set of flies consisting of 10 flies, five consecutive cycles are carried out. Before each cycle flies are gently shaken to the bottom of the vial

and allowed to walk up on the vertical walls of the vial driven by negative geotaxis. Cycle duration is 10 s. Diameter of the vials: 15 mm, length 72 mm.

Grooming assay. The ability of flies to clean themselves is determined as described in Phillis et al. (1993). Briefly, flies are dusted with a defined amount of dust (Reactive Yellow 86 (SIGMA R-2879)) and thereafter transferred to a vial with the bottom replaced with a fine mesh screen and shaken to remove "excess" dust. Afterwards, flies are allowed to groom for one hour. Differing from Phillis et al. (1993) flies are not screened visually for remaining dust but the remaining dust is quantified by washing it off and measuring the optic density of the wash.

Time to flight initiation. To quantify the altered flying behavior, spontaneous flight initiation is analyzed by releasing single flies on an plate illuminated from above (100 cm x 60 cm) and measuring the time before flight initiation. The experiment is terminated after 60 seconds.

OLFACTORY BEHAVIOUR

Odor avoidance assays: Odor avoidance assays similar to those described in Anholt et al. (1996) are performed. Two or 3 day old females are transferred in groups of 5 to clear plastic culture vials 3 h prior to the experiment. The vials (35 mm diameter) are marked with two lines, 3 and 6 cm from the bottom. Experiments are performed under red light. The stopper of the vial is soaked in the respective odorant diluted to the respective concentration with paraffin oil (Merck, Hohenbrunn, Germany). Afterwards, the flies are placed in the vial and the stopper is inserted to the 6 cm line and the vial is placed on its side. Flies are given 15 seconds to recover from the disturbance. Then every 5 sec the number of flies in the compartment more distant to the odorant soaked stopper is taken. After 10 counts the experiment is terminated. 8 or 10 replicate tests are done on three or four different days. The behavior of each line is quantified as the "avoidance score", calculated as the number of flies in the compartment more distant to the odorant soaked stopper, averaged over the 10 measurements (every 5 sec) and the 8 or 10 replicates. Since flies are tested in groups of 5 and the vial is divided in two compartments, a score of 2.5 indicates that the flies are equally distributed in the vial. Benzaldehyde, 4-Methylcyclohexanol (both Merck, Hohenbrunn, Germany) and Isoamylacetate (Aldrich, Steinheim, Germany) are used.

VISUAL BEHAVIOUR

***Optomotor response in walking**:* Optomotor response in walking is measured as described earlier (Heisenberg and Götz, 1975). Briefly, fixed flies walk on an air-supported styrofoam ball the rotations of which can be measured (Buchner, 1976). A grating of stripes is rotated around the fly and the difference in the number of counts monitoring revolutions of the styrofoam ball around the vertical axis following and against the pattern movement is measured. After 50 counts monitoring revolutions of the ball around its transverse horizontal axis (forward walking), a new measurement starts. In some experiments, conditions known to elicit maximal responses (24° patterns; 3 Hz contrast frequency; maximal pattern luminance) are used. In other experiments, optomotor responses to a range of contrast frequencies at maximal pattern luminance or to a range of pattern luminances at 3 Hz contrast frequency are measured (Figure 7A).

***Fixation in walking**:* Fixation in walking is measured as described earlier (Heisenberg et al., 1978). Briefly, flies with clipped wings are placed in the center of an illuminated arena (29 cm diameter) with two opposing black stripes of different width. In some experiments only one black stripe, covering half of the arena (180°) is presented. If not indicated otherwise, flies are scored when reaching one of 12 segments of a measuring circle (19 cm diameter). After each run, flies are transferred back to the center of the arena with a brush and a small vial. Flies perform 10 or 12 runs (Figure 7B). The fixation efficiencies given are normalized because randomly walking flies walk towards a larger stripe more frequently than towards a smaller stripe although their fixation efficiency is zero in both cases. Normalization is performed by subtracting the theoretical fixation value of a randomly walking fly and subsequent multiplication to reach 100 % (for example for two opposing 10° stripes and 10 runs: (X (number of runs towards stripes) - 1,67) x 12.

Forced-choice fixation Y-maze: In the forced-choice fixation Y-maze described in Bülthoff et al. (1982) flies walk through a maze in which they have to decide at 14 choice levels to walk towards a circular segment of either a black or a transparent rod (Figure 7C). The segments cover approximately 5° x 5° as seen by the fly. The results are calculated as fixation efficiencies ranging from 100% (absolute preference for black) over 0% (random choices) to -100% (absolute avoidance of black).

Landing response: When a flying fly intends to land it lowers the second and third pair of legs and stretches its forelegs above the head. This can be elicited by visual stimuli simulating an approaching object. Here, a spiral that generates an expanding visual

stimulus when rotated is used. The leg extension is visually recorded under a microscope (Fischbach, 1981) (Figure 7D). Each fly is tested ten times.

Visually induced head yaw, pitch and roll. Rotation of the visual field around a fly leads to syndirectional turning of the head (Hengstenberg, 1993). The angle of the head yaw, pitch or roll response is measured by gluing flies to a manipulator and placing them in an arena (Figure 7E). If not indicated otherwise, the stimulus is produced by a striped drum rotating around the fly (pattern wavelength $\lambda = 60°$; contrast frequency: 1.2 Hz). The flies are videotaped. Flies turn their head in the direction of the movement. The fly head reaches a steady state in which the position of the head is marked on the screen. Then the direction of the rotation of the striped drum is changed and the position of the head after it reached the steady state is marked. The angle between head positions for clockwise and counterclockwise stimulation is measured. Each fly performs three times. In other experiments, a range of contrast frequencies is tested.

To test the yaw response to progressive (front to back) and regressive (back to front) stimulation independently a 180° screen is used. To test the response to progressive stimulation the screen is installed to block the left side of the visual space of the fly and the drum was rotated in a way that on the right side front to back movement is seen by the fly (pattern wavelength $\lambda = 60°$; contrast frequency: 1.2 Hz). After the fly head reaches the steady state, the position of the head is marked on the screen. Then the direction of the rotation of the striped drum is changed and the screen is moved from the left side of the fly to the right side. The fly now sees front to back movement on the left side. Again the position of the fly head in the steady state is marked and the angle between the two marks is measured on the screen. Both marks represent responses to front to back movement, one on the left and one on the right side of the fly. The response to regressive stimulation is measured accordingly.

A different pattern is used to test the response to moving black to white edges and to moving white to black edges independently. To test the response to white to black edges, the pattern rotated around the fly consists of gray gradients from black to white and sharp white to black edges (pattern wavelength $\lambda = 60°$; contrast frequency: 1.2 Hz). After clockwise stimulation the pattern is changed to one with an opposite orientation of the gradient and the direction of the drum rotation is changed. In this way clockwise and counterclockwise stimulation consist only of white to black edges. Again the position of the steady state of the head is marked on the screen for both directions

and the angle between the two positions is measured. Responses to black to white edges are measured accordingly.

Figure 7

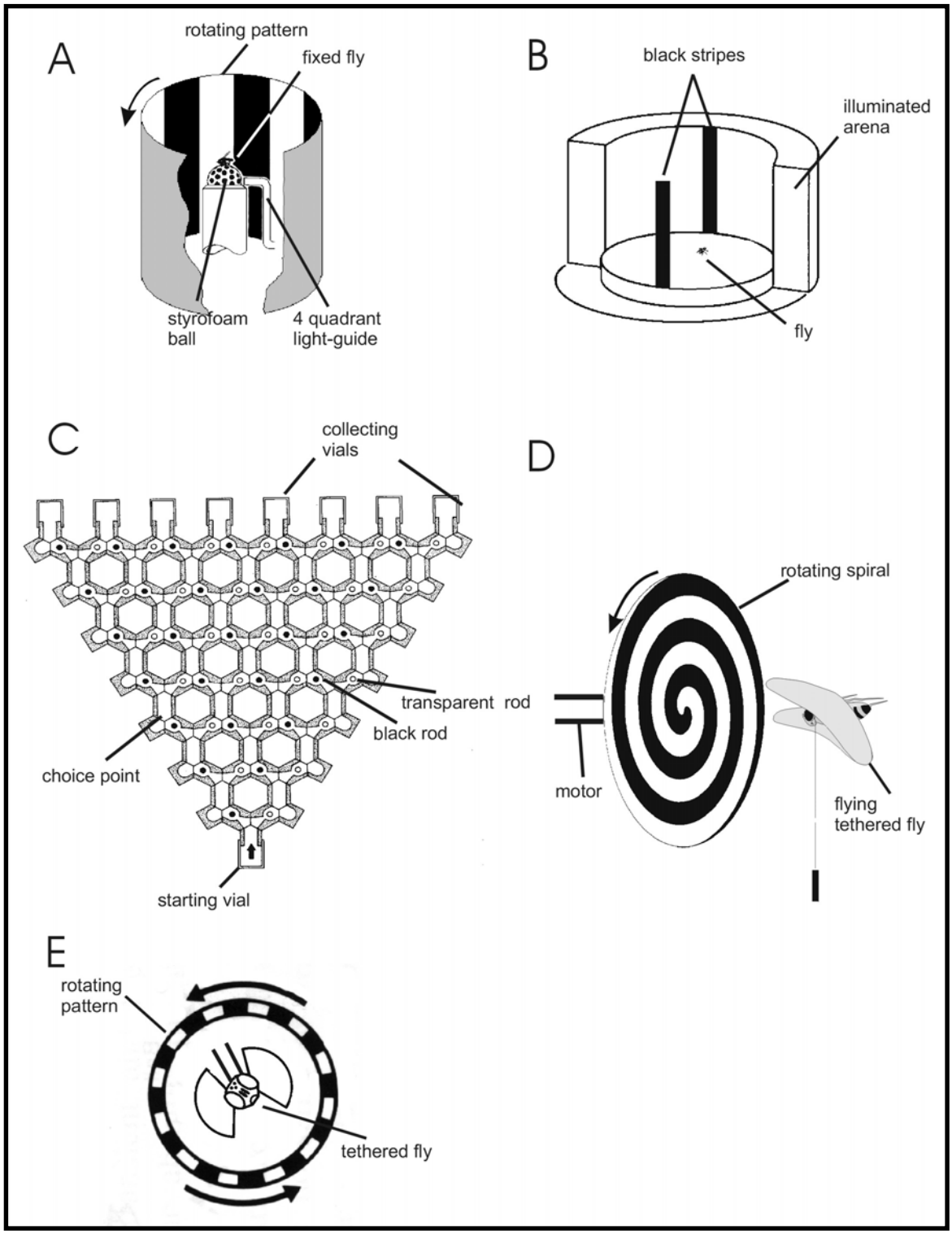
A
rotating pattern
fixed fly
styrofoam ball
4 quadrant light-guide
B
black stripes
illuminated arena
fly
C
collecting vials
transparent rod
black rod
choice point
starting vial
D
rotating spiral
motor
flying tethered fly
E
rotating pattern
tethered fly

Figure 7 (previous page): (**A**) Optomotor response in walking is measured as described earlier (Heisenberg and Götz, 1975). Briefly, fixed flies walk on an air-supported ball whose rotations can be measured (Buchner, 1976). A grating of stripes is rotated around the fly and the difference in the number of counts monitoring revolutions of the ball around the vertical axis following and against the pattern movement is measured. (**B**) Rotation of the visual field around a fly leads to syndirectional turning of the head (Hengstenberg, 1993). The angle of the head yaw, pitch or roll response is measured by gluing flies to a manipulator and placing them in an arena. (**C**) Fixation in walking is measured as described earlier (Heisenberg et al. 1978). Briefly, flies with clipped wings are placed in the center of an illuminated arena (29 cm diameter) with two opposing black stripes. Flies are scored when reaching one of 12 segments of a measuring circle (19 cm diameter). (**D**) When a flying fly intends to land it lowers the second and third pair of legs and stretches its forelegs above the head. This can be elicited by an expanding visual stimulus. Here, a spiral generates an expanding visual stimulus when rotated. The leg extension is visually recorded under the microscope (Fischbach, 1981) (**E**) The forced-choice fixation Y-maze described in Bülthoff et al. (1982) is used. Flies walk through a maze in which they have to decide at 14 choice levels to walk towards a circular segment of either a black or a transparent rod.

2.2. Immunohistochemistry

TNT expression patterns are examined in sections that are blocked for two hours with normal horse serum (1:50) in PBS plus 0,1% Triton X100 (PBT) and incubated with monoclonal anti-TNT antibodies (1:1000; kindly provided by Niemann, Hannover) in PBT overnight at 4 °C. A series of washes and incubation with a biotinylated anti-mouse antibody (1:200) for one hour at room temperature follows. Signal is detected using the Vectastain ABC elite kit (Vector Laboratories, Burlingame, California) following manufacturer's instructions.

ß-GAL expression patterns are examined in sections following the same protocol but using a monoclonal anti-*ß-GAL* antibody (1:200, Promega, Madison, Wisconsin).

For double stainings, flies are anaesthetized and brains are dissected in *Drosophila* Ringer by stripping off the head capsule including the eyes. Brains are fixed overnight in 2% para-formaldehyd at 4° C. The brains are stained using a polyclonal rabbit anti-*ß-GAL* antibody (1:1000, Cappel, ICN Biomedicals, Seven Hills, Australia) and a monoclonal mouse antibody labeling the whole neuropil (nc82, 1:7.5, Rein et al., 1999). As secondary antibodies an anti-rabbit antibody conjugated to Alexa488 (1:100, Molecular Probes, Eugene, Oregon) and an anti-mouse antibody conjugated to CY3.18 (1:250, Jackson Immuno Research, West Grove, Pennsylvania) are used. Optical sections are acquired using a Leica CLSM/Aristoplan confocal microscope equipped with a Zeiss objective lens (Plan Neofluar 25x) with a numerical aperture of 0.8. 0.8 µm are sampled in all directions.

Non-neuronal structures are in some cases erased from the figures using the Adobe Photoshop (Adobe Systems Inc., San Jose, California) stamp function.

3. Results

3.1. Comparison of effectors

Apoptosis inducers and cell toxins

To investigate how useful apoptosis inducers and cell toxins are for structure-function mapping in the *Drosophila* nervous system flies expressing the different effectors under *GMR*-GAL4 control were generated. *GMR*-GAL4 drives expression in eye discs of third instar larvae in all cells posterior to the morphogenetic furrow. Sixty hours after pupation, expression persists in photoreceptors and pigment cells, but can not be detected in cone cells. In eye discs, additional expression can be detected in ocellar precursor cells (Ellis et al., 1993). Expression in retinal photoreceptors and pigment cells persists to adulthood (Figure 8). No additional neuronal expression can be detected.

Figure 8

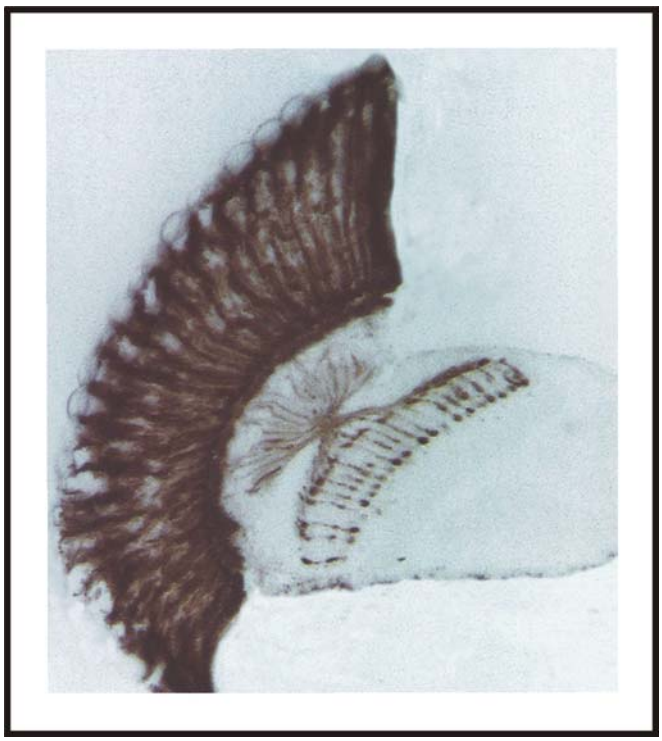

Figure 8: Expression pattern of the *GMR*-GAL4 line. A horizontal section of one eye and optic lobe is shown (10 µm section). Tetanus neurotoxin (TNT) expression is visualized with an anti-TNT antibody. The photoreceptors projecting from the retina to the lamina or the medulla and pigment cells in the retina are labeled.

Flies expressing a cold-sensitive variant of the protein synthesis inhibitor *ricinA* (mutant *rAcs*, inactive at 18°C) under *GMR*-GAL4 control survive to adulthood when reared at 18°C. When adult flies are shifted to 29°C for at least two days prior to

behavioral experiments these flies still show optomotor responses (Figure 9) indicating that sufficiently enough photoreceptors are still functional to provide dual input to movement detectors. Individuals expressing the apoptosis-inducing genes *reaper* (*rpr*) or *head involution defective* (*hid*) in a *GMR*-GAL4 dependent manner do not survive to adulthood (Table 2). Most individuals die shortly after pupariation although *GMR*-GAL4 is reported to drive expression only in the eye discs and in the ocellar precursor cells.

Table 2

	rdgC	***GMR***	**GH146**	**21D**	**TP849**	**3A**	**1187**
TNTE	viable	viable	viable	viable	viable	viable	viable
TNTC	viable	viable	viable	viable	n.d.	viable	n.d.
TNTG	viable	viable	n.d.	viable	n.d.	n.d.	n.d.
shi^{ts1}	n.d.	viable	viable	n.d.	n.d.	viable	viable
KIR1	viable	viable	n.d.	viable	n.d.	viable	viable
KIR14	viable	viable	n.d.	lethal	n.d.	viable	lethal
KIR15	viable	viable	n.d.	n.d.	n.d.	viable	viable
KIR16	viable	viable	n.d.	n.d.	n.d.	lethal	viable
KIR17	lethal	lethal	n.d.	n.d.	n.d.	lethal	lethal
hid	viable	lethal	n.d.	lethal	lethal	lethal	lethal
rpr	viable	lethal	n.d.	lethal	lethal	n.d.	n.d.
rAcs	n.d.	viable	n.d.	n.d.	n.d.	viable	n.d.

Table 2: Consequences of expressing a variety of effectors killing or functionally blocking neurons under the control of seven GAL4 lines expressing in sensory structures. Note that independent transformants of the same transgene can have different effects when crossed to the same GAL4 line. For example, the GAL4 line 1187 is viable in combination with KIR16 but not with KIR14 whereas 3A is viable with KIR14 but not with KIR16. For the GAL4 line *GMR* the viable combinations are color-coded. Those that produce the expected phenotype (blindness) are highlighted in blue and those that fail to do so in red. n.d. = not done.

Figure 9

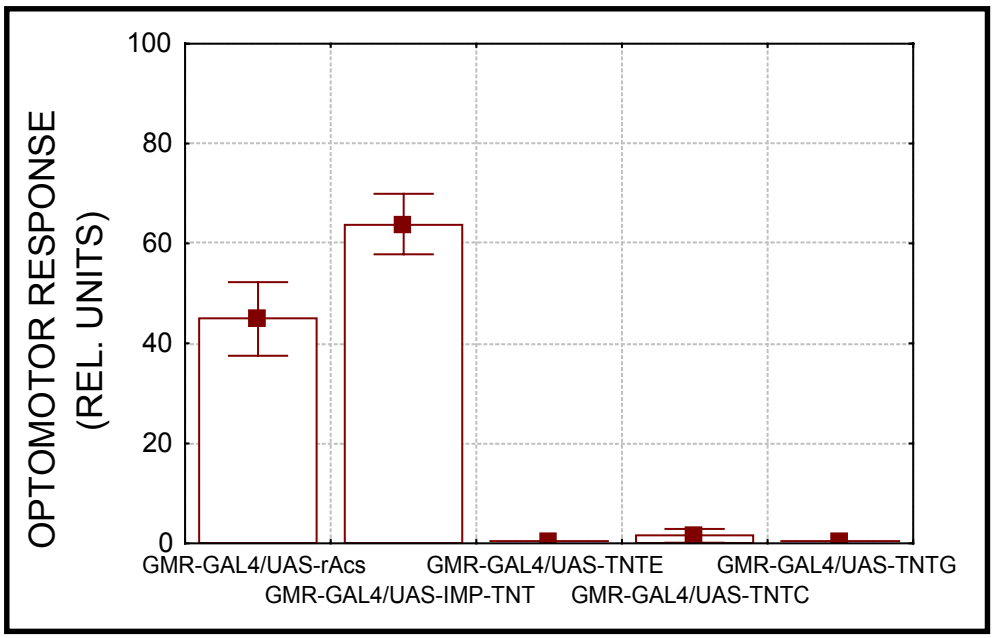

Figure 9: Optomotor response in walking of flies expressing toxins in photoreceptors. Flies expressing cold-sensitive ricin A chain (*rAcs*), or active (TNTE, TNTG, TNTC) or inactive (IMP-TNT) tetanus neurotoxin in photoreceptors (*GMR*-GAL4) were tested. For experimental details see Material and Methods. Error bars denote SEMs over flies. Values for *GMR*-GAL4/UAS-TNTE, *GMR*-GAL4/UAS-TNTG and *GMR*-GAL4, UAS-TNTC are not significantly different from zero ($P > 0.05$ in all cases). *rAcs* flies were reared at the permissive temperature (18°C, inactive toxin) and after eclosion were shifted to the non-permissive temperature (29 °C) for at least two days. The value for *GMR*-GAL4, UAS-*rAcs* is significantly different from the *GMR*-GAL4/UAS-IMP-TNTQ value and from zero ($P < 0.01$ in both cases; n = 4-6).

In contrast, a second GAL4 line, *rdgC*-GAL4, is viable in combination with either UAS-*rpr* or UAS-*hid* (Table 2). The *rdgC*-GAL4 construct, driving GAL4 expression under control of the 5' region of the *rdgC* gene, labels a subset of chemosensory neurons and campaniform sensilla (Figure 10). In all six legs, expression is driven in campaniform sensilla in the trochanter and femur that arborize in the respective neuromeres and in additional unidentified neurons further distal in the legs (Figure 10D). Surprisingly, immunohistochemical analysis of *lacZ* expression in UAS-*lacZ*/UAS-hid/*rdgC*-GAL4 flies revealed that the *rdgC*-GAL4-expressing campaniform sensilla in the legs were still present in these flies (data not shown).

Figure 10

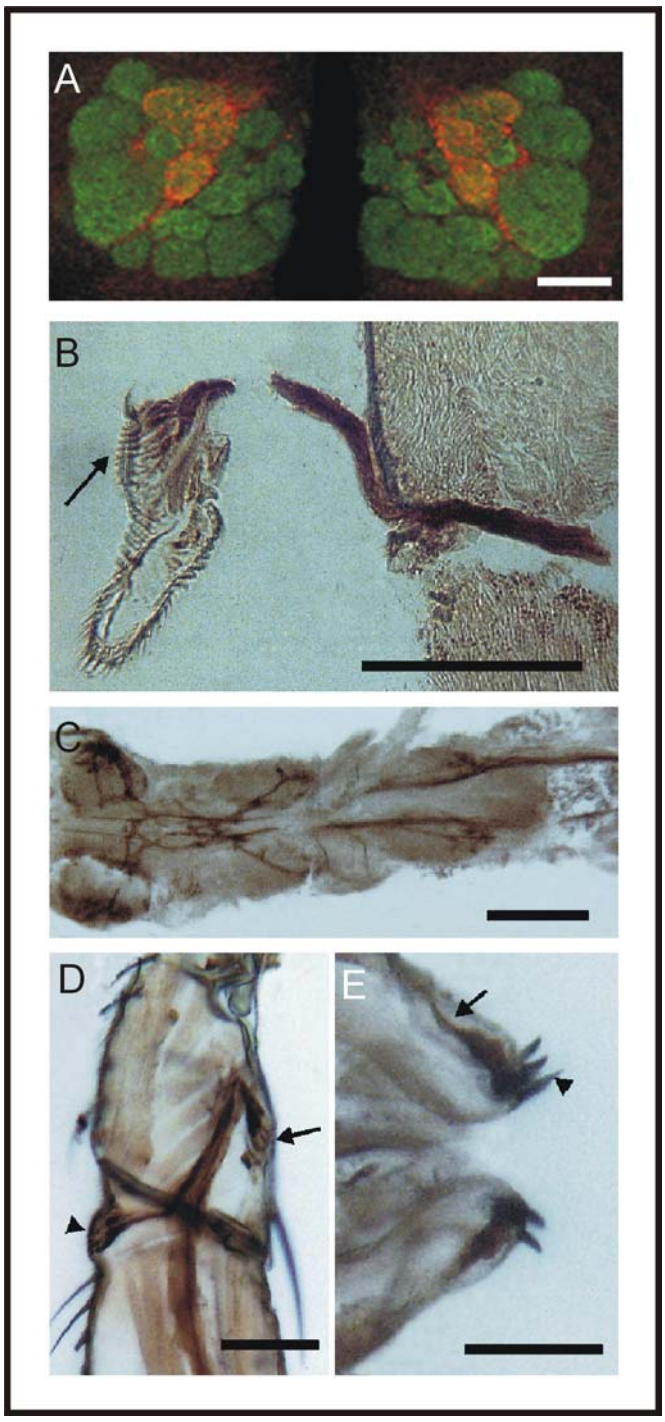

Figure 10: Expression pattern of the *rdgC*-GAL4 line, visualized using antibody against ß-GAL (A) or TNT (B-E). (**A**) Frontal section through antennal lobes of an *rdgC*-GAL4, UAS-*lacZ* fly. Double staining for a neuropil marker (nc82, green) and ß-GAL (red) is shown. Expression is driven in a set of at least four distinct glomeruli in each antennal lobe. (**B**) Haltere section (10 µm section) of an *rdgC*-GAL4/UAS-TNTE fly. The campaniform sensilla in one haltere (arrow) and, artificially detached, their projections in the haltere nerve passing inward are labeled. (**C**) Horizontal section of the thoracic ganglion (50 µm section) of an *rdgC*-GAL4/UAS-TNTE fly. For detailed description of expression pattern see Results. (**D**) Leg section (30 µm section) of an *rdgC*-GAL4/UAS-TNTE fly. Campaniform sensilla in the trochanter (arrow) and femur (arrowhead) and unidentified neurons further distal are shown. (**E**) Horizontal section of the abdomen (50 µm section) of an *rdgC*-GAL4/UAS-TNTE fly. The thorn bristles (arrowhead) and their arborizations (arrow) are shown.

These experiments illustrate two drawbacks of the use of cell toxins and apoptosis inducers for structure-function correlation in the nervous system. Firstly, non-neuronal expression may kill the flies like presumably in the case of *GMR*-GAL4/UAS-*rpr* and *GMR*-GAL4/UAS-*hid* flies. Secondly, the ability of the effectors to kill neurons is dosage-dependent.

Tetanus neurotoxin

Tetanus neurotoxin in contrast to *rAcs*, *rpr* or *hid* does not kill cells but blocks signal transmission at chemical synapses. Therefore, tetanus neurotoxin acts specifically on neurons. In addition, it is a highly potent toxin acting at low dosages (Schiavo et al., 1992a). To test and compare the capability of tetanus neurotoxin to block neuronal activity at a behaviorally relevant level, two visually guided behaviors (optomotor response and fixation) were measured in flies expressing tetanus neurotoxin in photoreceptors under *GMR*-GAL4 control. Optomotor responses were measured with tethered flies that walked on a styrofoam ball floating on a jet of air in the center of a rotating striped drum. Fixation was measured in flies with clipped wings walking freely on a platform surrounded by an illuminated cylinder with two opposing black stripes. Flies were scored when reaching one of 12 segments of a measuring circle (for experimental details see Materials and Methods). Flies carrying the *GMR*-GAL4 construct and any one of the three independent insertions of the UAS-TNT construct survived to adulthood. They were blind (Figure 9) indicating that photoreceptors are completely blocked by TNT expression irrespective of the insertion site of the UAS-TNT construct. In contrast, control flies of genotype *GMR*-GAL4/UAS-IMP-TNT showed no evidence of visual impairment.

The effects of expressing TNT using the three independent transformants were also compared in the *rdgC*-GAL4 line. The average walking speed between the three independent transformants is not different, when crossed to *rdgC*-GAL4 (Figure 11B). In contrast, there are significant differences in activity between *rdgC*-GAL4/UAS-TNTG, *rdgC*-GAL4, UAS-TNTC and *rdgC*-GAL4/UAS-TNTE flies, the latter being the most active (Figure 11A). This difference can not be explained by mutational effects of the UAS-TNT insertions, since the UAS-TNTC and the UAS-TNTG insertions in the absence of a GAL4 driver do not show a lower activity than UAS-TNTE (UAS-TNTE: mean=69.03, ±SEM=7.59, n=12; UAS-TNTC: mean=81.12, ±SEM=5.86, n=10; UAS-

TNTG: mean=82.15, ±SEM=3.33, n=11). More likely, different expression levels of toxin in the three types of flies is responsible for this effect.

Figure 11

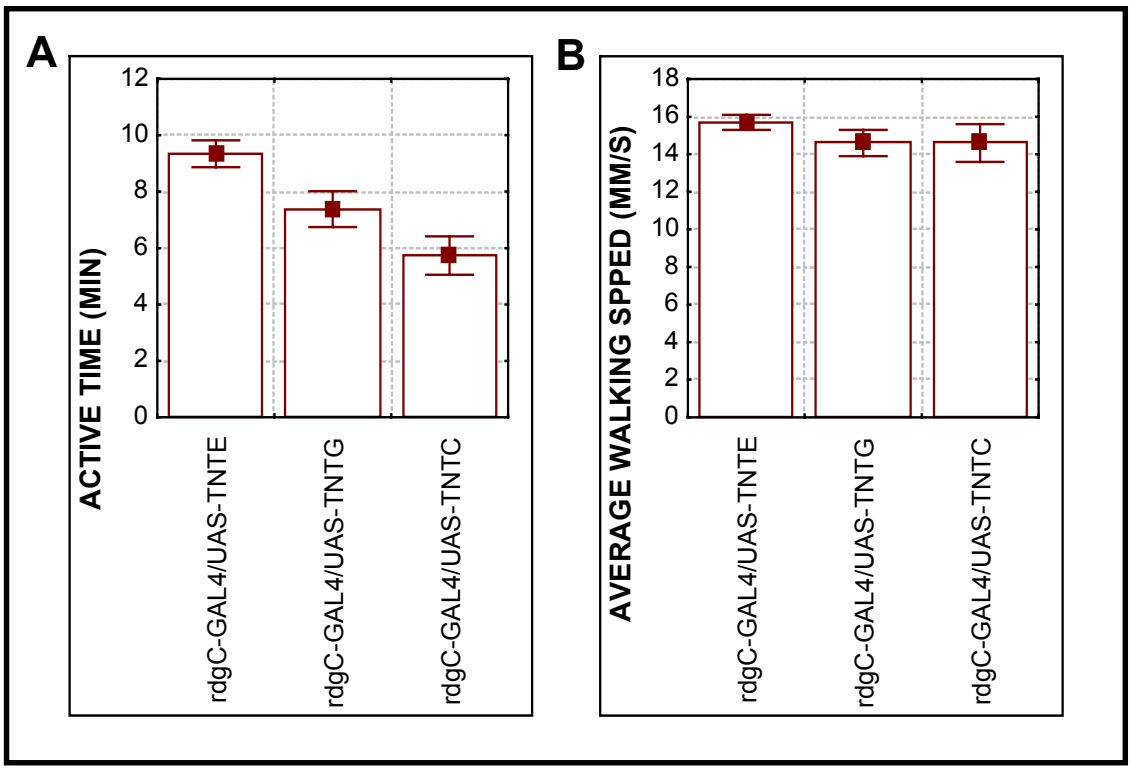

Figure 11: Comparison of the active time (**A**) and average walking speed (**B**) of three independent UAS-TNT transformants crossed to *rdgC*-GAL4. In combination with *rdgC*-GAL4/UAS-TNTG and *rdgC*-GAL4/UAS-TNTC flies are less active than *rdgC*-GAL4/UAS-TNTE flies (A; n=10; $P < 0,05$). In contrast, there is no difference between the average walking speed of the three genotypes (B; n = 10; $P > 0,05$).

Tetanus neurotoxin proved to be capable to fully block chemical synapses in *GMR*-GAL/UAS-TNT flies. However, the differences in activity between *rdgC*-GAL4/UAS-TNTG, *rdgC*-GAL4, UAS-TNTC and *rdgC*-GAL4/UAS-TNTE flies shows that there is some leakiness which can cause unspecific effects.

Human inwardly rectifying potassium channel

A disadvantage of tetanus neurotoxin is that it only blocks chemical synapses. Expressing a human inwardly rectifying potassium channel (KIR) suppresses the initiation of potentials. As a consequence expression of KIR in motorneurons results in an almost total absence of excitatory junctional currents in its target muscle (Baines et al. 2001). Since KIR interferes with the initiation of potentials rather than with synaptic transmission, it can be expected to block neurons irrespective of their synapse type. Five independent transformants of the UAS-KIR construct in combination which *GMR*-GAL4 were tested for their potential to block the chemical synapses of photoreceptors. Fixation of a black 180° stripe (one half of the arena black) in walking was tested. Only *GMR*-GAL4/UAS-KIR1 and *GMR*-GAL4/UAS-KIR14 flies are blind in this essay (Figure 12). Both genotypes are also blind in the head roll paradigm (Figure 40). *GMR*-GAL4/UAS-KIR15 and *GMR*-GAL4/UAS-KIR16 flies still show fixation of the black 180° stripe. *GMR*-GAL4/UAS-KIR17 flies are not viable. As mentioned above, KIR can be assumed to block both chemical and electrical synapses. To test this assumption a mixed synapse consisting of a chemical and an electrical component was investigated. The haltere campaniform sensilla, in which *rdgC*-GAL4 drives expression, detect angular rotations of the body during flight and mediate corrective reflexes. They are known to form mixed synapses with a flight motorneuron (Trimarchi and Murphey, 1997). Flies without halteres are unable to fly. To quantify the altered flying behavior spontaneous flight initiation was analyzed by releasing single flies on an illuminated plate and measuring the time before flight initiation. The experiment was terminated after 60 seconds. Flies without halteres were compared to flies in which either the chemical (*rdgC*-GAL4/UAS-TNTE) or the electrical ($shakB^2$) or both (*rdgC*-GAL4/UAS-KIR) components of the mixed synapses mediating the corrective reflexes were blocked (Figure 13). Flies without halteres never started flight. Flies in which either the electrical ($shakB^2$) or the chemical (*rdgC*-GAL4/UAS-TNTE) component of the haltere campaniform sensilla synapses were blocked showed a delayed start when compared to control flies (*rdgC*-GAL4/+ and UAS-TNTE/+). Blocking both the chemical and the electrical component of the synapses labeled in *rdgC*-GAL4 by expressing KIR results in flightlessness for two UAS-KIR transformants (UAS-KIR1 and UAS-KIR16), whereas two other transformants (UAS-KIR14 and UAS-KIR15) produced a less severe phenotype. As with the *GMR*-driver, *rdgC*-GAL4/UAS-KIR17 flies are not viable.

Figure 12

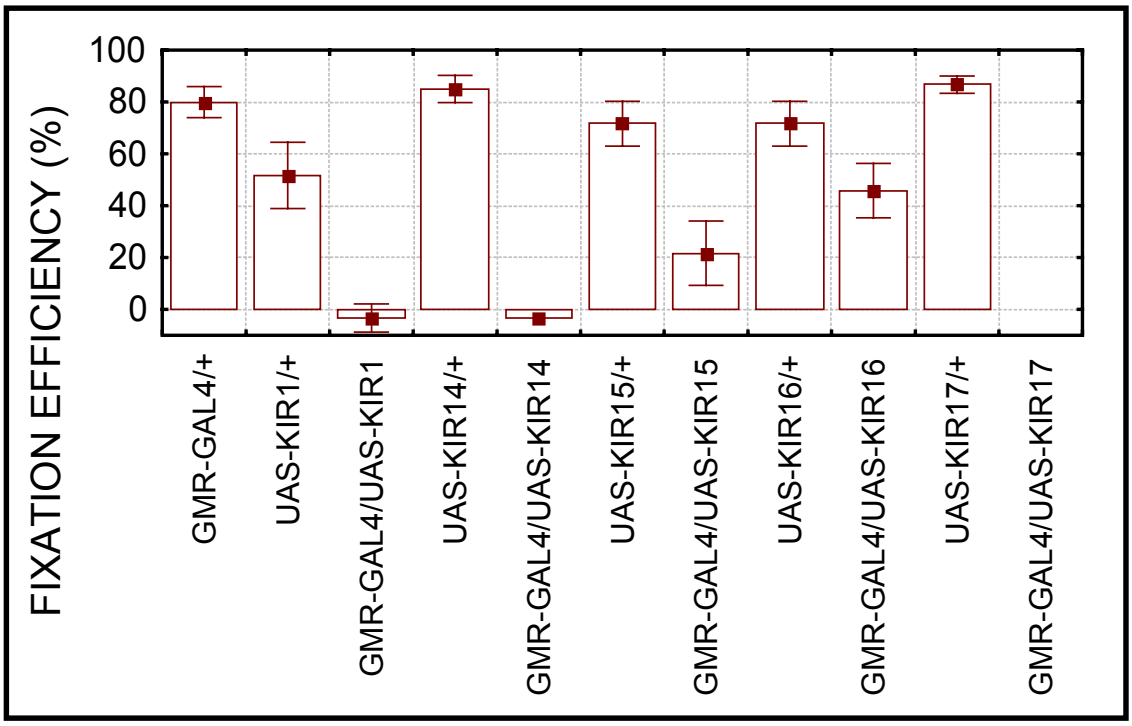

Figure 12: Fixation of a black 180° stripe of flies expressing an inwardly rectifying potassium channel (KIR) in photoreceptors (for experimental details see Materials and Methods). The fixation efficiency of *GMR*-GAL4 in combination with four independent transformants of UAS-KIR and the respective controls are shown. The efficiencies for *GMR*-GAL4/UAS-KIR1 and *GMR*-GAL4/UAS-KIR14 are not significantly different from zero ($P > 0,05$). *GMR*-GAL4/UAS-KIR17 flies are not viable. (n = 10; except for *GMR*-GAL4/UAS-KIR16, n = 4)

Figure 13

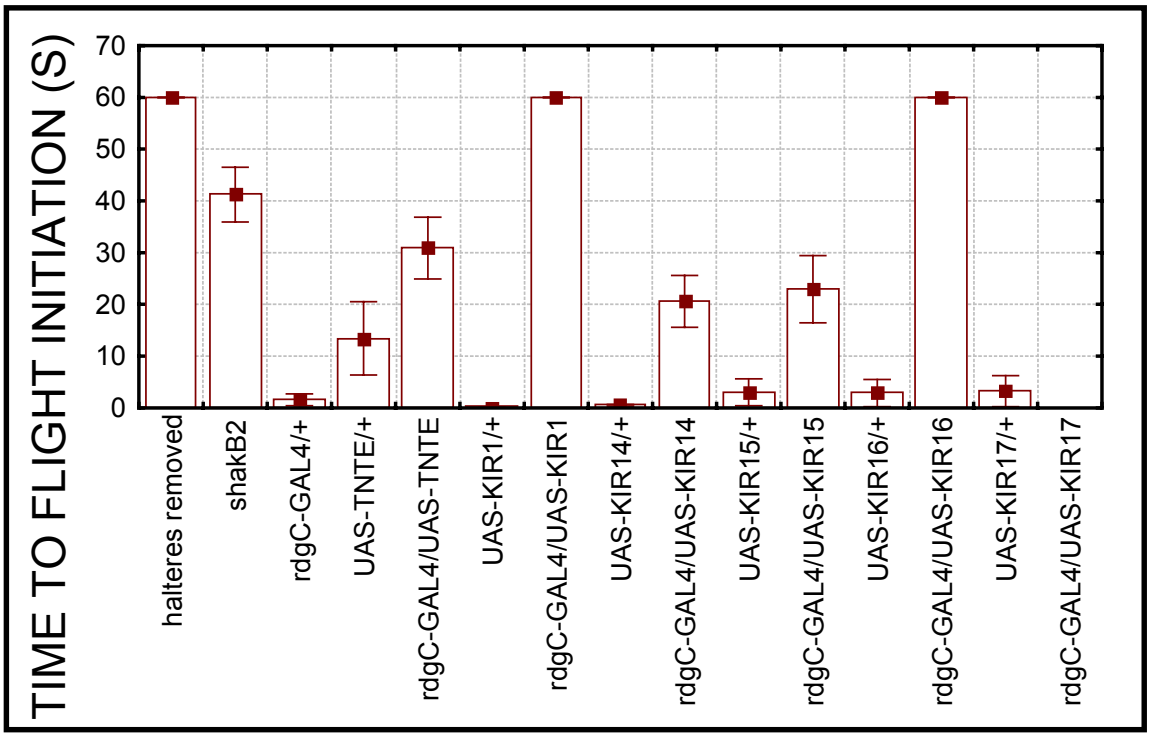

Figure 13: Time to flight initiation for flies in which either the electrical (*shakB*^{2}) or the chemical (*rdgC*-GAL4/UAS-TNTE) or both (*rdgC*-GAL4/UAS-KIR) components of the synapses in haltere campaniform sensilla are blocked. Four independent transformants of UAS-KIR and, in addition, flies with microsurgically removed halteres are tested. *shakB*^{2} and *rdgC*-GAL4/UAS-TNTE show intermediate values between genetic controls and flies with removed halteres. *rdgC*-GAL4/UAS-KIR1 and *rdgC*-GAL4/UAS-KIR16 flies are flightless just like the haltereless flies, whereas *rdgC*-GAL4/UAS-KIR14 and *rdgC*-GAL4/UAS-KIR15 flies are less impaired. *rdgC*-GAL4/UAS-KIR17 flies are not viable.

This results show, that KIR is capable to block both chemical and, in contrast to tetanus neurotoxin, mixed synapses. However, the effect of KIR, which is less potent than tetanus neurotoxin, was shown to be dosage-dependent.

3.2. Inducible and conditional systems

Mapping behavioral tasks to specific structures in the brain using the UAS/GAL4 method depends upon having GAL4 lines expressing in the structure under investigation. However, as shown above, many potentially useful lines cause lethality during development when crossed to effectors killing or blocking neurons. Even with UAS-TNTE many lines are not viable (Sweeney et al., 1995). In order to make more

GAL4 lines accessible to structure-function correlation, conditional or inducible systems are very useful.

3.2.1. Heat shock induced recombinase activity to induce tetanus neurotoxin expression

In collaboration with Sean Sweeney and Cahir O'Kane (Cambridge) an inducible system in which the TNT gene is separated from the UAS sequence by a heterologous gene (containing a stop codon) flanked by two *FRT* (>) sites (UAS>*STOP*>TNT construct) was designed. Recombination between the *FRT* sites, catalyzed by a heat shock inducible flp recombinase (hsFLP), brings TNT under UAS control (Smith et al., 1996). Flies carrying *GMR*-GAL4, hsFLP and UAS>STOP>TNT were analyzed immunohistochemically and behaviorally before and after heat shock, and compared to flies that carried *GMR*-GAL4, hsFLP and UAS>STOP>IMP-TNT. Both types of animals showed scattered background expression in the retina when raised at 18 °C and not heat shocked (Figure 14).

Figure 14

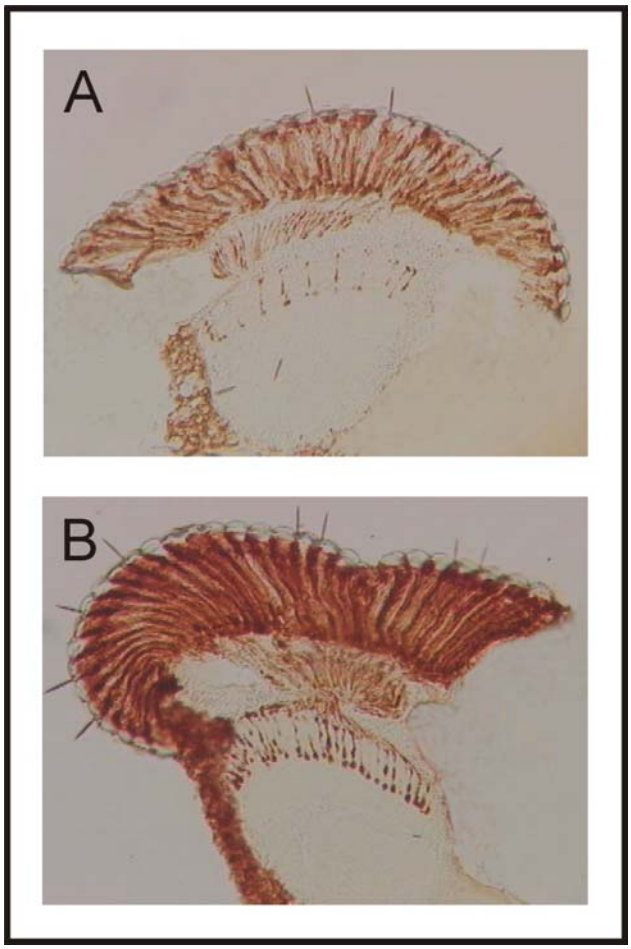

Figure 14: Heat shock induced TNT expression in photoreceptors. Horizontal sections of adult brains (10 µm sections) are shown. (**A**) Background expression in a fly carrying *GMR*-GAL4, hsFLP and UAS>STOP>TNT before heat shocks. TNT is located in a subset of photoreceptors projecting from the retina to the lamina (R1-6) or the medulla (R7, R8). (**B**) Expression in a fly originally carrying *GMR*-GAL4, hsFLP and UAS>STOP>TNT, after three 30 minute heat shocks in the adult fly. All photoreceptors terminating in the medulla express TNT.

In the fixation assay, both groups significantly preferred sectors with stripes over other sectors (Figure 15A/B). However, the leakiness of the hsFLP construct observed immunohistochemically also showed in behavior. Fixation was less strict in the flies containing the active TNT transgene (Figure 15B) compared to flies with the inactive construct (Figure 15A). After three 30 minute heat shocks on three successive days in adults, the expression pattern of TNT (Figure 14B) was indistinguishable from the expression pattern observed in *GMR*-GAL4/UAS-TNTE flies and flies showed no visually guided fixation behavior (Figure 15B) and no optomotor responses (data not shown). However, heat-shock induction of UAS>STOP>IMP-TNT had no effect on these behaviors. (Figure 15A).

Figure 15

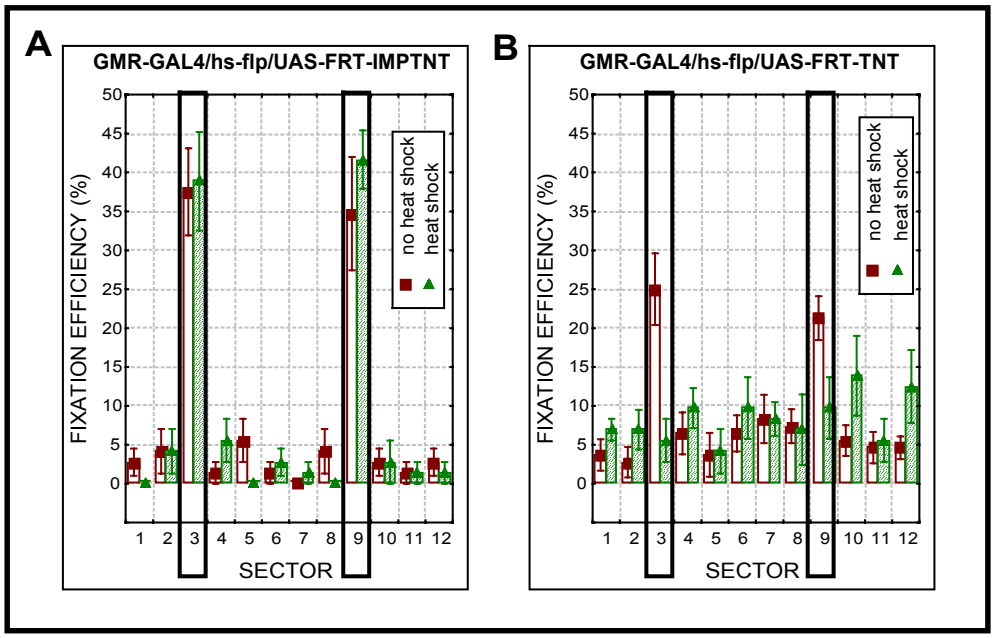

Figure 15: Fixation of black stripes in walking. (**A**) Flies carrying *GMR*-GAL4, hsFLP and UAS>STOP>IMP-TNT expressing an inactive form of the TNT (IMP-TNT) in photoreceptors after heat treatment were tested before (white columns) and again after three times 30 min of 37 °C (gray columns). Each fly was tested 12 times. Error bars denote SEMs. In sectors 3 and 9 black stripes (10° width) were presented. The preference for the black stripes ($P < 0.001$) is not reduced after heat treatment ($P > 0.05$). (**B**) Flies carrying *GMR*-GAL4, hsFLP and UAS>STOP>TNT expressing TNT in photoreceptors after heat shocks were tested in the same manner. Before application of the heat shocks flies showed a clear preference for the black stripes ($P < 0.001$) that was abolished after heat treatment ($P > 0.05$).

To quantify the background activity of flp, R7/R8 terminals in the medulla of flies containing *GMR*-GAL4, hsFLP and UAS>STOP>TNT raised at 18 °C were counted. Between 39 and 65 of the 1400 terminals per eye (n=5) expressed TNT, i.e., below 5% . After heat shocks of increasing length up to 30 minutes, the proportion of TNT-expressing photoreceptors increased . After two 30 minute heat shocks, most but not all photoreceptors expressed TNT (data not shown), indicating that three 30 minute heat shocks are the minimal amount of heat shock to induce recombination in all photoreceptors carrying the hsFLP and the UAS>STOP>TNT constructs.

Flies carrying *rdgC*-GAL4, hsFLP and UAS>STOP>TNT were analyzed immunohistochemically before and after heat shocking in adults. No induction of expression could be detected when the same heat shock regime applied successfully to flies that carried *GMR*-GAL4, hsFLP and UAS>STOP>TNT (three 30 minute heat shocks at three successive days) was used. The observed background expression in this case was higher and more variable than with the *GMR*-GAL4 driver (data not shown).

Since in two other lines (21D and *elav*-GAL4) expression also could not be induced in adult flies (data not shown), expression was induced by giving heat shocks to 3rd instar larvae. In 21D background expression in the absence of heat shocks was very low (Figure 16A), a non saturating heat shock (30 min) produced a mosaic (Figure 16B) and three 30 minute heat shocks in 3rd instar larvae induced expression with a high efficiency (Figure 16C). Results for *elav*-GAL4 were similar (data not shown).

Figure 16

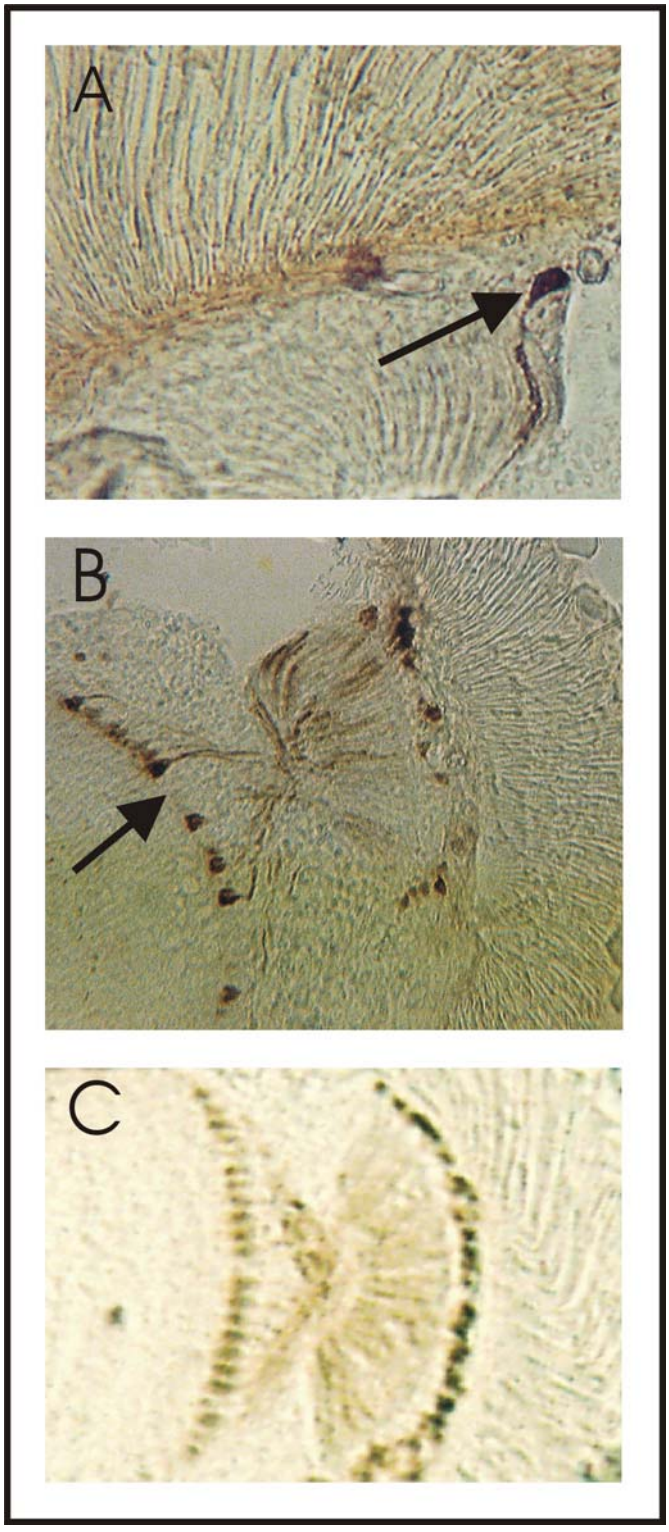

Figure 16: Heat shock induced TNT expression in L2 neurons. (**A**) Background expression in a fly carrying 21D, hsFLP and UAS>STOP>TNT before heat shocks. TNT is located in a single L2 neuron (arrow). (**B**) Expression in a fly carrying 21D, hsFLP and UAS>STOP>TNT after one 30 minute heat shock in third instar larvae. Expression is driven in a subset of L2 neurons but there are still gaps in the arborization layer in the medulla (arrow). (**C**) After three 30 minute heat shocks in third instar larvae (flies carrying 21D, hsFLP and UAS>STOP>TNT) all L2 cells express TNT.

The heat shock induced recombinase activity to induce tetanus neurotoxin expression seems to work reliably in cells undergoing mitosis. This allows to postpone

expression during development or to generate mosaics like shown here for the line 21D. In postmitotic cells the system only worked with one of the three GAL4 lines investigated.

3.2.2. Doxycycline dependent expression of *reaper* (*rpr*)

Flies were generated that, in the presence of doxycycline, express the apoptosis inducer *reaper* (*rpr*) in the eye (*GMR*-GAL4/UAS-rtTA*/tetO-*rpr*). Kept on normal food, theses flies showed no cell death in the eye (Figure 17A). However, when third instar larvae were subjected to food containing 20 µg doxycycline for one day, adult flies showed eye defects of different severities ranging from no to mild (Figure 17B) and severe (Figure 17C) defects. Most flies (roughly 70 %) showed no obvious defect and the eye shown in Figure 17C is the most severely affected.

Figure 17

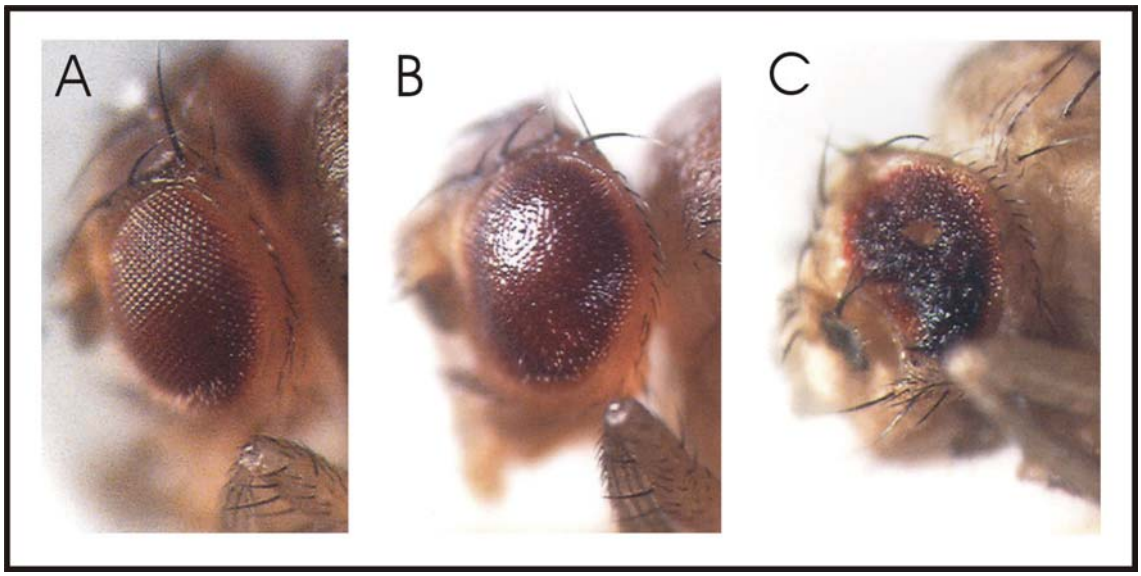

Figure 17: Eyes of flies that, in the presence of doxycycline, express the apoptosis inducer *reaper* (*rpr*) in the eye (*GMR*-GAL4/UAS-rtTA*/tetO-*rpr*) kept on normal food (**A**) or food containing 20 µg doxycycline for one day as third instar larvae (**B** and **C**).

This pilot experiment shows that doxycycline dependent expression of *reaper* (*rpr*) is a possible way to induce cell death although the application of doxycycline has to be optimized to reach more reliable results.

3.2.3. Expression of a semidominant temperature sensitive allele of *shibire* (shi^{ts1})

Two GAL4 lines that produce a severe behavioral defect when crossed to UAS-TNTE were used to investigate the properties of the semidominant temperature sensitive mutant allele of *shibire* (shi^{ts1}). *GMR*-GAL4/UAS-TNTE flies are blind due to expression in photoreceptors (Keller et al., 2002), whereas GH146/UAS-TNTE flies are blind to movement stimuli but not to stationary stimuli due to expression in lamina monopolar cells and other optic lobe neurons (Heimbeck et al., 2002).

Kitamoto (2001) tested flies driving shi^{ts1} expression under *GMR*-GAL4 control at a restrictive temperature of 30° C and at a permissive temperature of 19° C after being allowed to rest at the respective temperature for 10 minutes. At the restrictive temperature flies were blind whereas at the permissive temperature they behaved like the control flies. In my hands, UAS-shi^{ts1}/+; *GMR*-GAL4/+; UAS- shi^{ts1}/+ flies showed

no phototactic behavior at 18° C, which is lower than the described permissive temperature (Figure 18A). This is in sharp contrast to the findings of Kitamoto (2001).

UAS- *shi*^{ts1}/+; GH146-GAL4/+; UAS- *shi*^{ts1}/+ flies do not show the defect observed in GH146/UAS-TNTE flies even at a temperature as high as 37° C (Figure 18B). In both experiments flies were allowed to rest at the respective temperature for at least 10 min.

Figure 18

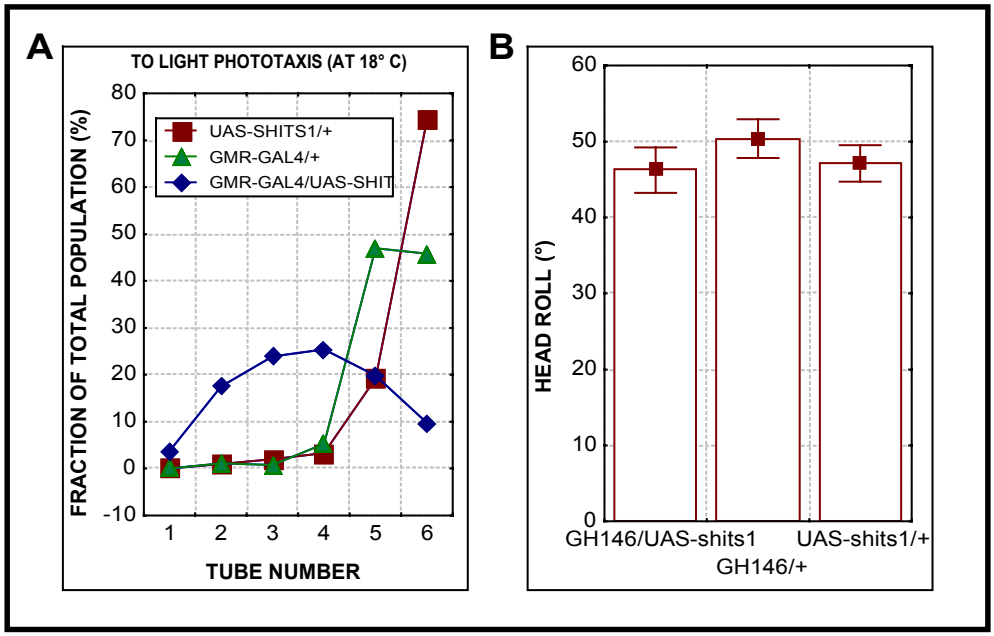

Figure 18: *shi*^{ts1} as a tool to block neurons. (**A**) Flies expressing *shi*^{ts1} in photoreceptors (UAS-*shi*^{ts1}/+; *GMR*-GAL4/+; UAS- *shi*^{ts1}/+) are tested for phototactic behavior at the permissive temperature of 18° C. UAS-*shi*^{ts1}/+; +/+; UAS-*shi*^{ts1}/+ and *GMR*-GAL4/+ flies serve as controls. (**B**) Visually induced head roll in flies expressing *shi*^{ts1} in lamina monopolar cells and other cells of the optic lobes (UAS-*shi*^{ts1}/+; GH146-GAL4/+; UAS-*shi*^{ts1}/+) is tested at the restrictive temperature of 37° C. UAS-*shi*^{ts1}/+; +/+; UAS-*shi*^{ts1}/+ and GH146/+ flies are used as controls. No significant difference between the three genotypes is found ($P > 0{,}05$).

These results illustrate how dosage dependent the effects of *shi*^{ts1} are. The dosage is presumably too high even at the permissive temperature in UAS-*shi*^{ts1}/+; *GMR*-GAL4/+; UAS- *shi*^{ts1}/+ flies, whereas in UAS- *shi*^{ts1}/+; GH146-GAL4/+; UAS-*shi*^{ts1}/+ flies even at the restrictive temperature the expected behavioral effect is not observed.

3.3. Behavioral analysis of *rdgC*-GAL4/UAS-TNT flies

The *rdgC*-GAL4 construct, driving GAL4 expression under control of the 5' region of the *rdgC* gene, labels a subset of chemosensory neurons and campaniform sensilla. The specificity of the expression pattern made it possible to study the behavioral relevance of the labeled structures. In this line dendritic fibers in the third antennal segment entering basiconic sensilla, and fiber endings approaching but not entering coeloconic sensilla are labeled, as well as unidentified cells in the second antennal segment. The antennal nerve diverges and some afferents extend into the antennal mechanosensory and motor center (AMMC), indicating that the unidentified cells in the second antennal segment may be mechanoreceptors, whereas the third antennal segment afferents project into four distinct antennal lobe glomeruli (Figure 10A). No staining is detected in other brain regions. In the abdomen, the chemosensory neurons of the thorn bristles of the vaginal plate that project to the abdominal ganglion (Taylor, 1989) are labeled (Figure 10E). Expression in mechanosensory neurons includes the campaniform sensilla of the halteres that project towards the thoracic ganglion (Figure 10B). The haltere nerves enter the central nervous system and join the haltere tract to extend anteriorly into the suboesophageal ganglion. The tract has small arborizations in both the meso- and metathoracic neuromeres (Figure 10C). In all six legs, expression is driven in campaniform sensilla in the trochanter and femur that arborize in the respective neuromeres and in additional unidentified neurons further distal in the legs (Figure 10D). Whether additional neurons contribute to the staining pattern is unclear. No *rdgC*-GAL4 driven expression could be detected in those regions where *rdgC* is most strongly expressed (ocelli, eyes, mushroom bodies; Steele et al., 1992).

Leg mechanoreceptors

In *rdgC*-GAL4/UAS-TNTE flies the effects of blocking the campaniform sensilla in the trochanter and femur on behaviors that involve coordinated leg movements can be studied. Two other GAL4 lines (C42 and C161) label a different subset of leg mechanoreceptors and can be tested in combination with UAS-TNTE for the same behaviors to compare the effects of blocking different subsets of leg mechanoreceptors.

rdgC-GAL4/UAS-TNTE flies are as active as control flies and their walking speed is not reduced when compared to *rdgC*-GAL4/+ or UAS-TNTE/+ flies (Figure

19A/B). This is different from earlier experiments in which *rdgC*-GAL4/UAS-IMP-TNTQ flies were used as controls and in which the activity was the same, whereas the average walking speed was slightly reduced (data not shown). *rdgC*-GAL4/UAS-TNTE flies show a defect in grooming behavior (Figure 19C), a severely increased copulation latency (Figure19D) and a reduced ability to walk on vertical surfaces (Figure 20).

Figure 19

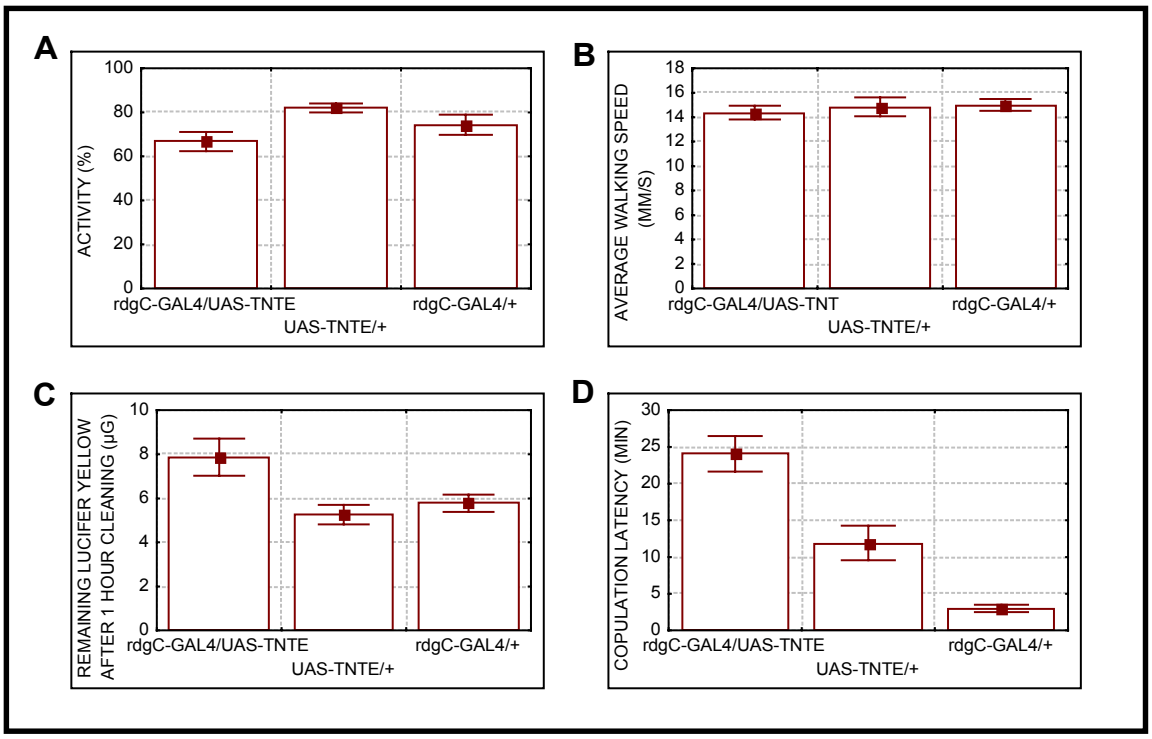

Figure 19: Activity (**A**), average walking speed (**B**), remaining lucifer yellow after 1 hour cleaning (**C**) and copulation latency (**D**) of *rdgC*-GAL4/UAS-TNTE flies and controls are shown. Neither the activity (A), nor the average walking speed (B) is significantly reduced in *rdgC*-GAL4/UAS-TNTE flies (n = 9 or 11; $P > 0{,}05$). There is significantly more lucifer yellow remaining on *rdgC*-GAL4/UAS-TNTE flies after 1 hour cleaning (n = 20; $P < 0{,}05$) (C) and their copulation latency is significantly higher than in controls (n = 19; $P < 0{,}005$) (D).

Figure 20

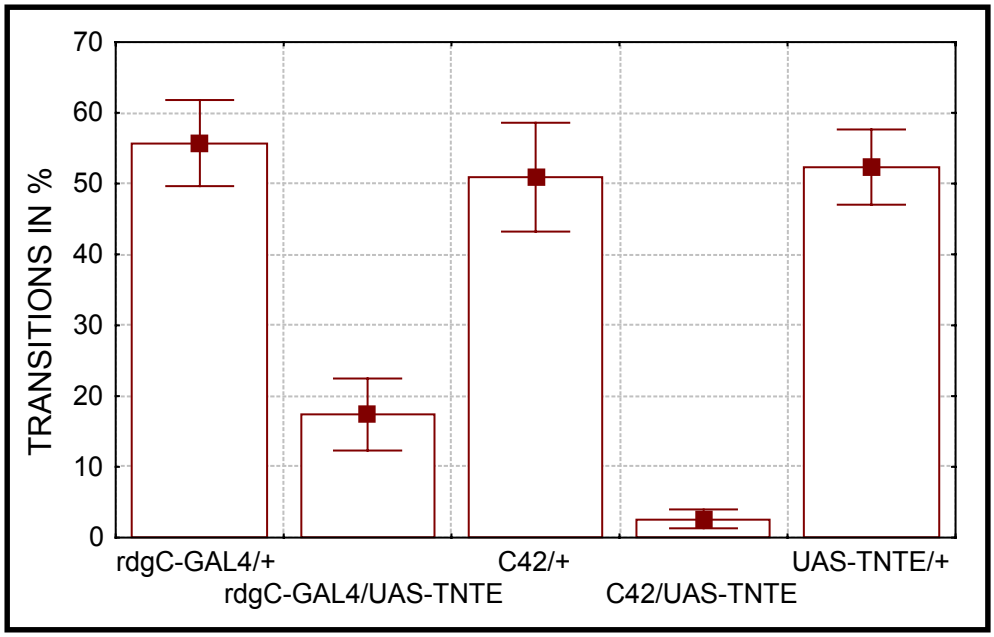

Figure 20: The ability of flies with blocked leg campaniform sensilla (*rdgC*-GAL4/UAS-TNTE) or femural chordotonal organs and tactile bristles (C42/UAS-TNTE) to walk on vertical surfaces. The percentage of transitions from the lower to the upper part of the test tube within 10 s is shown. n is the number of independent experiments. In each experiment 10 flies have been tested. The values of both experimental genotypes are different from the values of the controls ($P < 0{,}001$). C42/UAS-TNTE flies are not significantly more severely impaired than *rdgC*-GAL4/UAS-TNTE flies ($P > 0{,}05$). (*rdgC*-GAL4/+: n = 7; *rdgc*-GAL4/UAS-TNTE: n = 13; C42/+: n = 9; C42/UAS-TNTE: n = 3; UAS-TNTE: n = 12)

To compare the effect of blocking leg campaniform sensilla to the effect of blocking other mechanoreceptors in legs, two GAL4 lines driving expression in the femural chordotonal organ were crossed to UAS-TNTE. The expression pattern of C161 is described in great detail (Smith and Shepherd, 1996). Briefly, expression is restricted in the legs to sensory neurons associated with hair plates, a subset of campaniform sensilla, and the femoral chordotonal organ. In the wings, expression is seen in subsets of campaniform sensilla. In the abdomen, expression is seen in Wheeler's organ and in internal sensory neurons. The line C42 that originates from the same screen for expression in sensory neurons shows expression in the femoral chordotonal organ and in tactile bristles (David Shepherd, Southhampton, pers. comm.) (Figure 21).

Figure 21

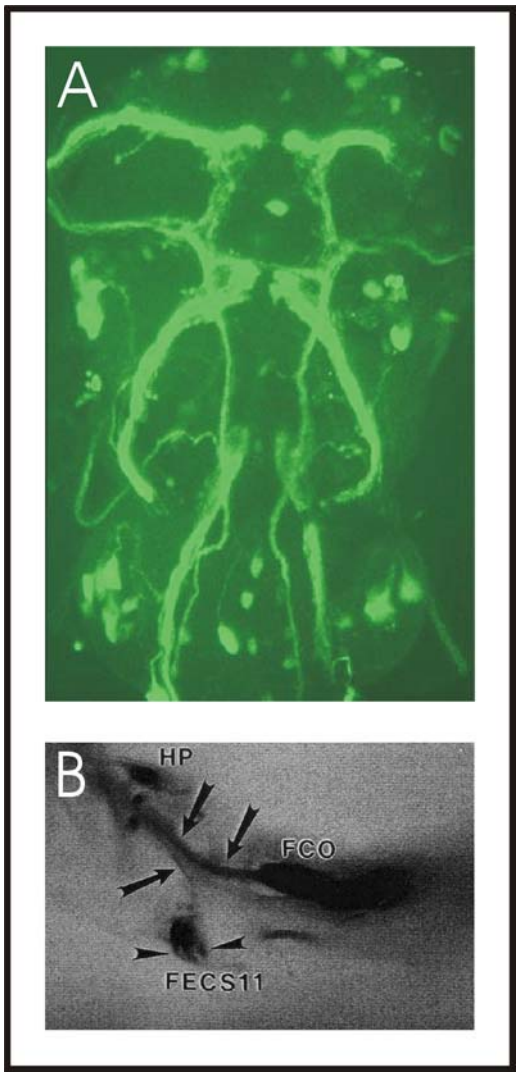

Figure 21: Expression pattern of C42 in the thoracic ganglion (A) and of C161 in the leg (B). (**A**) C42 expression in the thoracic ganglion is monitored by GFP expression. Sensory axons of the femural chordotonal organs in all three neuromeres are labeled. Figure taken from http://www.soton.ac.uk/~ds/c42.htm. (**B**) Photomicrograph of the coxa, trochanter, and femur of a C161/UAS-lacZ fly. ß-galactosidase expression is revealed by X-GAL staining in a hair plate (HP), in campaniform sensilla (FECS11) and in the femoral chordotonal organ (FCO). Figure taken from Smith and Shepherd (1996).

Both lines (C42 and C161) show a severely reduced activity and average walking speed when crossed to UAS-TNTE (Figure 22). C42/UAS-TNTE flies are severely reduced in their ability to walk on vertical surfaces (Figure 20).

Figure 22

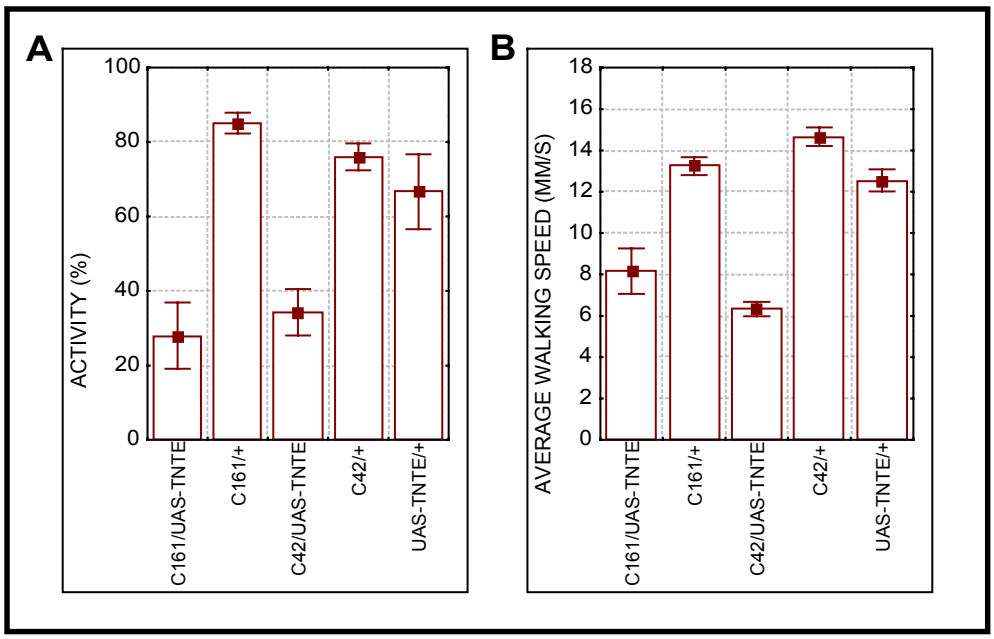

Figure 22: GAL4 lines C161 and C42 in combination with UAS-TNTE are tested for their activity (**A**) and average walking speed (**B**). Both lines show a severely reduced activity ($P < 0{,}005$ for C161/UAS-TNTE and $P < 0{,}05$ for C42/UAS-TNTE) and average walking speed ($P < 0{,}001$ in both cases) when driving TNT expression (n = 6; except UAS-TNTE/+: n = 8).

Campaniform sensilla in the legs obviously have little effect on normal walking behavior, whereas other leg mechanoreceptors play an important role in leg coordination during walking. However, more demanding tasks like walking on vertical surfaces are impaired in flies with blocked leg campaniform sensilla, too.

Haltere campaniform sensilla

rdgC-GAL4 drives expression in the haltere campaniform sensilla that form mixed synapses consisting of a chemical and an electrical component with a flight motorneuron (Trimarchi and Murphey, 1997). Flies without halteres are unable to fly. Expression of the KIR transgene that is capable of blocking both the electrical and the chemical signal transmission results in flight defects, as severe as haltere amputation (Figure 13), suggesting that halteres can not stabilize flight without sensory information obtained by campaniform sensilla.

Chemoreceptors

The fact that in the *rdgC*-GAL4 line antennal chemoreceptors projecting into a specific subset of antennal lobe glomeruli are labeled enabled me to study the chemospecificity of these receptors by testing the avoidance of different odors. Antennal chemoreceptors expressing a given receptor gene project into specific glomeruli within the antennal lobe (Vosshall et al., 2000). Therefore the labeled chemoreceptors may represent functional classes of chemoreceptors expressing the same receptor gene. To identify odors for which the labeled chemoreceptors are sensitive, the chemotactic response to different concentrations of three odors (benzaldehyde, 4-methylcyclohexanol and isoamylacetate) was tested (Figure 23). No significant difference between *rdgC*-GAL4/+ and *rdgC*-GAL4/UAS-TNTE flies was detected at all three tested concentrations (0.01%, 0.1%, 1%) of benzaldehyde and 4-methylcyclohexanol. At 0.3% and 1% isoamylacetate *rdgC*-GAL4/UAS-TNTE values were significantly higher than values of control stocks *rdgC*-GAL4/+ and UAS-TNTE/+, which did not differ significantly from random avoidance.

The chemoreceptors labeled in *rdgC*-GAL4 are therefore sensitive to isoamylacetate.

Figure 23

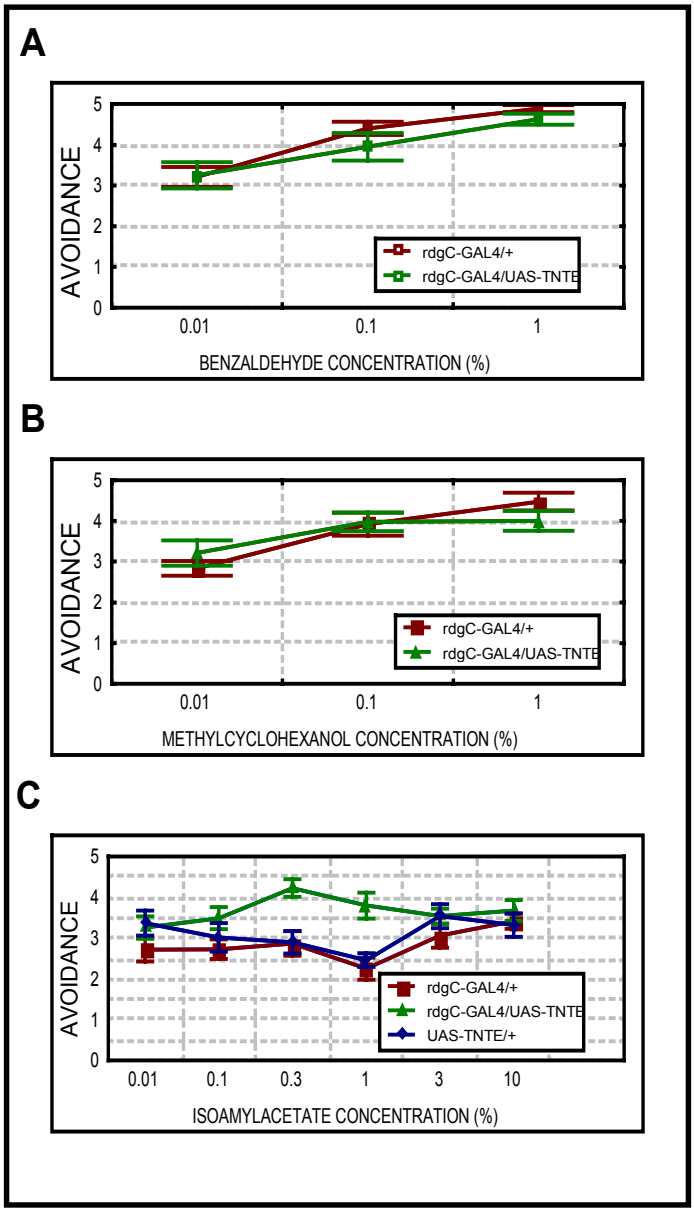

Figure 23: Olfactory avoidance scores of flies expressing TNT under the control of the *rdgC*-GAL4 driver (for experimental details see Materials and Methods). Avoidance scores for different concentrations of benzaldehyde (**A**; 0.01%, 0.1%, 1%), 4-methylcyclohexanol (**B**; 0.01%, 0.1%, 1%) and isoamylacetate (**C**; 0.01%, 0.1%, 0.3%, 1%, 3%, 10%) are shown. Each value represents the mean of 8 (benzaldehyde and 4-methylcyclohexanol) or 10 (isoamylacetate) tests. Each test consists of measurements of 5 flies at 10 time points each. Error bars denote SEMs over flies. The avoidance scores for all three tested concentrations of benzaldehyde and 4-methylcyclohexanol are not significantly different between *rdgC*-GAL4/+ and *rdgC*-GAL4/UAS-TNTE flies ($P > 0.05$ in all cases). For experiments with isoamylacetate (C) *rdgC*-GAL4/+ and UAS-TNTE/+ serve as controls. Only for 3 % and 10 % the avoidance scores of both control lines are different from 2.5 which is the score of randomly distributed flies ($P < 0.05$ in both cases). *rdgC*-GAL4/UAS-TNTE values are significantly different from 2.5 at all concentrations tested ($P < 0.05$ in all cases). At 0.3 % and 1 % *rdgC*-GAL4/UAS-TNTE values are significantly different from both control values ($P < 0.05$ in both cases).

3.4. Optic lobe interneurons

To study the processing of visual information and especially of visual motion information, flies in which columnar visual interneurons are blocked by tetanus neurotoxin were generated and tested for their capability to respond to different visual inputs. The following GAL4 lines driving expression in visual interneurons were investigated: C850, TP849, GH146 and 21D.

Line C850 is reported to drive expression in T1 cells and in at least one additional neuron type in the medulla and in the lobula complex (Ito et al., 1997). I was not able to reproduce the published expression pattern with three different stocks of line C850 obtained from Stefan Schneuwly (Regensburg), Chihiro Hama (Kobe) and Paul Garrity (MIT). Both Stefan Schneuwly and Chihiro Hama confirmed thereafter my finding that the obtained stocks of C850 do not drive expression in the adult brain. Line C850 obviously is not what it is supposed to be and can not be used to study information processing in the *Drosophila* visual system.

Line TP849 drives expression in a variety of cells in the optic lobes including glia cells at the first optic chiasm and the retina-lamina-margin as well as neuronal nuclei and arborizations in all visual neuropils including the lamina in which expression is driven in lamina monopolar cells. Expression is strongest in cell bodies at the rind of the lobula plate and in the lobula plate, lobula and the proximal medulla (Figure 24). There is no detectable expression in the central brain. TP849/UAS-TNTE flies show a variety of locomotor defects that make it impossible to test visually guided behaviors that involve walking or flight. The only behavior tested in TP849/UAS-TNTE flies was head roll. The flies showed no head roll (Figure 25), although they showed spontaneous head movements, indicating that this was not due to a disturbance in the motor output. The set of labeled neurons in TP849 therefore is necessary to detect visual motion.

Figure 24

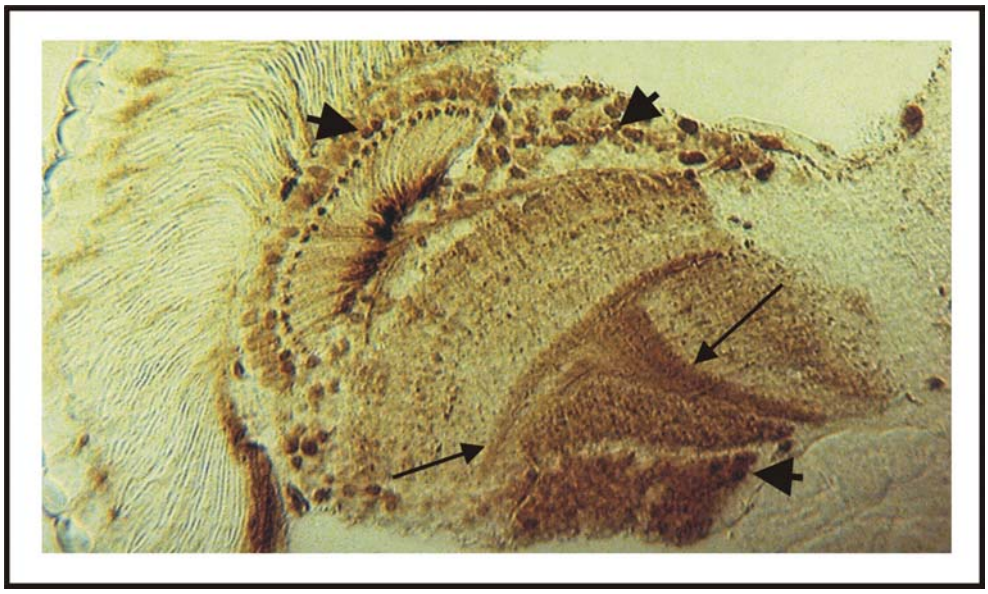

Figure 24: The expression of line TP849 in the optic lobe is visualized with an anti-TNT antibody. A horizontal section of one optic lobe of a TP849/UAS-TNTE fly is shown (10 µm section). Expression can be seen in a variety of cell bodies (arrowheads) at the retina-lamina-margin, at the rind of the medulla and at the rind of the lobula plate, where nuclear expression is strongest. All optic neuropils show staining at low level. In the lobula plate, the proximal lobula and the 8th layer of the medulla neuropil staining is strongest (arrows).

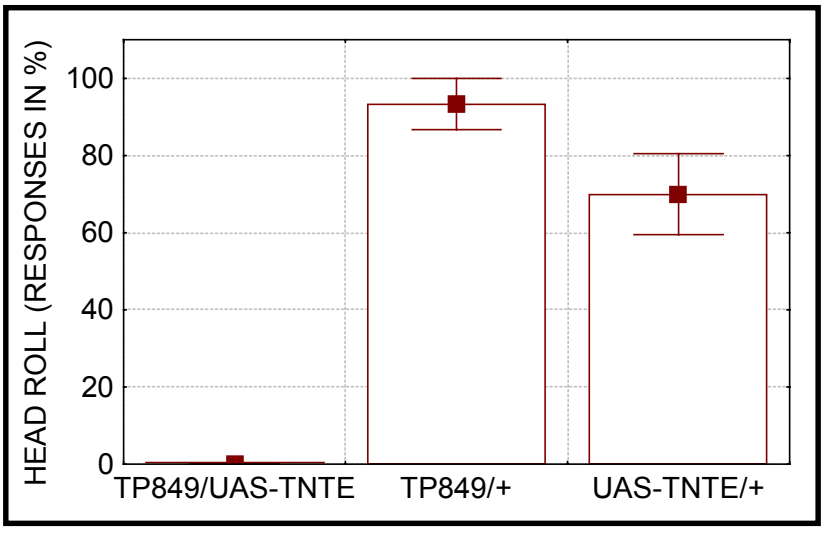

Figure 25: Head roll responses for TP849/UAS-TNTE, TP849/+ and UAS-TNTE/+ flies. The percentages of flies responding to a moving grating of 60° rotating around the fly are given (head roll angles of more than 15° are considered to be a response). TP849/TNT flies never respond to the stimulus, whereas the controls do (n=4; N=40).

In line GH146 expression is driven in a subset of lamina monopolar cells most likely L1 and L2 and in unidentified cells of the medulla and the lobula complex. In the lobula plate expression is detected in specific layers. Expression throughout the visual system shows an anterior/posterior gradient of intensity (Figure 26). Expression in the lamina monopolar cells is stronger than expression in the rest of the optic lobes. The defects in visually guided behaviors in GH146/UAS-TNTE are selective. In the behaviors depending on movement detection (optomotor response in walking, head roll, landing response) GH146/TNTE flies show no response (Figure 27A and data not shown). In the fixation paradigm that requires detection of stationary objects, GH146/UAS-TNTE flies show a defect only for 10° stripe width. For bigger stripes GH146/UAS-TNTE responses are not different from control fly responses (Figure 27B). These results show that the pathways through the lamina left unaffected in GH146/UAS-TNTE are not sufficient to detect visual motion although stationary stimuli can be detected.

Figure 26

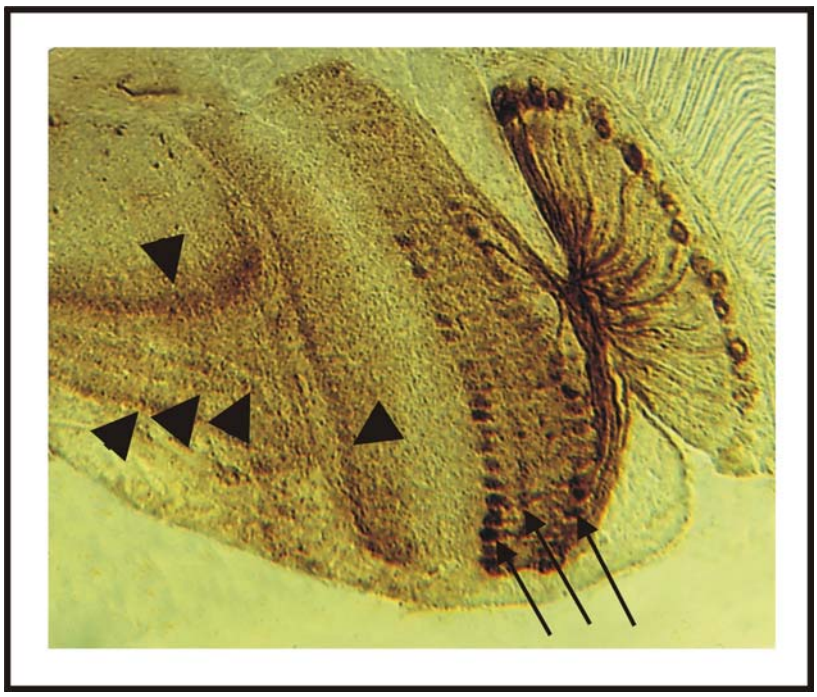

Figure 26: Expression of tau in optic lobes of GH146/UAS-tau flies is visualized using an anti-tau antibody. tau was used as a reporter to visualize the expression pattern, because expression is very weak and anti-TNT staining was faint. A horizontal section of one optic lobe of a GH146/UAS-TNTE fly is shown (10 µm section). Expression is driven in a subset of lamina monopolar cells arborizing in three layers of the medulla (arrows) most likely L1 and L2 and in unidentified cells of the medulla and the lobula complex (arrowheads). In the lobula plate expression is detected in specific layers(arrowheads). Expression throughout the visual system shows an anterior/posterior gradient of intensity.

Figure 27

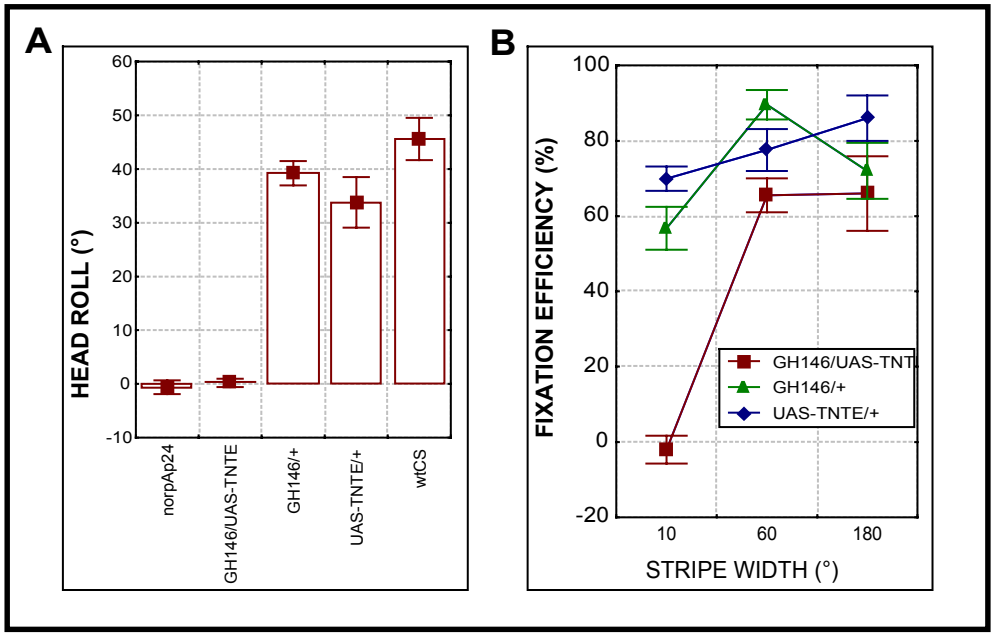

Figure 27: Visually guided behaviors in GH146/UAS-TNTE flies. (**A**) GH146/UAS-TNTE and *norpA*^{p24} flies show no significant head roll, whereas GH146/+, UAS-TNTE/+ and wtCS flies show a head roll response (n=5; $P > 0,05$). (**B**) GH146/UAS-TNTE flies show no fixation of two opposing 10° stripes but they fixate two 60° stripes or one 180° stripe. GH146/+ and UAS-TNTE/+ flies fixate all three stripes (n=10; $P > 0,05$).

In line 21D expression in the brain is driven exclusively in L2 lamina monopolar cells (Figure 28). The function of the L2 lamina monopolar cells, which represent one of four parallel pathways in the lamina, can be studied with 21D/UAS-TNTC flies in which L2 is blocked. 21D/UAS-TNTC flies show reduced optomotor responses in walking compared to 21D/UAS-IMP-TNTQ flies at a wide variety of pattern luminances and contrast frequencies (Figure 29).

Figure 28

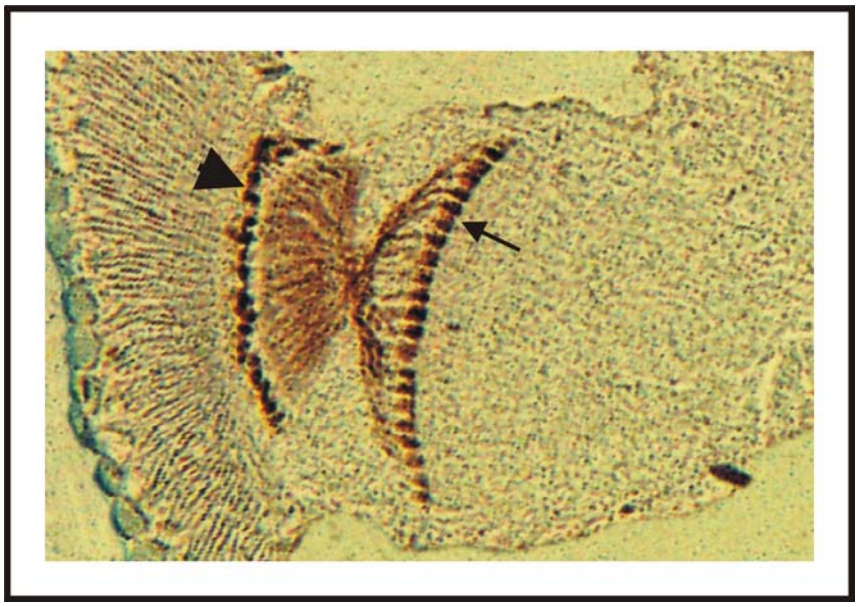

Figure 28: Expression of line 21D in the optic lobe is visualized with an anti-TNT antibody. A horizontal section of one optic lobe of a 21D/UAS-TNTE fly is shown (10 µm section). Expression can be detected exclusively in L2 lamina monopolar cells. The cell bodies (arrowhead) distal of the medulla as well as the arborizations in the third layer of the medulla (arrow) can be seen. No staining can be detected in the central brain.

Figure 29

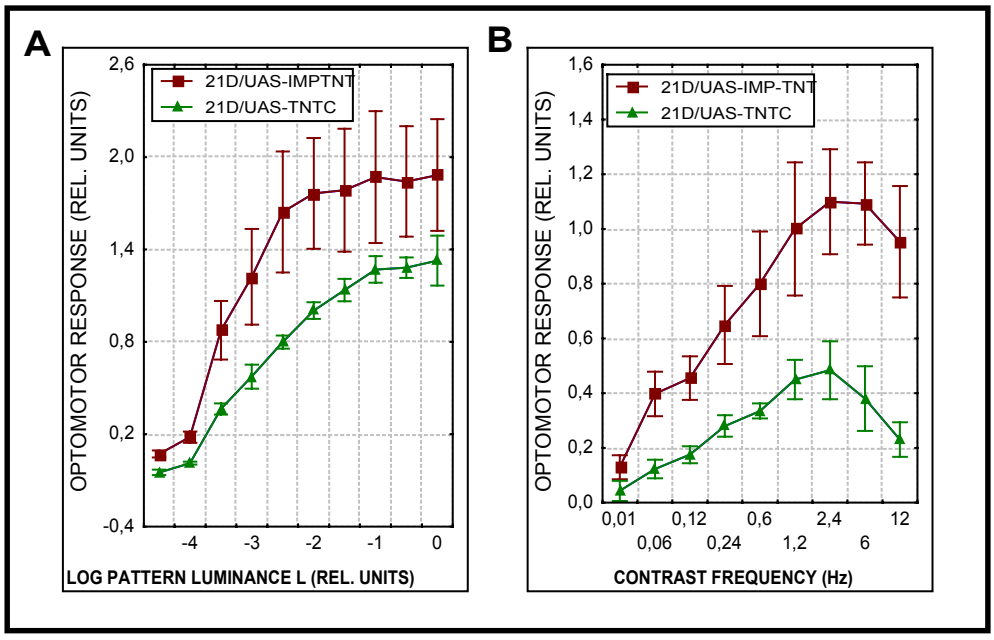

Figure 29: 21D/UAS-TNTC flies and 21D/UAS-IMPTNTQ flies are tested for optomotor responses in walking for 10 pattern luminances (**A**) and contrast frequencies ranging from 0,01 to 12 Hz (**B**). Under all conditions tested, 21D/UAS-TNTC flies showed a lower response than 21D/UAS-IMPTNTQ flies. (n = 6).

To test if L2 represents the input to one of two antiparallel unidirectional motion detectors, a less variable behavior that although depends on the ability to detect motion stimuli, the visually induced head yaw movement, was tested under progressive and regressive stimulation in 21D/UAS-TNTE, UAS-TNTE/+ and 21D/+ flies. Reactions to both kinds of stimuli are not different between flies with blocked L2 neurons and controls (Figure 30). In this experiment the response to whole field stimulation is not reduced in 21D/UAS-TNTE flies (Figure 30).

Figure 30

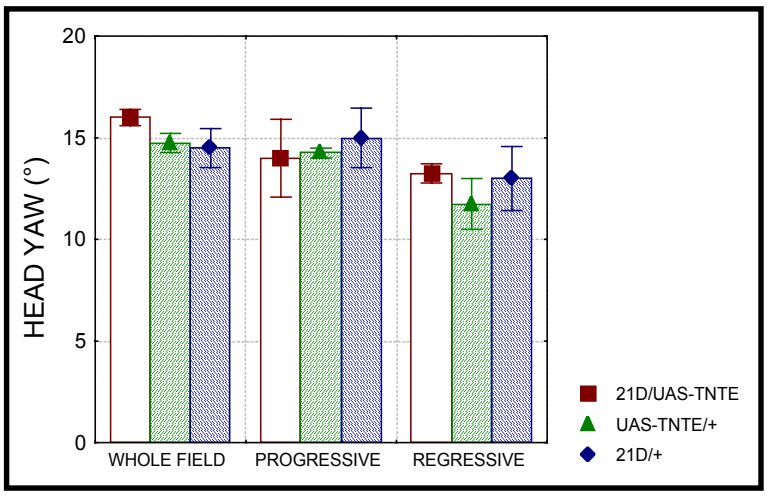

Figure 30: The head yaw responses of flies with blocked L2 lamina monopolar cells and controls. The moving grating was presented either in the whole visual field or as a progressive or regressive stimulus (for a detailed description of the experimental procedure see Material and Methods). For neither of the three stimulations 21D/UAS-TNTE flies showed a head yaw response significantly different from the controls (n = 4; $P > 0{,}05$)

Electrophysiological recordings indicated that motion detection in flies takes place in two separate ON and OFF channels (Franceschini et al., 1989; Horridge and Marcelja, 1990). This means that motion of light edges is processed in one channel and motion of dark edges in a separate parallel channel. To test if L2 feeds into an ON or OFF channel for motion detection, stimuli consisting only of moving edges of one type were used for head roll experiments. Again no difference between 21D/UAS-TNTE and controls could be found (Figure 31).

To rule out the possibility that the lack of defects seen in 21D/UAS-TNTE flies is due to the fact that tetanus neurotoxin is not sufficient to block L2 neurons, KIR, which blocks chemical and electrical synapses, was used as an effector. 21D/UAS-KIR1 flies did not show a reduced head roll (Figure 32), although UAS-KIR1 proved to be sufficient to block neurons in other experiments (Figure 12 and Figure 13).

Figure 31

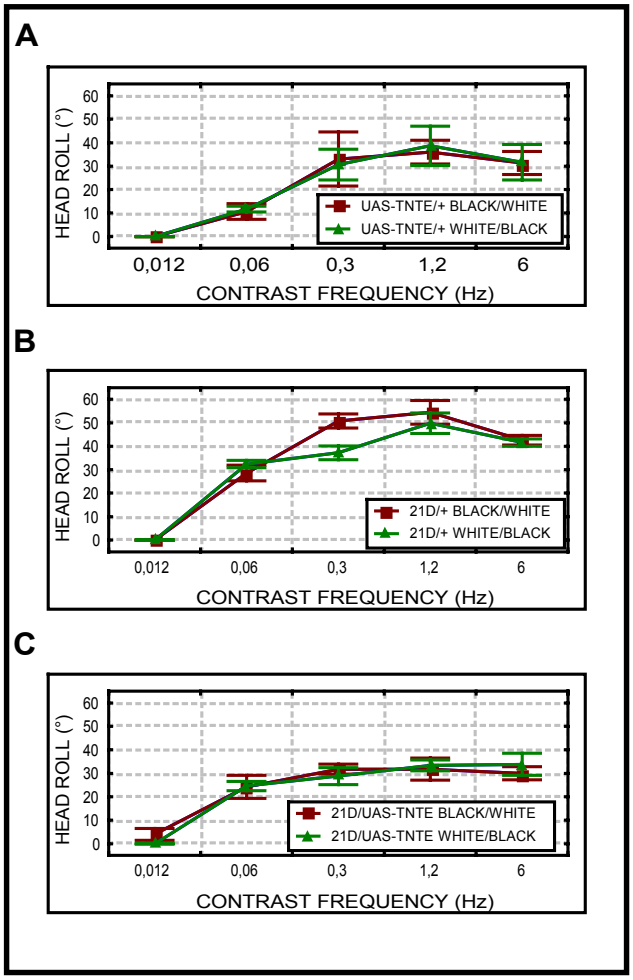

Figure 31: Head roll responses to stimuli that consist of a black to white gradient followed by a white to black edge or vice versa were tested for UAS-TNTE/+ flies (**A**), 21D/+ flies (**B**) and 21D/UAS-TNTE flies (**C**) (for a detailed description of the experimental procedure see Material and Methods). The responses to the black to white edges were not significantly different from the responses to the white to black edges except for 21D/+ flies at 0,3 Hz. At no contrast frequency was the response of the 21D/UAS-TNTE flies significantly different from the responses of the controls (n = 5; $P > 0,05$).

Figure 32

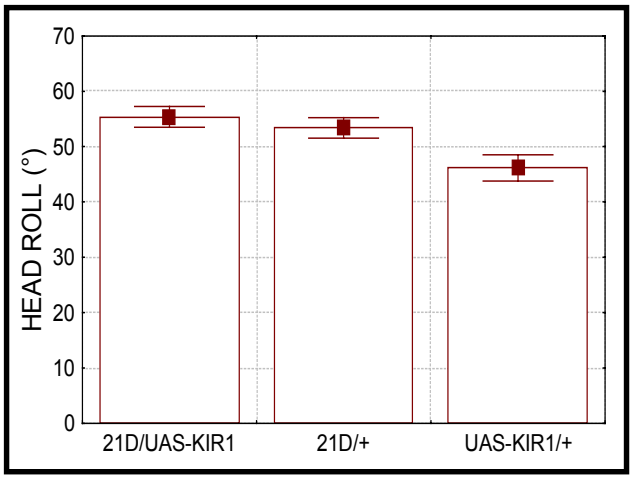

Figure 32: The head roll responses of 21D/UAS-KIR1 flies and the respective controls are shown. Responses are not significantly different between the experimental flies and the controls (n = 8; $P > 0,05$).

The detection of stationary objects of flies with blocked L2 neurons was tested for one black 10, 60 or 180° stripe and for two opposing 10 or 60° stripes or one 180° stripe (Figure 33). No defect could be detected in 21D/UAS-TNTE flies for any of the

tested conditions. This is in contrast to earlier experiments, in which 21D/UAS-TNTC flies showed a fixation efficiency lower than 21D/UAS-IMP-TNTQ flies for a single black 10 or 60° stripe (data not shown).

Figure 33

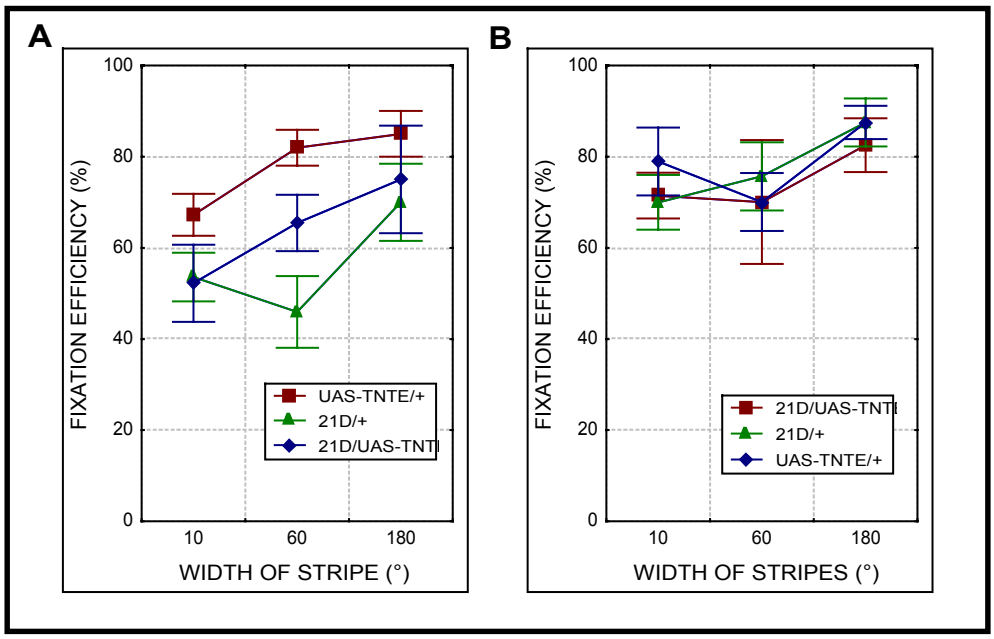

Figure 33: Fixation efficiencies for 21D/UAS-TNTE, 21D/+ and UAS-TNTE/+ flies. Fixation of a single stripe of 10, 60 or 180° width (**A**) as well as fixation of two opposing 10, 60 or one 180° stripe (**B**) is not significantly reduced in 21D/UAS-TNTE flies (N = 10; $P > 0{,}05$).

In summary, the L2 pathway is not necessary for motion detection and no functional specialization of the pathway was found.

3.5. Optic lobe output neurons

Characterization of *omb* mutants

Behaviors known to be defective in omb^{H31} are retested here and compared to the allelic combinations $Tp(1)bi^{D1}/omb^{H31}$ and $Df(1)rb^{5}/omb^{H31}$. $Df(1)rb^{5}/omb^{H31}$ flies have no HS and VS cells but other anatomical defects are less severe (see Introduction). The original mutant omb^{H31} is described to behave different from wildtype in a variety of motion-detection dependent and independent behaviors (see Introduction). These behavioral defects are believed to be causally related to the abnormalities described anatomically for omb^{H31}. omb^{H31} has no HS and VS cells, the two M-fibers are missing, the volume of the lobula plate is reduced by 30 %, the fiber number in the anterior optic tract is reduced from 1300 to 1000, the order of the inner optic chiasm (IOC) is disturbed, very rarely a preimaginal orientation of the medulla is observed in adult flies and cells labeled by a monoclonal antibody (nb169) lack their normally extensive arborizations in the lamina in omb^{H31}. The allelic combination $Df(1)rb^{5}/omb^{H31}$ also has no HS and VS cells but other anatomical defects are less severe (Brunner et al., 1992).

Only optomotor responses in flight and fast phototaxis have been tested in $Df(1)rb^{5}/omb^{H31}$. The courtship defect can not be tested in the allelic combination since $Df(1)rb^{5}/omb^{H31}$ males are not viable. The other three behaviors defective in omb^{H31} are tested here for three genotypes lacking HS and VS cells: omb^{H31}, $Tp(1)bi^{D1}/omb^{H31}$ and $Df(1)rb^{5}/omb^{H31}$. The difference in the fixation of one black 10° stripe between wtCS and omb^{H31} could not be reproduced (Figure 34A). Of the three genotypes that lack VS and HS cells $Tp(1)bi^{D1}/omb^{H31}$ and omb^{H31} fixate one black 10° stripe as good as the controls. Only $Df(1)rb^{5}/omb^{H31}$ shows a reduced value, although fixation is above random (Figure 34B). In addition, fixation efficiency of omb^{H31} is not different from fixation efficiency of wtCS for a variety of different patterns (data not shown).

Figure 34

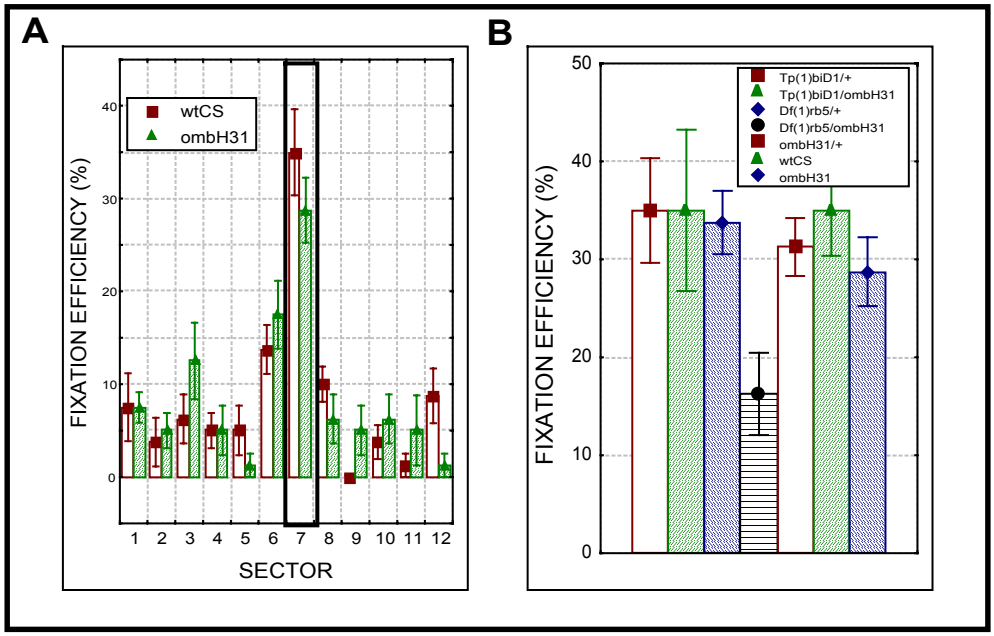

Figure 34: (**A**) The distribution of first choice walks in an illuminated arena with one black 10° stripe in sector 7 (black box). wtCS and the mutant strain omb^{H31} are tested. The fixation efficiency is not different between the two genotypes (n = 8; $P > 0{,}05$). (**B**) Efficiency of fixation of allelic combinations of the *omb* locus towards a single black 10° stripe. Efficiencies of three genotypes that lack VS and HS cells ($Tp(1)bi^{D1}/omb^{H31}$, $Df(1)rb^{5}/omb^{H31}$ and omb^{H31}) as well as of wtCS and control flies carrying the *omb* alleles in a single copy are shown. Only the value for $Df(1)rb^{5}/omb^{H31}$ is significantly lower than the values of the respective controls (n = 8; $P < 0{,}05$). Note that in contrast to all other fixation experiments, the measuring circle (like in the original study) had a diameter of only 10 cm (see Materials and Methods).

In the forced-choice fixation Y-maze wtCS flies turn towards the black dot with an efficiency of 70% whereas omb^{H31} avoid it with similar efficiency. The other two genotypes that lack HS and VS cells ($Tp(1)bi^{D1}/omb^{H31}$ and $Df(1)rb^{5}/omb^{H31}$) also avoid the black dot, although with much lower efficiency (Figure 35). $Df(1)rb^{5}/omb^{H31}$ avoids with an efficiency of 30% and $Tp(1)bi^{D1}/omb^{H31}$ with an efficiency of 15%. However, the allelic combinations avoid the black dot more efficient than all other visual mutants tested and all mutants selected for avoidance of the black dot in a large scale screen (Bülthoff, 1982).

Figure 35

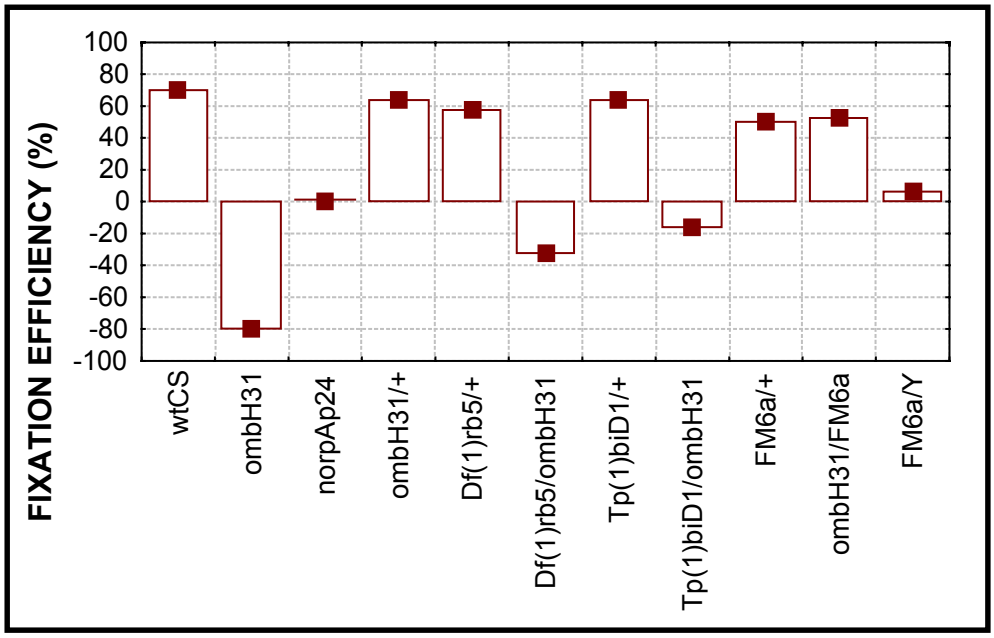

Figure 35: The fixation efficiencies in the forced-choice fixation Y-maze for 11 genotypes are shown. wtCS, omb^{H31}/+, $Df(1)^{rb5}$/+, $Tp(1)bi^{D1}$/+, FM6a/+, omb^{H31}/FM6a flies do prefer the black dot with efficiencies between 50 and 70 %. FM6a/Y flies do show a very weak fixation efficiency of 3 % and blind $norpA^{p24}$ flies do neither prefer nor avoid the black dots. omb^{H31} flies avoid the black dot with an efficiency of 80 %. The other two allelic combinations of the *omb* locus that lack HS and VS cells ($Df(1)rb^{5}/omb^{H31}$ and $Tp(1)bi^{D1}/omb^{H31}$) avoid the black dot with a much lower efficiency (32 and 16 %). (wtCS: n = 399; omb^{H31}: n = 340; $norpA^{p24}$: n = 715; omb^{H31}/+: n = 402; $Df(1)rb^{5}$/+: n = 846; $Df(1)rb^{5}/omb^{H31}$: n = 341; $Tp(1)bi^{D1}$/+: n = 901; $Tp(1)bi^{D1}/omb^{H31}$: 213; FM6a/+: n = 1378; omb^{H31}/FM6a: n = 867; FM6a/Y: n = 913)

Head yaw is reduced to about 75 % in $Tp(1)bi^{D1}/omb^{H31}$ and $Df(1)rb^{5}/omb^{H31}$ (Figure 36A). Head roll is absent in both genotypes (Figure 36C) and only $Tp(1)bi^{D1}/omb^{H31}$ shows a head pitch response different from zero (Figure 36B). A change in body position induced by the moving pattern in the head roll experiment was seen in all controls and wildtypes (Figure 37) but was absent for $Tp(1)bi^{D1}/omb^{H31}$ and $Df(1)rb^{5}/omb^{H31}$ (this response was not quantified).

Figure 36

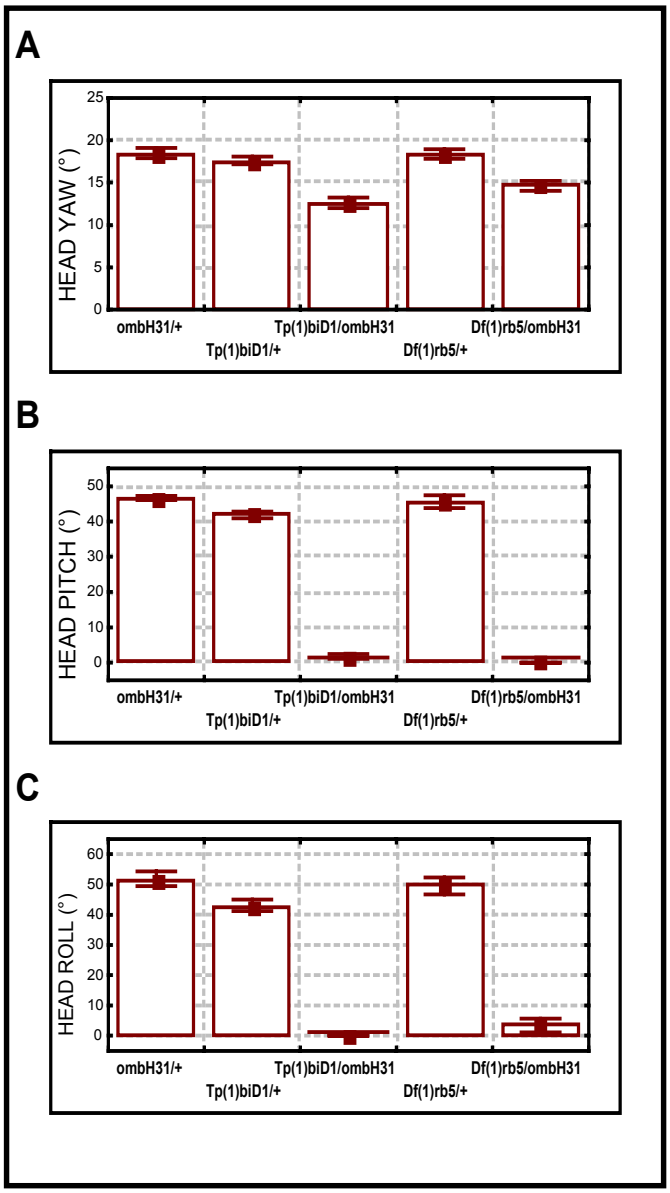

Figure 36: Head yaw, pitch and roll in two genotypes without VS- and HS-cells (*Tp(1)bi*^{D1}/*omb*^{H31}, *Df(1)rb*^{5}/*omb*^{H31}). (**A**) Head yaw is significantly reduced to about 75 % in both genotypes (P < 0,01). (**B**) Head pitch is reduced more severely in both genotypes (P < 0,001) however, *Tp(1)bi*^{D1}/*omb*^{H31} still shows a response just significantly different from zero (P < 0,01). (**C**) Both genotypes show no significant head roll response (P > 0,05). (n = 8; except yaw and pitch for *Tp(1)bi*^{D1}/*omb*^{H31} and *Tp(1)bi*^{D1}/+, n = 16)

Figure 37

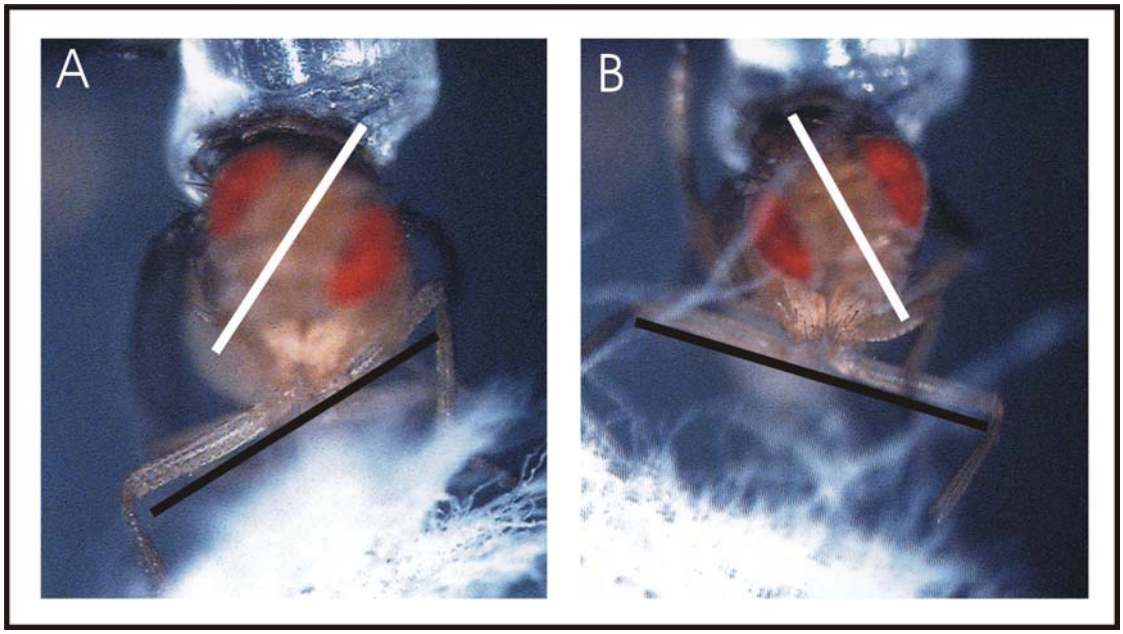

Figure 37: In response to a rotating environment flies show corrective movements (yaw, roll or pitch) of the head as well as changes in body position. This was first described for *Musca* by Gaffron (1933). Here the roll response is shown. In (**A**) a clockwise rotating pattern is used as a stimulus. The fly rolls its head in the direction of movement (white bar) and lowers the side of the body to which the pattern moves (black bar visualizes the line formed by the trochanders of the first legs). In (**B**) the pattern rotates in the opposite direction.

To better understand visually induced head position corrections (especially head roll that is zero in all allelic combinations of the *omb* locus tested) the basic properties of this response have been characterized. Head roll is contrast frequency dependent. The optimal response is reached at a frequency of 1.2 Hz (Figure 38A and B). The response also depends on the pattern contrast. A higher pattern contrast elicits a higher response (Figure 38A). Head roll responses to stimuli that consist of a black to white gradient followed by a white to black edge are not different from responses to stimuli that consist of a white to black gradient followed by a black to white edge at different contrast frequencies (Figure 38B). A grating of moving stripes (60° pattern wavelength) elicits the same head roll response as a random pattern, consisting of randomly distributed black and white squares of 15°. A single black stripe of 30° rotating around the fly elicits a response reduced to about 50% (Figure 38C). The wtCS flies (Figure 38C) show a maximal response of 52° to a moving grating of stripes, whereas the value for wtB flies under the same stimulus conditions is 72° (Figure 38A). Progressive and

regressive stimulation elicit head yaw responses that are reduced to about 80% compared to the head yaw induced by whole field stimulation (Figure 38D).

Figure 38

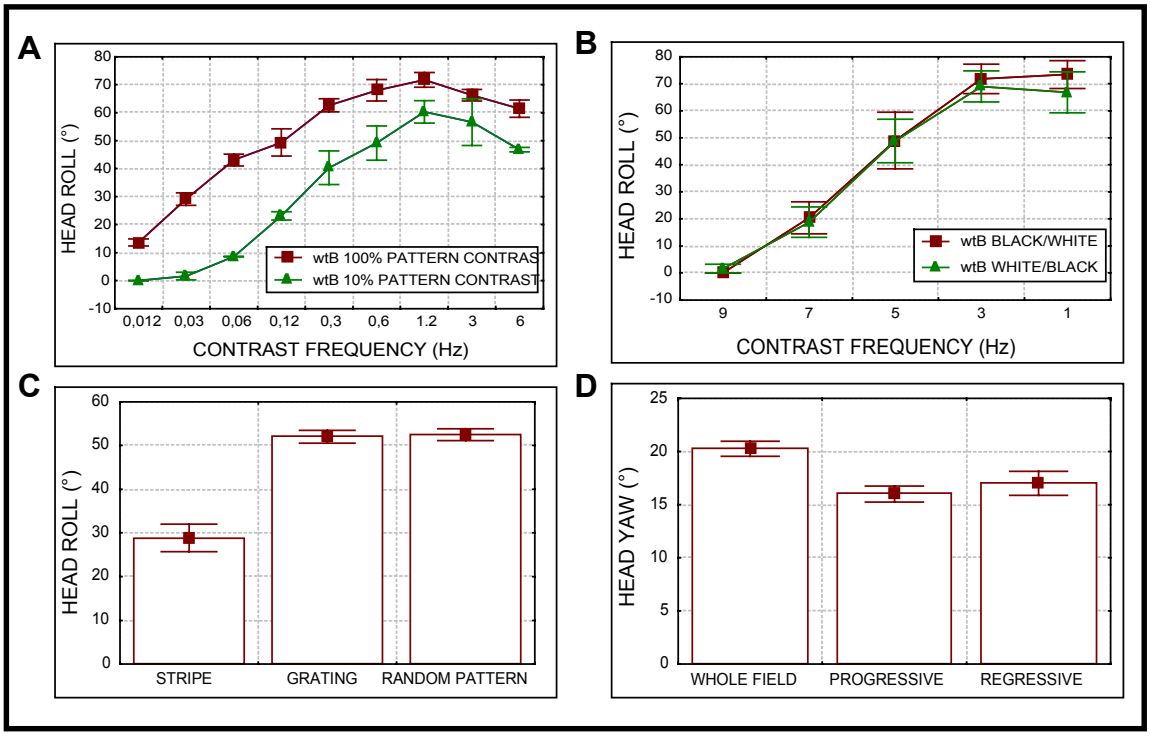

Figure 38: (**A**) Head roll responses of wtB flies to contrast frequencies ranging from 0,012 to 6 Hz. Two different pattern contrasts were tested. The lower contrast being ten times lower than the higher contrast. The responses to the lower contrast are significantly different from the responses to the higher contrast for all contrast frequencies except 1,2 Hz and 3 Hz, for which responses to both patterns reaches a maximum (high contrast: n = 8; low contrast: n = 5; $P < 0,05$). (**B**) Head roll responses of wtB flies to stimuli that consist of a black to white gradient followed by a white to black edge or vice versa are tested (for a detailed description of the experimental procedure see Material and Methods). The responses are not significantly different at all five contrast frequencies tested (n = 8; $P > 0,05$). (**C**) Head roll responses of wtCS flies to a single stripe, a grating of moving stripes and a random pattern are tested. The grating of moving stripes and the random pattern induce the same response, whereas a single stripe rotating around the fly elicits a response significantly reduced to about 50% (n = 8; $P < 0,001$). (**D**) Progressive and regressive stimulation elicit head yaw responses in wtCS flies that are significantly reduced to about 80% compared to the head yaw induced by whole field stimulation (for a detailed description of the experimental procedure see Material and Methods) (n = 8; $P < 0,05$).

Flies with functionally blocked VS cells

In a next step two GAL4 lines expressing in VS cells (1187 and 3A) are used to drive effectors believed to functionally knock out the neurons in which they are

expressed. The two behaviors most severely affected in the *omb* mutants (head roll and the forced-choice fixation Y-maze) were investigated in these flies.

Head roll is completely absent even in $Df(1)rb^5/omb^{H31}$ flies that (despite the absence of HS and VS cells) during flight show a wildtype response to progressive stimuli and 25 % of the wildtype response to regressive stimuli. Although in these flies the inner optic chiasm phenotype observed in omb^{H31} flies is gone, the number of fibers of the anterior optic tract connecting the optic lobes to the anterior central brain is, like in omb^{H31}, reduced by 300 (Brunner et al., 1992). To study the behavioral significance of the six VS cells, genetic modifications that functionally block VS cells in flies that do not lack the 300 cells of the anterior optic tract were used. Two GAL4 lines were available. Line 1187 expresses in the VS cells and in central brain structures (Kerscher et al., 1995; Figure 39), whereas line 3A labels the VS cells and additional lobula plate giant neurons with horizontal arborizations, as well as central brain structures (Scott, Stanford, pers. comm.; Figure 39). I expressed tetanus neurotoxin or the human inwardly rectifying potassium channel to functionally block the labeled cells in combination with both GAL4 lines. Neither tetanus neurotoxin nor the human inwardly rectifying potassium channel did have any effect on head roll responses when expressed in VS cells (Figure 40).

Figure 39

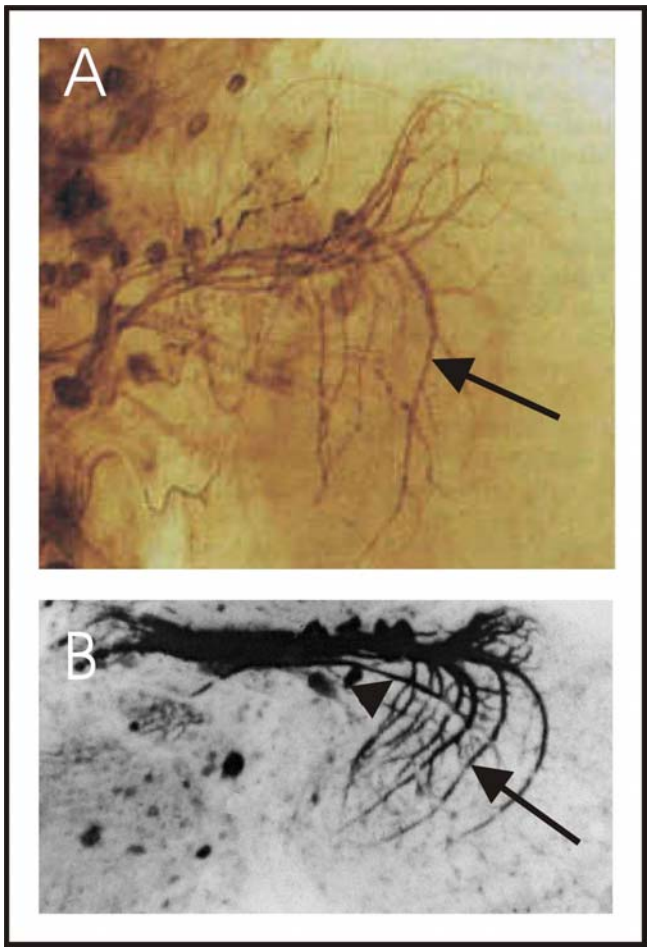

Figure 39: Expression pattern of GAL4 lines 1187 and 3A. (**A**) 1187 described in Kerscher et al. (1995) is a marker for VS cells. A whole mount preparation of a 1187/UAS-tau fly stained with anti-tau antibody is shown. The VS cells arborize in the most distal layer of the lobula plate (arrow). (Figure is taken from Kerscher et al. (1995)) (**B**) 3A labels VS cells (arrows) and additional lobula plate giant neurons with horizontal arborizations (arrowhead). A whole mount preparation of a 3A/UAS-lacZ fly stained with anti-ß-GAL antibody is shown.

Figure 40

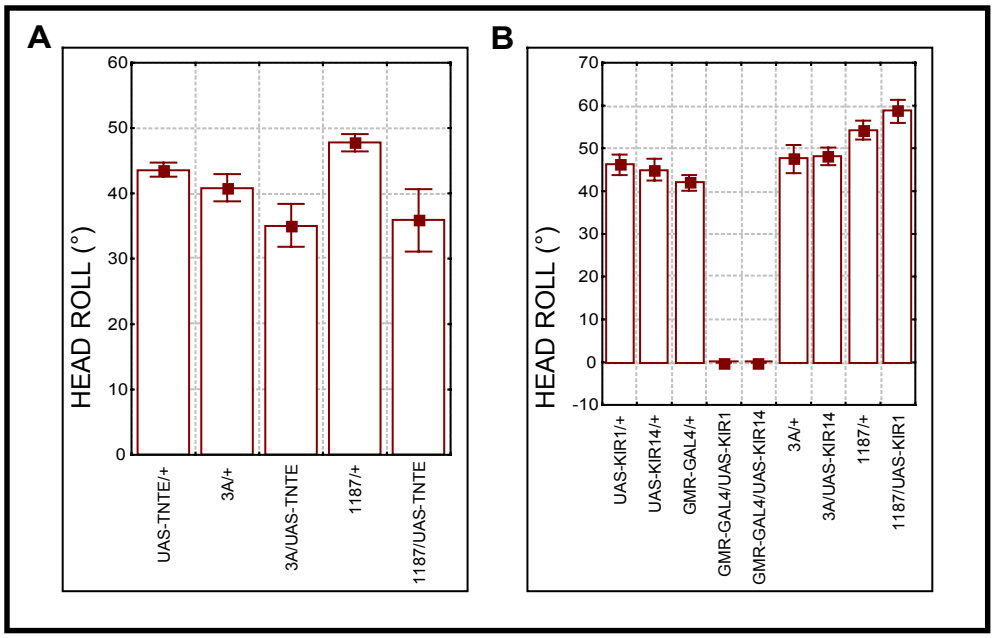

Figure 40: Head roll responses of flies expressing tetanus neurotoxin (A), or a human inwardly rectifying potassium channel (KIR) (B) in a 3A or 1187 dependent way. (**A**) 3A/UAS-TNTE and 1187/UAS-TNTE do show a head roll response that is not significantly lower than the respective controls (n = 8; $P > 0{,}05$). (**B**) *GMR*-GAL4/UAS-KIR1 and *GMR*-GAL4/UAS-KIR14 flies, expressing KIR in the photoreceptors show no significant head roll response (n = 8; $P > 0{,}05$). 3A/UAS-KIR14 and 1187/UAS-KIR1 flies, expressing KIR in VS cells show a head roll response, not different from the controls (n = 8; $P > 0{,}05$).

In the forced-choice fixation Y-maze omb^{H31} is severely more impaired than the allelic combinations, indicating that an anatomical defect that is less impaired in these combinations is responsible for that behavioral phenotype. However, I tested the fixation efficiency in the forced-choice fixation Y-maze for 3A/UAS-KIR14 and 3A/UAS-TNTE flies. Both genotypes did fixate the black bar with an efficiency similar to the control (Figure 41).

Figure 41

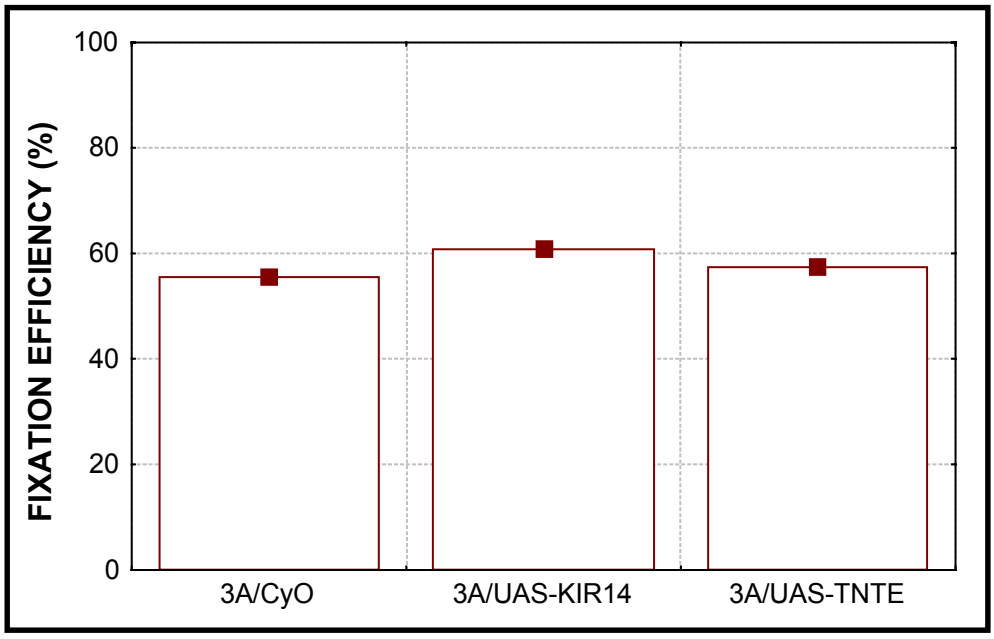

Figure 41: Fixation efficiencies in the forced-choice fixation Y-maze for 3A/UAS-KIR14 and 3A/UAS-TNTE flies. 3A/CyO flies serve as a control. All three genotypes fixate the black dots with similar efficiencies. (3A/CyO: n = 490; 3A/UAS-KIR14: n = 68; 3A/UAS-TNTE: n = 625)

Outputs other than HS and VS cells

The lines OK107 and J163 were initially not studied because of their expression patterns. Both lines have been identified in a behavioral screen of GAL4 lines in combination with UAS-TNT. Three behaviors have been tested for more than 150 genotypes: visually induced landing response, head roll and fixation of two opposing 60° bars in walking. This approach is less biased than the approach used in the lines above in which the known expression pattern in visual neurons allowed me to test specific hypothesis.

OK107/UAS-TNTE flies showed no landing behavior in response to an expanding visual stimulus (Figure 42B). However, they reliably extended their legs in response to a reduction in light intensity (data not shown). They also showed a head roll response (Figure 42A). Fixation in walking could not be tested due to walking difficulties in the experimental flies. However, observations of single flies performing in the fixation paradigm suggest, that OK107/UAS-TNTE flies do fixate 60° bars (data not shown). Expression in OK107 is driven in neurons connecting the lobula and

medulla with an optic foci in the ipsilateral central brain. In the central brain there is expression in the mushroom bodies and elsewhere (Figure 43).

Figure 42

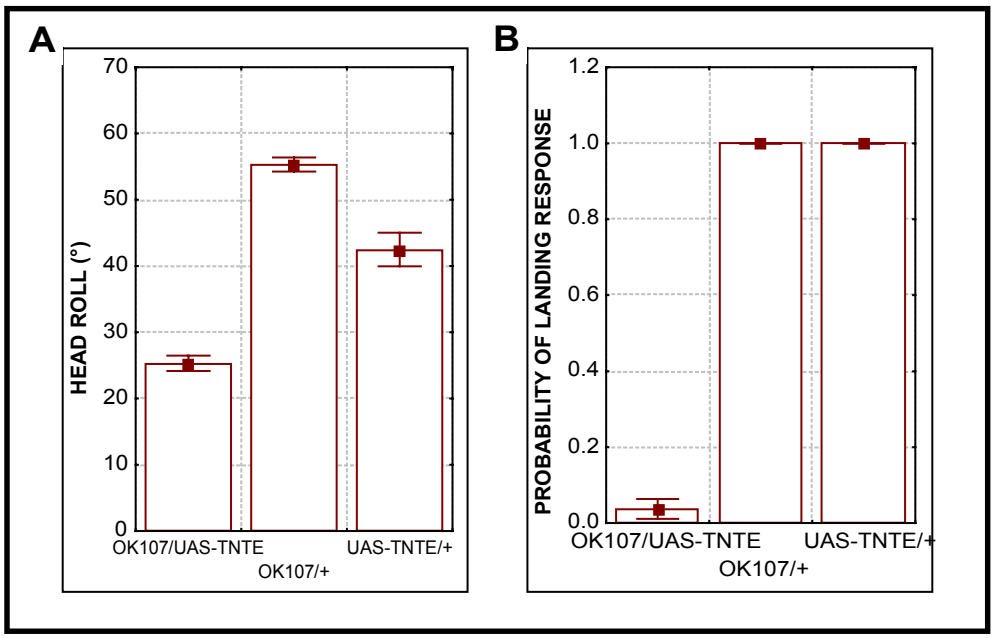

Figure 42: Head roll (A) and the probability of a landing response (B) of OK107/UAS-TNTE flies and controls. (**A**) Head roll response is reduced to about 50% compared to the responses of OK107/+ and UAS-TNTE/+ (n=6; $P < 0{,}001$). (**B**) OK107/UAS-TNTE flies show no landing response whereas in the controls the expanding stimulus elicited a response in all cases (n=8).

Figure 43

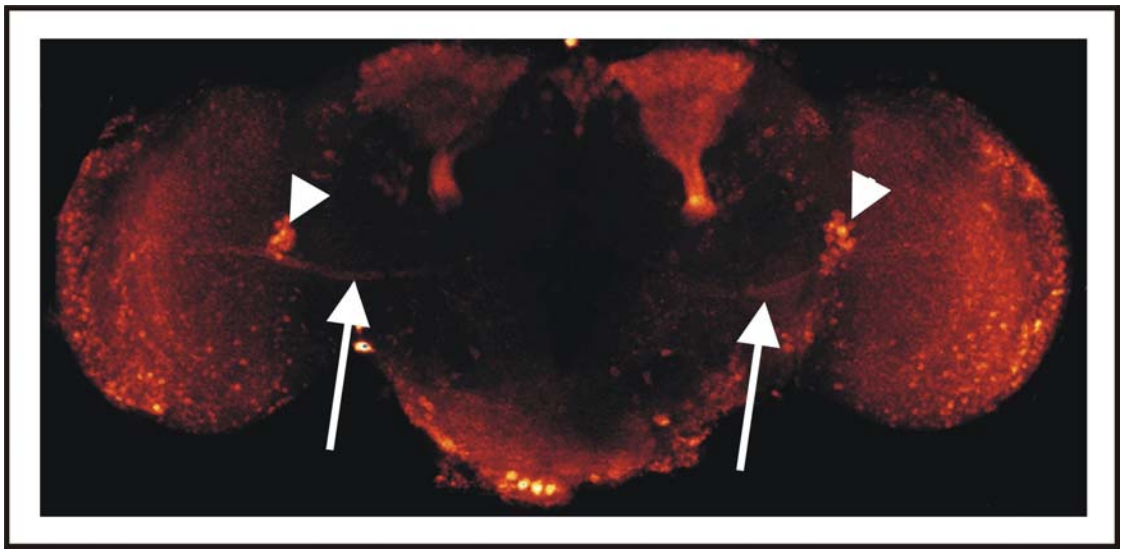

Figure 43: The expression in OK107 is visualized with anti-ß-GAL antibody. Expression is driven in neurons connecting the lobula and medulla to the ipsilateral central brain. Cell bodies are located between the optic lobe and the central brain (arrowhead). The axons project to the central brain (arrows) but do not cross the midline. Arborizations are seen in specific layers of medulla and possibly also lobula. In the central brain there is extensive expression in the mushroom bodies and elsewhere. If the heavily stained dots at the periphery of the optic lobes and the central brain are cell bodies or an artifact of the preparation can not be decided, however they are not distributed symmetrically.

J163/UAS-TNTE flies did neither prefer nor avoid the two black 60° bars (Figure 44A). Two opposing black 10° bars, however, were fixated by J163/UAS-TNTE flies, although fixation efficiency was reduced significantly compared to the controls (Figure 44A). Landing response and head roll were not abnormal in J163/UAS-TNTE flies (Figure 44B/C). J163 drives expression in few cell bodies ventrally proximal of the medulla (Figure 45). Few clustered cell bodies could be detected in the central brain (data not shown). Only anti-TNT expression patterns were examined. The more sensitive anti-tau staining would be useful to visualize the arborizations of these cells.

Figure 44

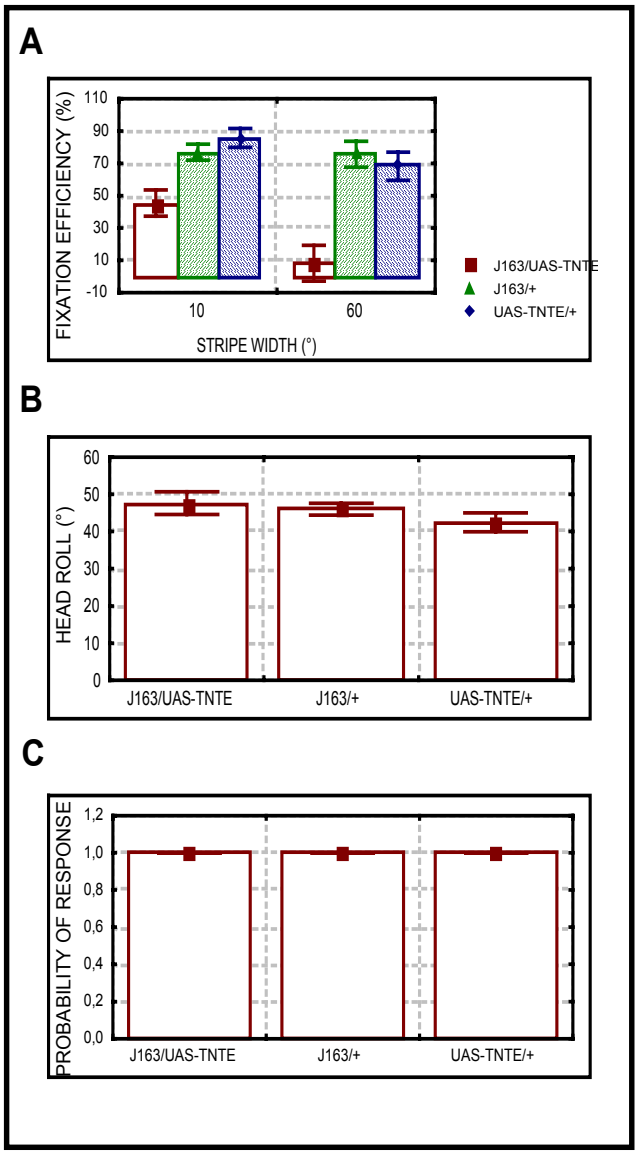

Figure 44: Fixation efficiency (A), head roll (B) and the probability of a landing response (C) of J163/UAS-TNTE flies and controls. (**A**) Fixation efficiency for two opposing 10° and 60° stripes. In both cases J163/UAS-TNTE flies have a significantly reduced fixation efficiency (n = 11 or 12 for 60° and 8 for 10°; $P < 0,01$). Only for 10° the fixation efficiency of J163/UAS-TNTE flies is significantly different from zero ($P < 0,05$). (**B**) Head roll responses are not significantly different between J163/UAS-TNTE flies and controls (n = 6; $P > 0,05$). (**C**) The landing response can be elicited as reliable in J163/UAS-TNTE flies as in controls (n = 8).

Figure 45

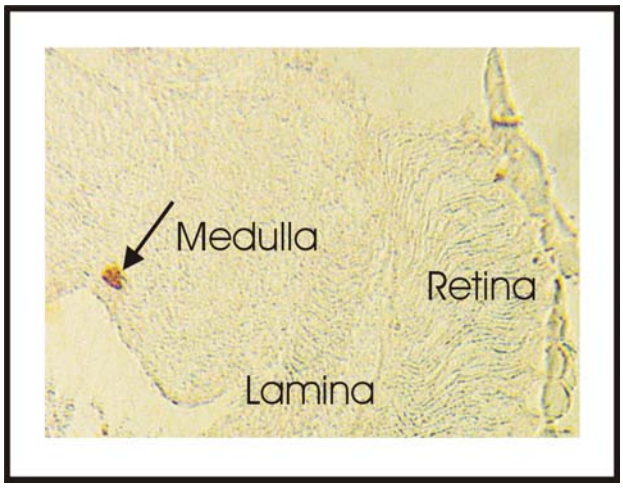

Figure 45: The expression of line J163 is visualized with an anti-TNT antibody. A horizontal section of one optic lobe of a J163/UAS-TNTE fly is shown (10 µm section). Expression can be detected in few cell bodies ventrally proximal of the medulla (arrow).

The expression pattern of *ato*-GAL4 is described in great detail in Hassan et al. (2000) (Figure 46). Expression is driven in one dorsal cluster (DC) of cells adjacent to the lobula (Figure 46A) and in two small groups of ventral cells (VLC and VBC) (Figure 46D). Some axons of the dorsal cluster project ipsilaterally over the lobula, whereas most axons form a bundle that is a component of the dorsal commisure and project to the contralateral lobula and medulla (Figure 46A). One of the smaller ventral clusters is located in the central brain (VBC) and the other in the lobula (VLC). The VLC forms an extensive network of fibers in the ventral lobula (Figure 46D). *ato*-GAL4/UAS-TNTE flies did not show the eclosion phenotype described for *ato*-GAL4/UAS-*rpr* flies (Hassan et al., 2000). In fact they showed no phenotype at all and behaved not different from controls in fixation of two 10° bars, head roll and landing response (Figure 47). *ato*-GAL4/UAS-TNTE flies were very reluctant to fly both tethered and free.

Figure 46

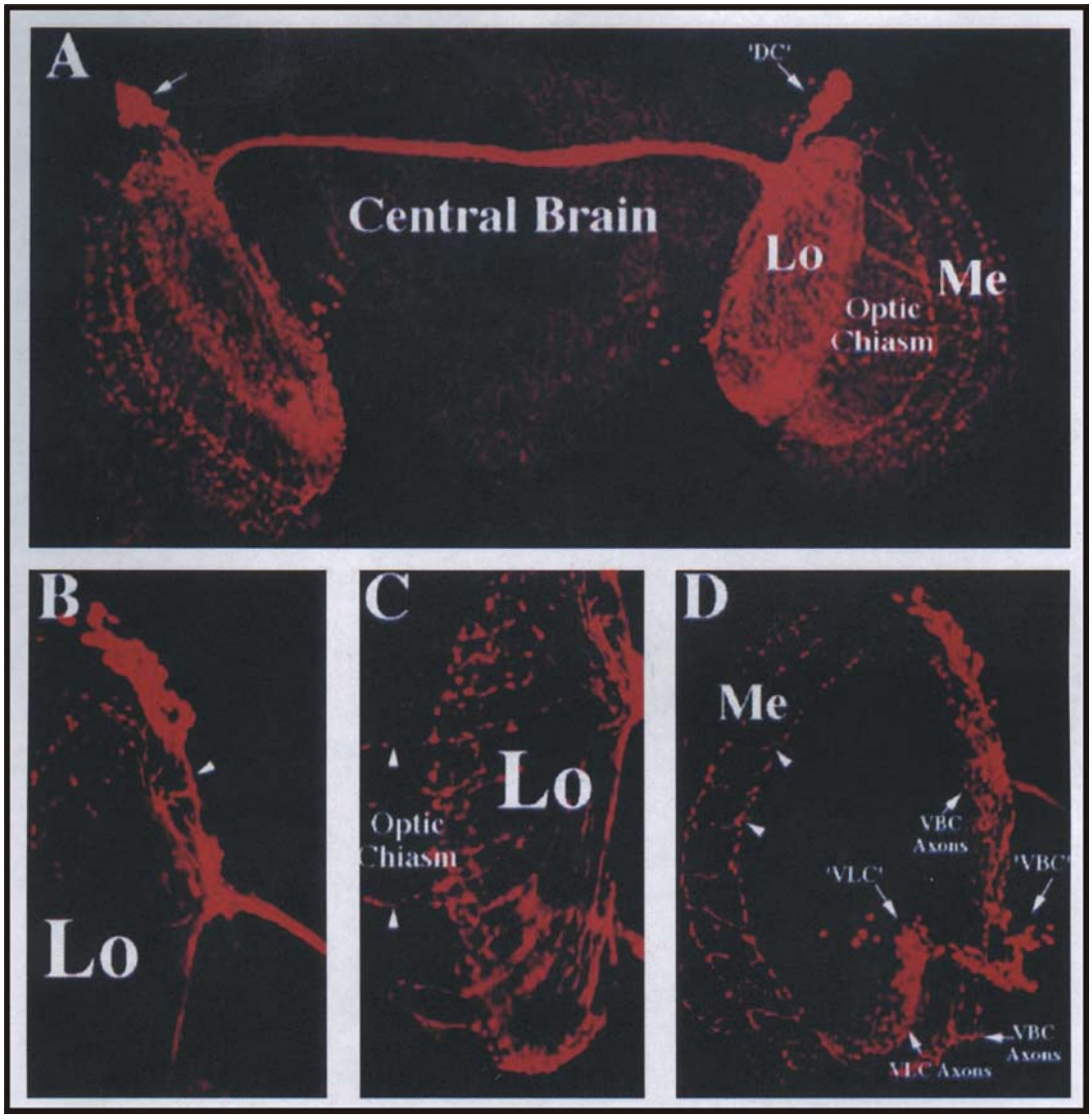

Figure 46: The expression pattern of *ato*-GAL4 is visualized with an anti-lacZ antibody. The figure is taken from Hassan et al. 2000. (**A**) Expression in the two dorsal clusters (DC) as well as in a group of ventral cells is seen. The commisure and extensive arborizations in the optic lobes are labeled. (**B**) A DC is shown at higher magnification. The descending bundle of axons, as well as fibers running between the cluster and the dorsal lobula (arrowhead) are labeled. (**C**) Fibers form a regular pattern in the lobula with some fibers exiting the lobula, crossing the optic chiasm and innervating the medulla (arrowheads). (**D**) The two VCs and their axons are shown. VBC axons innervate the brain lobula border. VLC axons form a dense network of fibers in the ventral lobula. In addition the grid-like pattern of fibers generated by the branching (arrowheads) of the DC axons that cross the optic chiasm are labeled. (Lo = lobula; Me = medulla; DC = dorsal cluster; VLC = ventral lobula cluster; VBC = ventral brain cluster)

Figure 47

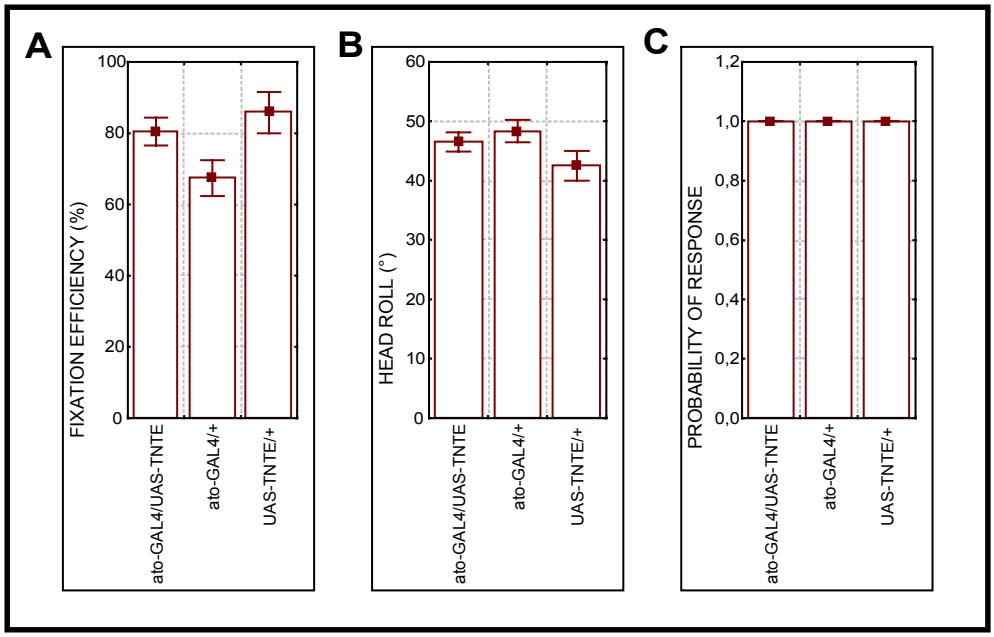

Figure 47: Fixation efficiency of two opposing 10° stripes (**A**), head roll response (**B**), and probability of landing response (**C**) of *ato*-GAL4/UAS-TNTE flies and controls are shown. *ato*-GAL4/UAS-TNTE values for fixation (n = 8), head roll (n = 6) and landing response (n = 8) are not significantly different from control values ($P > 0,05$).

No behavioral function could be assigned to the neurons labeled in *ato*-GAL4. The very few neurons stained in J163 seem to play a role in establishing preferences for black pattern, whereas the immunopositive cells in OK107 are crucial for the induction of the landing response by expanding patterns but not for the detection of visual motion.

4. Discussion

4.1. Comparison of effectors

Apoptosis inducers and cell toxins

The apoptosis inducers and cell toxins (*rpr*, *hid*, rAcs) proved to be of limited use for structure-function mapping in the *Drosophila* nervous system. Both UAS-*rpr* and UAS-*hid* killed flies when driven by *GMR*-GAL4. This is unexpected because flies carrying a *GMR*-*rpr* insertion survive well and show a dosage dependent ablation of the eyes (White et al., 1996). A possible explanation is that the UAS/GAL4 system induces effector expression at higher levels compared to the direct expression of the effector fused to the relevant promoter. When UAS-*rpr* or UAS-*hid* are expressed under the control of *rdgC*-GAL4 flies survive and do not show ablation of the leg campaniform sensilla or the behavioral defects of *rdgC*-GAL4/UAS-TNT flies. While *rpr* has been successfully used in *Drosophila* to ablate eclosion hormone cells and produce discrete deficits in eclosion behavior (McNabb et al., 1997), resistance to apoptosis induction by either *rpr* or *hid* alone has been described for embryonic central nervous system midline cells. In the latter case expression of *rpr* and *hid* in combination is sufficient to induce apoptosis (Zhou et al., 1997). The same may be true for the campaniform sensilla. Alternatively, the dosage of the apoptosis inducers expressed with *rdgC*-GAL4 is not high enough to cause programmed cell death. *GMR*-GAL4*,* UAS-*rAcs* flies survived at the toxin non-permissive temperature. After being shifted to the toxin permissive temperature for two days or more, rAcs expression failed to blind flies completely. Therefore, some photoreceptor function survived. Considering that *sine oculis* flies with fewer than ten ommatidia per eye still show optomotor responses (Götz, 1983), a small number of functional photoreceptors, or large numbers of photoreceptors that were less functional, would probably be enough to produce the observed behavior.

Tetanus Neurotoxin

In contrast, all three insertions of UAS-TNT block visual behavior completely in combination with *GMR*-GAL4, showing that all express TNT at high enough levels to fully block synaptic transmission in photoreceptors. However, the three insertions produce behavioral phenotypes of different severity when expressed under control of the *rdgC*-GAL4 construct. Since the expression patterns of the three insertions are indistinguishable (data not shown), I consider two possible explanations. First, the weak

insertions may not produce enough toxin under the control of this specific GAL4 line to fully block the neurons expressing it. Second, expression below the immunohistological detection level may occur in unidentified neurons and may in some insertions be higher than in others, leading to additional defects not directly related to the observable expression pattern. An intracellular concentration of TNT as low as 10 nM is effective in inhibiting evoked neurotransmitter release in the buccal ganglion of *Aplysia californica* within two hours (Schiavo et al., 1992a). This concentration is well below the detection limits of the avidin-biotin method used here to detect TNT. This method is much less sensitive than the Peroxidase-Antiperoxidase method (Sternberger and Sternberger, 1986) that has a detection limit of 0.1 mg/ml (Fritz et al., 1992). Taking MW = 50 000 as the molecular weight of TNT this corresponds to 2 µM which is 200 times higher than the concentration needed to block *Aplysia* neurons, making it highly likely that immunopositive neurons are fully blocked. Therefore I favor the explanation for the behavioral differences that the leakiness of the UAS-TNT construct at the three genomic locations is different, generating different levels of undetected TNT expression in unknown regions of the nervous system. This line of arguments suggests that the reduction of active walking time in *rdgC*-GAL4, UAS-TNTC and *rdgC*-GAL4/UAS-TNTG flies is due to expression below the detection level and not caused by the visualized expression pattern of TNT. However, since the active walking time of the three lines carrying UAS-TNT as heterozygotes does not differ, this undetected driver-dependent expression is different from the leaky TNT expression of the UAS-TNT insertions in the absence of any driver, proposed by Scholz et al. (2000). Therefore, in the subsequent studies the UAS-TNTE transformant as the line with the least sub-detection threshold expression was used.

Human inwardly rectifying potassium channel

A drawback of TNT is that it only blocks chemical synapses. Electrical synapses may occur frequently in the *Drosophila* nervous system. Cobalt-coupling (the ability of cobalt ions to pass from one neuron to another) is a phenomenon typical for *Diptera* (for example: Milde and Strausfeld, 1986). It is believed to be an indication, that the coupled neurons share gap junction-like apposition areas (Strausfeld and Bassemir, 1983). In invertebrates gene products of the *innexin* gene family form gap junctions (Phelan et al., 1998). In *Drosophila* there are five *innexin* genes (Curtin et al., 1999; Thomas, 1980; Watanabe and Kankel, 1992). In the adult nervous system only two *innexins* are

expressed in a very restricted patter, whereas in the pupae most neurons express *innexins* (Curtin et al., 1999; Crompton et al., 1980; Watanabe and Kankel, 1992). By blocking the initiation of action potentials signal transmission can be blocked independent of the synapse type. I therefore tested six independent transformants of a human inwardly rectifying potassium channel (KIR) in combination with *GMR*-GAL4 and *rdgC*-GAL4. In both combinations the resulting phenotypes varied depending on the transformant used. Interestingly, the most severe phenotypes in combination with *rdgC*-GAL4 were more severe than the one observed in *rdgC*-GAL4/UAS-TNTE flies. This is consistent with the fact that the campaniform sensilla which are labeled in *rdgC*-GAL4 flies form mixed synapses consisting of a chemical and an electrical component and therefore can not be fully blocked by tetanus neurotoxin.

In summary, TNT is very useful because it blocks chemical synapses very efficiently, however, neurons forming gap junctions can only be blocked completely by KIR. Considering only chemical synapses, KIR is less potent than TNT and requires higher GAL4 expression levels.

4.2. Conditional and inducible systems

Heat shock induced recombinase activity to induce tetanus neurotoxin expression

By inducing TNT expression using the flp/FRT system in adult flies, I have shown that the blindness observed in *GMR*-GAL4/UAS-TNT flies was not due to TNT expression before adulthood. I thus conclude that the blocking of neurotransmitter release in fully differentiated photoreceptors is responsible for the observed behavioral phenotype, and that this can be achieved by flp expression in these adult postmitotic neurons. However, with the *rdgC*-GAL4 line, flp-mediated somatic recombination in adult neurons was not inducible for reasons that are unclear. Therefore, the flp/FRT system is not universally applicable in the nervous system. However, the approach can clearly be used to postpone TNT expression to later stages in cases where early TNT expression may be lethal or damaging, although it remains to be seen whether even that application will work in all neurons or with all GAL4 lines. A further potential use facilitated by the somatic recombination event that turns on TNT expression is clonal analysis of the functions of small numbers of neurons by giving non-saturating heat shocks during development resulting in mosaics as shown here for line 21D.

Doxycycline dependent expression of *reaper* (*rpr*)

The two major advantages of the doxycycline dependent expression are the quantitative control of expression and the reversibility. Both advantages are lost when the apoptosis inducer *reaper* (*rpr*) is used as an effector, because *reaper* does not show graded effects and the action of *reaper* is not reversible. However, in this study reaper was used because the effect of reaper (cell death) is easy to observe. The preliminary experiment described here shows that the activity of rtTA* (Stebbins et al., 2001) can be controlled by doxycycline administration. This is in contrast to earlier experiments using tTA (Bello et al., 1998; data not shown). For an application of the rtTA* transgene in structure-function correlation, effectors that allow to take advantage of the graded expression and the reversibility (TNT/KIR/*tra*/*etc*.) need to be cloned in a tetO construct.

Expression of a semidominant temperature sensitive allele of *shibire* (shi^{ts1})

Generating mosaics of temperature dependent neurons by expressing a semidominant allele of *shibire* did not result in the expected temperature sensitivity of behaviors. When shi^{ts1} expression was driven in photoreceptors by *GMR*-GAL4, even at 18° C these flies did not show phototactic behavior. This is in contrast to Kitamoto (2001) who showed that *GMR*-GAL4 driven shi^{ts1} expression leads to temperature dependent blindness in adult flies. A possible explanation for this contradictionary result is that different insertions of the *GMR*-GAL4 construct driving GAL4 expression at different levels may have been used in this study and by Kitamoto (2001). When shi^{ts1} expression is driven by GH146, the phenotype observed in GH146/UAS-TNTE flies (blindness to motion), can not be observed even at a temperature as high as 37° C [Kitamoto (2001) used 30° C as a restrictive temperature]. The most straight forward explanation is that GAL4 expression level is not sufficiently high in the optic lobe cells in GH146. Indeed the level of expression in the optic lobe cells in GH146 is much lower than in the projecting neurons, where GH146 also drives expression (Heimbeck et al., 2002) and behavioral effects of shi^{ts1} are observed (Schwärzel, Würzburg. pers. comm.). Taken together, these results show that the method of generating mosaics of temperature dependent neurons by expressing a semidominant allele of *shibire* can only be applied to a limited number of GAL4 lines that drive expression at a level sufficient to block neurons at the restrictive temperature, but not at the permissive temperature. Only in experiments of which the outcome is known beforehand, it can be decided if the expression level is in the right range.

4.3. Functional specialization of mechanoreceptors

Leg mechanoreceptors

Expression in *rdgC*-GAL4/UAS-TNT flies is driven in chemosensory and mechanosensory neurons. The identified mechanosensory structures expressing TNT are campaniform sensilla in the halteres and legs. Three types of mechanoreceptors (chordotonal organs, campaniform sensilla and bristles) are found in the *Drosophila* leg. A global loss of mechanosensory input is believed to lead to severe coordination defects (Kernan et al., 1994). Therefore, one or more classes of mechanoreceptors are likely to be necessary for coordinated locomotion. Blocking the leg campaniform sensilla (*rdgC*-GAL4/UAS-TNTE) leads to no alteration in average walking speed and in walking activity. However, in more advanced paradigms involving coordinated leg action like walking on vertical surfaces and grooming behavior flies with blocked campaniform sensilla performed significantly worse than the controls. Blocking the second major mechanosensory structure in legs, the femural chordotonal organ, (C42/UAS-TNTE and C161/UAS-TNTE) severely affects average walking speed and walking activity. Results from C161/UAS-TNTE are difficult to interpret since expression is driven in a wide variety of mechanosensory structures. In contrast, behavioral abnormalities in C42/UAS-TNTE flies can be expected to be associated with the femural chordotonal organ since the only other mechanosensory structures expressing TNT in these flies are tactile bristles. The mutant *hdc* in which the tactile bristles are non-functional shows no locomotor defects (Buchner, Würzburg, pers. comm.), therefore they seem not to play a role in mediating locomotor patterns but rather mediate responses to external stimuli. Following this line of arguments, all locomotor defects observed in C42/UAS-TNTE flies can be attributed to the block of the chordotonal organ. C42/UAS-TNTE flies were not only tested for their average walking speed and walking activity but also for their ability to walk on vertical surfaces. They proved to be severely impaired. These findings indicate that the femural chordotonal organ provides mechanosensory feedback required for normal walking (Strauss and Heisenberg, 1990). This is consistent with the fact, that TNT expression in the femural chordotonal organ blocks the resistant reflex, that normally excites the tibial extensor motor neurons when the femoral-tibial joint is flexed (Reddy et al., 1997). In contrast, the leg campaniform sensilla are dispensable for normal walking but are important for leg coordination during grooming and walking on

vertical surfaces and may also play a role in courtship which is also affected in *rdgC*-GAL4/UAS-TNTE flies.

Haltere campaniform sensilla

Haltere campaniform sensilla form mixed synapses, consisting of a chemical and an electrical component, with a motorneuron that innervates a small flight steering muscle. The electrical component of the synapse was shown to be abolished in the *shakingB* mutant (Trimarchi and Murphey, 1997). The monosynaptic connection between sensory- and motorneuron allows quick reflexes, correcting for angular rotations of the body during flight, sensed by the haltere campaniform sensilla. Excitatory postsynaptic potentials at this mixed synapse are dominated by the electrical component (Fayyazuddin and Dickinson, 1999). Nevertheless, the results suggest that haltere campaniform sensilla can mediate the corrective signals necessary for flight at least to some degree using exclusively the electrical or chemical components of their synapses. This still holds true even if the flight defect observed in *rdgC*-GAL4/UAS-TNTE flies is not due to the expression in haltere campaniform sensilla but to expression elsewhere. As expected a complete block of the haltere campaniform sensilla by expressing KIR results in flightlessness. Whether the prolonged copulation latency in *rdgC*-GAL4/UAS-TNTE flies is due to the described locomotor defects or the chemosensory defects (see below) can not be decided.

4.4. Specificity of chemoreceptors

Since the antennal chemoreceptors labeled in the *rdgC*-GAL4 line project into a specific subset of antennal lobe glomeruli, they can be expected to be sensitive to specific classes of odors (see Vosshall et al., 2000). The responses to three odors at different concentrations were tested and, remarkably, a modified response was detected for isoamylacetate, but not for the other two odors tested. The specificity of this observed phenotype makes it likely that expression in the subset of visualized chemoreceptors contributes to this alteration in behavior, and that some or all of the labeled chemoreceptors respond specifically to isoamylacetate. The experimental flies are neither anosmic for isoamylacetate nor are they less sensitive to it. They show a largely dosage independent avoidance of isoamylacetate at the concentrations tested, whereas the two control lines are repelled by the odor only at the two highest concentrations tested (3 % and 10 %). Isoamylacetate is known to be repulsive only at

high concentrations, but at concentrations lower than the ones used here, it is an attractant (Ayyub et al., 1990). It is likely that the random distribution in the controls is a balance between attraction and repulsion at this concentration range, and that TNT expression in the labeled neurons increases avoidance by interfering with specific attraction to this odor.

4.5. Information processing in the lamina

The expression throughout the visual system in GAL4 line GH146 makes it impossible to make any statements about behaviors that depend on functional lamina monopolar cells L1 and L2. However, the reverse statement, that behaviors unaffected in GH146/UAS-TNTE flies are independent of L1/L2 still holds true. Fixation of 60° or 180° stripes is unaffected in GH146/UAS-TNTE. For these behaviors, L1/L2 lamina monopolar cells are therefore not necessary. The photoreceptors R7 and R8 which pass through the lamina without synaptic contacts can mediate fixation behavior in walking flies (Coombe, 1984). However, this requires 110° stripes. The fixation of the 60° stripes therefore may be mediated by the L3 neurons or by amacrine cells alpha , the only neurons apart from L1 and L2 that receive R1-6 input. This could be tested in GH146/UAS-TNTE flies in an *sev* background in which in addition to the neurons labeled in GH146 the R7 and R8 pathway would be blocked. This experiment could rule out effects of the genetic background which is different in the flies tested here and by Coombe (1984).

Flies with blocked L2 neurons still show optomotor responses in walking, visually induced head roll and visually induced landing response. This shows that L2 is not necessary for motion detection. Some electrophysiological evidence from large flies suggests that both large monopolar cells L1 and L2 are not on the optomotor pathway (Coombe et al., 1989; Laughlin, 1984; Riehle and Franceschini, 1984). In addition, in the *Drosophila* structural mutant *vam* there is poor correlation between large monopolar cell degeneration and the strength of the optomotor response (Coombe et al., 1989) and the optomotor response in *Drosophila* is strongly polarization sensitive (Wolf et al., 1980), although the large monopolar cells show no polarization sensitivity (Coombe et al., 1989). The experiments shown here give the first direct genetic evidence that L2 is not necessary for motion detection by showing that both 21D/UAS-TNTE as well as 21D/UAS-KIR1 flies do still show all the movement detection dependent behaviors tested. However, the optomotor response in walking is reduced in 21D/UAS-TNTC flies

at all pattern luminance's and contrast frequencies tested. This data has to be taken with caution for three reasons: (1) UAS-TNTC has shown to have unspecific effects in other paradigms (Figure 29). (2) The UAS-TNTC construct is not tested for dominant effects. (3) The results of the optomotor response in walking measurements are extremely variable. Nonetheless, the clear trend indicates that although L2 is obviously not necessary for movement detection, it may still be involved to some degree in the response.

Electrophysiological recordings of a directionally selective interneuron (H1-cell) in large flies in response to motion stimuli gave indirect evidence about the intrinsic properties of the elementary movement detectors in flies (reviewed in Franceschini et al., 1989). One conclusion drawn from recordings of H1-cell responses is, that retinal input signals segregate into separate channels, which then feed two antiparallel movement detectors driving the H1-cell with opposite polarities (Riehle and Franceschini, 1984). I found no impairment in the response to regressive or progressive motion stimuli in flies with blocked L2 lamina monopolar cells. If there are two antiparallel movement detectors, than L2 does not provide input to one of it. Another conclusion drawn from electrophysiological recordings by some researchers is, that motion detection in flies takes place in two separate ON and OFF channels (Franceschini et al., 1989; Horridge and Marcelja, 1990). Other investigators, however, find no indication that flies process motion information in independent ON and OFF channels (Egelhaaf and Borst, 1992). The data presented here shows that L2 cells in *Drosophila* are neither necessary for seeing motion of light edges nor for seeing motion of dark edges. Data from *Drosophila*, including the data presented here, does not support the idea that retinal input signals segregate into separate channels, which then feed independent movement detection channels.

In large flies, already in the second visual neuropil, the medulla, directionally sensitive small field elements have been identified (DeVoe and Ochleford, 1976; DeVoe, 1980; Gilbert et al., 1991) and in *Drosophila*, deoxyglucose labeling experiments showed that in the medulla patterns specifically activated by retinotopic motion are found (Buchner et al., 1984; Bausenwein et al., 1992). Therefore elementary movement detection may take place already in the lamina. Connections between columns upstream of the medulla are provided by L4 cells that connect neighboring columns and the large field amacrine cells alpha that pool information from 6-20 visual sampling units. The only input to L4 is provided by L2 which can be assumed to be

blocked in 21D/UAS-TNTE and 21D/UAS-KIR1 flies. If it is true that these flies are still capable of detecting motion, then motion detection is mediated by the matrix of amacrine cells alpha. Amacrine cells alpha pool information from 6-20 visual sampling units. If it is true that these cells mediate motion detection, this would help to explain that spatial receptive fields of motion sensitive neurons adjust according to ambient light (Schuling et al., 1989; Pick and Buchner, 1979; Srinivasan and Dvorak, 1980).

4.6. Optic lobe output neurons

Characterization of *omb* mutants

In the mutant omb^{H31} a variety of motion-vision dependent and motion-vision independent behaviors is known to be abnormal (see Introduction). These behavioral defects are assumed to be causally related to the anatomical defects described for this mutant. Especially the impairment in responses to visual motion was correlated with the lack of VS and HS cells. VS and HS cells are known to respond to visual motion in *Dipterans* (reviewed in: Dahmen et al., 2001; van Stevenick et al., 2001; Warzecha and Egelhaff, 2001). This correlation was challenged by the finding that other mutants of the *omb* locus which also lack VS and HS cells show a less severe impairment in there responses to visual motion (Brunner et al., 1992). I retested omb^{H31} flies for their optomotor response in walking, head yaw, pitch and roll, fixation of one black stripe and the behavior in the forced-choice fixation Y-maze. omb^{H31} behaved as described previously in all paradigms except the fixation of a black stripe in walking where it proved to be not different from the control (Figure 34). All motion-vision dependent behaviors turned out to be as impaired as in the original description of the line, indicating that the structures defective in these flies are crucial for these responses.

In $Df(1)rb^5/omb^{H31}$ flies, only the OLR3 of the regulatory region of the *omb* gene is removed in both copies (Figure 6), yet these flies have no HS and VS cells (Brunner et al., 1992).

Surprisingly, in such mutants the optomotor phenotype is much less pronounced than in omb^{H31}. $Df(1)rb^5/omb^{H31}$ shows a wildtype response to progressive stimuli during flight and 25 % of the wildtype response to regressive stimuli. The original omb^{H31} mutant shows a 50 % response to progressive an no response to regressive stimuli in the same experiment. In addition, the defect in fast phototaxis observed for omb^{H31} is abolished in $Df(1)rb^5/omb^{H31}$. The other behaviors found to be defective in omb^{H31} have not been tested. The most straight forward interpretation of the behavioral

differences between omb^{H31} and $Df(1)rb^5/omb^{H31}$ is that the anatomical defects occurring in *omb* mutants in addition to the lack of HS and VS cells are less severe in $Df(1)rb^5/omb^{H31}$ flies. This was shown to be true for the disruption of order in the IOC (Brunner et al., 1992).

Here, head yaw, pitch and roll of $Df(1)rb^5/omb^{H31}$ and its behavior in the forced-choice fixation Y-maze have been tested. The flies show a head yaw reduced to about 75 % and no roll or pitch (Figure 36). The mild defects these flies show in the head yaw and in optomotor yaw responses during flight (Brunner et al., 1992) show that the loss of HS and VS cells is not the cause of the low optomotor yaw and head yaw values in omb^{H31}. On the other hand, responses to vertical movements are absent in both genotypes (omb^{H31} and $Df(1)rb^5/omb^{H31}$). Therefore this defect may be causally related to anatomical defects observed in both genotypes like the absence of HS and VS cells or the 300 fibers of the anterior optic tract (Brunner et al., 1992). The absence not only of the head turning movements but also of the corrective changes in body position (Figure 37) indicates, that the structures defective in theses flies do not provide exclusive input to the motorneurons innervating head muscles. More likely, they feed into descending neurons that supply neck motorneurons and also leg motor neuropils. Descending neurons that supply neck motorneurons and flight motor neuropils have been described in *Calliphora* (Gronenberg et al., 1995).

Both omb^{H31} and $Df(1)rb^5/omb^{H31}$ flies avoid the black dots that are preferred by wildtype and control flies in the forced-choice fixation Y-maze. omb^{H31} shows an avoidance as strong as the preference of the wildtype. This shows that detection of the black dot is not affected in the mutant. Two possible alternative explanations could account for the mutant phenotype. There could be two parallel visual pathways, one mediating attraction to a black pattern and the other mediating avoidance of the same pattern. If the pathway mediating attraction but not the one mediating avoidance would be blocked in omb^{H31} this could result in avoidance of these patterns. This would not explain the fixation of a wide variety of black patterns in fixation experiments both in walking (Figure 34) and flight (Wolf, Würzburg, pers. comm.). Alternatively, the change in preference could be not due to impairments in the processing of sensory information but due to motivational differences or alterations in decision making processes. Decision making is usually modulated by a number of factors and a variety of factors differ between the fixation in walking paradigm and the forced-choice fixation Y-maze (different overall light intensity; walking in a tube (6 mm diameter)

versus walking on an open platform; wings intact versus wings cut off; duration of experiment: seconds versus 12 hours; etc.). Each of these differences or a combination of them could account for the interesting finding that *omb* mutants do fixate like wildtype flies in one experimental setup, whereas they show antifixation in another setup. Whatever the factors causing the avoidance of the black dot are, the fact that mutants of the *omb* locus that lack HS and VS cells but are different with respect to other anatomical abnormalities show very different scores (ranging from 16 % to 80% (Figure 35)) indicates that the lack of these cells is most likely not the cause of the antifixation.

Taken together these results suggest that the loss of HS and VS cells is not the exclusive cause of the Y-maze phenotype nor of the low optomotor yaw and head yaw values in omb^{H31}. On the other hand, the absence of responses to vertical movements may be causally related to the loss of HS and VS cells or to any other anatomical defect found in both omb^{H31} and $Df(1)rb^{5}/omb^{H31}$ flies.

Flies with functionally blocked VS cells

To test if the loss of the vertical-motion-sensitive VS cells or another anatomical defect in $Df(1)rb^{5}/omb^{H31}$ flies is responsible for the absence of the head roll response, two GAL4 lines (3A and 1187) labeling these cells were used to drive tetanus neurotoxin or KIR. In combination with UAS-*rpr* or UAS-*hid* both GAL4 lines were not viable. Tetanus neurotoxin blocks signal transmission at chemical synapses. To my knowledge no anatomical study revealed the existence of electrical synapses in VS cells. However, there is evidence for electrical synapses from cobalt coupling experiments. Cobalt coupling is the ability of cobalt ions to pass from one neuron to another. Electron microscopy has shown, that coupled neurons share gap junction-like apposition areas (Strausfeld and Bassemir, 1983). Cobalt-coupling between VS cells and their postsynaptic partners has been reported (Milde and Strausfeld, 1986). VS cells may therefore form mixed synapses consisting of a chemical and an electrical component or electrical synapses with one or all of its postsynaptic partners. At mixed synapses tetanus neurotoxin expression can be expected to produce a phenotype observable at the behavioral level as in the campaniform sensilla of the halteres (see above). The human inwardly rectifying potassium channel (KIR) blocks neurons irrespective of the synapse type. Tetanus neurotoxin expression in VS cells in the two independent GAL4 lines does not abolish head roll. More strikingly, the two

transformants KIR1 and KIR14 that both proved to efficiently block photoreceptors, do not produce any effect on head roll response in combination with VS cell drivers.

These surprising results give evidence that VS cells although sensitive to vertical motion are not necessary for the head roll responses elicited by vertical motion. This situation is reminiscent of the finding that omb^{H31} flies lacking VS cells react normally to translatory large field movements with a lift/thrust response (Heisenberg et al., 1978), although VS cells respond with the same amplitude to rotatory and translatory vertical movements (Holger Krapp, Cambridge, pers. comm.). Alternative structures mediate the head roll response in flies with blocked VS cells. Pflugfelder and Heisenberg speculated about the existence of smaller twin fibers (hs and vs) that have been described in Musca (Pierantoni, 1976). These twin fibers could be missing in the *omb* mutants, whereas in 1187 and 3A they are not labeled and therefore are not blocked by TNT or KIR expression. Since vs cells are postsynaptic to the same cells that synapse with VS cells (Bishop and Bishop, 1981), the existence of these twin fibers (and their absence in *omb* mutants) would fully explain the findings described here. Alternatively, one of the other described anatomical abnormalities in *omb* mutants may be responsible for the lack of head roll responses in omb^{H31}, $Tp(1)bi^{D1}/omb^{H31}$ and $Df(1)rb^{5}/omb^{H31}$. The reduction of the fiber number in the anterior optic tract from 1300 to 1000 is a good candidate. The reduction is observed in both omb^{H31} and $Df(1)rb^{5}/omb^{H31}$ (Brunner et al., 1992) and in other insects visual interneurons in the anterior optic tract that respond to moving stimuli have been described (Collett, 1972). However, it can not be excluded that undescribed structural abnormalities in the *omb* mutants (for example the lack of other motion sensitive lobula plate neurons (like h cells, CH cells, V1-V3 cells, H1-H3 cells, WF cells) that could account for the 30% reduction in lobula plate volume) cause the defects in *omb* mutants possibly in combination with the lack of VS and HS cells.

To exclude a role of the VS cells in the forced-choice fixation Y-maze flies with functionally blocked VS cells were tested in this paradigm. These flies were unaffected in this paradigm. This is not surprising considering that most likely this behavioral abnormality is not caused by an impairment in the processing of visual information but in motivation or decision making (see above).

The findings presented here show that VS cells are neither necessary for head roll responses nor for fixation in the Y-maze.

Outputs other than HS and VS cells

HS and VS cells provide the central brain with information about large field movements. Although many other types of optic lobe output neurons have been described to be motion-sensitive (i.e. h cells, CH cells, V1-V3 cells, H1-H3 cells, WF cells, AOT fibers), all other aspects of the visual surround that initiate or alter behavioral responses have to be propagated from the optic lobes to the central brain, too. Here, three lines labeling output neurons from the optic lobes other than VS and HS cells have been investigated. Only one of the lines (*ato*-GAL4) has been studied because of its expression pattern. *ato*-GAL4 expression is driven in heterolateral neurons connecting the two lobulae. Some visually guided behaviors have been tested in flies expressing TNT in these neurons but none of the tested behaviors was defective in *ato*-GAL4/UAS-TNTE flies. The labeled neurons are not necessary for landing response, head roll or fixation.

The other two lines (OK107 and J163) were found in a new type of behavioral screen, in which numerous GAL4 lines were crossed to UAS-TNT without prior knowledge of their expression pattern. The surviving lines were tested for three visually induced behaviors. This approach is more unbiased and similar to the classical genetic screens routinely performed in *Drosophila*. The difference, however, is, that here I screened for necessary structures rather than for necessary genes.

OK107/UAS-TNTE flies do show a behavioral response to large field movement but no significant landing response induced by an expanding visual stimulus. When a fly attempts to land, it lowers its second and third pair of legs and completely extends its forelegs. The landing response can be induced by an expanding pattern as well as by reduction of the light intensity. Reducing light intensity reliably induces landing responses in OK107/UAS-TNTE flies, proving that exclusively the detection of expanding stimuli is blocked in these flies. A detailed comparison between the movement detection systems underlying the optomotor and the landing response in the housefly indicated that a common set of movement detectors mediates both the optomotor course control and the landing response induced by an expanding visual stimulus (Borst and Bahde, 1987). Therefore the blocking of neurons downstream of the movement detection system and upstream of the neurons mediating leg extensions is most likely the cause of the behavioral defect in OK107/UAS-TNTE flies. An intriguing candidate are the optic lobe output neurons projecting from the medulla, in which motion sensitive small field elements are described (DeVoe and Ochleford, 1976;

DeVoe, 1980; Gilbert et al., 1991), to the posterior central brain where these cells may provide input to descending neurons. In some insects optic lobe output neurons sensitive for expanding stimuli have been found (i.e. in locust (Gabbiani et al., 1999) or *Manduca* (Wicklein and Strausfeld, 2000)). Perhaps the optic lobe output neurons labeled in OK107 are the equivalent cells in *Drosophila*. Optomotor responses can be mediated by very few visual sampling units (Götz, 1983), whereas to identify the more complex expanding visual stimulus presumably more visual sampling units are needed. Therefore an alternative explanation of the OK107/UAS-TNTE phenotype is that expression in OK107 is driven in medulla small field elements and that expression there is inhomogeneous resulting in few columns left unaffected. These few columns would be sufficient to mediate the head roll response but not the landing response. However, no indication for an inhomogeneous expression is available.

The cells in which expression is driven in line J163 are not described in detail. However, expression in J163 is extraordinarily specific and therefore the behavioral phenotype of J163/UAS-TNTE flies is interesting. Expression is restricted to few cells with cell bodies proximal of the medulla. Blocking these cells results in abnormal fixation whereas head roll and landing response are unaffected. Two opposing black 60° stripes are not fixated, whereas two opposing black 10° stripes are fixated, although with decreased efficiency. Since detection of 10° stripes can be assumed to be more demanding than detecting of 60° stripes, the behavioral phenotype is unlikely to be caused by a defect in the visual system. The cells labeled in J163 are most likely not involved in the detection of the pattern but in the decision making process.

The results for OK107 and J163 show that a behavioral screen for structures necessary for a certain behavior is a promising approach that leads to interesting and maybe unexpected results. This approach supplements the strategy used with the other lines described in this thesis. These lines were chosen because of their expression pattern and then used to test theories about the labeled cells in the processing of sensory information. Both approaches gave interesting results, however to make sure that the neurons under study are truly blocked the right effector has to be chosen. In addition, an inducible system would make even more GAL4 lines accessible to this method. However, the resolution of the analysis presented here is at the cellular level and therefore unmatched in most other neuronal systems. Therefore, a refinement of the genetic tools for structure-function correlation in the Drosophila brain is definitely worthwhile.

5. Zusammenfassung

Die vorliegende Arbeit vergleicht Transgene, die in *Drosophila* Neuronen exprimiert wurden, um diese abzutöten oder zu blockieren. Tetanus Neurotoxin erwies sich als sehr effizient, um chemische Synapsen zu blockieren. Synapsen, die aus einer chemischen und einer elektrischen Komponente bestehen, ließen sich dagegen mit einem ektopisch exprimierten humanen Kalium-Kanal zuverlässiger ausschalten.

Es wurden drei Möglichkeiten verglichen, eine zeitliche Kontrolle über die Funktion von Neuronen zu erlangen. Keines der getesteten Systeme erwies sich als universell anwendbar, aber die durch Rekombination induzierte Tetanus Neurotoxin Expression ist ein vielversprechender Ansatz.

Die aus dieser vergleichenden methodischen Studie gewonnenen Ergebnisse wurden angewendet, um die Rolle von Neuronen in sensorischen Systemen bei der Verarbeitung verschiedener sensorischer Informationen zu untersuchen.

Chemische und mechanische Rezeptorneuronen konnten den olfaktorisch gesteuerten Verhaltensweisen beziehungsweise den lokomotorischen Leistungen, denen sie zu Grunde liegen, zugeordnet werden.

Hauptthema der Arbeit ist die Suche nach Neuronen, die an der Bewegungsdetektion im visuellen System beteiligt sind. Dabei zeigte sich, daß weder L2 noch L4 Neuronen im ersten visuellen Neuropil essentiell für die Detektion von Bewegung sind. Vielmehr deuten die Ergebnisse darauf hin, daß die Bewegungsdetektion über das Netzwerk der amacrinen Zellen (α) erfolgt. Die für vertikale Bewegung sensitiven VS Zellen in der Lobula Platte erwiesen sich als nicht notwendig für die Verhaltensreaktionen auf vertikale Bewegungsreize. Daraus folgt auch, daß in der Strukturmutante *optomotor blind* das Fehlen der VS Zellen nicht ursächlich für die stark eingeschränkten Reaktionen auf vertikale Bewegung ist. Ein anderer Defekt in *optomotor blind* muß dafür verantwortlich sein.

Die Arbeit zeigt das große Potential der beschriebenen Methoden zur Untersuchung der Informationsverarbeitung im Nervensystem von *Drosophila*. Einzelne Neuronengruppen konnten komplexen Verhaltensweisen zugeordnet werden und Theorien über die Informationsverarbeitung konnten in Verhaltensexperimenten mit transgenen Fliegen getestet werden. Eine weitere Verfeinerung der Methodik zur genetischen Intervention wird das *Drosophila* Gehirn zu einem noch besseren Modell für die Informationsverarbeitung in Nervensystemen machen.

6. Summary

Different transgenes that can be expressed in neurons to kill or block them were compared. Tetanus neurotoxin blocked chemical synapses very efficiently. Synapses consisting of a chemical and an electrical component were blocked more reliably by expressing a human inwardly rectifying potassium channel.

To gain temporal control over neuronal function, three genetic tools have been investigated. None of the systems is without drawbacks, however, the heat shock induced recombination to induce tetanus neurotoxin expression is a promising approach.

The knowledge gained from the comparative methodological study was used to investigate the role of neurons in sensory systems in processing different sensory informations.

Receptor neurons sensitive for chemical or mechanical stimuli were correlated to specific olfactory behaviors or locomotor tasks.

The main topic of this thesis is the much discussed question, which neurons are involved in motion processing in the visual system of flies. Neither L2 nor L4 neurons in the first visual neuropil are essential for motion-detection. The results indicate that maybe motion is detected by the network of amacrine cells (α). The vertical motion-sensitive VS cells VS in the lobula plate are not necessary for behavioral responses to vertical motion. This finding implies that the lack of VS cells in the structural mutant *optomotor blind* is not causally related to the altered responses to motion stimuli. Other abnormalities in *optomotor blind* are responsible for this behavioral phenotype.

This work shows the potential of the described methods in studying information processing in the *Drosophila* brain. Groups of neurons were correlated to complex behavioral responses and theories about information processing were tested by behavioral experiments with transgenic flies. The refinement of the genetic tools to interfere with neuronal function will make the *Drosophila* brain an even better model to study information processing in nervous systems.

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Erklärung gemäß §4 der Promotionsordnung für die Fakultät für Biologie der Bayerischen Julius-Maximilians-Universität Würzburg vom 15. März 1999:

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig angefertigt habe und keine anderen Hilfsmittel als die angegebenen angewandt wurden. Alle aus der Literatur entnommenen Stellen und Abbildungen sind als solche kenntlich gemacht. Die Dissertation wurde weder vollständig noch teilweise einer anderen Fakultät vorgelegt.

Würzburg, den 17.04.02

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LIST OF PUBLICATIONS

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

Kretzschmar D, Poeck B, Roth H, Ernst R, Keller A, Porsch M, Strauss R, Pflugfelder GO. 2000. Defective Pigment Granule Biogenesis and Aberrant Behavior Caused by Mutations in the Drosophila AP-3ß Adaptin Gene ruby. Genetics 155:213-223

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