The Central Control of Gap Climbing Behaviour in *Drosophila melanogaster*

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Tilman Triphan
aus Werneck

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Mitglieder der Promotionskommission:

Vorsitzender: .....

Gutachter: Prof. Dr. Roland Strauß

Gutachter: Prof. Dr. Wolfgang Rössler

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1. Introduction

1.1 *Drosophila melanogaster* as a Model Organism

Drosophila melanogaster's story as model organism started at the beginning of the 20th century, when Thomas Hunt Morgan began experimenting with the fly. The short generation time, approx. ten days at 25°C (Ashburner et al., 2005), the high number of progeny and the low cost of keeping the flies are big advantages over mammals.

After the discovery of the *white* mutant (Morgan, 1911), the foundation for using *Drosophila melanogaster* as a model organism was laid. Morgan discovered that genes are carried on chromosomes in a linear order with a defined distance and was later on awarded with the Noble Price in Medicine for his discoveries. The next big step was in the late 60th, when Seymour Benzer of the California Institute of Technology coined the term of "behavioural genetics", i.e. the idea that behaviour in animals is influenced by genes. Benzer and Konopka discovered that the circadian rhythm of activity is under the control of certain genes, e.g. the *clock* gene (Konopka & Benzer, 1971). The attribution of certain behaviour to certain brain areas was introduced by the screen for structural brain mutants by mass histology (Heisenberg und Böhl 1979). In the following years, lots of genes that are involved in behaviour had been found. In parallel, Christiane Nüsslein-Volhard identified genes that control development (Nüsslein-Volhard & Wieschaus, 1980). Later on in 1995 she received together with Eric Wieschaus and Edward Lewis the Noble Price in Physiology and Medicine for her work.

In 1982, Gerald Rubin and Spradling developed the possibility to generate transgenic flies by the help of transposons (Rubin & Spradling, 1982; Spradling & Rubin, 1982). With the method of germline transformation, the option existed to directly mingle with the flies' genome.

This possibility was further improved by Brand and Perrimon in 1993, when they introduced the two component GAL4/UAS system. The idea of this is to keep the effector and the expression pattern separated and only to combine them in the experimental crossing. That way, also effectors that have a negative effect can be kept as a stable line. In recent years, this system has been improved and became widespread used (Duffy, 2002) The GAL4/UAS system has been used to kill or silence specific cells, to rescue in a tissue specific way or to visualize expression patterns. Some examples will be explained in the following. With the additional GAL80 component it is possible to further sharpen the GAL4 expression pattern by preventing the activity of GAL4. This is even possible in a temporally controlled manner to restrict the expression of genes to specific periods in the development (McGuire et al., 2003).

By the advent of the lexA system (Lai & Lee, 2006), a second binary system, independent of GAL4/UAS, the *Drosophila* toolkit was further enhanced.

As reporters, initially lacZ (Brand & Perrimon, 1993) was used and later the green fluorescent protein GFP (Yeh et al. 1995). The UAS-Cameleon2.1 allowed to monitor Ca^{2+}-levels and thereby the activity of neurons (Diegelmann et al. 2002).

These methods can now be used to restore gene functions in flies with mutant background. By using specific GAL4-drivers, the rescue can be performed in specific subsets of neurons to prove the necessity of the rescued gene in these neurons for the tested behaviour. One example for this kind of partial rescue showed the necessity of *rutabaga* in the mushroom bodies for odour learning (Zars et al. 2000).

On the effector side, various tools have been developed, starting with the use of tetanus toxin (TNT) to inhibit neurons by cleavage of synaptobrevin (Sweeney et al. 1995). The next step was a dominant negative form of dynamin called *shibire*^{ts}, which allows a reversible silencing of neurons at high temperature (Kitamoto et al. 2001). As an effective counterpart, trpA1, *Drosophila*'s homologue to the transient receptor protein in mammals can be used. This channel is voltage- and temperature-gated and is needed for regulation of thermotaxis (Hamada et al., 2008). Since the advent of the Channelrhodopsin, a directly light-activated cation-selective ion channel (Nagel et al., 2003), the possibility exists to directly activate specific neurons by blue light. This has already been used in appetitive and aversive learning experiments (Schroll et al., 2006). Another method is the use of RNAi (Fire et al., 1998; Boutros et al., 2004), to specifically inactivate certain genes in certain regions of the brain. In the last years, several stock centres for RNAi lines have been established (Dietzl et al., 2007).

In 2000, the whole fly genome has been published (Adams et al., 2000), giving access to the modern methods of bioinformatics. The Basic Local Alignment Search Tool (BLAST) allows to compare gene sequences (Altschul et al., 1990), the databank FlyBase (flybase.org) offers a plethora of background information for different genes (Ashburner & Drysdale, 1994; The FlyBase Consortium, 2003). In conclusion, *Drosophila* is an extremely useful model organism for the study of neuronal function.

1.2 The Central Brain of *Drosophila melanogaster*

The adult *Drosophila* brain can be subdivided in three different neuromeres: the protocerebrum, the deuterocerebrum and the tritocerebrum (Bullock & Horridge, 1965). The protocerebrum is the largest part of the brain and in itself consists of the optic lobes on both sides and the mushroom bodies and the central complex in the central region of the brain (Caellerts et al., 2001). The central complex, which is sometimes called central body in other insect species, is located at the sagittal midline and is symmetrically organized (Power, 1943), a feature which separates it from other neural centres like the mushroom bodies, the antennal lobes and the optical lobes (Renn et al., 1999). Because of this special design, the central complex has early been suggested to play a role in inter-hemisphere coordination (review Homberg, 1987). It is comprised of four interconnected neuropiles: the ellipsoid body, the fan-shaped body, the protocerebral bridge and the paired noduli (**Figure 1**). Columnar small-field elements link the different substructures or regions in the same substructure while tangential large-field neurons form strata perpendicular to the columns (Hanesch et al., 1989). The input to the central complex comes primarily via tangential neurons from the ventral lobes and from the lateral triangles – both are accessory areas of the central complex.
The protocerebral bridge consists of a set of 16 glomeruli, eight on each side of the midline. The horizontal fibre system, a set of isomorphic neurons connects each glomerulus to one of eight distinct segments of the fan-shaped body by means of a cross-over scheme (Hanesch et al., 1989).

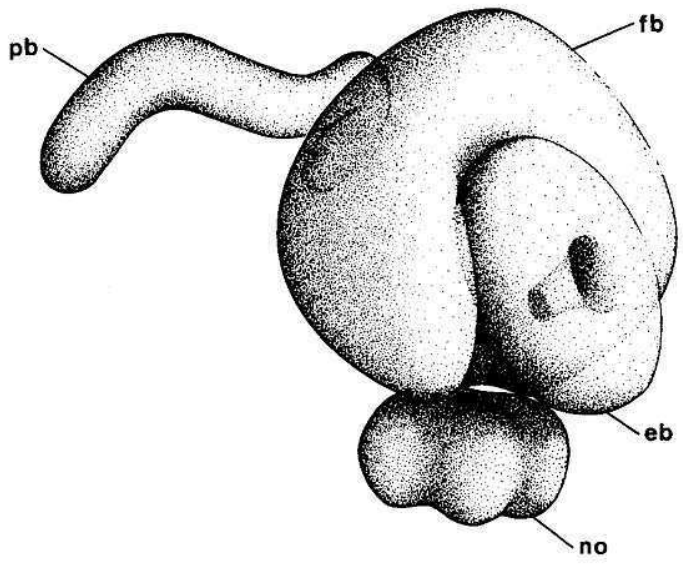

Figure 1 The central complex

The central complex of *Drosophila melanogaster* consists of four neuropils. From caudal to frontal there is the protocerebral bridge (pb), the fan-shaped body (fb) and the ellipsoid body (eb). Ventral of the fb and the eb there are the paired noduli (no).
Figure taken from (Hanesch et al., 1989)

The input to the central complex is thought to be essentially visual. The central complex is needed for visual orientation (Neuser et al., 2008), to learn visual object features in the flight simulator (Liu et al., 2006) and mediates information about visual polarization in the grasshopper (Heinze & Homberg, 2007). By electrophysiological methods, mechanosensory input has been proven in the central complex homologue of the locust (Homberg, 1994). Most experiments point to a role of this brain area as a pre-motor centre (Heisenberg, 1994; Strauss, 2002a). When analysing the walking behaviour of *Drosophila* strains with defects in the central complex, the walking defects were early attributed to the structural changes in the central complex structures, establishing its role as a centre for higher level motor control. The mirror-symmetrical structure of the central complex with its fibres crossing the midline implicates very obviously an important function in right-left-bargaining, i.e. the exchange of information between and adjustment of activity from the both halves of the brain as well as of the body (Strauss et al., 1992, 1993, 2002a; Heisenberg, 1994). And flies with a defective central complex are indeed unable to compensate for asymmetries in locomotion and do walk in circles (Strauss, 2002a; Pielage et al. 2002).

Finally, the central complex' prominent role in insect locomotion gets obvious when looking at structural mutants of this region (Strauss, 2002a) When screening for walking deficits in almost 11.000 EMS mutated flies, lines with a visible disruption in the central complex architecture where over-represented (30 of 230 lines, 13%) in comparison to other neuropiles (Strauss, 1995). In flight, animals with a defect in the central complex also show problems (Ilius et al., 1994). When looking in more detail, most of the specific phenotypes can be attributed to a single substructure of the central complex.

1.2.1 The Ellipsoid Body

The ellipsoid body is the frontal-most neuropil of the central complex. It is roughly toroid-shaped and gets tangential input via four sets of ring neurons from the lateral triangles and columnar input primarily from the bridge and fan-shaped body. The ellipsoid structure is unique in dipterans, in other insects its homologue structure is ventrally open and has a half-circle structure. Flies in which the ellipsoid body is structurally altered, show reduced or even no persistence of orientation towards a temporally invisible target (Mronz, 2004). To further analyse this spatial working memory, ellipsoid body defective lines where tested in the detour paradigm. Flies were put into a virtual-reality arena, which displays a Buridan's paradigm-like situation e.g. two stripes opposite to each other. Flies readily patrol between those stripes. When the fly crossed an invisible midline, the stripes disappeared and were replaced by a new

stripe orthogonal to the walking direction. After the fly approached the new stripe, it disappeared as well and wild-type flies return to their previous target with a high probability. Ellipsoid body defective lines, in contrast, show no preference for the direction of their previous target after the detour. By expression of Tetanus toxin in subsets of the ring neurons, the detour behaviour can be destroyed as it is in structural mutants (Neuser et al., 2008). Mutants of the *ignorant* gene, which codes for the serine protein kinase S6KII also fail to perform in this setup. The *ignorant* gene had been shown previously to have a learning and memory phenotype in operant learning in the heat box (Putz et al., 2004). When expressing wild type *ignorant* in a subset of the ellipsoid body ring neurons, the memory could be rescued in $ignorant^{58/1}$ mutant flies.

1.2.2 The Fan-Shaped Body

The fan-shaped body resides posterior to and in close contact to the ellipsoid body and gets prominent input from the ventral lobes via tangential neurons. In a columnar fashion it is connected to all other neuropils of the central complex. The fan-shaped body plays an important role in operant visual learning in the flight simulator. By expression of tetanus toxin (Sweeney et al., 1995) in either one of six distinct subsets of neurons branching as parallel, horizontal strata in the fan-shaped body, the memory for a particular object feature was abolished. The fifth layer is needed to learn and/or recall the edge orientation of a punished object, and layer one the elevation above the horizon (Liu et al., 2006). In *rutabaga* flies, that have a learning phenotype in visual pattern orientation at the flight simulator (Eyding, 1993), expression of *rutabaga* in the aforementioned layers of the fan-shaped body specifically rescued memory for either one of the distinct object features.

1.2.3 The Protocerebral Bridge

The protocerebral bridge is the caudal-most part of the central complex. The appearance resembles that of a bicycle's handlebar (Hanesch et al., 1989). The bridge consists of 16 glomeruli in a row, eight on each side of the midline. The bridge is connected to the fan-shaped body by the horizontal fibre system, that forms bilaterally the w-, x-, y-, z-system of fibre bundles on its way from the bridge to the fan-shaped body (Hanesch et al., 1989). The projection pattern of these neurons connects the 16 glomeruli of the protocerebral bridge with the eight fans of the fan-shaped body in a cross over scheme and terminates in accessory areas called the ventral bodies. The tracks originating from the innermost three glomeruli will cross

the midline to the contralateral side of the fan-shaped body while the outer five will stay on the ipsilateral side. In the ventral bodies, only the outermost glomerulus will stay on the ipsilateral side, all other fibres will terminate in the contralateral ventral body.

The protocerebral bridge plays a role in various behaviours, such as keeping up the motivation for approaching a landmark, e.g. in Buridan's paradigm. In flies with a structural defect in the protocerebral bridge, the walking activity quickly declines (Strauss et al., 1992).

An other function that has been attributed to the protocerebral bridge is the control of step length. With higher step frequency wild-type flies also raise their step length (Strauss and Heisenberg, 1990). Structural mutants of the protocerebral bridge like *no bridge* (Strauss et al., 1992) and the *eyeless* allele ey^{JD} (Callaerts et al., 2001) fail to increase step lengths along with stepping frequency. As the duration of the swing phases is normal, an intact protocerebral bridge might be necessary for a wild-type like leg swing speed (Strauss et al., 1992, review Strauss, 2002a). Also other structural mutants of the protocerebral bridge like *tay bridge*^{1} (*tay*^{1}), *ocelliless*^{1} (*oc*^{1}) and *central complex*^{KS181} (*cex*^{KS181}) show similar phenotypes (Leng & Strauss, 1999). Interestingly, evolutionarily less developed insects, like stick insects, do not raise their step length with increasing stepping frequency.

Considering the anatomical and behavioural data already existing, Strauss (2002b) designed a model to explain the function of the protocerebral bridge in behaviour. The basic idea is that the bridge mediates the increase in step length when flies are walking directly towards a landmark. All structural mutants of the protocerebral bridge are significantly slower in Buridan's paradigm. Their inability to increase their step length together with the stepping frequency leads to a lower overall speed (Strauss 1992, 2002a, 2002b). Also the approaches to the landmarks are less straight than in wild-type flies. Moreover, their walking activity declines over time.

According to Strauss (2002b), the hypothetical function of the bridge might be the following:

The azimuth position of the landmark is represented on the ipsilateral side of the bridge on which the object appears on the retina. As the fibres of the horizontal fibre system cross the midline of the brain in the anterior chiasm dorsally of the ellipsoid body, the step length on the contralateral side would be increased by the activity of one of those fibres. The difference in step size on both sides would lead to a turn towards the landmark. A frontal landmark would be represented in the innermost glomeruli on both sides, thereby increasing the step size on both sides – on direct approaches, the speed would be highest. Landmarks in the posterior visual area (110° and up) have an aversive effect on the flies (Mronz, 2004). This can be explained by the fact that they would be represented by the outermost glomerulus on

the ipsilateral side. As this glomerulus – in contrast to the others – innervates the ventral body on the ipsilateral side, also the ipsilateral step side would be increased, leading to aversive walking behaviour with regard to the landmark.

One function that has recently been attributed to the protocerebral bridge is the sky compass in locusts. Many animals use the polarization pattern of the blue sky as a compass cue for orientation (Wehner, 2001). The plane of polarization of the E-vector depends on the position of the sun and varies systematically around the sun and over the sky. This cue can be used for orientation by many insects (Wehner, 1976). In the locust, the E-vector orientation is detected by photoreceptors in the dorsal rim area of the eye and integrated in the central complex (Vitzthum et al., 2002). The map of the zenithal E-vector orientations is represented in the columnar organisation of the locust protocerebral bridge and lower division of the central body (the ellipsoid body homologue; Heinze & Homberg, 2007). In a natural environment under the open sky, the activity of the polarization sensitive neurons is directly related to the orientation of the locusts head.

1.3 Climbing in Insects

All necessary neuronal circuitry needed for coordinated walking can be found in the thoracic ganglion of the fly. In decapitated flies, a little amount of octopamin applied on the neck connective will elicit coordinated but not targeted walking behaviour (Yellman et al., 1997).
This holds not true when walking is on difficult terrain. As the walking behaviour has to be adapted to the surrounding environment all the time, the controlling influence of the brain gains more and more importance. Gaps or clefts in the walkway are of eminent difficulty. Especially a natural environment like the habitat of stick insects, could be described composed of gaps and obstacles with hardly any flat surface (Bläsing et al., 2006)
Gaps pose a considerable risk on the insect, as they are more difficult to detect. They do not give a strong visual stimulus as they don't stand out of the horizon like obstacles and the tactile stimulation is more of a missing stimulus predicting future lack of ground contact (Bläsing et al., 2006). In most cases, only small gaps, i.e. up to one step length have been used in investigations (Cruse, 1976, 1979; Pearson & Franklin, 1984; Dürr, 2001; Watson et al., 2002a, 2002b). An exception to this is Pick and Strauss (2005). They show that *Drosophila melanogaster* can cross width of more than one body length with an astonishing manoeuvre. More details on this behaviour will follow in the following parts.

1.3.1 Stick Insect

In the stick insect *Aretaon asperrimus*, Bläsing and Cruse (Bläsing & Cruse 2004a, 2004b) tested gaps of up to three times the step length. In order to investigate the insects' behavioural adaptations to crossing the gap, they carried out a detailed analysis of locomotor patterns, either while the insects were walking on a flat surface or during climbing. If the gap gets too broad, normal walking behaviour can not be used, as the normal positions of ground contact for the legs would be in the void, so in the gap crossing behaviour, normal walking behaviour is slightly modified. The first front leg stepping into the gap elicits a reduction in walking speed and changes in the swing trajectories of all following steps into the gap. No reaction will be detected after lowering an antenna into the gap. After detecting the gap, legs that swing into the void will perform oscillating searching movements (Bläsing & Cruse, 2004a) Also the antennae will explore the space in front of the gap and eventually will make contact to the other side. The swing phase duration and extreme positions of single legs will be altered during the whole climbing process. The far edge of the gap is detected by tactile stimuli as has been shown in ablation experiments of the antennae. Once the stick insect makes contact with

the other side, be it with its antennae or the front legs, this marks a “point-of-no-return” from which on the climbing behaviour can not be stopped anymore.

1.3.2 *Drosophila melanogaster*

Fruit flies can cross gaps of more than 4.0 mm with a body size of about 2.5 mm (Pick & Strauss, 2005). The probability to engage in climbing at a certain gap length is dependent on the actual gap size, if the gap gets too broad, no climbing behaviour will be initiated. The gap width is measured visually. Blind flies manage to cross small gaps of up to 2.0 mm by normal walking, but fail at larger gaps (Pick & Strauss, 2005). The distance estimation depends on the motion vision system R1-R6, but not on colour vision. Flies also showed the same climbing initiation when the binocular region of one eye (Pick & Strauss, 2005) is covered with black paint, ruling out binocular disparity and vergence. The next step was to fix the head to the thorax to rule out peering and bobbing movements as they exist e.g. in mantids and locusts (Collet, 2002). Also with this treatment, the climbing initiation was unchanged. On the distal side, the opposing front wall is used, as the front wall presented alone will elicit normal climbing initiation, while at the top side alone the climbing probability was lowered. When decorating the opposing side with vertical stripes to increase parallax motion signals, the rate of climbing initiation could be increased. As only vertical and not horizontal stripes were effective, this hinted to an extraction of gap-width information by parallax motion generated by the walking mechanics during the approach (Pick & Strauss, 2005).

The rate of climbing initiation stays high until the just manageable gap width of 4.0 mm and then decreases at broader gaps. When looking at unsuccessful attempts i.e. climbing is initiated but the fly does not manage to cross the gap, the rate is highest at the just manageable gap size of 4.0 mm.

In order to cross a gap, a fly has to grab the opposing side with its front legs, thereby forming a kind of a “bridge”. To facilitate this in wide gaps, it has to increase the reach of its front legs. For each pair of legs, there are adaptations and optimisations to reach this goal. The hind legs move as close as possible to the rim of the gap, thereby moving the tip of the abdomen into the gap. The mid legs stretch, lifting up the body and thereby giving the front legs a better working space. The front legs finally are stretched and extended.

These adaptations can be seen as separable subunits. When testing several lines from the Strauss screen for locomotor mutants (Strauss, 2002b), Pick & Strauss, (2005) found mutant lines with specific defects in climbing adaptations. One line did rarely engage in climbing, even at small gaps, despite the fact that those flies are not smaller than wild-type flies and that

they were able to cross gaps in principle. The second mutant has problems in the parameter climbing position. These flies engage in climbing while still over solid ground, thereby giving away about 1 mm of their optimal reach. The third mutant fails to lift up its body with its mid legs. As most climbing attempts that way are targeted more to the ground than to the opposing side, the climbing success is equally bad as in the other two mutants. The rate of initiation in these latter mutant lines is close to those of wild-type flies.

2. Material and Methods

2.1 Fly Keeping and Histology

2.1.1 Fly Keeping and Preparation

Flies were raised on standard medium containing water, cornmeal, soy bean, agar, molasses, yeast and methyl-4-hydroxy-benzoate as preservative at 25°C, 60% humidity and a 14h/10h light/dark cycle. The light phase started at 7AM. Generation time is ten days at this temperature. If not otherwise indicated, three to five day old flies were used for all experiments. For Gap Climbing, Buridan's Paradigm and the Fast Geotaxis paradigm, the wings of the flies were shortened at least 12 hours before the experiment to one third of their original length to prevent the flies from flying away. The clipping was done with an iridectomy scissors under cold anaesthesia (4°C). A stream of dry air prevented condensation of water at the cold plate. After the operation, the flies were kept on food for a minimum of 12 hours to recover.

2.1.2 Paraffin Sections

Paraffin sections are a method to analyse structural defects in the *Drosophila* brain. All structural mutants and HU treated animals have been checked by this method to confirm the anatomical phenotype. The exact method is described by Heisenberg & Böhl (1979). Anaesthetized flies are run in a small collar, the head always in the same orientation. Up to 15 heads can be processed in a single collar this way. When checking individual flies after a behavioural experiment, easily identifiable marker flies like e.g. *sine oculis* (Milani, 1941) can be placed at distinct positions in the collar. The flies are then treated for 4 h in Carnoy's fixative ($6/10^{th}$ ethanol, $3/10^{th}$ chloroform, $1/10^{th}$ acetic acid). After that there are three ethanol steps (2x 30min, 1x 60min) to remove residual water. Finally the collar is kept in methyl benzoate over night. The methyl benzoate is then supplanted by paraffin at 63°C (1h 1/1 methyl benzoate/paraffin, 8x 20min paraffin). Lastly, the collars are encased in paraffin. After the paraffin has hardened, the heads can be broken off the collar and will stick in the paraffin block. After trimming, the block with the row of heads can be sliced in 7 µm sections. After removing the paraffin with xylol at 63°C the sections can either be stained with antibodies or covered with the embedding medium Enthelan and a cover glass and directly examined at in the fluorescence microscope. In the latter case, the pigments of the

eyes will stain the neuropil green and cell bodies yellow as seen under short wavelength blue light.

2.1.3 Ablation of the Mushroom Bodies with Hydroxyurea

The ablation of the mushroom bodies was done following the protocol published by de Belle & Heisenberg (1994). The mushroom bodies, a paired neuropil in the brain of *Drosophila melanogaster* consists of approximately 2500 parallel Kenyon fibres (de Belle & Heisenberg, 1994) that are derived from four neuroblasts. These neuroblasts are mitotically active during the first 4 to 5 hours after larval hatching. If the larvae are treated with hydroxyurea – a powerful antineoplastic drug - during this time window, the mushroom bodies can be ablated with little to no damage to other parts of the developing brain (de Belle & Heisenberg, 1994).
Newly hatched larvae are collected and put into a small pot containing yeast paste and 60 mg/ml HU. After 4 h, the larvae are washed out of the yeast paste and transferred to normal food vials. A control group is treated similarly, but without HU in the yeast paste. The survival rate of HU-flies is more than 90%. In most animals the mushroom bodies are completely reduced, taken aside about 50 larval Kenyon cells which survive during metamorphosis. All animals have been checked by paraffin histology to confirm the absence of the mushroom bodies.

2.2 Behavioural Experiments

2.2.1 Fast Phototaxis

For the fast phototaxis experiments, the Benzer counter current apparatus (Benzer, 1967) was used. Groups of not more than 50 flies were food deprived for 6 h but had access to water. For the experiment, the flies were filled into the starting tube. The flies were shaken to the ground and the apparatus was placed on a flat, dark surface, the far end pointing towards a light source. To adapt the paradigm for mutants with defects in walking speed rather than visual mutants, the time for each transition towards light was shortened to 6 s (Benzer, 1967 used 30s). After that time, the upper part was moved to the right, thereby taking all the fast flies to the next tube. The flies were then shaken down again. The performance index was measured by looking at five consecutive trials. A fly in the rightmost tube would be assigned a value of 100, a fly still in the starting tube would receive a rating of 0.

2.2.2 Fast Geotaxis

In this paradigm, the walking speed on a vertical surface is measured (Strauss & Heisenberg, 1993). Single flies with clipped wings (see Preparation) were put in a translucent, cylindrical polystyrene fly bottle of 100 mm height and 49 mm diameter. The vials were covered with black lids and tests were performed on a black background in ambient light. After gently shaking down the fly to the bottom of the vial, the time needed from the start of the ascent at the wall until crossing a marker ring 82 mm above the ground was measured. Only straight runs were taken, if the fly jumped onto the wall, stopped during upward walking or walked in spirals, the walk was discarded. As flies still not always walk perfectly straight upwards, for each single fly ten valid measurements were obtained and only the fastest speed was kept. For each genotype, at least ten individual flies were measured. The fastest runs were averaged for a mean maximum speed for that strain.

2.2.3 Buridan's Paradigm

The Buridan's paradigm can be used to analyse walking behaviour, orientation and activity at the same time (Götz, 1980). Two dark vertical stripes of 12° horizontal and 50° vertical viewing angle, seen from the centre of the arena, are presented opposite to each other on a translucent cylinder illuminated by Tungsten ring lights from behind. Single flies with clipped wings walk on a platform of 85 mm diameter which is sitting in the middle of the cylinder. The walking platform is surrounded by a water barrier to keep the flies from escaping. Each fly spends 15 min in the arena. Within the parameters that are extracted are total track length, mean walking speed, the latter taken from all transitions between the two objects only, the angle of orientation towards the objects and the walking activity i.e. the percentage of time spent walking. It is also possible to look at 3-min bins for these parameters in order to see temporal changes, e.g. a decay of walking activity over time.

2.2.4 Distance Estimation

For the distance estimation experiment, a four-arm walking-platform was used. (Götz et al., 1994, Schuster et al., 1996, Schuster et al., 2002) In the experiment, flies are confronted with two pairs of visual objects that have the same viewing angle when seen from the centre of the arena and only vary in their distance to the centre of the platform. In this situation, wild-type flies will preferably patrol between the set of closer landmarks. Not only real objects can be used in this setup, but also virtual ones that are simulated on the screen of the LED arena in

dependency of the position of the fly. The flies do not prefer real objects over virtual ones or vice versa (Schuster et al., 2002). So the fly can sometimes choose between two real reference objects (B and B') and two virtual objects (A and A') (**Figure 2**). In the standard experiment, the diameter of the virtual cylinder would be 50 mm and for the cylinder with the real objects it would be 200 mm, the diameter of the LED arena, but also different settings have been used. It is possible to show to sets of virtual landmarks. Seen from the centre of the arena, both sets of landmarks are 12° wide and 48° high. The position of the landmarks is in elongation of the arms of the platform.

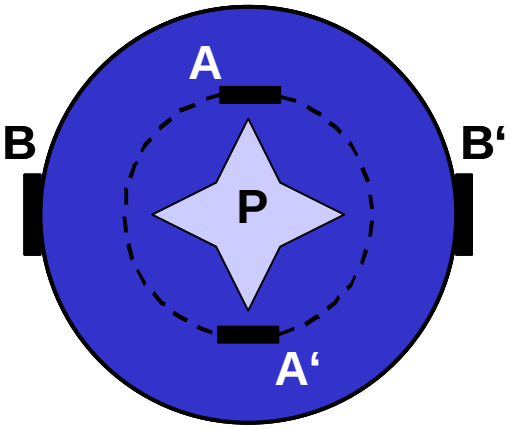

Figure 2 The four-arm walking-platform
The outer set of landmarks (B, B') is at 200 mm from the centre of the walking platform (P), the virtual inner ones are at 50 mm. Seen from the centre of the arena, both sets of landmarks are 12° wide and 48° high. Modified from Mronz (2004).

2.2.5 High-Speed Video-Setup

The detailed analysis of the climbing problems in the different mutant lines was assessed with the high speed video setup established by Pick & Strauss (2005). A small black polycarbonate plastic block (dimensions: 34 x 10 x 4 mm^3) was put in the centre of a 88 mm plastic petri dish. In the middle of the block is a 5 mm deep gap of widths ranging from 1 mm to 6 mm. A water barrier at the inner rim of the petri dish confines a single fly with clipped wings to the block. A 10 cm white cardboard cylinder surrounds the petri dish and shields the fly from outside visual stimuli. Two synchronous DALSA CA-D1 high speed video cameras monitor an area of about 1 cm^3 around the gap from the side (through a small opening) and from above (through a ring light that illuminates the arena). Both cameras can record sequences of 256x256 pixel of 8-bit greyscale images at a frame rate of 200 fps. They typically approach the gap several times per minute and readily cross it, if manageable (Pick & Strauss, 2005).

2.2.6 Direct Observation of Gap Crossing

For an initial quantitative screen for climbing initiation and success a similar setup without cameras was used. The fly was observed from the side through a tilted dissection microscope

(Zeiss OPMI 1-F). This is sufficient to see the ability of the fly to cross gaps of a given size and also allows scoring the initiation of climbing behaviour, albeit not as reliable as with the high-speed setup. The reason for an unsuccessful attempt can not be determined with this setup. For each genotype and gap size, ten approaches for at least ten single flies were evaluated. An approach was either scored as “successful crossing”, “turning around” or “walking down into the gap”. Rarely, other events like “falling down while trying to cross” or “jumping away” were also noticed. The latter was always in a seemingly undirected manner and was likely to be a flight start. In the evaluation, the probability to elicit a climbing attempt for all the approaches was scored for different gap sizes, normally reaching from 2 mm to 6 mm. Also the rate of success for the attempts was calculated.
For gap sizes that turned out to be a challenge (approx. 50% success) the high speed setup was used to further analyse the cause of the climbing problems.

2.2.7 Evaluation of the Climbing Behaviour

For reasons of comparability, the videos had to meet the following criteria to be analysed: during the approach, the fly had to cross two imaginary lines on top of the climbing block, 7 mm and 1 mm away from the front of the gap. To gauge whether a climbing attempt had occurred, the movements of the legs were scored. The most important criterion was the leg-over-head behaviour (Pick & Strauss, 2005). It is defined as lifting at least one front leg above a tangential plane touching the head at the ocelli. As this kind of leg movement does not occur during normal walking, it is a strong indicator for a climbing attempt. Some mutants like *tay bridge*^{1} or *no bridge*^{KS49} have problems in lifting their front legs to the required height. Therefore, additional indicators were established, all having in common to break the rules of walking defined by Cruse (1979). When both front legs are in the air at the same time or when a single front leg is moved upward a second time before touching the ground, this was also scored as a climbing attempt. Within broader gaps (4.0 mm and up), the flies sometimes show front leg actions towards the bottom of the gap. To rule out those reactions, flies had to touch the proximal wall at least three times after climbing initiation to be treated as a climbing attempt.
The analysis of the videos was achieved with a custom written Delphi program. The origin of the coordinate system was manually placed in the upper proximal edge of the gap. For each climbing attempt, the first and the last leg-over-head stroke was evaluated. At those points in time, the positions of the abdomen, the head and the hindlegs were recorded for both camera views so that the position of the fly was derived in three dimensions. The resulting values

were exported to Excel or Origin for further analysis. Form these data the distance of the fly to the gap was evaluated. Also body angles of the fly in relation to the plane of the gap and the angular deviation from the direct way over the gap were analysed.

2.3 Statistics

All statistics have been done with the software Statistica (Version 7) by StatSoft. To test for normal distribution, the Shapiro-Wilk test was used. If a set of data contained at least one group with not normally distributed values, non parametric statistics were used for the whole set. For pairwise comparisons of normally distributed data, the Student's t-test (t-test) was used, for nonparametric data the Mann-Whitney U test (U-test) was used. Correction for multiple testing was done by applying Bonferroni correction. Comparisons between multiple groups were done by either ANOVA (parametric) or the Kruskal Wallis test of ranks (nonparametric). As post-hoc test for ANOVA, the Tukey HSP test was used. For the Kruskal Wallis test, corrected U-tests were used. To indicate significance of test results, $p<0.05$ was named "significant" and $p<0.01$ "highly significant". The number of flies of one certain genotype was abbreviated by N, the total number of single experiments by n.

2.4 Fly Lines

2.4.1 Wild-type Strains

Line	Chromosome	References
Canton Special (CS)	*wild-type*	Würzburg stock collection
Wild-type Berlin (WTB)	*wild-type*	Würzburg stock collection

2.4.2 Classical Mutant Lines

Line	Chromosome	References
climbing sisyphus (csi)	X	Strauss, 2002b
ellipsoid body open^{678} (ebo^{678})	X	Ilius et al., 1994, Strauss & Heisenberg, 1993
ignorant$^{58/1}$ (ign$^{58/1}$)	X	Neuser et al., 2008
ocelliless^{1} (oc^{1})	X	Bedichek & Patterson, 1934
sine oculis (*so*)	II	Milani, 1941
tay bridge^{1} (tay^{1})	X	Poeck et al., 2008
tay bridge^{2} (tay^{2})	X	Poeck et al., 2008
tay bridge^{2} (tay^{3})	X	Poeck et al., 2008
C(1)DX, *yellow white forked*	X	Bloomington stock center
yellow, forked (*y f*)	X	Bloomington stock center
yellow, crossveinless, vermilion, forked, carnation (*y cv v f car*)	X	Bloomington stock center

2.4.3 Transgenic Fly Lines

Line	Chromosome	References
P{tay}^{D1}	III	Poeck et al., 2008
UAS-*otd*	II	Bloomington stock center
UAS-*tay*	II	Poeck et al., 2008
UAS-TNTE	II	Sweeney et al., 1995
007Y-GAL4	III	Renn et al., 1999, Poeck et al. 2008
078Y-GAL4	III	Renn et al., 1999
210Y-GAL4	III	Renn et al., 1999, Poeck et al. 2008
c232-GAL4	III	Renn et al., 1999, Neuser et al. 2008
c320-GAL4	III	Aso et al., 2009
c819-GAL4	III	Renn et al., 1999, Neuser et al. 2008
elav-GAL4	III	Luo et al., 1994
mb247-GAL4	III	Zars et al., 2000, Poeck et al. 2008
NP2320-GAL4	III	Liu et al., 2006
NP3124-GAL4	III	Liu et al., 2006
hs-GAL4	III	Bloomington stock center
tubGAL80^{ts}	III	McGuire et al., 2003

3. Results

3.1 The Function of *tay bridge* in Walking and Optomotor Compensation

The mutant *tay bridge*^{1} (*tay*^{1}) was isolated in a screen for walking mutants by R. Strauss (2002b). About 11,000 EMS treated flies were screened in the fast phototaxis behaviour and the slowest were kept. From these 2000 males, 1200 stable C(1)DX balancer lines were generated and flies from these lines were retested in fast phototaxis in groups of 50 flies. Lines with a mean number of transitions of at least 20% below wild-type were then tested in negative geotaxis and Buridan's paradigm. After this screening process, 230 lines with stable walking defects were kept.

tay^{1} flies exhibit a structural phenotype in the protocerebral bridge, a constriction at the sagittal midplane (Poeck et al., 2008). This suggests that the latero-lateral connections between the two hemispheres of the bridge might be reduced in this mutant. On the level of light microscopy, no further anatomical abnormalities can be detected in the brain of *tay*^{1} flies. A second overall anatomical phenotype of *tay*^{1} is the abnormal wing posture. In the resting position, the wings are not parallel to the body axis but stick out at an angle. *tay*^{1} flies show a more than 70% performance reduction in the fast phototaxis behaviour (Poeck et al., 2008, R. Strauss, personal communication) To check for visual-system defects, *tay*^{1} flies were tested for their mean maximum speed in the fast geotaxis paradigm, where they reached only a fraction of the wild-type speed (Poeck et al., 2008).

The Buridan's paradigm finally showed several problems in the *tay*^{1} mutants. The mean walking speed of *tay*^{1} is about half of the wild-type speed (Poeck et al., 2008) and the walking activity, i.e. the percentage of time spent in walking, is dramatically reduced in comparison to the wild-type flies.

3.1.1 Identification of the *tay bridge* Gene

The *tay* gene was identified by Poeck et al. (2008). By analysing the recombination frequency between the *tay*^{1} gene (using the protocerebral bridge structural phenotype) and the five visible marker mutations *yellow, crossveinless, vermilion, forked* and *carnation* (*y cv v f car*), all located on the X-chromosome, *tay*^{1} was mapped to position 50 ± 2 cM. With subsequent complementation analysis, this area was further narrowed down to an interval of about 200 kb. In addition, two lethal EMS mutations (EM26 and EM34, Katzen, 1990) mapped to this region and turned out to be allelic to *tay*^{1}, hence they were called *tay*^{2} and *tay*^{3}, respectively.

Direct genomic sequencing showed a 431 bp deletion in exon 3 of CG9056 in the *tay*^{3} allele. In the *tay*^{2} allele, a replacement of 7 by 8 bp in exon 3 of CG9056 was found.

To ultimately proof the identity of *tay* with the annotated gene CG9056, a genomic fragment comprising the putative *tay* coding region and an about 1 kb large flanking sequence proximal and distal to it was cloned into a P-element transformation vector. Two independent insertions on the third chromosome, P{*tay*}^{D1} and P{*tay*}^{D2} reverted all anatomical as well as the behavioural phenotypes of *tay* in all alleles when analysing mutant males (Poeck et al., 2008). All the behavioural testing and the anatomy based on the collar method (Heisenberg & Böhl, 1979) has been done as part of this thesis.

3.1.2 Buridan's Paradigm

In the Buridan's paradigm, the total covered distance in all three alleles of *tay* is drastically reduced when compared to wild-type CS flies (**Figure 3, Table 1**). The lethal alleles can only be tested in the heterozygous state over *tay*^{1}.

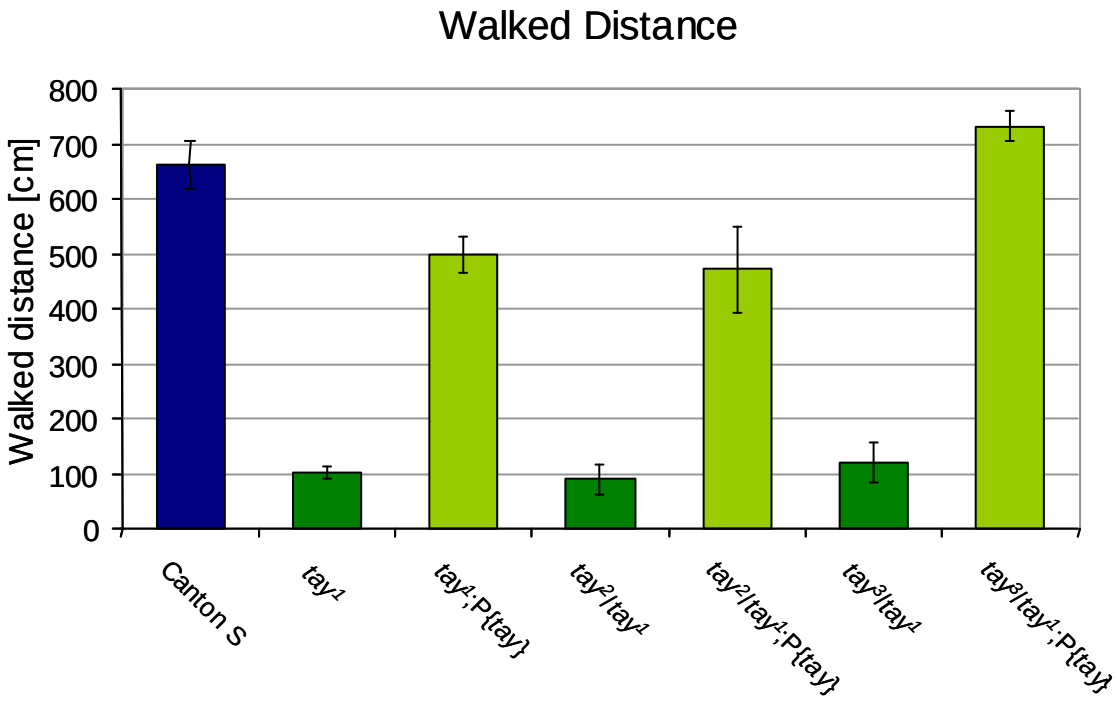

Figure 3: Walked distance in Buridan's paradigm

The total walked distance in Buridan's paradigm in 15 min is significantly reduced in all alleles of *tay bridge*. By giving back the *tay* gene the wild-type behaviour is restored. Bars denote mean values and the standard error of the mean.

N= 13, 10, 18, 10, 10, 10, 10

	Canton S	*tay*^{1}	*tay*^{1}; P{*tay*}^{D1}	*tay*^{2}/*tay*^{1}	*tay*^{2}/*tay*^{1}; P{*tay*}^{D1}	*tay*^{3}/*tay*^{1}	*tay*^{3}/*tay*^{1}; P{*tay*}^{D1}
Canton S		0.000126	0.051359	0.000126	0.042669	0.000126	0.909298
tay^{1}	0.000126		0.000126	0.999995	0.000126	0.999917	0.000126
tay^{1};P{*tay*}^{D1}	0.051359	0.000126		0.000126	0.999034	0.000126	0.001004
tay^{2}/*tay*^{1}	0.000126	0.999995	0.000126		0.000126	0.998873	0.000126
tay^{2}/*tay*^{1};P{*tay*}^{D1}	0.042669	0.000126	0.999034	0.000126		0.000129	0.001301
tay^{3}/*tay*^{1}	0.000126	0.999917	0.000126	0.998873	0.000129		0.000126
tay^{3}/*tay*^{1};P{*tay*}^{D1}	0.909298	0.000126	0.001004	0.000126	0.001301	0.000126	

Table 1 Walked distance in Buridan's paradigm, ANOVA with Tukey post-hoc test

Walked distance is severely reduced in *tay*^{1} and the heterozygous combinations *tay*^{1}/*tay*^{2} and *tay*^{1}/*tay*^{3} when compared to the wild-type CS ($p<0.001$). All alleles of *tay* are significantly the same ($p>0.995$). When the *tay* gene is reintroduced, the walked distance increases, not being significantly different from wild-type in two of three cases.

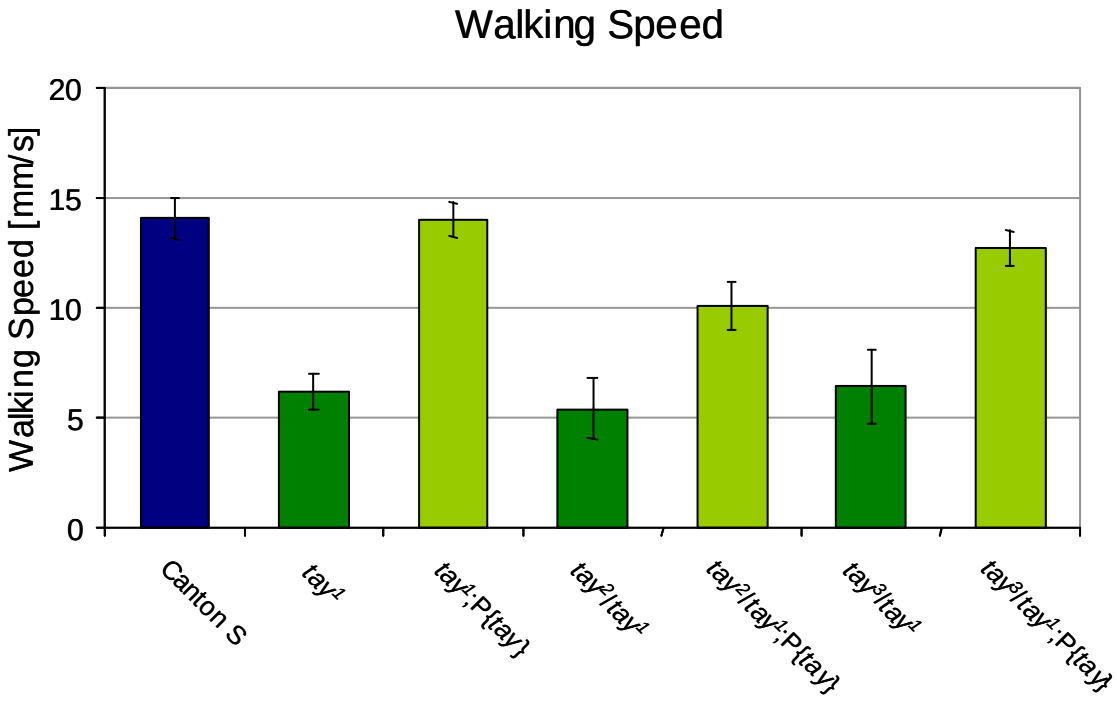

Figure 4: Walking speed in Buridan's paradigm

tay mutant flies walk slower than wild-type flies in Buridan's paradigm. This can also be rescued by P{*tay*}^{D1}. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 18, 10, 10, 10, 10

When reintroducing the *tay* gene with the P{*tay*}^{D1} construct, this phenotype can be recovered and there is no significant difference to the wild-type CS (Statistics see **Table 1**). The short path is due to two problems in *tay*^{1}: the low walking speed and the low activity. The walking speed of *tay*^{1} is less than half of normal flies (**Figure 4, Table 2**). The activity, i.e. the percentage of time spent in walking is also significantly reduced (**Figure 5, Table 3**). The lethal alleles *tay*^{2} and *tay*^{3} were tested in heterzygosity over *tay*^{1}.

	Canton S	*tay*^{1}	*tay*^{1}; P{*tay*}^{D1}	*tay*^{2}/*tay*^{1}	*tay*^{2}/*tay*^{1}; P{*tay*}^{D1}	*tay*^{3}/*tay*^{1}	*tay*^{3}/*tay*^{1}; P{*tay*}^{D1}
Canton S		0.001439	1.000000	0.000140	0.201811	0.000333	0.985806
tay^{1}	0.001439		0.000246	0.808009	0.633804	0.990892	0.018499
tay^{1};P{*tay*}^{D1}	1.000000	0.000246		0.000126	0.092296	0.000139	0.967769
tay^{2}/*tay*^{1}	0.000140	0.808009	0.000126		0.064904	0.995451	0.000483
tay^{2}/*tay*^{1};P{*tay*}^{D1}	0.201811	0.633804	0.092296	0.064904		0.260284	0.654723
tay^{3}/*tay*^{1}	0.000333	0.990892	0.000139	0.995451	0.260284		0.003458
tay^{3}/*tay*^{1};P{*tay*}^{D1}	0.985806	0.018499	0.967769	0.000483	0.654723	0.003458	

Table 2: Walking speed in Buridan's paradigm, ANOVA with Tukey post-hoc test

The walking speed is also severely reduced in *tay*^{1}, *tay*^{2}/*tay*^{1} and *tay*^{3}/*tay*^{1} ($p<0.005$). When restoring the gene, the walking speed increases to wild-type levels in *tay*^{1} and *tay*^{3}/*tay*^{1} and to an intermediate level in *tay*^{2}/*tay*^{1}.

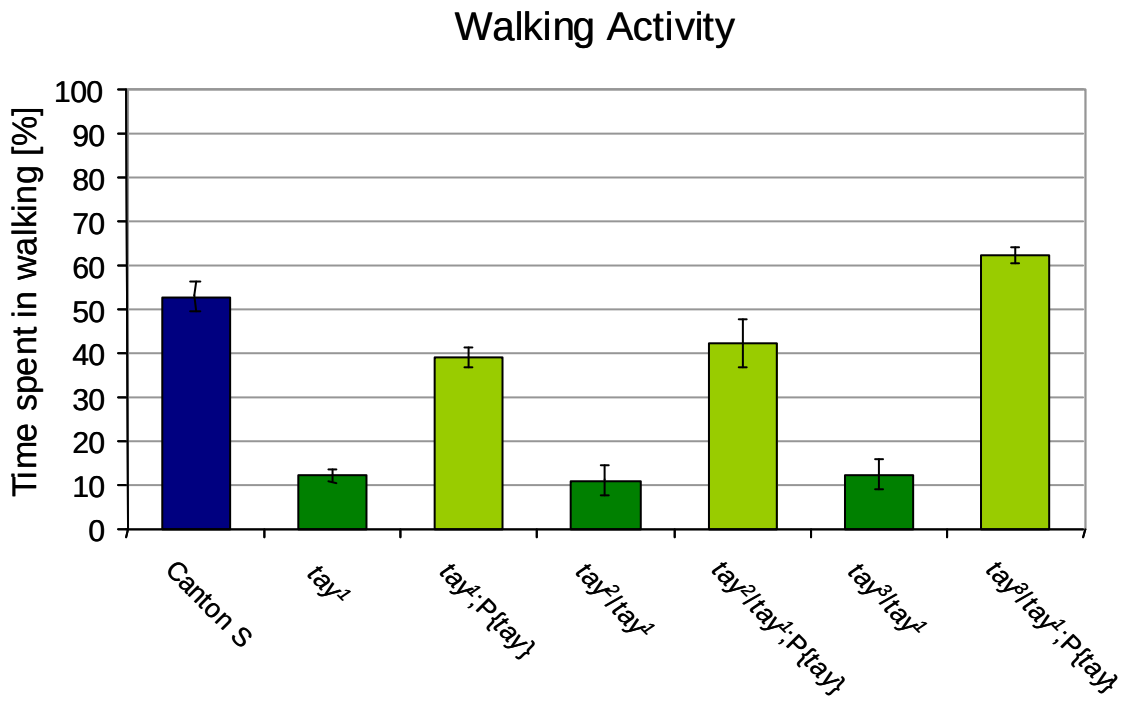

Figure 5: Walking activity in Buridan's paradigm

The time spent in walking is highly significantly reduced in reduced in *tay*^{1}, *tay*^{2}/*tay*^{1} and *tay*^{3}/*tay*^{1} ($p<0.001$). With the genomic rescue, this can be brought back to wild-type level. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 18, 10, 10, 10, 10

3.1.3 Optomotor Compensation

Parallel to those experiments, it was tested whether *tay* mutants are affected in optomotor compensation during walking (Strauss et al., 1997). This paradigm can be used to elicit curve walking or turning on the spot. *tay*^{1} and the two lethal alleles heterozygous over *tay*^{1} show a certain degree of compensation, but the flies were not able to reach wild-type performance. In this setup, giving back the genomic sequence caused a complete rescue of the phenotype (**Figure 6, Table 4**) as well.

	Canton S	*tay*^{1}	*tay*^{1}; P{*tay*}^{D1}	*tay*^{2}/*tay*^{1}	*tay*^{2}/*tay*^{1}; P{*tay*}^{D1}	*tay*^{3}/*tay*^{1}	*tay*^{3}/*tay*^{1}; P{*tay*}^{D1}
Canton S		0.000126	0.026086	0.000126	0.284772	0.000126	0.457445
tay^{1}	0.000126		0.000126	0.999992	0.000126	1.000000	0.000126
tay^{1};P{*tay*}^{D1}	0.026086	0.000126		0.000126	0.990561	0.000126	0.000134
tay^{2}/*tay*	0.000126	0.999992	0.000126		0.000126	0.999961	0.000126
tay^{2}/*tay*;P{*tay*}^{D1}	0.284772	0.000126	0.990561	0.000126		0.000126	0.001539
tay^{3}/*tay*	0.000126	1.000000	0.000126	0.999961	0.000126		0.000126
tay^{3}/*tay*;P{*tay*}^{D1}	0.457445	0.000126	0.000134	0.000126	0.001539	0.000126	

Table 3: Walking activity in Buridan's paradigm, ANOVA with Tukey post-hoc test

Finally, the walking activity is reduced in *tay*^{1}, *tay*^{2}/*tay*^{1} and *tay*^{3}/*tay*^{1} ($p<0.001$). Also here, the phenotype can be rescued by reintroducing the *tay* gene by p{tay}^{D1}.

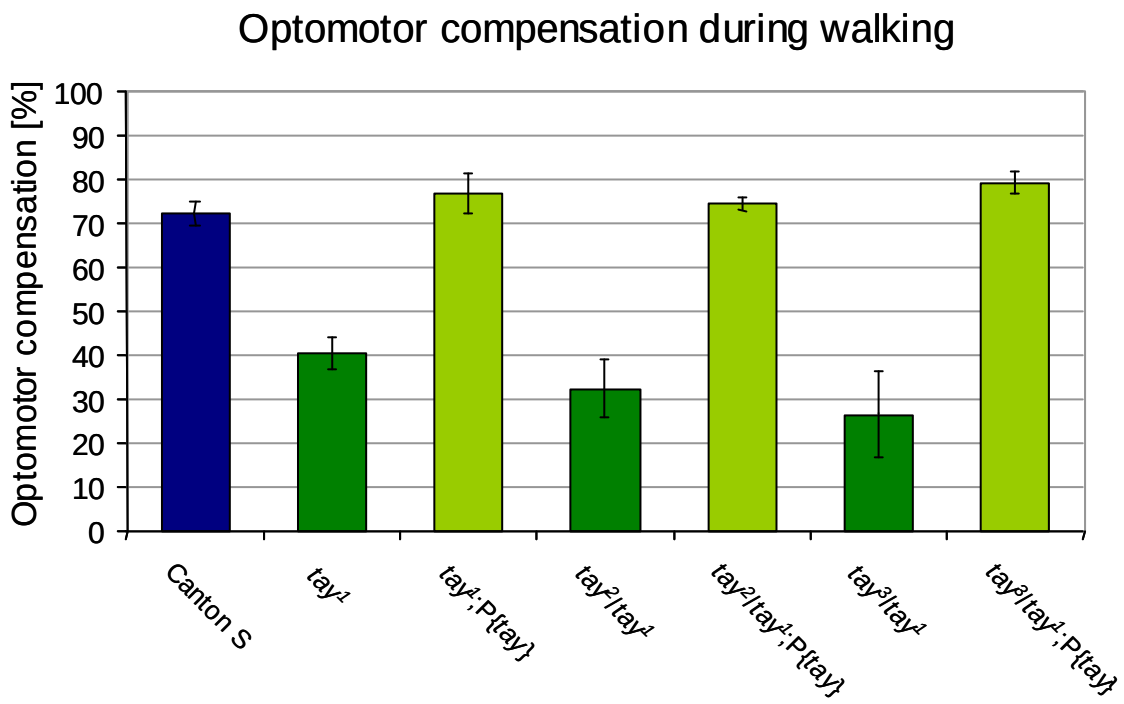

Figure 6: Optomotor compensation during walking

Wild-type flies tend to follow optomotor stimulation. In *tay*^{1}, *tay*^{2}/*tay*^{1} and *tay*^{3}/*tay*^{1}, the efficiency of this behaviour is significantly reduced. In genomic rescue flies of *tay* the compensation is back at wild-type level. Bars show mean values and the whiskers denote the standard error of the mean.

N= 12, 10, 10, 11, 10, 10, 10

	Canton S	*tay*^{1}	*tay*^{1}; P{*tay*}^{D1}	*tay*^{2}/*tay*^{1}	*tay*^{2}/*tay*^{1}; P{*tay*}^{D1}	*tay*^{3}/*tay*^{1}	*tay*^{3}/*tay*^{1}; P{*tay*}^{D1}
Canton S		0.001517	0.998795	0.000143	0.995950	0.000129	1.000000
tay^{1}	0.001517		0.000616	0.945207	0.000449	0.597287	0.002724
tay^{1};P{*tay*}^{D1}	0.998795	0.000616		0.000133	1.000000	0.000129	0.998990
tay^{2}/*tay*	0.000143	0.945207	0.000133		0.000131	0.989273	0.000170
tay^{2}/*tay*;P{*tay*}^{D1}	0.995950	0.000449	1.000000	0.000131		0.000128	0.996602
tay^{3}/*tay*	0.000129	0.597287	0.000129	0.989273	0.000128		0.000131
tay^{3}/*tay*;P{*tay*}^{D1}	1.000000	0.002724	0.998990	0.000170	0.996602	0.000131	

Table 4: Optomotor compensation during walking, ANOVA with Tukey post-hoc test

The failure to compensate for optomotor stimuli in *tay* mutants can be rescued by the genomic P{*tay*}^{D1}construct.

3.1.4 Pan-neuronal rescue

After the *tay* gene had been identified, the next step was to perform a differential rescue to find out, in which brain areas and times of expression Tay is needed. For this approach, Poeck et al. (2008) cloned the cDNA (LD22609; Stapleton et al., 2002) of *tay* into the pUAST vector (Brand & Perrimon, 1993) and established several independent transgenic lines (UAS-*tay*). As a first step, a pan-neuronal expression of *tay* via the elav-GAL4 (Luo et al., 1994) driver line was tried out. Surprisingly, this line did not rescue the gross morphological defect in the protocerebral bridge found in tay^1. When testing those flies in the Buridan's paradigm, the inclination to walk was not higher than in the mutant flies (**Figure 7, Table 5**). The walking speed, however, was higher than in tay^1 and not significantly different from wild-type (**Figure 9, Table 6**). As the overall walking speed of the rescued flies was also not significantly different from tay^1, this constitutes only a partial rescue. We were also unable to rescue the optomotor compensation defect of walking tay^1 flies with this driver (**Figure 10, Table 7**).

Figure 7: Walked distance

Only the 007Y-GAL4 line can rescue the walked distance in tay^1 mutant flies, when driving UAS-*tay*. Other lines with expression in the protocerebral bridge fail to accomplish this task. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 10, 14, 10, 15, 12

	Canton S	*tay*^{1}	*tay*^{1};elav>*tay*	*tay*^{1}; 007Y>*tay*	*tay*^{1}; 078Y>*tay*	*tay*^{1}; 210Y>*tay*
Canton S		0,000130	0,000134	0,396341	0,000294	0,000135
tay^{1}	0,000130		0,998049	0,000245	0,375628	0,974472
tay^{1};elav>*tay*	0,000134	0,998049		0,001542	0,723062	0,999763
tay^{1};007Y>*tay*	0,396341	0,000245	0,001542	0,	0,037606	0,002075
tay^{1};078Y>*tay*	0,000294	0,375628	0,723062	0,037606		0,850259
tay^{1};210Y>*tay*	0,000135	0,974472	0,999763	0,002075	0,850259	

Table 5: Walked distance, ANOVA with Tukey post-hoc test

The total walked distance is only rescued with the 007Y-GAL4 line. *tay*^{1}/Y;UAS-*tay*/II;elav-GAL4/III flies are not different from the mutant *tay*^{1}.

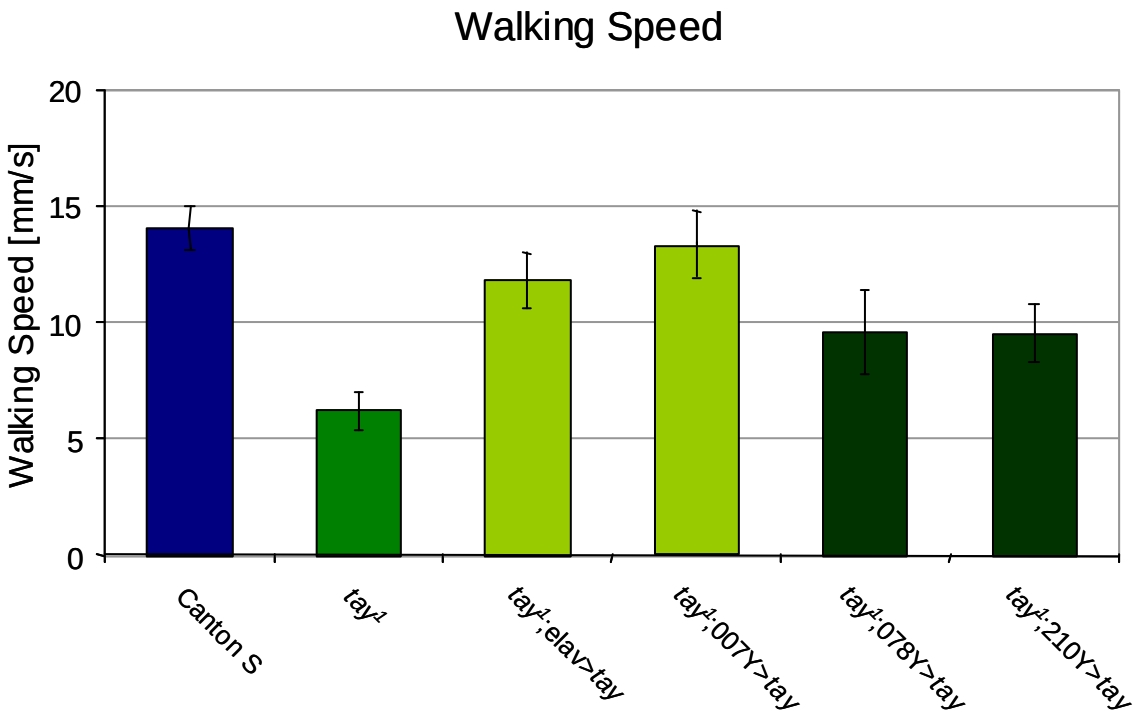

Figure 8: Walking speed

The reduced speed in *tay*^{1} bridge flies can fully be rescued with 007Y-GAL4>UAS-*tay*. elav-GAL4>UAS-*tay* gives only a partial rescue. Bars show mean values and the whiskers denote the standard error of the mean.
N= 13, 10, 10, 14, 10, 15, 12

	Canton S	*tay*^{1}	*tay*^{1};elav>*tay*	*tay*^{1}; 007Y>*tay*	*tay*^{1}; 078Y>*tay*	*tay*^{1}; 210Y>*tay*
Canton S		0,014651	0,548209	0,999969	0,072463	0,079884
tay^{1}	0,014651		0,583534	0,010902	0,965487	0,984536
tay^{1};elav>*tay*	0,548209	0,583534		0,582853	0,933953	0,920435
tay^{1};007Y>*tay*	0,999969	0,010902	0,582853		0,061836	0,071792
tay^{1};078Y>*tay*	0,072463	0,965487	0,933953	0,061836		0,999999
tay^{1};210Y>*tay*	0,079884	0,984536	0,920435	0,071792	0,999999	

Table 6: Walking speed, ANOVA with Tukey post-hoc test

The walking speed is only fully rescued in *tay*^{1}/Y;UAS-tay/II;007Y-GAL4/III flies.

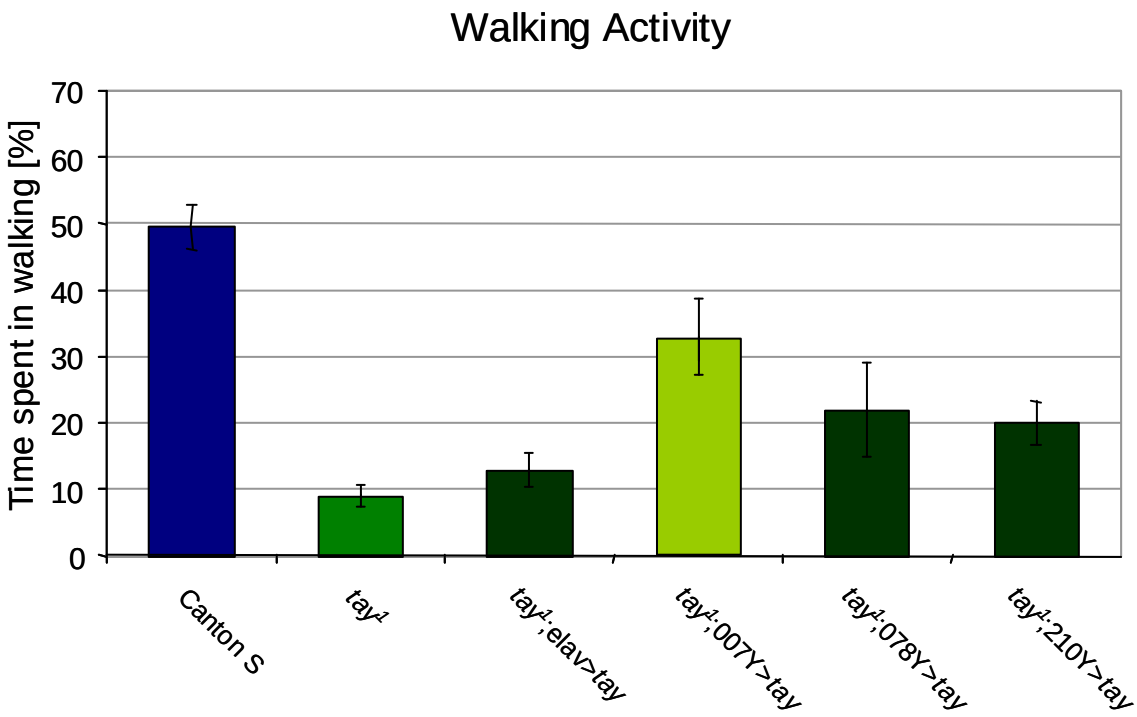

Figure 9: Walking activity

The time spent in walking is partially rescued in *tay*^{1},007Y-GAL4>UAS-*tay* flies. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 10, 13, 10, 15, 12

	Canton S	*tay*^{1}	*tay*^{1};elav>*tay*	*tay*^{1}; 007Y>*tay*	*tay*^{1}; 078Y>*tay*	*tay*^{1}; 210Y>*tay*
Canton S		0.000129	0.000129	0.009634	0.000133	0.000132
tay^{1}	0.000129		0.999995	0.002223	0.427248	0.716016
tay^{1};elav>*tay*	0.000129	0.999995		0.006295	0.574880	0.824574
tay^{1};007Y>*tay*	0.009634	0.002223	0.006295		0.214558	0.129687
tay^{1};078Y>*tay*	0.000133	0.427248	0.574880	0.214558		0.998990
tay^{1};210Y>*tay*	0.000132	0.716016	0.824574	0.129687	0.998990	

Table 7: Walking activity, ANOVA with Tukey post hoc test

The time spent in walking is partially rescued in *tay*^{1},007Y-GAL4>UAS-*tay*.

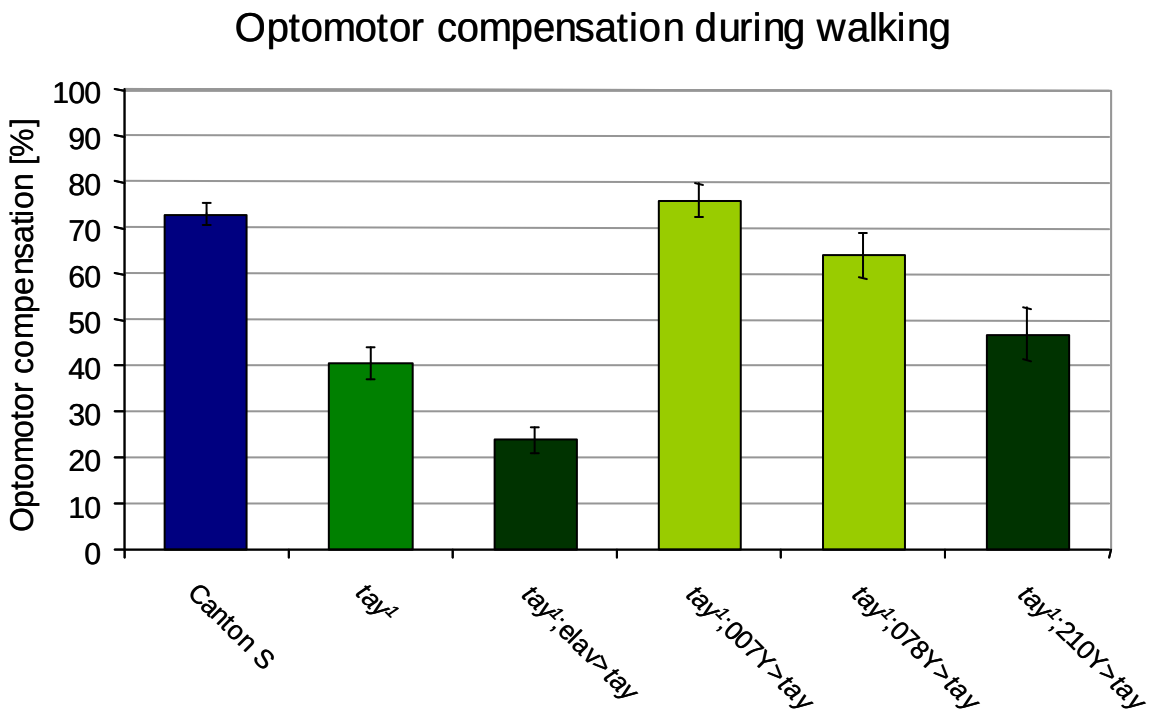

Figure 10: Optomotor compensation

The ability to compensate for optomotor stimuli is rescued in *tay*^{1},007Y-GAL4>UAS-*tay* and *tay*^{1},078Y-GAL4>UAS-*tay*. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 12, 10, 10, 10, 11

	Canton S	*tay*^{1}	*tay*^{1};elav>*tay*	*tay*^{1}; 007Y>*tay*	*tay*^{1}; 078Y>*tay*	*tay*^{1}; 210Y>*tay*
Canton S		0.000139	0.000136	0.995542	0.596558	0.000281
tay^{1}	0.000139		0.062311	0.000137	0.002295	0.875870
tay^{1};elav>*tay*	0.000136	0.062311		0.000136	0.000136	0.002274
tay^{1};007Y>*tay*	0.995542	0.000137	0.000136		0.338192	0.000185
tay^{1};078Y>*tay*	0.596558	0.002295	0.000136	0.338192		0.041832
tay^{1};210Y>*tay*	0.000281	0.875870	0.002274	0.000185	0.041832	

Table 8: Optomotor compensation, ANOVA with Tukey post-hoc test

The ability to compensate for optomotor stimuli is rescued in *tay*^{1};007Y-GAL4>UAS-*tay* and *tay*^{1};078Y-GAL4>UAS-*tay*.

In summary, pan-neural expression with elav-GAL4 slightly improved walking speed but did not improve the other behavioural phenotypes (**Figures 7-10, Tables 5-8**). The structural phenotype of the protocerebral bridge was also not repaired in that crosses. These results are most likely attributable to the relatively weak expression strength of the elav-GAL4 line (Kretzschmar et al., 2005).

3.1.5 Specific Rescue Experiments

As the pan-neuronal expression did not give any promising results, it was decided to focus on drivers with stronger expression, primarily in the protocerebral bridge. The line 007Y-GAL4 (Renn et al., 1999) is one of those lines. It has strong expression in the protocerebral bridge, additional expression can be seen in the ellipsoid body, in two layers of the fan-shaped body, in the dorsal parts of the noduli and in the mushroom bodies (Poeck et al., 2008, **Figure 11**).

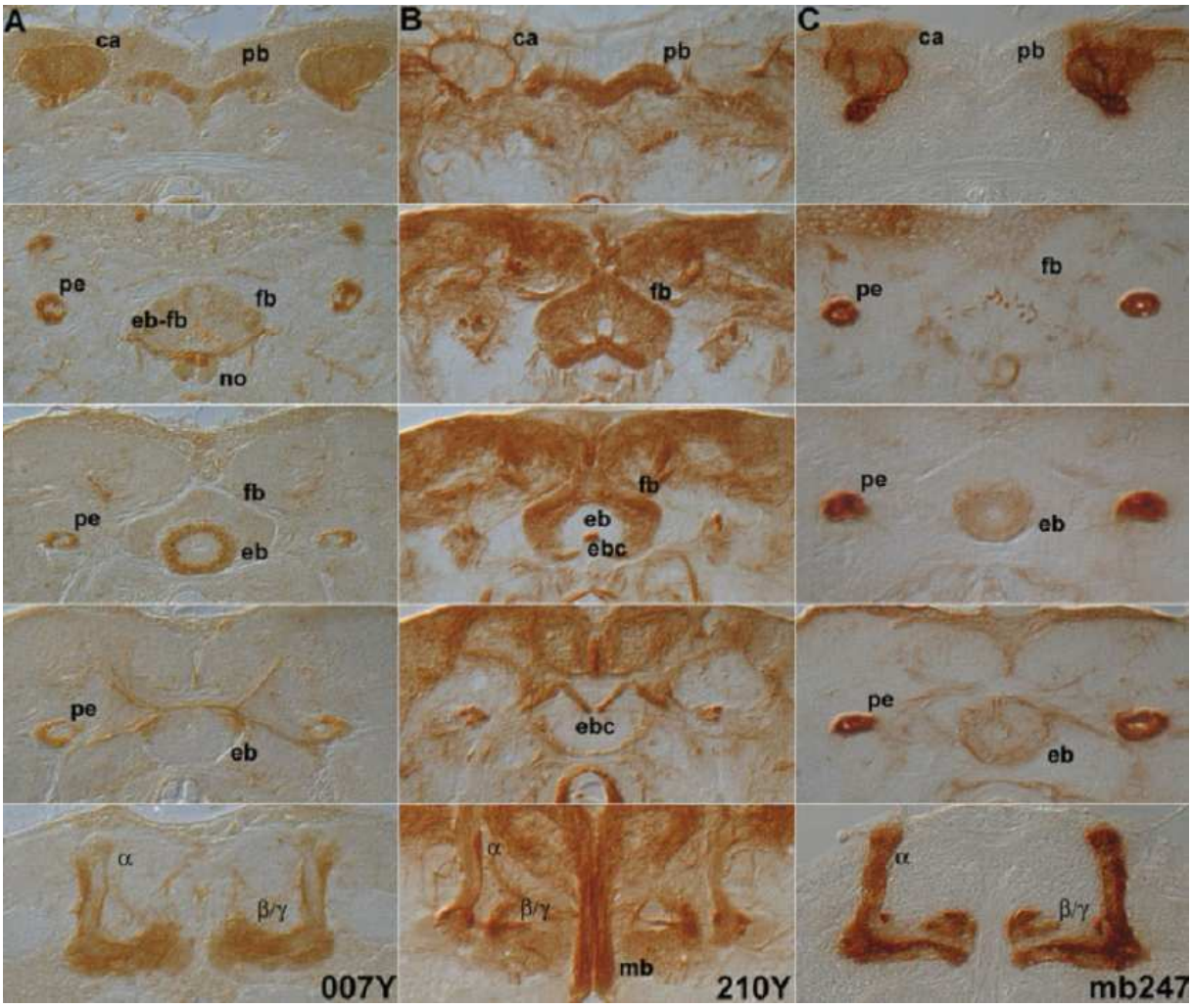

Figure 11: Expression pattern of different GAL4 lines used in the rescue experiments

Expression pattern of (A) 007Y-GAL4, (B) 210Y-GAL4, and (C) mb247-GAL4. The GAL4-lines were crossed to UAS-tau-GFP and 7µm-frontal paraffin sections were stained with an antibody against bovine TAU.
pb, protocerebral bridge; fb, fan-shaped body; eb, ellipsoid body; no, noduli; eb-fb, ellipsoid- and fan-shaped body connecting neurons; ebc, ellipsoid-body canal. Constituents of the mushroom bodies are labeled with ca, calyx, pe, peduncle, and α,β,γ, α-, β-, γ-lobes. mb, median bundle.
Figure taken from Poeck et al. (2008).

The 007Y-GAL4 line expresses also in the w-, x-, y- and z-bundles of the horizontal fibre system connecting bridge and fan-shaped body (Hanesch et al., 1989). By induction of UAS-*tay* using 007Y-GAL4 in the tay^1 mutant background, the protocerebral bridge phenotype can be rescued (Poeck et al., 2008). The overall walking activity of tay^1/Y; UAS-*tay*/II; 007Y-GAL4/III in Buridan's paradigm was significantly improved as compared to the mutant and not different from wild-type (**Figure 8, Table 6**). Also walking speed of the rescued flies was at wild-type levels and thereby clearly faster than that of tay^1 flies (**Figure 9, Table 7**). Additionally, 007Y-GAL4 rescued flies are completely normal when compensating for optomotor stimuli; they follow the pattern in a wild-type manner (**Figure 10, Table 8**). All in all, expression of the *tay* cDNA under the control of 007Y-GAL4 rescues the neuroanatomical defects as well as all behavioural parameters of Buridan's paradigm and the optomotor compensation.

Next, it had to be tested whether this result is due to the expression of *tay* in the protocerebral bridge. A second GAL4 line - 210Y-GAL4 (Renn et al., 1999) - with strong expression in the protocerebral bridge has been used to this end. Expression additional to the bridge can be found in the fan-shaped body and the median bundle and weakly in other brain areas (Poeck et al., 2008, **Figure 11**). The expression pattern of 210Y-GAL4 is distinctly different from that of 007Y-GAL4. While in the latter, the w-, x-, y- and z-bundles are strongly stained, there is no staining in this bundles in 210Y-GAL4. Also the staining within the bridge is different. In 007Y-GAL4, one can see the glomerular structure of the bridge while in 210Y-GAL4, the bridge has a somewhat compact appearance. This suggests that 210Y-GAL4 stains the latero-lateral connections while 007Y-GAL4 stains the columnar elements connecting the bridge to other parts of the central complex and possibly the Horizontal Fibre System (Poeck et al., 2008). In tay^1/Y; UAS-*tay*; 210Y-GAL4/III animals, neither the structural defect of the protocerebral bridge nor the behavioural defects are rescued (**Figures 7-10, Tables 5-8**). Walking activity, walked distance and walking speed are not significantly different from tay^1 in the Buridan's paradigm. The same is true for optomotor compensation. Furthermore we tested the line 078Y-GAL4 (Renn et al., 1999; Scholz et al., 2000). The expression pattern of this line is almost indistinguishable from 007Y-GAL4 in spatial terms, but weaker (H. Scholz, personal communication). Only the optomotor compensation was rescued in this line (**Figure 10, Table 8**).

Other GAL4 lines expressing in different parts of the central complex were tested as well. c232-GAL4 and c819-GAL4 (Renn et al., 1999; Neuser et al. 2008) both express in the ellipsoid body, NP2320-GAL4 and NP3124-GAL4 (Liu et al., 2006) have expression in different areas of the fan-shaped body. None of these lines was able to rescue the structure of the protocerebral bridge. Also the travelled distance in Buridan's behaviour was rescued in none of the lines (**Figure 12, Table 9**). The walking speed in the ellipsoid body rescue lines was not different from wild-type but also not different from the mutant (**Figure 13, Table 10**). The walking activity in *tay*^{1}/Y; UAS-*tay*; c232-GAL4/III was significantly different from wild-type as well as from the mutant (**Figure 14, Table 11**). The optomotor compensation finally in *tay*^{1}/Y; UAS-*tay*; c232-GAL4/III was not significantly different from wild-type as well as from the *tay*^{1} mutant (**Figure 15, Table 12**). In summary, none of these lines gave a clear rescue in any of the analysed behaviours, implying that most parts of the central complex aside from the protocerebral bridge play only a minor role in the behavioural defects of the *tay bridge*^{1} mutant.

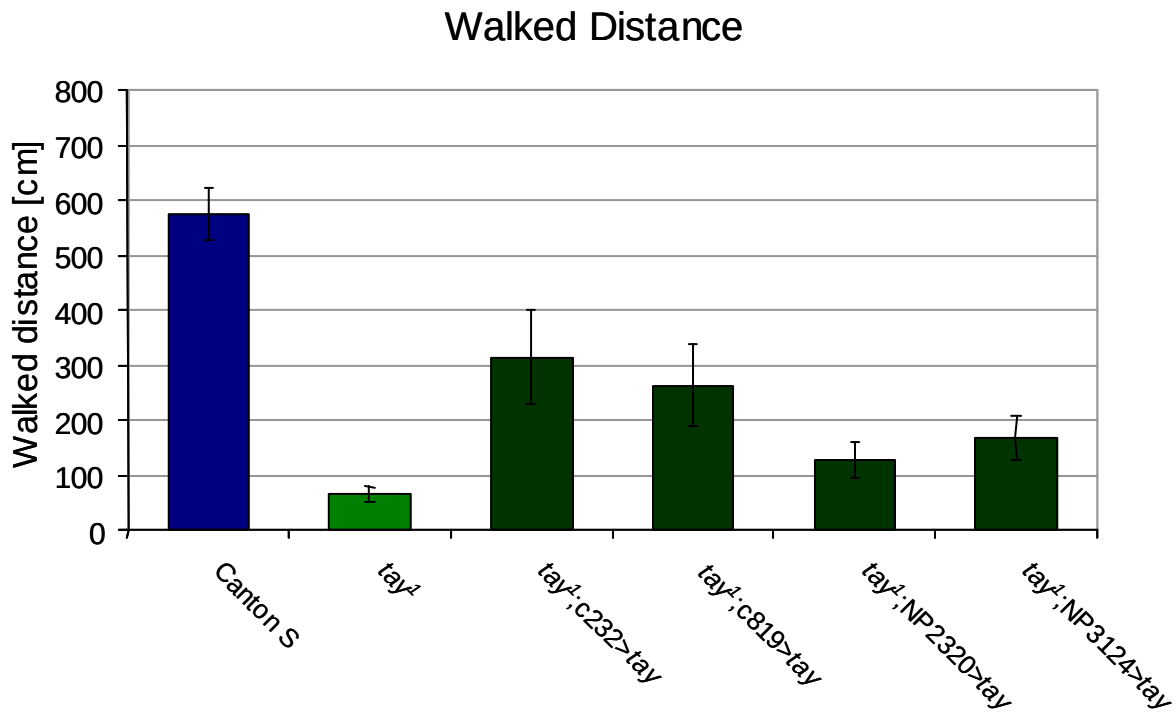

Figure 12: Walked distance

None of the used ellipsoid body or fan-shaped body driver lines can rescue the walked distance of *tay*^{1} Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 13, 10, 10, 11

	Canton S	*tay*^{1}	*tay*^{1}; c232>*tay*	*tay*^{1}; c819>*tay*	*tay*^{1}; NP2320>*tay*	*tay*^{1}; NP3124>*tay*
Canton S		0.000133	0.000847	0.000353	0.000133	0.000134
tay^1	0.000133		0.070347	0.352023	0.999476	0.979641
tay^1;c232>*tay*	0.000847	0.070347		0.988395	0.198242	0.346411
tay^1;c819>*tay*	0.000353	0.352023	0.988395		0.601982	0.793154
tay^1;NP2320>*tay*	0.000133	0.999476	0.198242	0.601982		0.999283
tay^1;NP3124>*tay*	0.000134	0.979641	0.346411	0.793154	0.999283	

Table 9: Walked distance, ANOVA with Tukey post-hoc test

All crosses to the ellipsoid body and fan-shaped body driver lines used are still highly significantly different from the wild-type and not significantly different from the *tay*^{1} mutant.

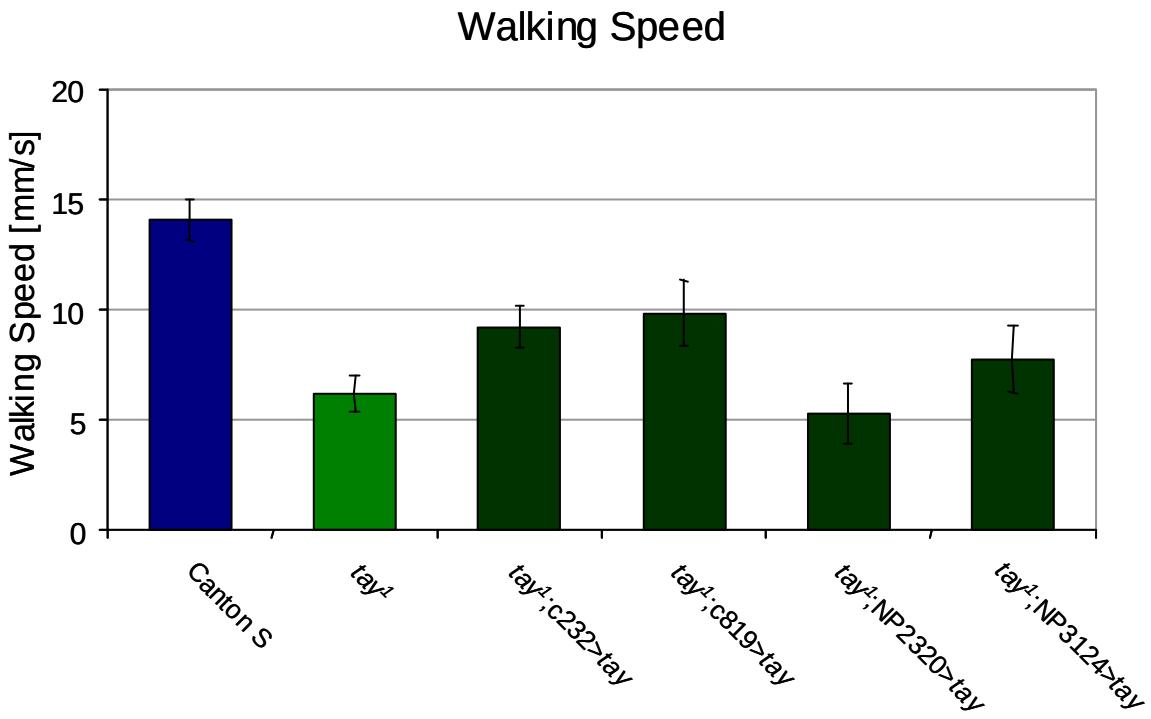

Figure 13: Walking speed

The ellipsoid body lines partially rescue the speed phenotype in *tay*^{1}. Expression of UAS-*tay* in the fan-shaped body gives no improvement. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 13, 10, 10, 11

	Canton S	*tay*^{1}	*tay*^{1}; c232>*tay*	*tay*^{1}; c819>*tay*	*tay*^{1}; NP2320>*tay*	*tay*^{1}; NP3124>*tay*
Canton S		0.003722	0.056794	0.281262	0.000203	0.001189
tay^1	0.003722		0.883550	0.585045	0.760444	0.997018
tay^1;c232>*tay*	0.056794	0.883550		0.989369	0.171584	0.636968
tay^1;c819>*tay*	0.281262	0.585045	0.989369		0.064498	0.330751
tay^1;NP2320>*tay*	0.000203	0.760444	0.171584	0.064498		0.953346
tay^1;NP3124>*tay*	0.001189	0.997018	0.636968	0.330751	0.953346	

Table 10: Walking speed, ANOVA with Tukey post-hoc test

Flies with expression of the Tay protein in the ellipsoid body are not significantly different from either wild-type or *tay*^{1} mutant regarding their walking speed in Buridan's behaviour. Expression in the fan-shaped body does not change the *tay*^{1} phenotype.

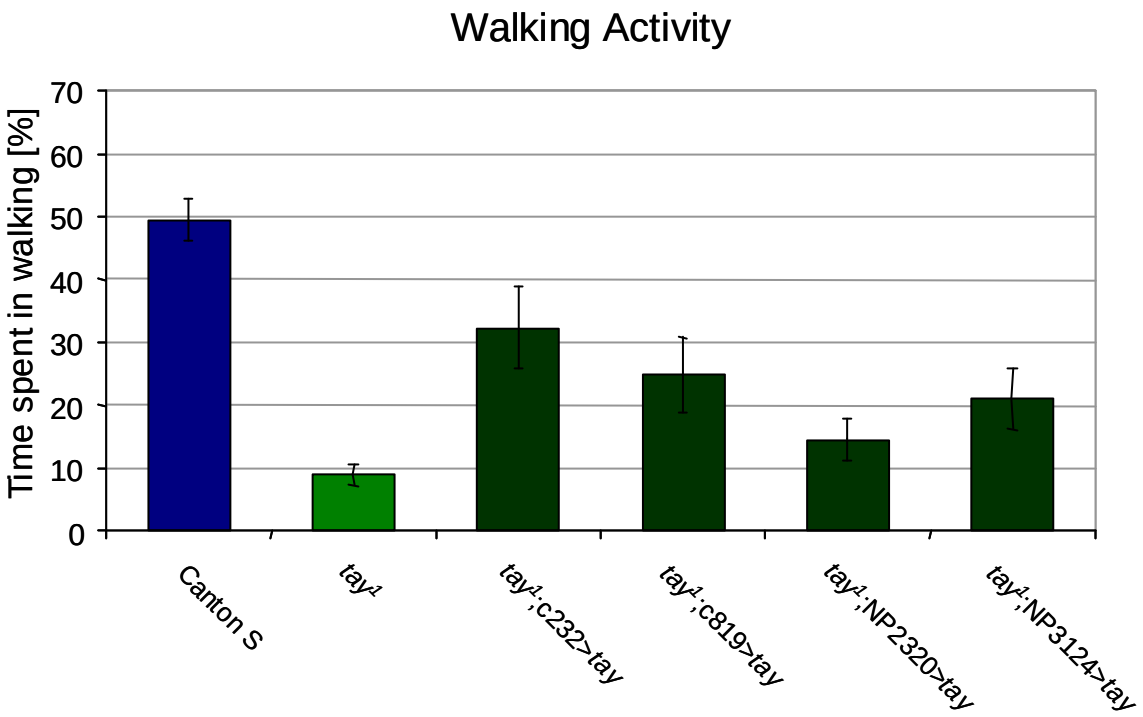

Figure 14: Walking activity

The walking activity can partially be rescued by c232-GAL4. The other rescue lines behave like the mutant. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 13, 10, 10, 11

	Canton S	*tay*^{1}	*tay*^{1}; c232>*tay*	*tay*^{1}; c819>*tay*	*tay*^{1}; NP2320>*tay*	*tay*^{1}; NP3124>*tay*
Canton S		0.000133	0.025568	0.001756	0.000139	0.000200
tay^{1}	0.000133		0.020471	0.398212	0.999107	0.859672
tay^{1};c232>*tay*	0.025568	0.020471		0.851683	0.080971	0.333794
tay^{1};c819>*tay*	0.001756	0.398212	0.851683		0.673144	0.968295
tay^{1};NP2320>*tay*	0.000139	0.999107	0.080971	0.673144		0.977010
tay^{1};NP3124>*tay*	0.000200	0.859672	0.333794	0.968295	0.977010	

Table 11: Walking activity in Buridan's paradigm, ANOVA with Tukey post-hoc test

Only in the c232-GAL4 line, a partial improvement of the *tay*^{1} phenotype can be seen.

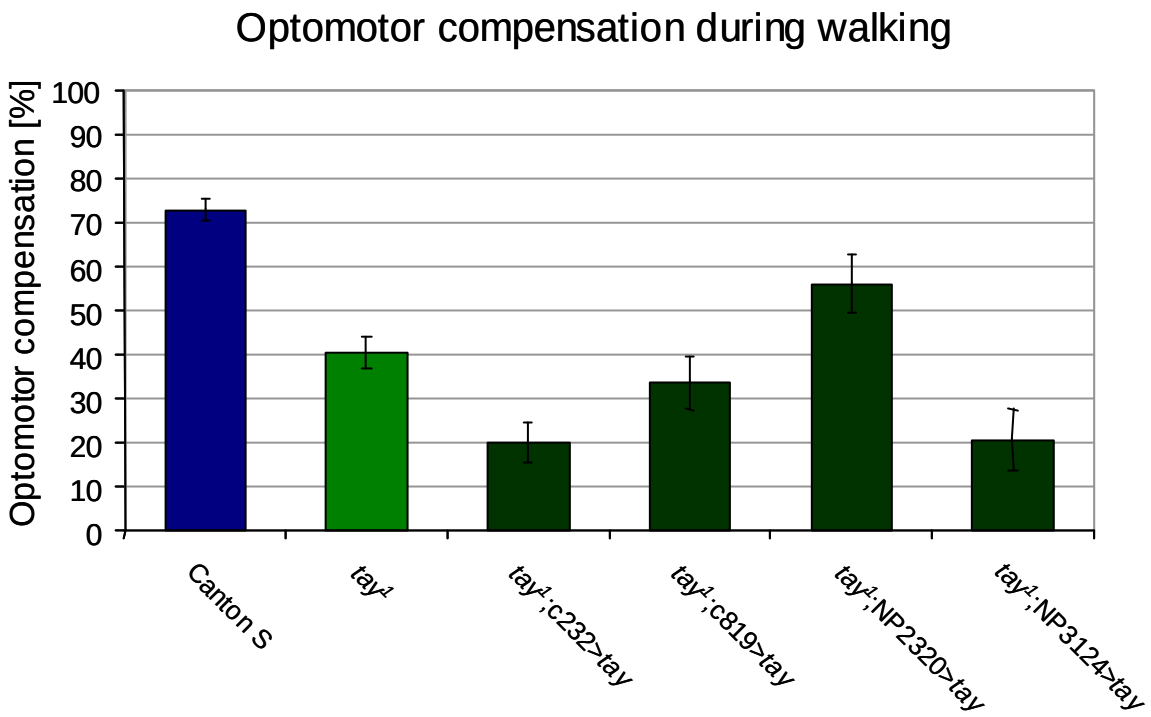

Figure 15: Optomotor compensation

The optomotor compensation during walking can partially be rescued by expression in NP2320-GAL4. Bars show mean values and the whiskers denote the standard error of the mean.

N= 12, 10, 12, 12, 10, 11

	Canton S	*tay*^{1}	*tay*^{1}; c232>*tay*	*tay*^{1}; c819>*tay*	*tay*^{1}; NP2320>*tay*	*tay*^{1}; NP3124>*tay*
Canton S		0.000819	0.000132	0.000139	0.228910	0.000132
tay^1	0.000819		0.079752	0.935779	0.347681	0.111363
tay^1;c232>*tay*	0.000132	0.079752		0.409023	0.000243	0.999999
tay^1;c819>*tay*	0.000139	0.935779	0.409023		0.039270	0.493216
tay^1;NP2320>*tay*	0.228910	0.347681	0.000243	0.039270		0.000361
tay^1;NP3124>*tay*	0.000132	0.111363	0.999999	0.493216	0.000361	

Table 12: Optomotor compensation, ANOVA with Tukey post-hoc test

When expressing UAS-*tay* under the control of NP2320-GAL4, the optomotor compensation is neither significantly different from wild-type nor from *tay*^{1}.

Besides the strong expression in the protocerebral bridge, 007Y-GAL4 also has expression in the ellipsoid body, the noduli and the mushroom bodies (**Figure 11**). To test whether the expression in the protocerebral bridge was the core part in the rescue with 007Y-GAL4, we used mb247-GAL (Zars et al., 2000) as a control. This line also expresses in the aforementioned brain areas with the exception of the protocerebral bridge (Poeck et al., 2008). When testing *tay*^{1}/Y; UAS-*tay*/II; mb247-GAL4/III flies in the Buridan's paradigm, the walking activity is at an intermediate level between wild-type and *tay bridge*^{1}. The walking speed is not significantly different to the mutant and strongly reduced in comparison to the wild-type. Also the structural phenotype is not rescued in these flies (Poeck et al., 2008). Unexpectedly, the optomotor compensation was at wild-type levels.

To test the mushroom bodies are necessary for optomotor compensation during walking, I chemically ablated this structure by the administration of the cytostatic substance hydroxy-urea (de Belle & Heisenberg, 1994) to *tay*^{1}/Y; UAS-*tay*/II; mb247-GAL4/III flies as 4h to 5h old larvae. Sham-treated animals were taken as control. All hydroxyurea treated flies were inspected for proper ablation of the mushroom bodies by paraffin histology after the behavioural tests. In Buridan's paradigm, all phenotypes remained unchanged; there was no significant difference between *tay*^{1} and the mb247-GAL rescue line with the different treatments. (**Tables 13-15**, ANOVA with Tukey post-hoc test, $p>0.05$), with the exception of walking activity in the mushroom-body ablated flies. As the ablation is known to increase walking activity (Mronz, 2004), this might be explained by the known effect. The ablation of the mushroom bodies did not alter the ability of mb247-rescue flies to compensate for optomotor stimuli (Tab. 34, ANOVA with Tukey post-hoc test, $p<0.001$). The expression shared by 007Y-GAL4 and mb247-GAL4 in a different brain area is therefore needed to rescue the optomotor compensation.

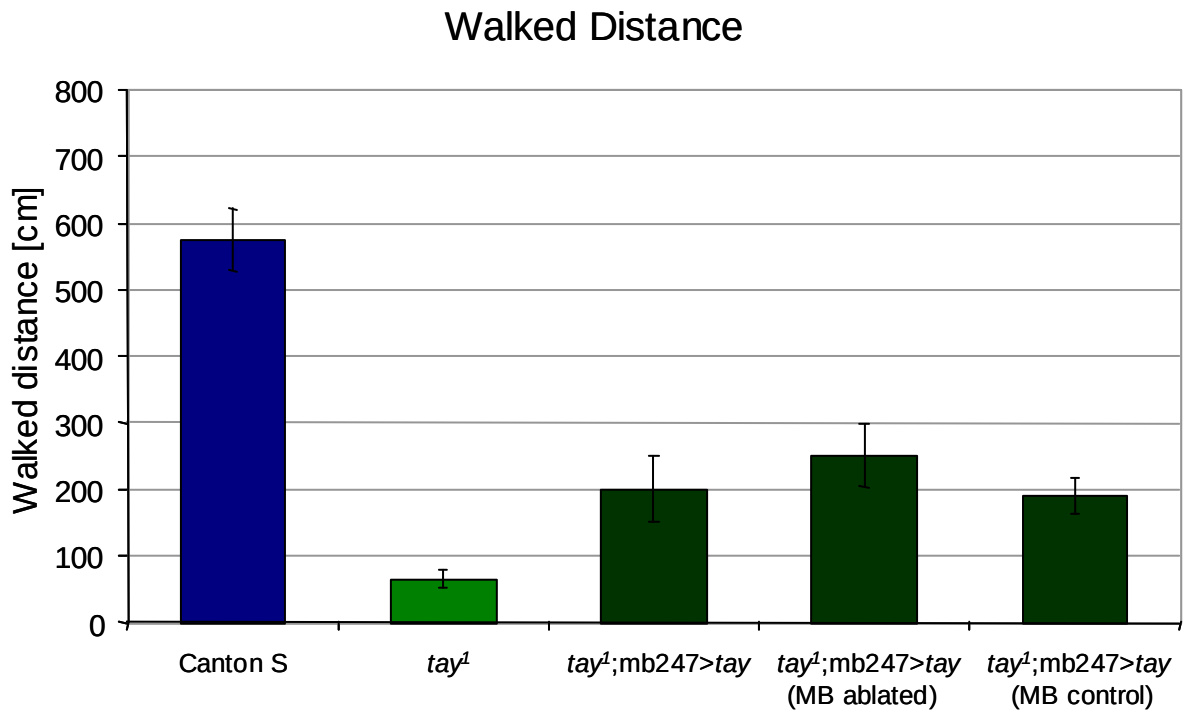

Figure 16: Walked distance

The total walked distance can not be rescued with mb247-GAL4. An ablation of the mushroom bodies does not change this result. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 10, 15, 14

	Canton S	*tay*^{1}	*tay*^{1};mb247>*tay*	*tay*^{1};mb247>*tay* (HU)	*tay*^{1};mb247>*tay* (KO)
Canton S		0.000129	0.000129	0.000129	0.000129
tay^{1}	0.000129		0.453188	0.055872	0.460016
tay^{1};mb247>*tay*	0.000129	0.453188		0.897125	0.999787
tay^{1};mb247>*tay* (HU)	0.000129	0.055872	0.897125		0.754011
tay^{1};mb247>*tay* (KO)	0.000129	0.460016	0.999787	0.754011	

Table 13: Walked distance, ANOVA with Tukey post-hoc test

When UAS-*tay* is expressed under the control of mb247-GAL, the walked distance is at *tay*^{1} level.

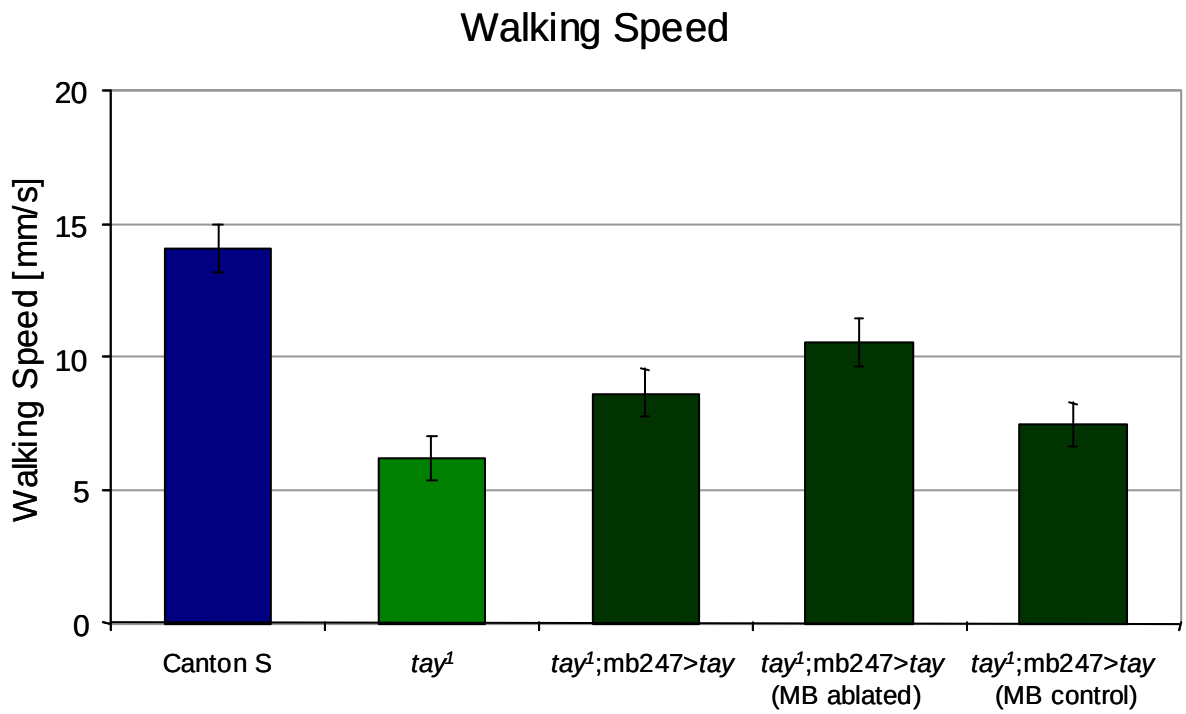

Figure 17: Walking speed

The walking speed is slightly improved in mushroom-body ablated flies in comparison to the mutant. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 10, 15, 14

	Canton S	*tay*^{1}	*tay*^{1};mb247>*tay*	*tay*^{1};mb247>*tay* (HU)	*tay*^{1};mb247>*tay* (KO)
Canton S		0.000183	0.001933	0.057503	0.000149
tay^{1}	0.000183		0.912621	0.096164	0.999992
tay^{1};mb247>*tay*	0.001933	0.912621		0.546479	0.867398
tay^{1};mb247>*tay* (HU)	0.057503	0.096164	0.546479		0.056020
tay^{1};mb247>*tay* (KO)	0.000149	0.999992	0.867398	0.056020	

Table 14: Walking speed, ANOVA with Tukey post-hoc test

Walking speed is neither significantly different from wild-type nor from *tay*^{1} in mushroom-body ablated flies that express tay under the control of mb247-GAL4.

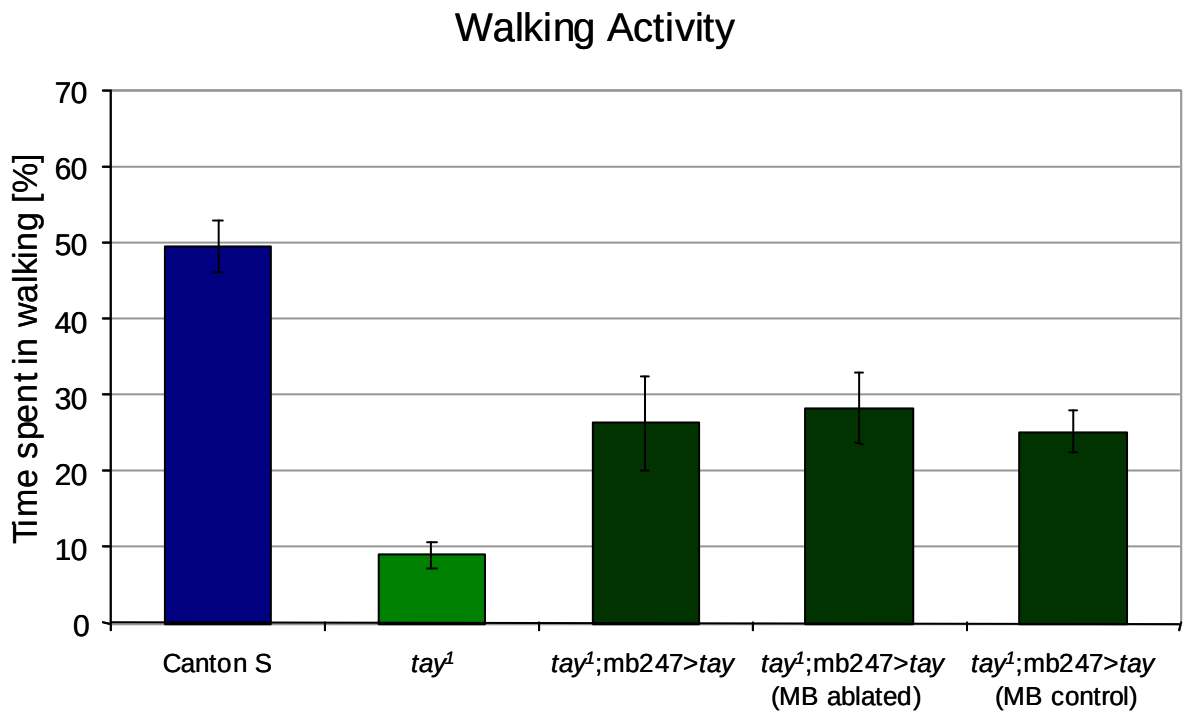

Figure 18: Walking activity

Walking activity is slightly improved in mushroom-body ablated flies. Bars show mean values and the whiskers denote the standard error of the mean.

N= 13, 10, 10, 15, 14

	Canton S	*tay*^{1}	*tay*^{1};mb247>*tay*	*tay*^{1};mb247>*tay* (HU)	*tay*^{1};mb247>*tay* (KO)
Canton S		0.000129	0.000727	0.000710	0.000196
tay^{1}	0.000129		0.134284	0.035806	0.126331
tay^{1};mb247>*tay*	0.000727	0.134284		0.997194	0.999590
tay^{1};mb247>*tay* (HU)	0.000710	0.035806	0.997194		0.973342
tay^{1};mb247>*tay* (KO)	0.000196	0.126331	0.999590	0.973342	

Table 15: Walking activity, ANOVA with Tukey post-hoc test

In *tay*^{1};mb247>*tay* flies with ablates mushroom bodies, the walking activity is weakly significant different from *tay*^{1} but still highly significant different from wild-type.

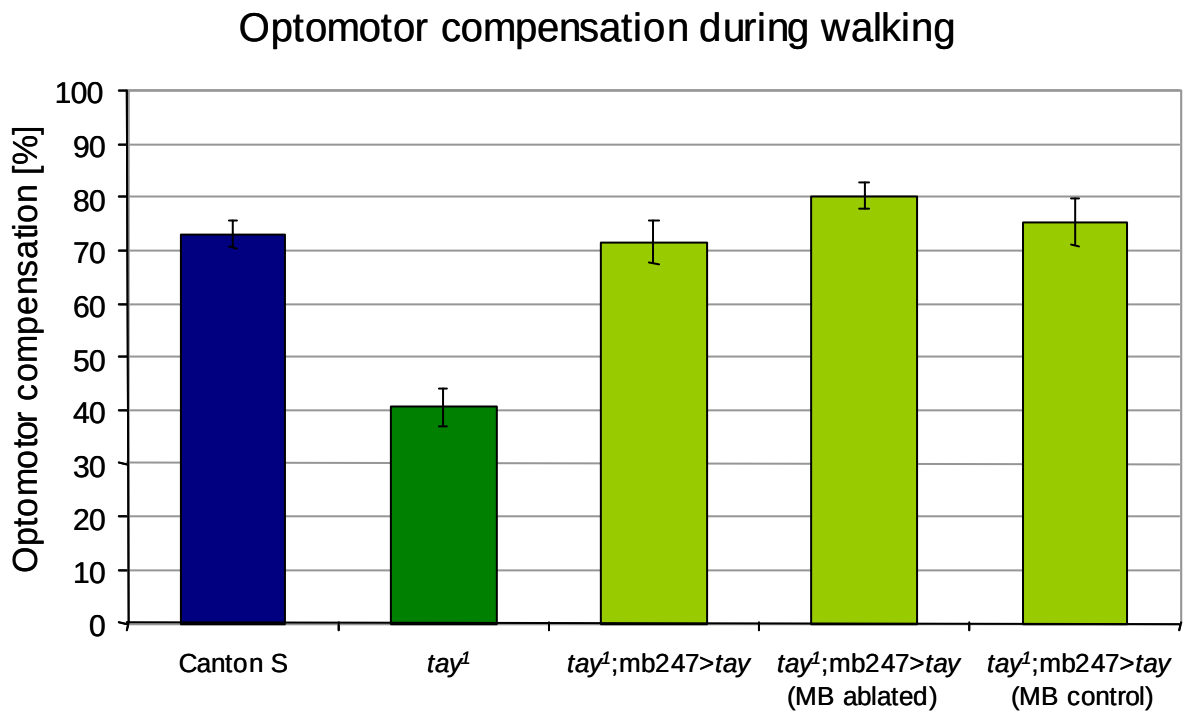

Figure 19: Optomotor compensation

The optomotor compensation during walking is at wild-type level with mb247-GAL4. Interestingly, the rescue remains even after the mushroom bodies have been ablated. Bars show mean values and the whiskers denote the standard error of the mean.

N= 12, 10, 10, 10, 11

	Canton S	*tay*^{1}	*tay*^{1};mb247>*tay*	*tay*^{1};mb247>*tay* (HU)	*tay*^{1};mb247>*tay* (KO)
Canton S		0.000131	0.998588	0.573141	0.988145
tay^{1}	0.000131		0.000131	0.000130	0.000130
tay^{1};mb247>*tay*	0.998588	0.000131		0.443580	0.944626
tay^{1};mb247>*tay* (HU)	0.573141	0.000130	0.443580		0.856434
tay^{1};mb247>*tay* (KO)	0.988145	0.000130	0.944626	0.856434	

Table 16: Optomotor compensation, ANOVA with Tukey post-hoc test

The optomotor compensation is clearly at wild-type levels with mb247-GAL4, regardless of the existence of mushroom bodies.

3.2 Climbing behaviour of *ocelliless*^{1} and *tay bridge*^{1} flies

3.2.1 Climbing behaviour of *ocelliless*^{1} flies

Earlier results had shown that structural mutants of the protocerebral bridge like e.g. *ocelliless*^{1} (*oc*^{1}), *tay bridge*^{1} (*tay*^{1})and *no bridge*^{KS49} (*nob*^{KS49}) all have difficulties in crossing broader gaps (R. Strauss, personal communication; **Figure 21 & Figure 28**). To investigate the cause for this reduced performance, the behaviour of *oc*^{1} has been studied in detail. These flies lack the three simple eyes on the frontal top of the head (Bedicheck, 1934). Finkelstein et al. (1990) found that *oc* is allelic to *orthodenticle* (*otd*) a gene required in *Drosophila* development. Hirth et al. (1995) later detected the severe structural defect in the protocerebral bridge. Only the outermost glomerulus on each hemisphere is present, otherwise the protocerebral bridge is missing. Some individuals show additional fragments of bridge material.

When analysing the behaviour of *oc*^{1} with the high speed video camera setup, it became obvious that climbing attempts were targeted into the void with a certain high probability (**Figure 20**).

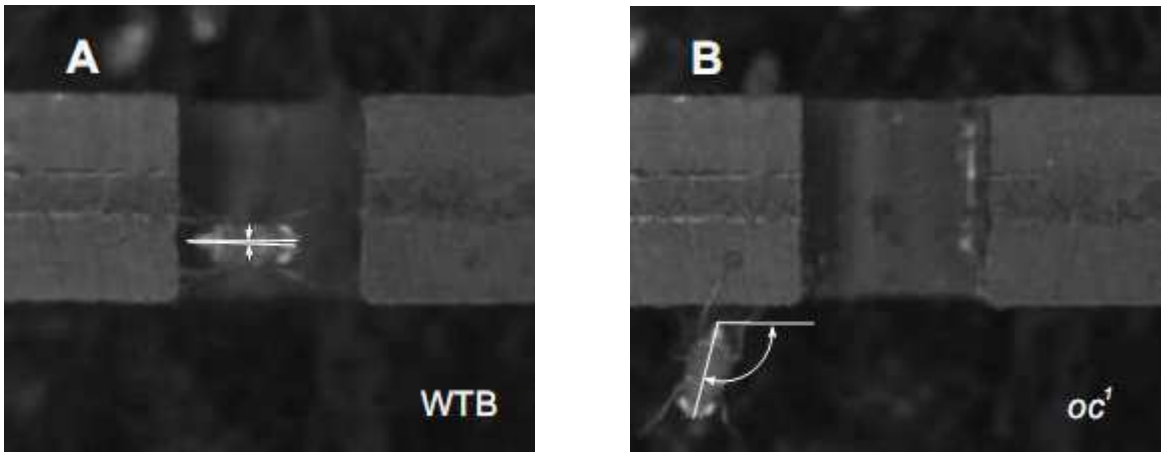

Figure 20: *ocelliless* flies sometimes lose orientation while climbing.

Climbing attempts that are correctly executed regarding the hind leg, middle leg or front leg actions are completely erroneous in regard of the body orientation towards the opposing side. The white angle depicts the absolute body angle, the deviation from the optimal climbing direction. The gap size is 3.5 mm in both examples.

To quantify this behaviour, the deviation of the body angle has been quantified in relation to the optimal climbing direction at the last leg-over-head stroke before either making contact with the opposite side or giving up the climbing attempt (**Figure 22**). In some cases, deviations of more than 90° were found (**Figure 20B**, **Figure 24**).

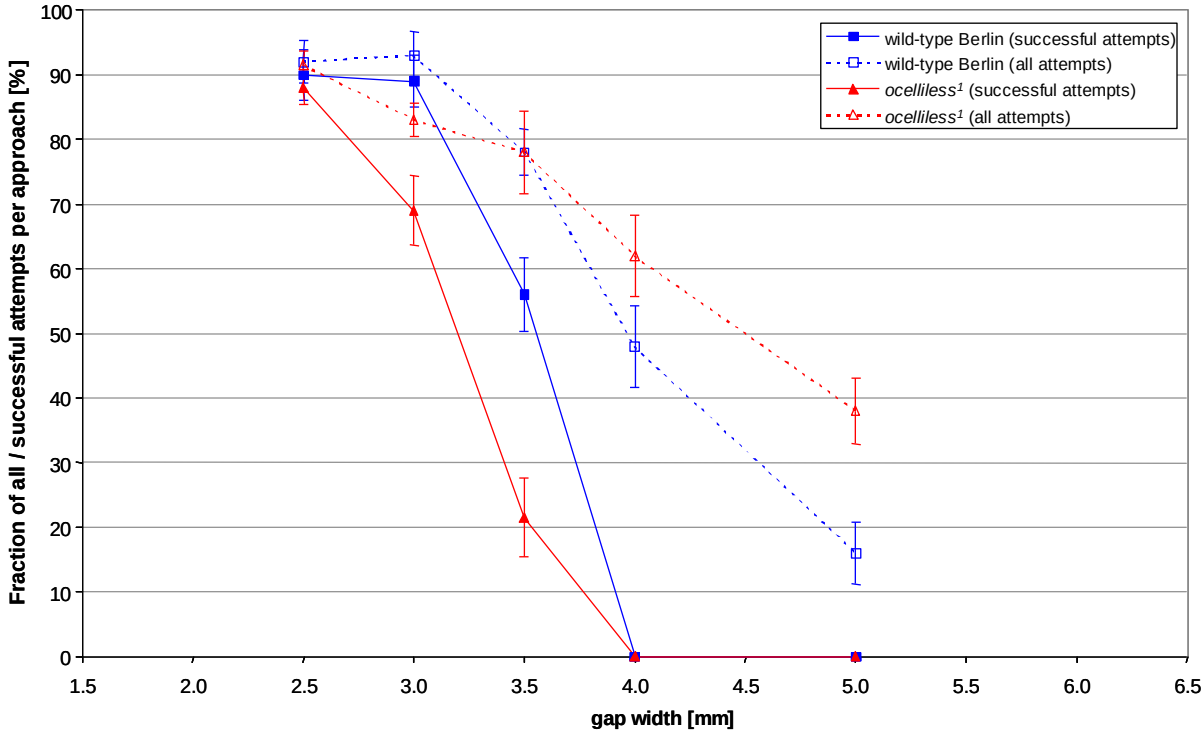

Figure 21: *ocelliless*^{1} flies experience problems particularly at wide gaps

While showing a normal performance at small gaps of up to 3.0 mm, the performance of *oc*^{1} flies drops when being challenged with wider gaps. The rate of climbing initiation is similar to that of wild-type flies. Solid lines show the success rate, i.e. the percentage of approaches to the gap that result in crossing, broken lines show initiation rate, i.e. the percentage of approaches where a climbing attempt is elicited. The figure shows means and SEMs.

N= (18, 19), n = (179, 188)

The median of this deviation is about 0° for both the wild-type Berlin (1.13±10.28°) and *oc*^{1} (1.79±46.16°) (**Figure 23**, but the total distribution is far broader in *oc*^{1}. To better demonstrate the variation, the median absolute angular deviation is used hereafter. The rate of climbing initiations at the 3.5 mm gap itself is not reduced in comparison to the wild-type (**Figure 21**).

In order to analyse which percentage of their body lengths was actually utilized by the flies to get to the other side, the cosine of the deviation angles is calculated. That way, only the portion of the body length contributing in reaching the opposite wall would be taken into account. Small error angles have only a very small influence, but large angles (>45°) will have a strong impact. Error angles of more than 90° will even get a negative sign, as the fly will climb away from the target region. As a result, *oc*^{1} flies used on average only about 75% of their body length to reach the opposite side (**Figure 25**).

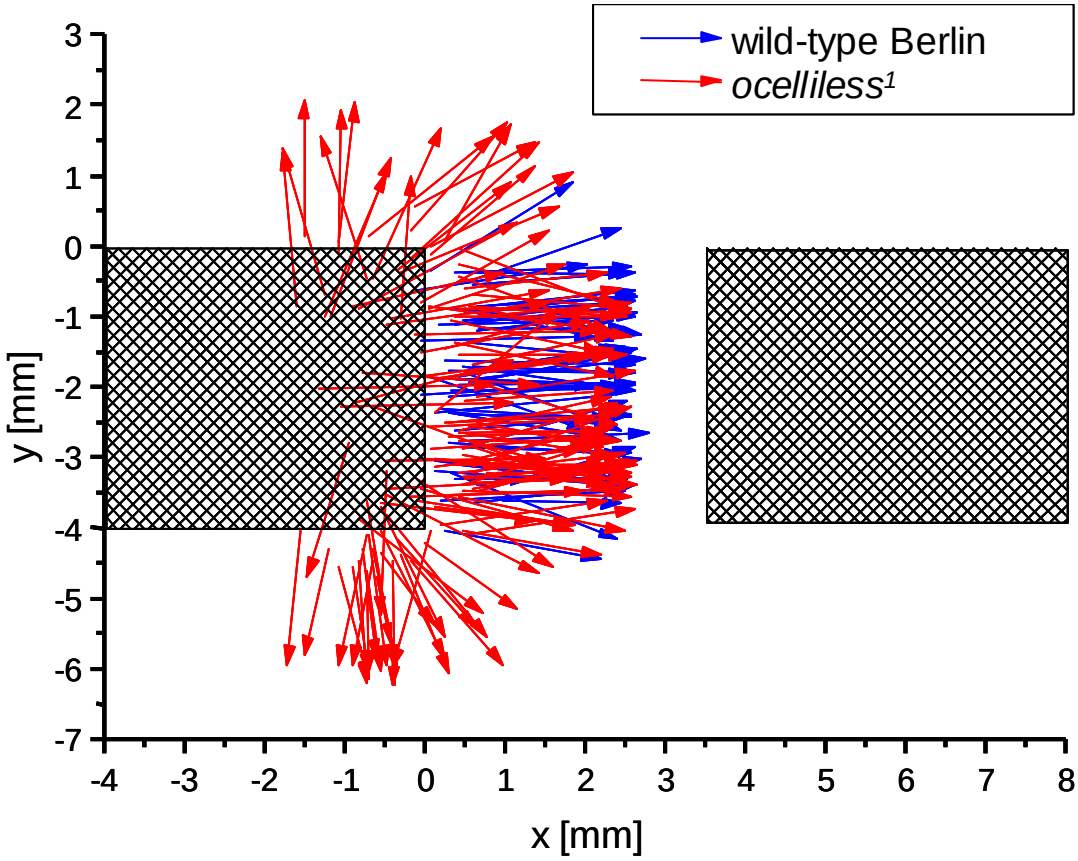

Figure 22: Body orientation of *oc*^{1} and wild-type Berlin flies during the last leg-over-head stroke before making contact to the other side or giving up the attempt

Each arrow represents one climbing attempt; the arrowheads symbolize the position of the head, the ends of the arrows represent the abdomen position. It is immediately obvious that attempts of wild-type flies are more or less parallel to the optimal climbing direction while attempts of *oc*^{1} flies show a wide scatter. Some of the attempts even deviate more than 90°.

N,n (wild-type Berlin) = 10,58; N,n (*oc*^{1}) = 19,134

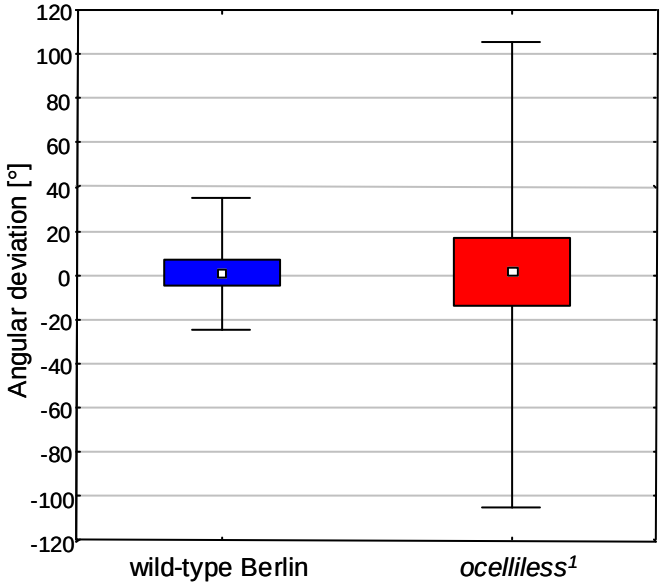

Figure 23: Median angular deviation

The median angular deviation is about 0° for both wild-type (1.13±10.28°) and *oc*^{1} (1.79±46.16°). However, the angular scatter in the *oc*^{1} data is huge. This graph shows the same dataset as shown in **Figure 22**.

Boxes show 25%- and 75%-quartiles, whiskers show the whole range of the data. The median is depicted by the small rectangle.

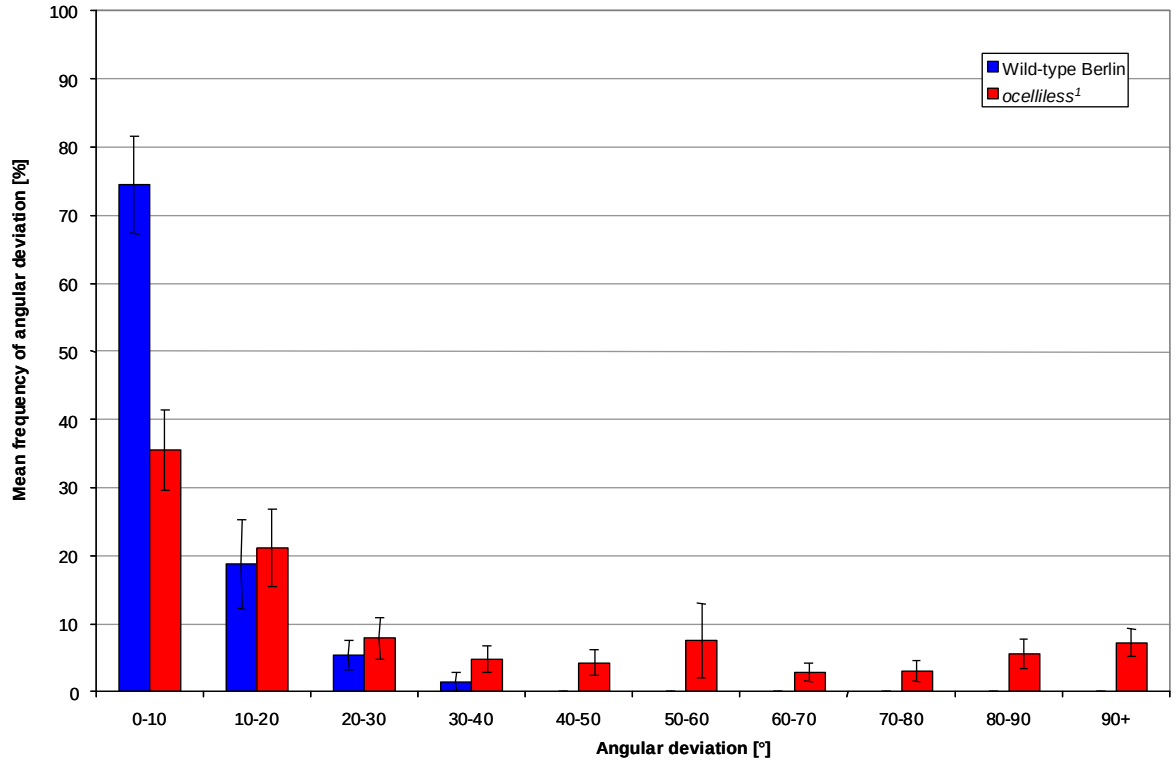

Figure 24: Mean frequency of angular deviation

In wild-type Berlin flies, more than 90% of all climbing attempts fall into the group of error angle of less than 20°. In *oc*^{1} flies, the error angles are distributed over a wide range, even deviations from the optimal climbing direction of more than 90° can be seen. This graph shows the same dataset as shown in **Figure 22**. Bars denote means, error bars SEMs.

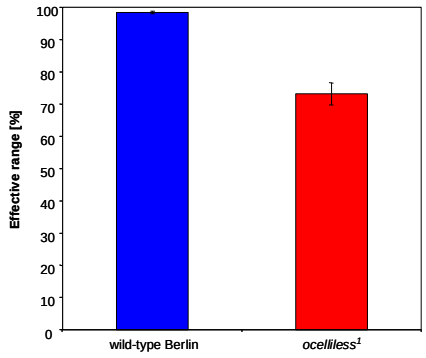

Figure 25: Effective range

The effective mean percentage of the body length that is used for climbing can be calculated by taking the cosines of the error angles. Low error angles have nearly no influence on the climbing efficiency, but as the angles get higher, it will get harder for the mutant flies to cross the gap. At 90°, 0% of a fly's body length is used to get to the other side. This graph shows the same dataset as shown in **Figure 22**. Bars denote means, error bars SEMs.

To test for an influence of the missing ocelli in this behaviour, wild-type Berlin males were tested with their ocelli occluded by application of a light-tight black paint (Schmincke Aerocolor 28870). The coverage of the ocelli was inspected again after the experiment, so that data of flies which had scratched off the paint could be discarded. There was no difference in the climbing direction between flies with covered ocelli and control flies (**Figure 26**).

Next the influence of the mushroom bodies on this behaviour was analysed. These brain structures can be conveniently ablated by administering HU to newly hatched larvae (de Belle & Heisenberg, 1994). Flies with ablated mushroom bodies perform equally well as wild-type Berlin flies (**Figure 26**). Sham-treated controls were dispensable as there was no phenotype.

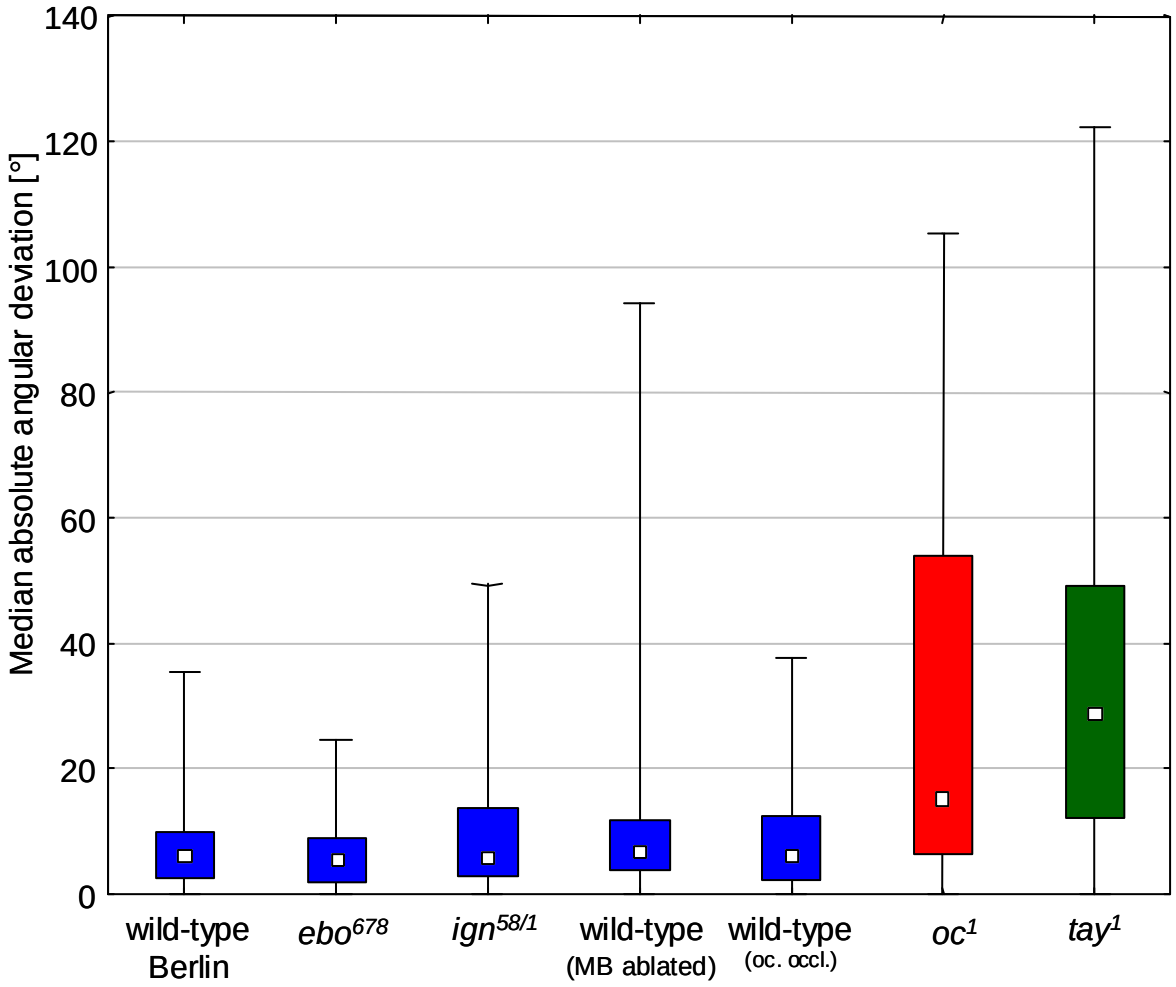

Figure 26: Median absolute body angles taken from climbing a 3.5mm gap.

Wild-type flies show a narrow distribution of absolute body angles, about 75% of all attempts have error angles of less than 10°. In *oc*^{1} mutants, the error angles are far more distributed. This phenotype is not caused by the missing ocelli, as wild-type flies with covered ocelli still perform in a wild-type fashion. Also it is not mediated by an Ignorant-dependent short-term memory, as *ign*$^{58/1}$ mutants show no sign of the phenotype. The mushroom bodies are also dispensable for this behaviour as mushroom body ablated flies also perform normal. As there was no phenotype, the handling control was dispensable. Just *tay*^{1}, another structural mutant of the protocerebral bridge, shows a comparable phenotype. All other genotypes are not significantly different (Kruskal W, p = 0.135) from wild-type. *tay*^{1} and *oc*^{1} are not significantly different from each other (p=0.064). Both, *oc*^{1} and *tay*^{1} are highly significantly different from wild-type (p < 0.001 for either genotype). Boxes show 25- and 75% quartiles, whiskers show the whole range of the data.

N=(10,9,12,15,6,18,18); n=(58,100,92,42,91,134,82)

It was also tested whether S6KII dependent working- / short-term-memory might be needed to perform well in this behaviour. The *ignorant*$^{58/1}$ mutant (*ign*$^{58/1}$), which lacks the S6KII kinase, fails in the detour paradigm (Neuser et al., 2008). Since one possible reason for the failure of protocerebral-bridge defective flies might have been a loss of an orientation memory for the climbing direction, it seemed reasonable to test the memory mutants for their climbing behaviour. Flies might judge the gap as manageable in an earlier planning phase (and store the direction) but than loose direction during the execution phase. *ign*$^{58/1}$ flies showed a clearly wild-type level of scatter at the 3.5 mm gap (**Figure 26**, p = 0.135).

In the same paper of Neuser et al. (2008) it was also shown that the ellipsoid body is important for keeping orientation during walking, when the target gets out of sight. To test whether the ellipsoid body also influences the direction of climbing, structural mutants as well as flies that express tetanus toxin in the ellipsoid body were tested. Neither the structural mutant *ebo*^{678} (Ilius et al., 1994, Strauss & Heisenberg, 1993) nor flies that express TNT under the control of the c232-GAL4 driver in the ring neurons of the ellipsoid body (Renn et al., 1999, Neuser et al. 2008) show an abnormal behaviour in their climbing (**Figure 27**). In conclusion, the working memory for directions found for the visual orientation during walking is not involved in climbing behavior.

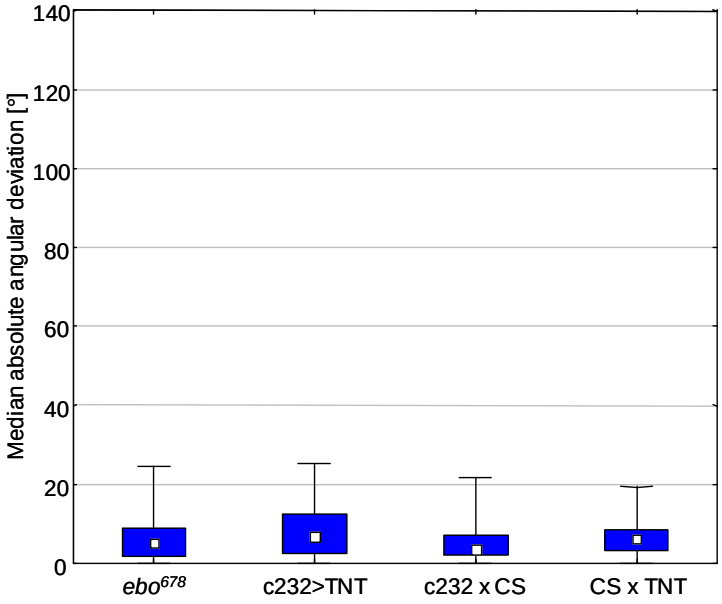

Figure 27: An intact ellipsoid body is not needed for a small scatter in climbing direction.
Flies with a disrupted ellipsoid body still perform well in gap crossing. *ebo*^{678} possess a structurally abnormal ellipsoid body. Also expression of tetanus toxin in the ring neurons of the ellipsoid body to shut down synaptic transmission does not influence the behaviour (Kruskal Wallis test, p = 0.092). Boxes show 25- and 75% quartiles, whiskers show the whole range of the data.

Genotypes :
c232>TNT: +/Y;UAS-TNT/+;c232-GAL4/+
c232xCS: +/Y;+/+;c232-GAL4/+
CS x TNT : +/Y ;UAS-TNT/+;+/+

N=(9,7,7,7); n=(100,64,67,59)

To test whether the structural defect in the protocerebral bridge is the cause for the distinct behaviour in *oc*^{1}, we wanted to partially rescue the *oc*^{1} bridge using a cDNA transgene of *otd*. To express *otd* in an *oc*^{1} background during a certain time frame in development, either 007Y-GAL4/tubGAL80^{ts} or hs-GAL4 where used. In both combinations, the flies either still had an *oc*^{1}-like bridge or were developmentally lethal, depending on the point in time and the duration of the expression of *otd*. We decided to focus on other mutants with a disruption of the protocerebral bridge. One of this mutants, *no bridge*^{KS49} (*nob*^{KS49}), shows climbing attempts into the void like *oc*^{1}, but the probability of a climbing attempt or even an approach to the gap is very low, a statistical analysis would have been too time consuming. So the main focus was put on the *tay*^{1} mutant, as there were rescue constructs for this line available (**see chapter 3.1 on *tay bridge***).

3.2.2 Climbing behaviour of *tay bridge*^{1} flies

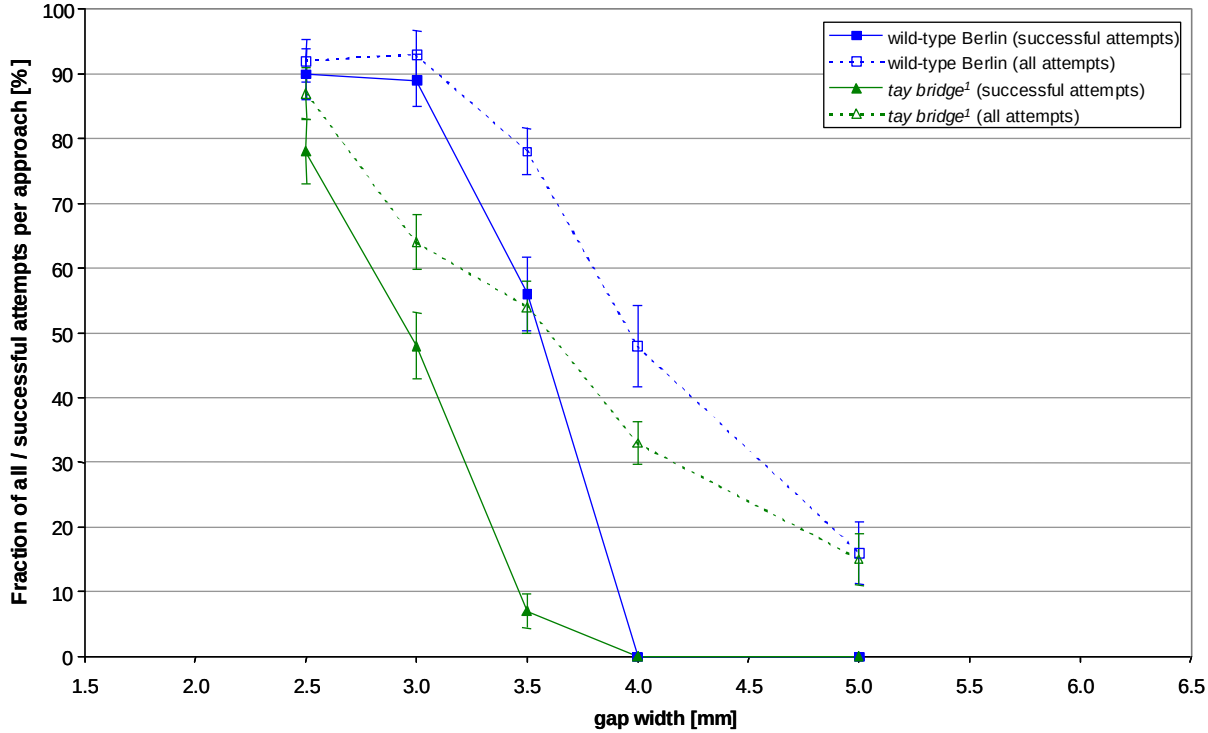

Figure 28: *tay bridge*^{1} mutant flies fail at broad gaps not different from *oc*^{1} flies.

Like *oc*^{1} flies (**Figure 21**), *tay*^{1} flies can cross small gaps. But as the gaps get broader, the rate of success drops. In contrast to *oc*^{1}, *tay*^{1} shows a lower tendency to initiate climbing. Another problem (that can not be inferred from this graph) is their lower activity and the thereby lower chance of an approach to the gap. The graph shows means and SEMs.

N= (18, 10), n = (179, 97)

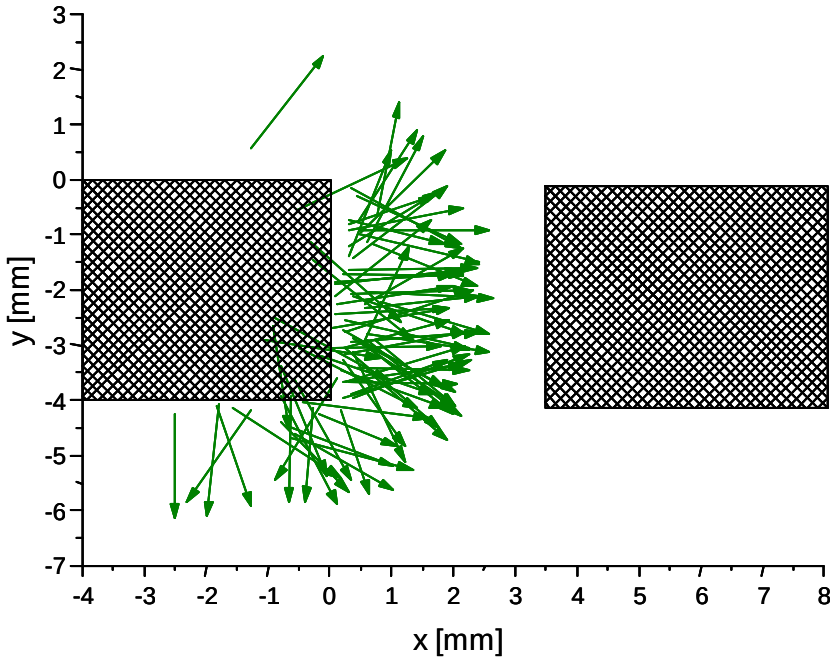

Figure 29: *tay*^{1} mutant flies fail to keep the correct climbing direction not different from *oc*^{1}.

When looking at the distribution of climbing attempts in *tay*^{1} mutant flies, one can see a similar phenotype as in *oc*^{1}. In both protocerebral bridge mutant lines, there are climbing attempts that are going completely astray.

N=18; n=82

A disadvantage of *tay*[1] in comparison to *oc*[1] is their lower probability to initiate climbing (**Figure 28**) and the overall reduced activity of the flies. It takes far longer time to get a certain number of climbing attempts or even approaches to the gap. Nevertheless, *tay*[1] flies show a similar distribution of error angles as *oc*[1] flies when trying to cross a gap (**Figure 29**).

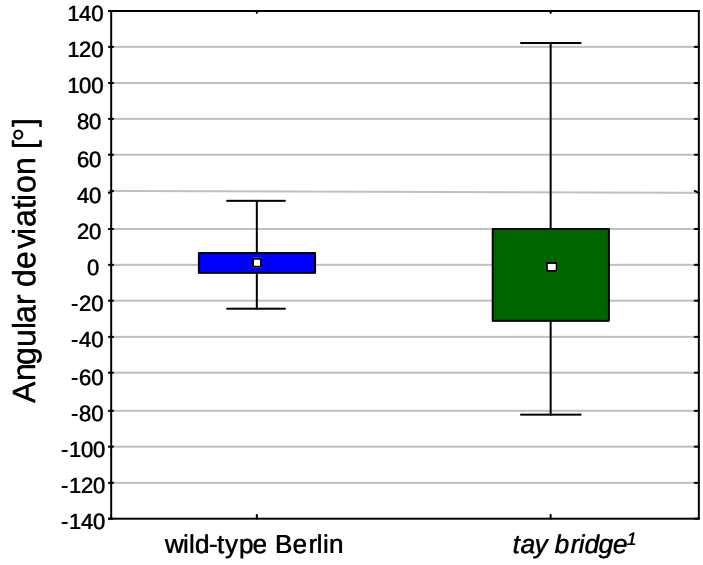

Figure 30: Median angular deviation

In *tay*[1] as in *oc*[1], the median angular deviation (-1.38±45.81) is close to 0° but the distribution is much broader than in wild-type Berlin. This graph shows the same dataset as in **Figure 29**. Boxes show 25- and 75% quartiles, whiskers show the whole range of the data. The median is depicted by the small rectangle.

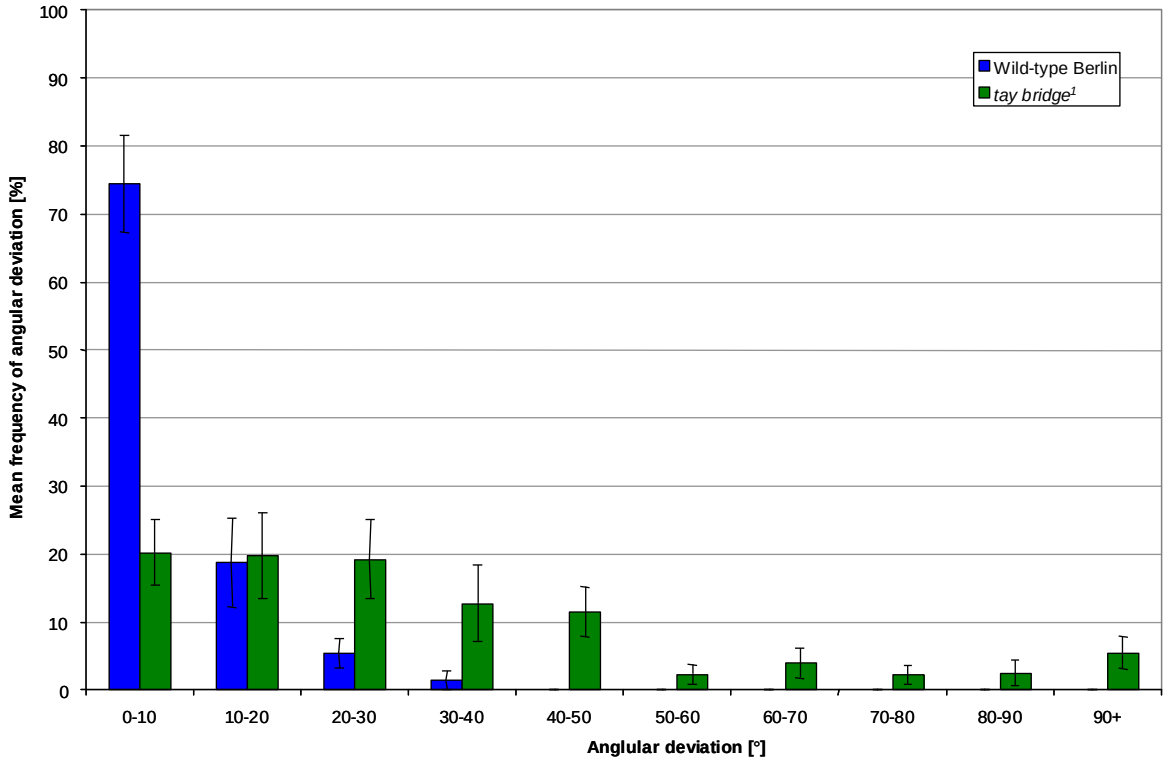

Figure 31: Mean frequency of angular deviation

The distribution of error angles in *tay bridge*[1] looks very much like that of *oc*[1]. This graph shows the same dataset as shown in **Figure 29**. Bars denote means, error bars SEMs.

3.2.3 Partial Rescue Experiments

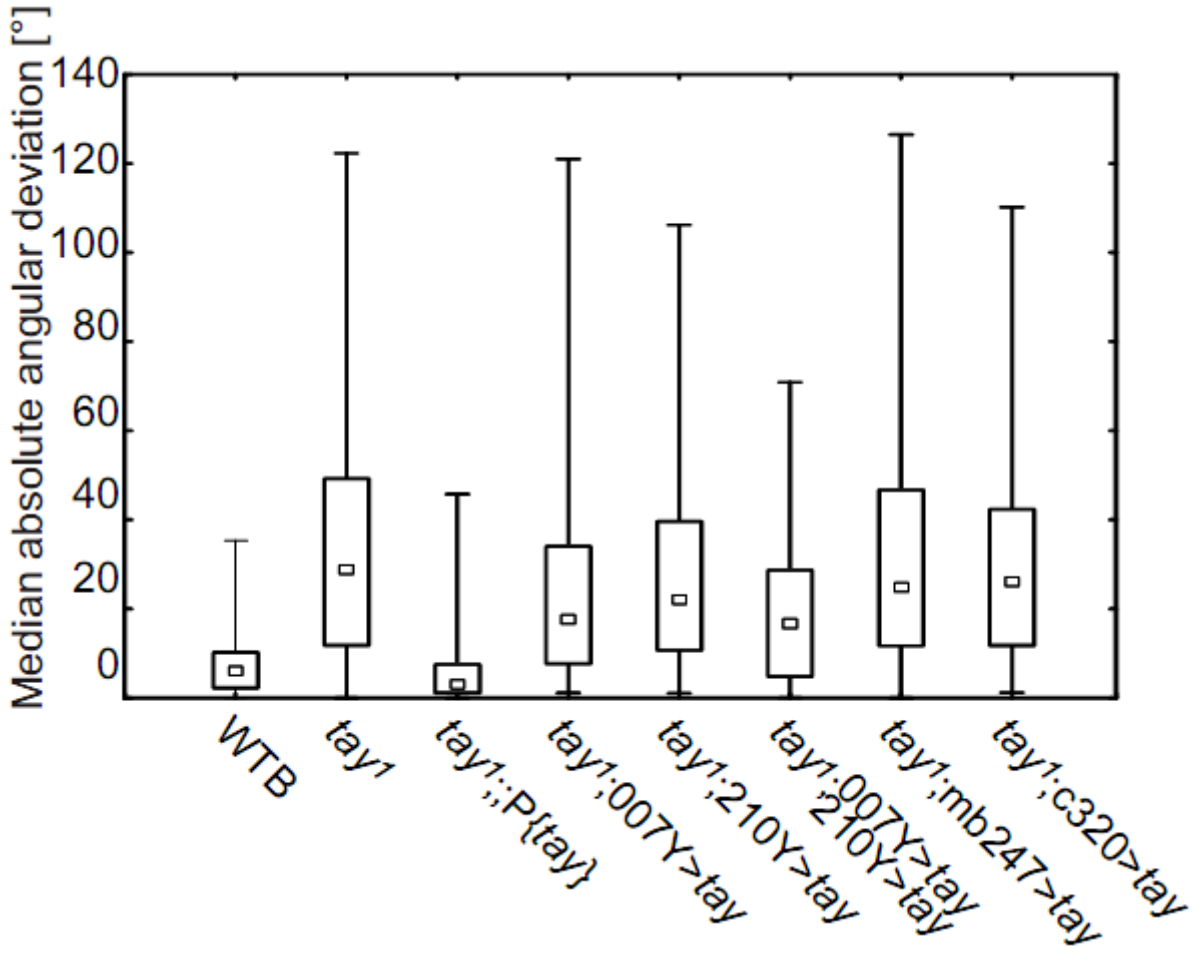

Figure 32: The median absolute body angle in *tay bridge*^{1} mutant flies, WT Berlin, and various genomic and partial rescue attempts

In *tay*^{1} mutant flies the absolute median body angle is more than 30°. The genomic rescue with P{tay}^{D1} is complete, the median absolute angular deviation is back to wild-type level. All attempts to rescue with different GAL4-lines driving UAS-*tay* did not result in a significant rescue. Boxes show the 25- and 75% quartiles, whiskers show the entire range of the data. For statistics see **Table 17**.

N=(10,18,8,32,11,18,7,7); n=(58,82,94,173,67,84,39,55)

Several rescue constructs for *tay* were at hand, both genomic and UAS-*tay*. Like in Buridan's behaviour, the genomic rescue by the P{*tay*}^{D1} construct completely reverts the structural as well as the behavioural phenotype (**Figure 32**; p = 1 against WTB). The next obvious guess was a partial rescue approach using the line 007Y-GAL4, as this line had given a full rescue for the structural phenotype as well as for the behaviour in Buridan's behaviour and optomotor compensation. The 007Y-GAL4 expression has been discussed above. Unexpectedly, the scatter in *tay*^{1}/Y;UAS-*tay*/II;007Y-GAL4 flies with restored protocerebral bridges was not significantly reduced in comparison with the *tay*^{1} mutant (**Figure 32**; p = 0.782).

Despite the negative outcome of the statistical tests it was nonetheless obvious that the quality of the climbing attempts had changed in the 007Y- and 210Y-rescue flies. Despite the fact that they were showing a high deviation from the direct path, a higher percentage at least pointed to the opposite side. The attempts were therefore classified into two categories: attempts that target the opposite side and attempts that miss the other side of the gap (**Figure 33**). The latter category was further binned into 20°-categories by the excess deviation by which the longitudinal body angle had missed the opposite side. Attempts pointing at the distal side have (at least in principle) a chance to succeed, whereas attempts directed into the void are certainly bound to fail (**Figure 34**). The excess deviation of *tay*^{1}/Y;UAS-*tay*/II;007Y-GAL4 flies is not significantly different from wild-type (p=0.367) but highly significantly different from the *tay*^{1} mutant (p=0.027).

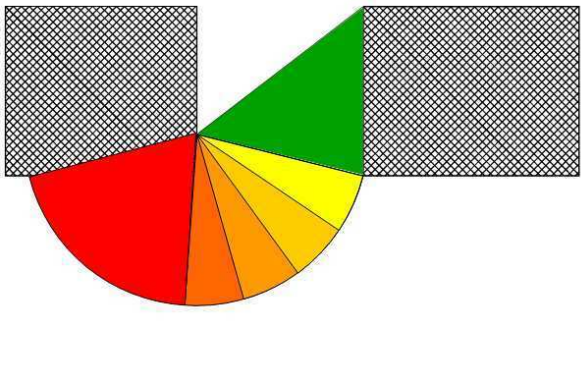

Figure 33: Definition of the excess deviation

The excess deviation describes the "futility" of a climbing attempt. This takes into account that an attempt with a large error angle that is nevertheless still targeted at the other side has a higher chance for successful crossing than an attempt with a lower angle that is clearly missing the opposite wall.

To control for the additional expression of 007Y-GAL4 in the mushroom bodies, UAS-*tay* was driven with the mushroom body driver mb247-GAL4 line. This expression alone does neither rescue the structural defect in the protocerebral bridge nor the angular scatter (**Figure 32**; p=1 against *tay*^{1}) or the excess deviation (**Figure 34**; p=1 against *tay*^{1}).

Other bridge drivers tested were 210Y-GAL4 (Renn et al., 1999, Poeck et al. 2008) and c320-GAL4 (Aso et al., 2009). Both lines express in the adult bridge but fail to rescue the structure of the protocerebral bridge. This might be due to expression in the wrong time-window during development and / or in the wrong subset of cells. The climbing behaviour in c320-rescue flies is at mutant levels, neither the scatter (**Figure 32**; p=1 against *tay*^{1}) nor the excess deviation is rescued (**Figure 34**; p=1 against *tay*^{1}).

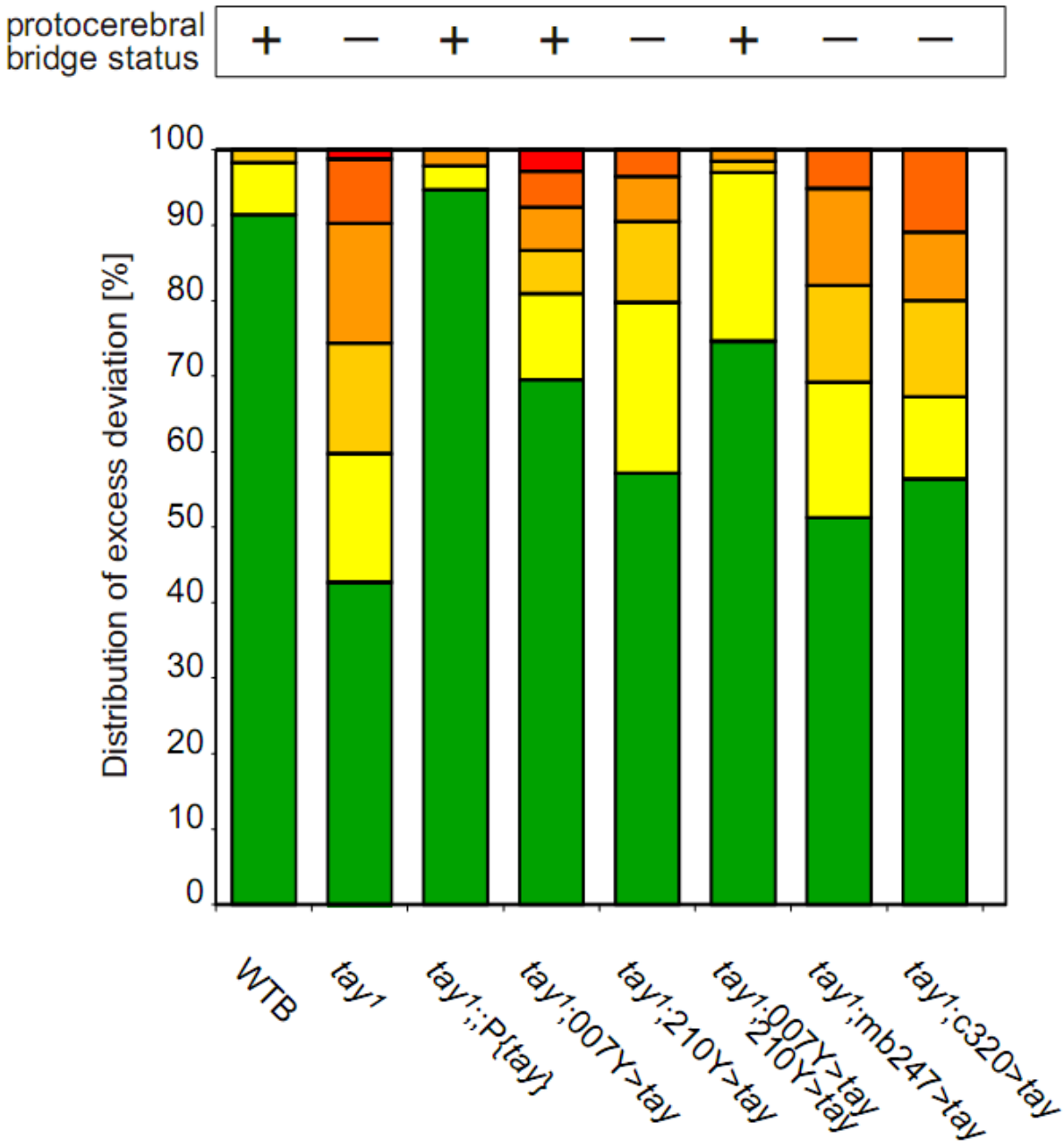

Figure 34: Excess deviation in *tay bridge*^{1}, WT and various rescue constructs.

More than 50% of all climbing attempts of *tay*^{1} flies miss the opposite side. About 20% of the attempts show a considerable deviation of more than 40°. Shown is the excess deviation, i.e. the residual angles between the closer side edge of the gap and the additional deviation of the body axis if there is any. If the body angle is within the limits given by the edges of the other side, the event falls into the category 0°. The genomic rescue is complete, while in the 007Y-driven and the double rescue with 007Y-GAL4 and 210Y-GAL4, a significant improvement to the mutant can be seen. 210Y-GAL4 alone shows an intermediate improvement. All other genotypes are not significant different from *tay*^{1}. The top row shows the status of the protocerebral bridge. A "+" indicates wild-type bridge, a "-" indicates mutant bridge. See **Table 18** for statistics.

Same data as in **Figure 32**

The 210Y-GAL4 rescue gave a different picture. Although the primary scatter is not significantly improved when compared to *tay*^{1} flies (**Figure 32**; p=1), the excess deviation shows intermediate improvement (Fig.2C; p=1 against *tay*^{1}, p=0.062 against WTB).

The effect of both drivers seems to be additive, when expressing UAS-*tay* under the control of 007Y-GAL4 plus 210Y-GAL4 in the same fly. The excess deviation is not significantly different from WTB (p=1), but highly significantly different from tay^1 (**Figure 34**; p=0.004).

3.2.4 Rescue Flies at the Reduced-Visibility Paradigm

As the 007Y-GAL4 rescue did not restore the wild-type precision of the climbing direction, the significant improvements in climbing performance might be attributed to a work-around solution. This might be a visual targeting mechanism that helps the flies to target the front surface of the landing site. The front surface has a stronger influence on the flies' behaviour than the top surface of the climbing block as wild-type flies show a lower initiation rate for climbing, if only a top surface (**Figure 35**) is presented (Pick & Strauss, 2005).

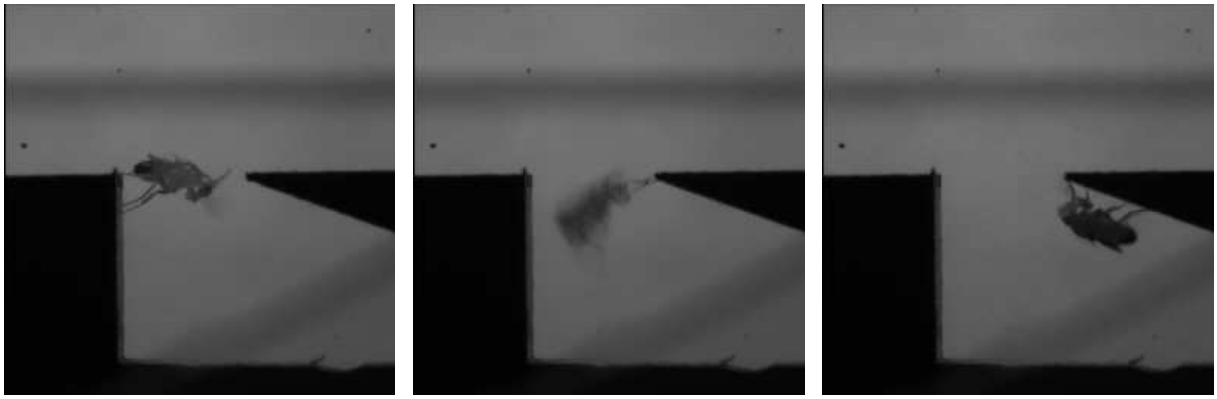

Figure 35: Wild-type fly crossing special gap

When no solid opposite wall is presented, the rate of climbing initiation is decreased. Nevertheless, flies will still show climbing attempts and do succeed in crossing.

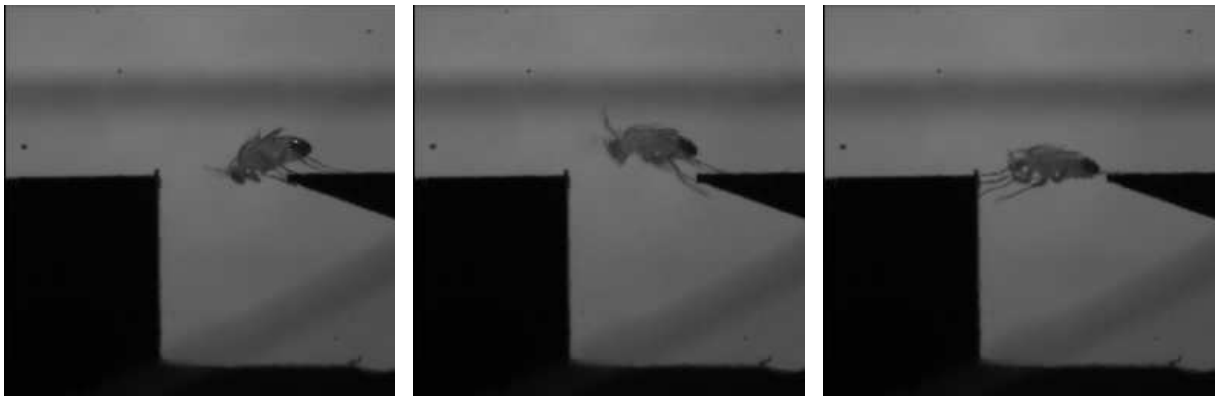

Figure 36: Crossing is also possible in the opposite direction

It is even possible for wild-type flies to cross the gap into the opposite direction, starting from the overhanging side. This happens only in rare cases as it is very difficult for the fly to position the middle legs efficiently.

Wild-type flies are also able to cross this kind of gap when they attach their front legs to the upper side of the gap. Crossing events in the other direction – from the overhanging cliff to the solid side – are also possible but happen only rarely as it is difficult for the fly to push up the body with the middle legs in this position (**Figure 36**).

When statistically comparing the angular deviation or the excess deviation in the wild-type, both are indistinguishable from data acquired at the solid gap (compare **Figure 32** & **Figure 34** to **Figure 37** & **Figure 38** respectively, p=1, p=1 for WTB).

As expected, *tay*^{1} mutant flies show a high scatter and excess deviation also in the modified paradigm. It is noteworthy however, that the behaviour does not worsen any further (**Figure 37** & **Figure 38**; both p=1 against *tay*^{1} tested in the normal paradigm).

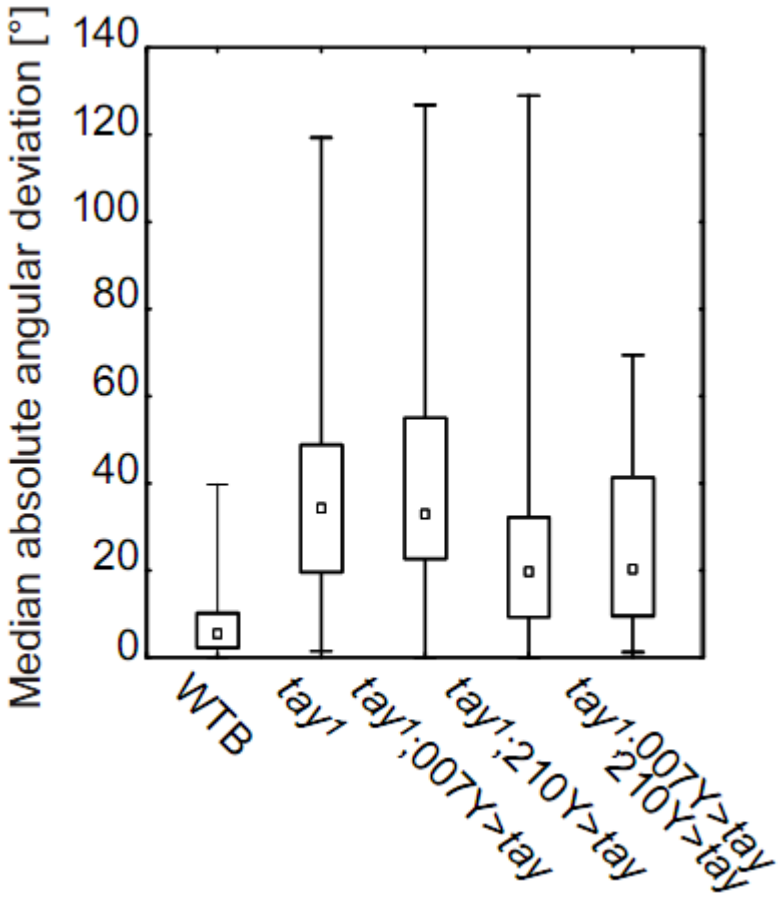

Figure 37: Median absolute angular deviation at the reduced visibility gap

When no opposing side is presented, still none of the rescue groups is significantly different from *tay*^{1} ($p > 0.05$ against *tay*^{1}). All groups are still highly significantly different from the wild-type ($p < 0.001$). Boxes show 25%- and 75%-quartiles, whiskers show the whole range of the data. Medians are depicted by the small boxes. See **Table 17** for statistics.

N=(12,11,15,10,18)
n=(80,53,75,52,90)

When testing now *tay*^{1}/Y;UAS-tay/II;007Y-GAL4 in this modified setup, the excess deviation went back to mutant level (**Figure 37**; p=1 against *tay*^{1}). By reducing the visibility of the opposite side the work-around solution of visual targeting becomes non-functional. In contrast, the intermediate rescue effect of 210Y-GAL4 remains the same in the diving board paradigm as in the standard block assay (**Figure 37**; p=1 against 210Y-GAL4 at the normal gap). With expression of both drivers, 007Y-GAL4 and 210Y-GAL4 in the same flies the

rescue falls back to the intermediate level of 210Y-GAL4 alone (**Figure 37**; p=1 against 210Y-GAL4 at the reduced visibility gap).

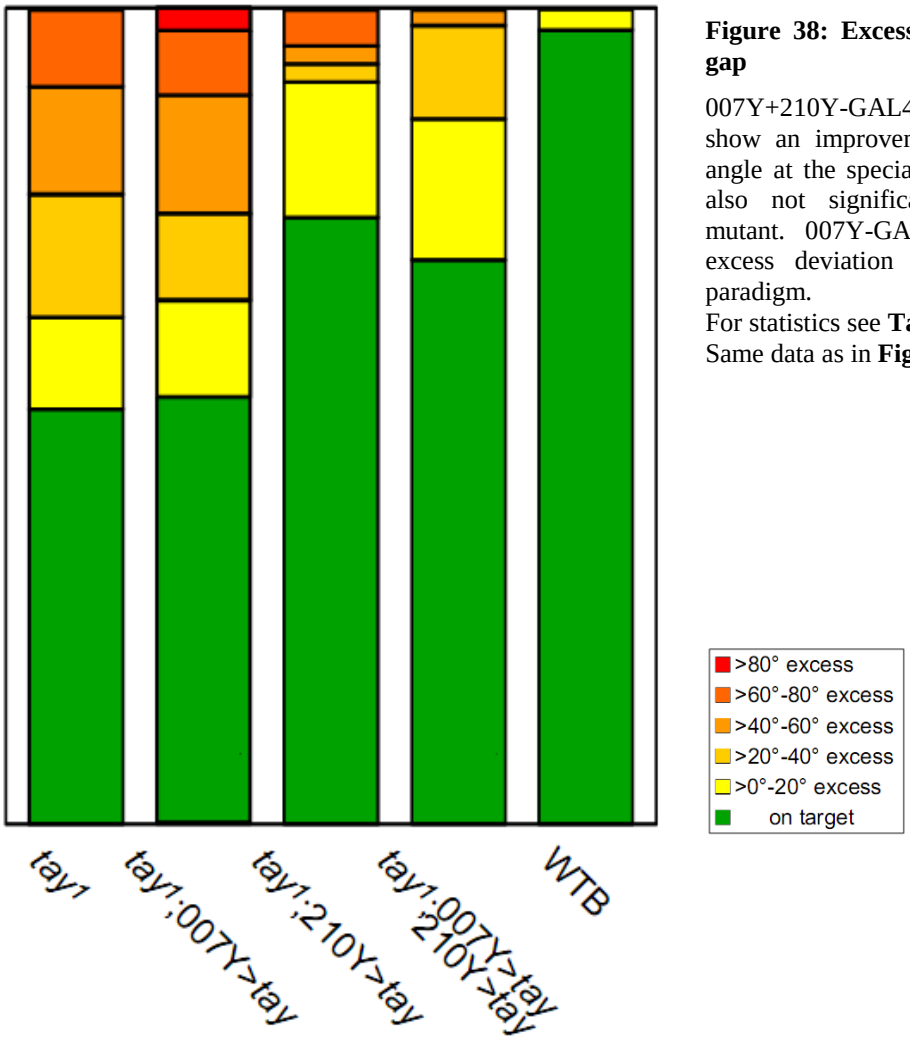

Figure 38: Excess deviation at the special gap

007Y+210Y-GAL4 and 210Y-GAL4 both show an improvement of the residual error angle at the special gap, but both groups are also not significantly different from the mutant. 007Y-GAL4 does not rescue the excess deviation in the reduced visibility paradigm.
For statistics see **Table 18**.
Same data as in **Figure 37**

In summary, the restoration of the protocerebral bridge in *tay*^{1} flies by the genomic construct P{*tay*}^{D1} resulted in a full rescue whereas the expression of UAS-*tay* via 007Y-GAL4 did rescue the bridge but not the robust alignment of climbing as it is found in WTB flies. However, flies with an intact protocerebral bridge do target the other side as the improvements in the parameter excess deviation show. The assumed basis is visual targeting as the advantage gets lost upon removing the opposite side surface of the landing site. The situation is different in 210Y-rescue flies. Their behavioral rescue effect is additive to the 007Y-partial rescue (as seen in double driver flies) and stable against reducing the visibility of the target side.

Table 17: Statistics for angular deviation

Kruskal Wallis analysis of ranks with correction for multiple comparisons

		210Y (r.v.)	007Y (r.v.)	*tay*^{1}(r.v.)	WTB (r.v.)	c320	mb247	007Y+210Y	210Y	007Y	p{*tay*}	*tay*^{1}	WTB	**R**	**N | n**
		536.43	707.75	678.89	265.44	603.84	579.21	462.31	589.38	527.36	213.11	627.26	277.50		
tay^{1};UAS-*tay*;GAL4	full block														
														WTB	10 | 58
													0.000000	*tay*^{1}	18 | 82
												0.000000	1.000000	p{*tay*}	8 | 94
											0.000000	0.782448	0.000001	007Y	32 | 173
										1.000000	0.000000	1.000000	0.000000	210Y	18 | 84
									0.573252	1.000000	0.000006	0.041991	0.028849	007Y+210Y	11 | 67
								1.000000	1.000000	1.000000	0.000000	1.000000	0.000037	mb247	7 | 39
							1.000000	0.561271	1.000000	1.000000	0.000000	1.000000	0.000000	c320	7 | 55
	reduced visibility bock														
						0.000000	0.000002	0.003116	0.000000	0.000000	1.000000	0.000000	1.000000	WTB (r.v.)	12 | 80
					0.000000	1.000000	1.000000	0.003652	1.000000	0.066498	0.000000	1.000000	0.000000	*tay*^{1} (r.v.)	11 | 53
				1.000000	0.000000	1.000000	1.000000	0.000035	0.782856	0.000509	0.000000	1.000000	0.000000	007Y (r.v.)	15 | 75
			0.011930	0.348636	0.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.000000	1.000000	0.000008	210Y (r.v.)	18 | 90
		1.000000	0.265142	1.000000	0.000002	1.000000	1.000000	1.000000	1.000000	1.000000	0.000000	1.000000	0.000041	007Y+210Y (r.v.)	10 | 52

Table 18: Statistics for excess deviation

Kruskal Wallis analysis of ranks with correction for multiple comparisons

		210Y (r.v.)	007Y (r.v.)	*tay*^{1}(r.v.)	WTB (r.v.)	c320	mb247	007Y+210Y	210Y	007Y	p{*tay*}	*tay*^{1}	WTB	**R**	**N | n**
		488.56	604.60	605.11	356.31	580.00	589.95	453.04	549.15	507.49	371.18	646.45	383.38		
tay^{1};UAS-*tay*;GAL4	full block														
														WTB	10 | 58
													0.000009	*tay*^{1}	18 | 82
												0.000000	1.000000	p{*tay*}	8 | 94
											0.018485	0.026655	0.367050	007Y	32 | 173
										1.000000	0.003279	1.000000	0.061830	210Y	18 | 84
									1.000000	1.000000	1.000000	0.003858	1.000000	007Y+210Y	11 | 67
								1.000000	1.000000	1.000000	0.005634	1.000000	0.044225	mb247	7 | 39
							1.000000	1.000000	1.000000	1.000000	0.001665	1.000000	0.023895	c320	7 | 55
	reduced visibility bock														
						0.000796	0.002783	1.000000	0.001556	0.008711	1.000000	0.000000	1.000000	WTB (r.v.)	12 | 80
					0.000094	1.000000	1.000000	0.332062	1.000000	1.000000	0.000197	1.000000	0.004312	*tay*^{1} (r.v.)	11 | 53
				1.000000	0.000007	1.000000	1.000000	0.143327	1.000000	1.000000	0.000015	1.000000	0.000962	007Y (r.v.)	15 | 75
			0.159119	0.404359	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.003139	1.000000	210Y (r.v.)	18 | 90
		1.000000	1.000000	1.000000	0.803970	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.162710	1.000000	007Y+210Y (r.v.)	10 | 52

3.3 *Climbing sisyphus*

3.3.1 Introduction

The mutant line *climbing sisyphus* was generated in Roland Strauss' screen for mutants with slow walking behaviour (Strauss, 2002b). For this screen, several thousand flies, F1 male offspring of ethyl methanesulfonate (EMS) treated parent flies, were generated. EMS is an organic compound that can induce point mutations by guanine alkylation. F1 males were tested in the Fast Phototaxis paradigm and the slowest flies were used to establish lines. Only lines with the mutation on the X-chromosome were kept and further analysed. When looking at the gross morphology, *climbing sisyphus* shows no apparent anatomical phenotype (R. Strauss, personal communication).

3.3.2 Gap Crossing Paradigm

In this PhD thesis, some of the walking defective mutant lines were analysed in the gap crossing paradigm.

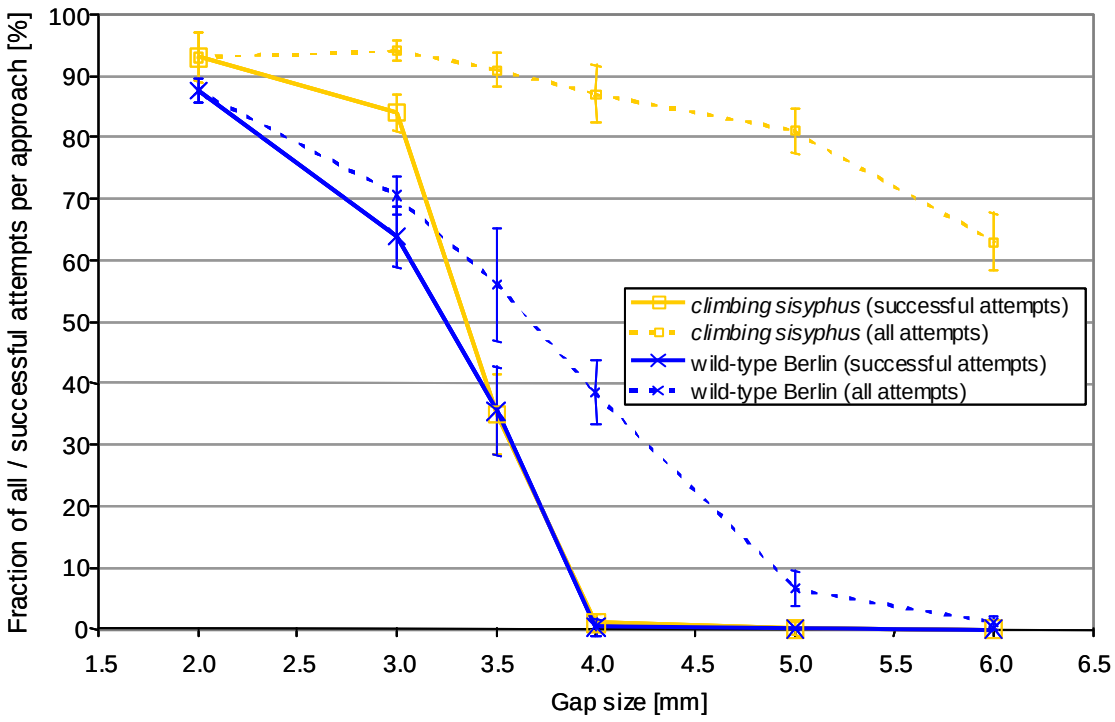

Figure 39: Climbing results for *climbing sisyphus*

Wild-type Berlin flies show a decreasing number of climbing attempts as the gap size increases. At 4.0 mm, the rate of success is nearly zero, the rate of climbing attempts is at about 40%. *climbing sisyphus* flies show a similar climbing success, nevertheless the rate of climbing attempts is substantial higher. Even at 6.0 mm, a gap of clearly insurmountable width, *climbing sisyphus* flies will initiate climbing in more than 50% of all approaches.

Finding the climbing phenotype in *climbing sisyphus* was somehow a lucky punch by virtue of the alphabet, as the internal stock name is A5. The mutant flies show a rate of success in climbing that is at the same level as wild-type. Regarding the climbing initiation, *climbing sisyphus* nevertheless looks dramatically different from wild-type flies. At 6.0 mm gap width, a distance that is clearly insurmountable by climbing flies, *climbing sisyphus* mutants will still show a much higher climbing initiation as compared to the wild-type flies. After seeing that behaviour, we decided to call the mutant *climbing sisyphus* after the character well-known from the Greek mythology.

Regarding the success of climbing attempts, *climbing sisyphus* and wild-type flies are virtually at the same level. Both show a high rate of success at small gaps, which drops at 3.5 mm gap width and reaches almost zero at 4.0 mm. Broader gaps can not be crossed by either *climbing sisyphus* nor wild-type flies. When looking at the climbing initiation, things are dramatically different. Wild-type flies reduce their probability for climbing at 4 mm and further down at 5.0 mm. At 6.0 mm hardly any climbing attempt is elicited any more. *climbing sisyphus*, in contrast, has a higher probability of climbing even at 3.5 mm and the probability stays high, even at clearly insurmountable gaps of 6.0 mm width. Nevertheless, the rate of initiation clearly drops at higher gap width, albeit at a far lower rate than in wild-type flies.

3.3.3 Fast Geotaxis

As climbing behaviour is a quite time consuming single-fly behavioural test, we decided to look for a faster way to map the location of *climbing sisyphus*. Older data generated by R. Strauss when first characterizing the walking mutants from his screen show a deficit in fast geotaxis (R. Strauss, personal communication). As *climbing sisyphus* was also isolated from this screen, a defect in walking besides the gap climbing defect was also to be expected. When testing now *climbing sisyphus* in the fast geotaxis, the walking phenotype could be reproduced in *climbing sisyphus* flies. In the mean speed as well as in the mean maximum strain speed there is a significant difference (t-Test, $p<0.001$) between wild-type Berlin and *climbing sisyphus* flies.

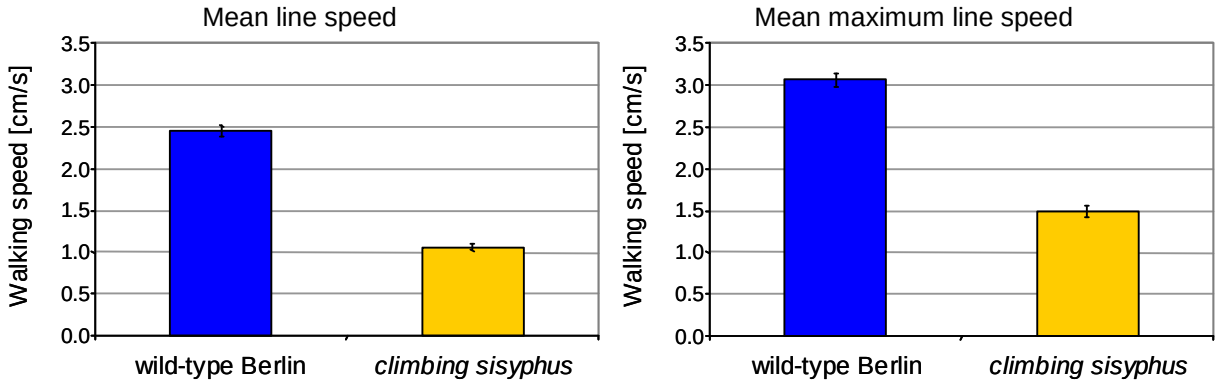

Figure 40: Fast Geotaxis in *climbing sisyphus* mutants

In the fast geotaxis paradigm, at least 18 flies per genotype with each ten runs were tested. The left graph shows the mean strain speed, i.e. the mean of mean speeds, the right graph shows the mean maximum strain speed, i.e. the mean of the fastest runs in each single fly. There is a significant difference between wild-type and *climbing sisyphus* in both mean and maximum speed (t-test, $p<0.001$, N = 20, 18).

3.3.4 Optomotor Compensation

To rule out basic vision problems, *climbing sisyphus* flies were also tested in the optomotor paradigm. The overall compensation is somewhat lower than in wild-type flies but the difference is statistically not significant (t-test, $p = 0.073$). The motion vision in *climbing sisyphus* flies seems to be intact, the climbing phenotype has to be attributed to other reasons.

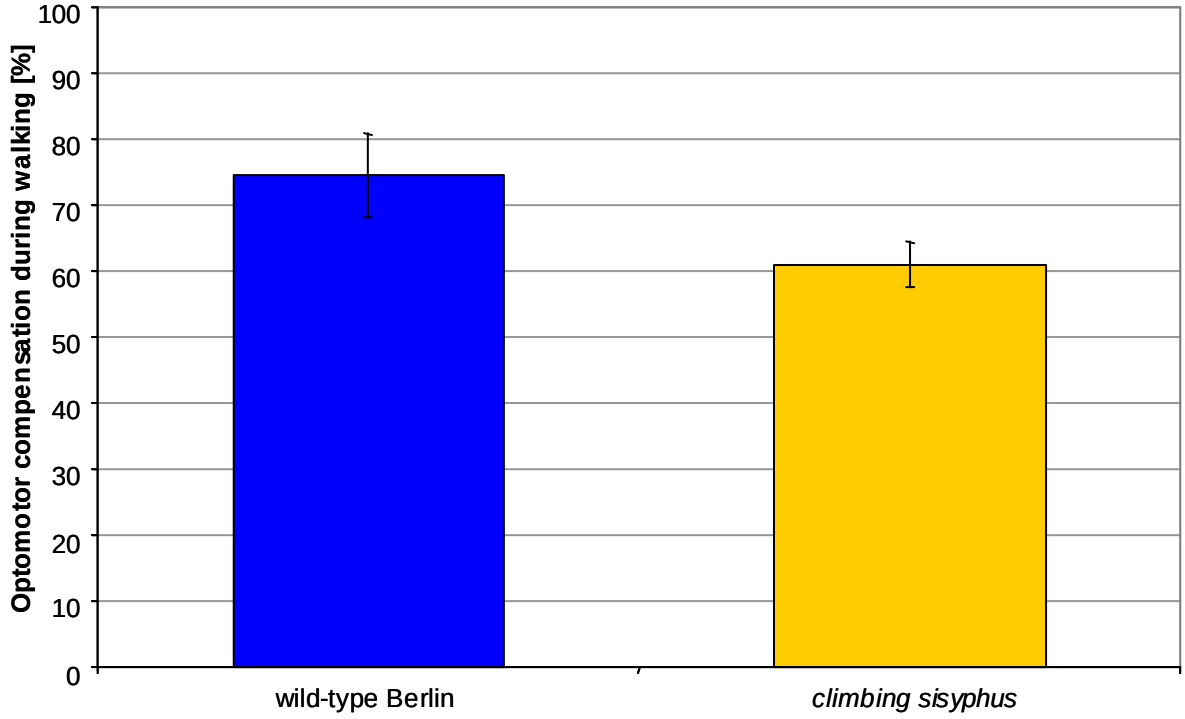

Figure 41: Optomotor compensation during walking

Both *climbing sisyphus* and wild-type flies show a strong tendency to follow the optomotor stimulus. Although there is a tendency, the difference in compensation proofed not to be statistically significant (t-test: $p = 0.073$, N = 10; 10)

3.3.5 Distance Estimation

The next step was to check for distance estimation. Wild-type flies, when challenged with two pairs of objects distinguished only by the distance from the midpoint of the walking platform, tend to patrol between the closer ones and will rarely visit the distant objects (Schuster et al., 2002). In an initial experiment, a distance of 50 mm for the closer objects and 200 mm for the distant ones was used. At these settings, the performance index for wild-type flies is 0.80, i.e. in nine out of ten approaches the fly will chose the closer object. For *climbing sisyphus*, the results are the same (PI=0.77), indicating that under these conditions the mutant has no distance-estimation phenotype (U-Test, p=0.772). Schuster et al. (2002) had shown that the relative rather than the absolute difference between two sets of objects are evaluated by the fly. Therefore, also different settings were tried. For the closer landmarks, 140 mm were used and 200 mm for the distant ones. This is comparable to the relevant difference in gap width between 3.5 mm and 5.0 mm, where *climbing sisyphus* flies show a higher rate of climbing initiation at 5.0 mm as wild-type flies at 3.5 mm. In the second experiment it was more difficult for the flies to distinguish between the two sets of landmarks, as the relative distance between them has decreased. Nevertheless, wild-type flies still show a significant preference for the closer objects (PI=0.39) and there is no significant difference to the mutant behaviour (PI=0.31, U-Test p=0.308). This shows that *climbing sisyphus* mutants have a considerable or even completely normal ability to discriminate between different distances.

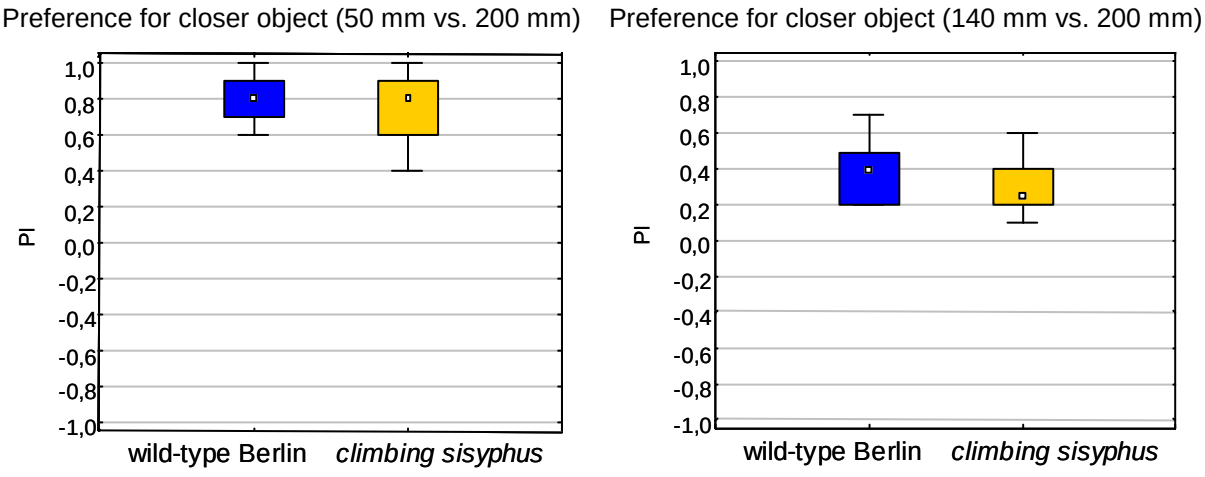

Figure 42: Distance estimation

At a setting of 50 mm for the closer and 200 mm for the distant objects, *climbing sisyphus* flies are not distinguishable from wild-type flies in their performance (U-Test, p= 0.772, N = 10, 10). Approximately 90% of all walks will be targeted to the closer stripes. At a setting of 140 mm to 200 mm, the performance index is lower for both wild-type and *climbing sisyphus* flies. The difference in relative distance referring to the middle of the walking platform has decreased. Yet, there still is no significant difference between the two genotypes (U-Test, p= 0.308, N = 10, 10). Boxes show 25%- and 75%-quartiles, whiskers denote the whole range of the data, small boxes show the medians.

3.3.6 Buridan's Paradigm

In order to further characterize the *climbing sisyphus* mutant, the flies were tested in Buridan's paradigm. When only looking at the total distance walked, *climbing sisyphus* flies are not distinguishable from wild-type flies. Both genotypes walk about 600 cm in the 15 min of the experiment (wild-type: 591.0 ±63.8 cm; *climbing sisyphus*: 576.1 ±81.5 cm). But the two strains differ in their ways how they cover this distance. Wild-type flies walk with nearly double the speed of *climbing sisyphus* flies (18.55 ±1.29 mm/s vs. 9.52±1.20 mm/s). *climbing sisyphus* partially compensates for that deficit by a somewhat higher walking activity (54.6 ±4.7%) as compared to wild-type flies (36.2 ±3.7%).

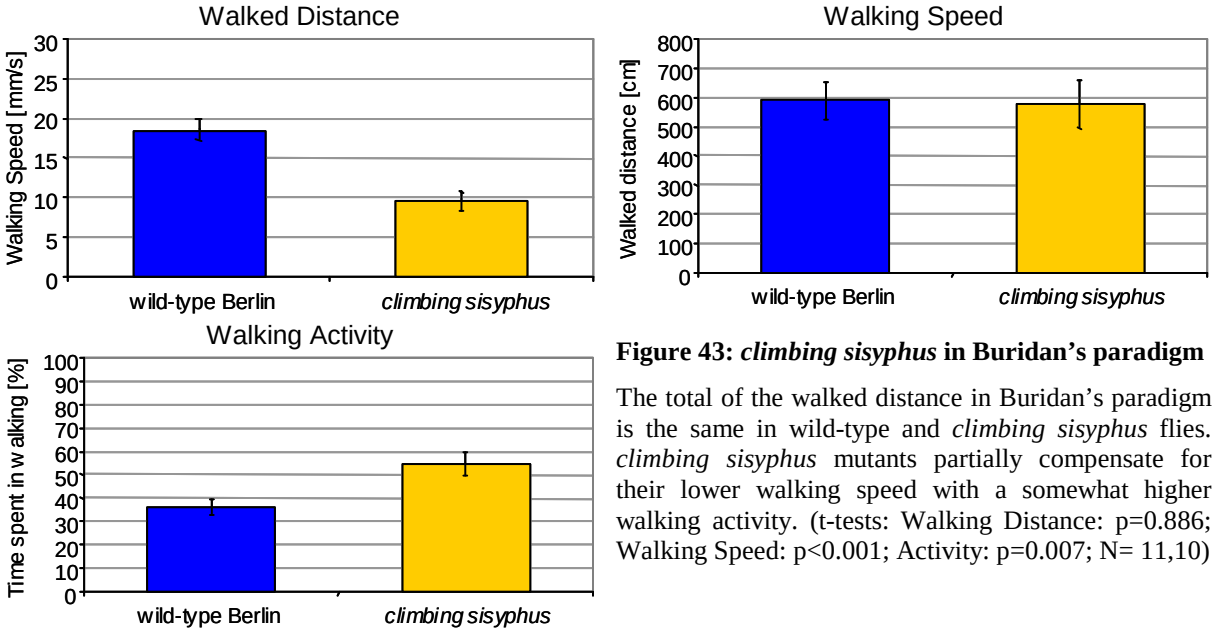

Figure 43: *climbing sisyphus* in Buridan's paradigm

The total of the walked distance in Buridan's paradigm is the same in wild-type and *climbing sisyphus* flies. *climbing sisyphus* mutants partially compensate for their lower walking speed with a somewhat higher walking activity. (t-tests: Walking Distance: $p=0.886$; Walking Speed: $p<0.001$; Activity: $p=0.007$; N= 11,10)

When evaluating the orientation towards the stripes, it was noticed that *climbing sisyphus* does orient towards objects, but less precisely than the wild-type.

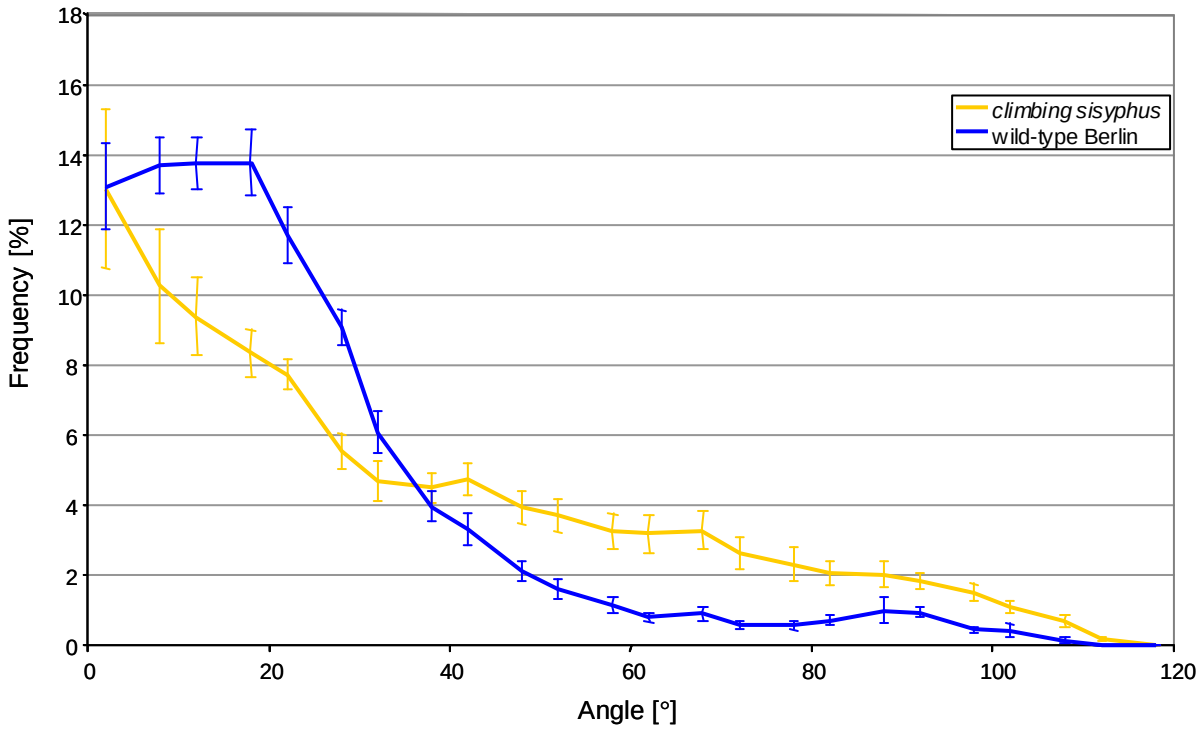

Figure 44: Orientation in Buridan's paradigm

In comparison to wild-type, *climbing sisyphus* flies show a somewhat broader distribution of error angles towards the stripes. The data show the distribution of error angles between the path increments taken every 0.2 s and the direct path to the angular-wise closer of the two landmarks (4500 readings in each 15min recording). Shown are mean values and their SEMs. Same dataset as in **Figure 43.**

3.3.7 Attempted Mapping of *climbing sisyphus*

After looking into the performance of *climbing sisyphus* flies in different paradigms, mapping the mutation causing the *climbing sisyphus* phenotype was attempted. Two paradigms seemed appropriate to use, climbing and fast geotaxis. Disregarding the high amount of experimental time needed, it was nonetheless wanted to use the single-fly climbing assay, as this was the most interesting phenotype. After all, other phenotypes might be caused by second site mutations and will not necessarily lead to the mapping of the climbing defect. As a second test the fast geotaxis paradigm allows testing a high number of flies in a relatively short time. For the complementation test, *climbing sisyphus* males were crossed to *y f* or *y cv v f car* virgins. The progeny was analysed for crossing-over events between the *climbing sisyphus* X-chromosome and the marker X-chromosome and flies with crossing-over events were tested in climbing and fast geotaxis.

Unfortunately, the marker mutations have influence on the walking speed in fast geotaxis themselves. Therefore, a lot of intermediate results were found. The climbing behaviour turned out to be highly variable at the level of the individual fly as well. Neither the climbing assay nor the Fast Geotaxis paradigm gave a consistent result for the gene locus of the

climbing sisyphus mutation. Mapping over deficiencies was not successful either, as the 40 deficiency strains from the Bloomington X-chromosome kit are each on unknown and different genetic backgrounds. For the time being the mapping had to be abandoned.

4. Discussion

4.1 The Neuronal Control of Gap Crossing Has a Modular Structure

Gap crossing behaviour in *Drosophila melanogaster* offers the unique possibility to study decision making processes and the orchestration of simpler motor actions into complex motor tasks in a genetically tractable animal. Pick and Strauss (2005) had shown that the decision to initiate climbing behaviour at a gap in the walkway is dependent on visual gap size estimation. Parallax motion gathered during the approach of the gap is evaluated to infer the gap size. Gap crossing is initiated predominantly at surmountable gaps; insurmountably broad gaps are usually not tried to overcome. Rather, the flies will walk down the vertical path into the gap or turn around. Pick and Strauss had identified a mutant line G74, which doesn't initiate gap climbing at surmountable gaps despite the fact that this mutant flies are able to climb in principle. These flies are not blind either and they possess a normal body size. In the current thesis a mutant line has been studied with the opposite behaviour: *climbing sisyphus* flies will initiate climbing at gaps which are clearly insurmountable. The finding nicely completes a collection of control modules that can be assessed with the help of mutants. The *climbing sisyphus* data are discussed below.

For the execution of climbing behaviour the flies orchestrate several motor actions in order to get out the maximum possible reach. The hind legs go iteratively closer to the edge in order to move the body as far as possible into the gap. The middle legs prop up the body in order to get the front legs into a favourable position for reaching out to the opposite side of the gap. The front legs ultimately stretch out as far as possible to get a hold of the opposite edge. They perform a unique searching behaviour that has been termed "leg-over-head-strokes" by Pick and Strauss (2005) and that cannot be seen in normal walking. Several mutant lines had been described earlier that efface specific units of the sequence of climbing motor programs. Flies of the line O151 fail to lean out into the gap and perform gap climbing while still having the body over solid ground instead of out in the gap. Flies of the line D44 fail to lift up the body with their middle legs. Rather, the body is held in a downward-tilted position and front legs are far less likely to reach the opposite edge (Pick & Strauss, 2005). In the current study a new set of lines is analysed, which define a novel module of control: the direction of the longitudinal body axis has to be controlled in the x-y plane as well and not just in the x-z plane (the problem of D44 flies). The novel set of mutants fails in a spectacular and common way in targeting the opposite side of the gap: After a correct decision to climb the flies with

defective protocerebral bridge are losing their direction and may even climb into the void at positions where there is no opposite side. Three lines with bridge defects have been studied: *no bridge*^{KS49}, *tay bridge*^{1} *(tay*^{1}*)* and *ocelliless*^{1} *(oc*^{1}*)*. One of these lines, *tay*^{1}, has been analysed at the molecular level to use it as a tool for gap crossing analysis.

4.2 The Molecular Analysis of *tay bridge* and its Function in Walking and Orientation

tay^{1} has been isolated in a screen for defective walking behaviour by R. Strauss and analysed at the molecular level by B. Poeck (Poeck et al. 2008). The behavioural analysis of *tay* constructs has been performed in the framework of this thesis. Walking behaviour was quantified in the Buridan's paradigm (Götz 1980; Strauss and Heisenberg 1993) and the ability to compensate for optomotor stimulation in a walking paradigm described by Strauss et al. (1997). Three alleles of *tay* became available but only *tay*^{1} was homozygously viable. The other two alleles *tay*^{2} and *tay*^{3} have been tested heterozygously over *tay*^{1}. Two different genomic rescue lines have been tested in addition to provide proof for the the identity of the cloned gene with the *tay* gene. The genomic rescue constructs rescued the structural phenotype of the protocerebral bridge as well as all of the known phenotypes in walking, climbing and optomotor compensation behaviour. The gene responsible for the phenotypes has been found. In the course of the analysis partial rescue experiments have been conducted using the UAS-*tay* construct in combination with different GAL4-driver lines. The experiments helped to answer the question in which neuropilar regions of the brain the *tay* gene has to be expressed in a *tay*^{1} mutant background in order to return to a wild-type behaviour.

4.2.1 *tay*^{1} Rescues in Buridan's Paradigm

The *tay*^{1} mutant shows several defects in walking and object orientation behaviour as it is tested in Buridan's paradigm. Their walking speed is reduced in comparison to the wild-type and they exhibit a lower walking activity. Surprisingly, an expression of *tay* in a *tay*^{1} mutant background with the pan-neural driver elav-GAL4 (Robinow and White, 1991; Luo et al., 1994) did only improve the walking speed but not the lack of activity. The structural defect in the protocerebral bridge was also still prominent. This might be explained by low expression strength of elav-GAL4 in those structures of the brain in which *tay* is needed. Several GAL4 driver lines with a more confined but stronger punctual expression were able to express *tay* in

levels sufficient to induce rescues for certain behaviours. The 007Y-GAL4 line rescues walking speed and activity phenotypes in the Buridan's paradigm as well as in optomotor compensation and it is the only driver line to restore the anatomical integrity of the protocerebral bridge. Expression of *tay* in the ellipsoid body by the driver lines c232-GAL4 or c819-GAL4 partially improved the walking speed, but to an far lesser extent than 007Y-GAL4.

4.2.2 *tay*^{1} Rescues in Optomotor Compensation

The pan-neuronal driver elav-GAL4 did not give a rescue of the *tay*^{1} phenotype in optomotor compensation, whereas the more restricted 007Y-GAL4 line was able to restore optomotor compensation to wild-type levels. Surprisingly, expression of UAS-*tay* by the mb247-GAL4 line was able to fully rescue the optomotor compensation as well while leaving the protocerebral bridge in its typical *tay*^{1} status with a sagittal constriction. This cross was meant as a control for the mushroom body expression in 007Y-GAL4. Still, mushroom bodies were highly unlikely to be responsible for the rescue of optomotor compensation during walking. Therefore, this neuropil was chemically ablated in *tay*^{1};UAS-*tay*;mb247-GAL4 larvae to test whether hitherto unknown expression of mb247-GAL4 outside of the mushroom bodies would be causal for this rescue. Both the mushroom bodies ablated flies and the control group that received the same treatment, except for the hydroxy urea, showed wild-type performance. The rescue was to be attributed to a set of neurons outside the mushroom bodies. Neither the protocerebral bridge nor the mushroom bodies are necessary for optomotor compensation during walking. The mb247-expression was scrutinized and a faint expression in the fan-shaped body and in two descending neurons found. The fan-shaped body neurons are the likely candidate for the rescue of optomotor compensation as there is an overlap in expression with line 007Y-GAL4 (Kirsa Neuser, personal communication, Poeck et al. 2008). The partial improvements found in the optomotor performance of *tay*^{1},np2320-GAL4>UAS-*tay* with expression in the fan-shaped body might be an additional hint into this direction.

4.3 Protocerebral Bridge Mutants in Climbing Behaviour

Three mutant strains with structural defects in the protocerebral bridge have been analysed in the framework of this thesis. All have in common that they are frequently losing their orientation towards the opposite side of the gap after having taken a correct decision to climb. In the *no bridge*^{KS49} mutant, the overall probability for climbing attempts or even approaches to the gap was so low that a statistical analysis of the behaviour would have been too time consuming. But from the rare events it was clear, that they initiate climbing into the void a well (data not shown). In *oc*^{1} flies the initiation is much higher. A partial rescue of the bridge status in *oc*^{1} flies was tried by expressing a cDNA transgene of *otd*. Driving UAS-*otd* by different GAL4 lines with expression in the protocerebral bridge and at different time points as well as durations in development, turned out to be developmentally lethal for the flies in all cases (data not shown).

By driving UAS-*tay* under the control of 007Y-GAL4, the structure of the protocerebral bridge was rescued but the scatter in climbing behaviour was not reduced to the wild-type level. However, concomitantly with the protocerebral bridge, a visual targeting mechanism could be restored that improves the excess deviation. This visual targeting is without function when the visibility of the opposite side is greatly reduced; the 007Y-GAL4 rescue flies were no longer able to target the landing site. In 210Y-GAL4 flies, a different system is partially rescued. The moderate improvement was unaffected by reducing the visibility of the target side and it is additive to the rescue caused by 007Y-GAL4. This was deduced from the behaviour of the flies that express UAS-*tay* under the control of both drivers.

4.4 *climbing sisyphus* in Gap Climbing

The *climbing sisyphus* mutant (previously called A5) was isolated in a screen for defective walking behaviour (Strauss, 2002). Up to now, no anatomical or neuroanatomical defects are known and the gene has not been identified at the molecular level. Besides the striking phenotype in climbing, *climbing sisyphus* flies are also defective in their vertical upward walking speed in the fast geotaxis paradigm. The respective mean strain speed is reduced to about 50% in comparison to the wild-type. In Buridan's paradigm, the total distance covered by *climbing sisyphus* is the same as by wild-type Berlin. The somewhat slower walking speed is compensated for by the mutant flies' higher activity, i.e. they spend a significantly higher fraction of the time in the experiment in walking. The applied EMS mutagenesis introduces point mutations by the alkylation of guanine. Due to the method it cannot be excluded at the

moment that more than one mutation might be causative for the different phenotypes of *climbing sisyphus*.

The flies show an extremely valuable phenotype for the further analysis of decision processes. While most mutant strains like *tay*^{1}, *no bridge*^{KS49}, or G74 (Pick & Strauss, 2005) have a lower probability to initiate a climbing attempt, this mutant line readily tries to cross even gaps of clearly insurmountably width. Interestingly, the climbing behaviour is not elicited by merely stepping into the void at gaps of any size. Rather, there is a reduction in willingness to initiate climbing as the gaps get broader, which is shifted, however, by several millimetres towards the clearly insurmountable gap width. In the determined range the decline reaches from a rate of more than 90% at gaps up to 3.5 mm to about 60% at 6.0 mm gap width. Wild-type flies hardly show any climbing attempts at this latter gap size at all. They have every right to do so as the success rate for crossing drops to a meagre 1% already at 4.0 mm wide gaps. The climbing abilities of *climbing sisyphus* are not any better than those of wild-type flies regardless of the gap size.

Figure 45: *climbing sisyphus* fly trying to cross a 5 mm gap

climbing sisyphus flies will even show climbing attempts at a clearly insurmountable gap width.

One possible explanation for the unadapted initiation rate might be defects in the evaluation of visual information. To check for this, *climbing sisyphus* flies were tested in several visual paradigms. The optomotor compensation during walking is not significantly altered compared to the wild-type background Berlin. As flies can compensate for optomotor stimulation, this mutant strain is certainly not blind. Moreover, the climbing performance of *climbing sisyphus* would not have been expected for blind flies, as flies without vision do not show gap-crossing initiation at gaps broader than 2.5 mm (Pick and Strauss, 2005). To analyse the visual capabilities of *climbing sisyphus* in further detail, their distance estimation has been tested in the four-arm walking paradigm (Schuster et al., 2002). In this setup, two pairs of visual

objects are presented to single flies, a pair of two close ones directly opposite to each other and a pair of distant ones with their connecting axis orthogonal to the one of the first (see Material & Methods). The distribution of visits is depended on the relative distance between the two sets of objects. The smaller the relative difference gets, the more equal will be the number of visits. The absolute distance is not relevant (Schuster et al., 2002). Two configurations were used in the present study, 50 mm vs. 200 mm distance and 140 mm vs. 200 mm distance. In both configurations, *climbing sisyphus* flies were not significantly different from the wild-type Berlin flies. The second configuration is close to the difference between 3.5 mm and 5 mm in the gap climbing paradigm. While in wild-type flies, the probability to initiate a climbing attempt drops from 56.0±5.3% at 3.5 mm to 6.5±2.2% at 5.0 mm gap width, initiation in *climbing sisyphus* was still 81.0±4.6% at the 5.0 mm gap. But as they perform well in the distance estimation paradigm, basic distance estimation is expected to be intact in *climbing sisyphus*. The problem of *climbing sisyphus* flies might be attributed to a defect in a hypothetical specific small scale differentiation of distances (gaps appear smaller than they actually are), a problem in the reference system of what the flies can actually achieve (flies appear to themselves bigger or more capable than what they are), or in the decision making machinery itself (wrong decisions upon correct inputs).

Because of the interesting phenotypes, it was desirable to identify the gene in order to study it at the molecular level. The X-chromosomal gene causes no apparent structural change in the neuroanatomy or the morphology of *climbing sisyphus* flies, at least not at the level of the light microscope (R. Strauss, personal communication). Because of this, assessment of behaviour was tried as an indicator for the presence or absence of the mutant phenotype. Two paradigms were used for the mapping, the climbing behaviour and the fast geotaxis for its relative simplicity. The main problem with recombination mapping turned out to be the missing clear cutline between mutant and wild-type behaviour. Various intermediate stages existed. The necessity for a common genetic background of the strains became very obvious, and prohibited the use of deficiency lines (deficiency kit for the X-chromosome, Bloomington stock centre). Various additional problems in behaviour would occur due to the hemizygous genes within the deficiency.

4.5 Synopsis and Future Prospects

Gap crossing behaviour in *Drosophila melanogaster* offers the unique possibility to study decision making for adaptive behaviour and the orchestration of simpler motor actions into complex motor tasks in a genetically tractable animal. In this thesis two modules have been analyzed. (1) For the understanding of decision making processes at the gap, *climbing sisyphus* offers the unique dimension of a hypermotivated specimen in addition to the known hypomotivated mutant lines. It is shown here that the flies are not affected in basic capabilities and likely to exhibit central decision making problems or defects in their self-concept. (2) A module for sustaining the direction during the complex motor task has been defined and concomitantly destroyed in all three protocerebral bridge mutant strains under study. The rescue of tay^{1} was key in identifying a visual targeting mechanism that has been rescued concomitantly with the structure of the protocerebral bridge. The mechanism gets lost when the visual input is diminished. (3) The rescue experiments of tay^{1} prove functions of the protocerebral bridge in increasing the walking speed and the control of walking activity. Only the 007Y-rescue restored the integrity of the protocerebral bridge and the above behavioural deficits. (4) The rescue experiments of tay^{1} allowed to separate protocerebral bridge functions. The bridge is not involved in the compensation of optomotor stimuli while walking. Rather, a network acting through the fan-shaped body is the likely candidate for this function. The fan-shaped body has been implicated before with left-right bargaining between body or brain sides (Strauss, 2002b).

The best possible partial rescue of tay^{1} did not rescue the full extent of the orientation in Buridan's paradigm nor in the gap crossing paradigm, the genomic rescue did, however. The residual function is therefore traceable and should be used for further mapping of brain functions with additional GAL4-lines. The further analysis of *climbing sisyphus* depends on the availability of deficiency lines with defined background. Those lines are becoming available by the DrosDel consortium (Ryder et al., 2004).

5. Summary

In this work, a behavioural analysis of different mutants of the fruit fly *Drosophila melanogaster* has been carried out. Primarily, the gap climbing behaviour (Pick & Strauss, 2005) has been assayed as it lends itself for the investigation of decision making processes and the neuronal basis of adaptive behaviour. Furthermore it shows how basic motor actions can be combined into a complex motor behaviour. Thanks to the neurogenetic methods, *Drosophila melanogaster* has become an ideal study object for neurobiological questions.

Two different modules of climbing control have been examined in detail. For the decision making, the mutant *climbing sisyphus* was analysed. While wild-type flies adapt the initiation of climbing behaviour to the width of the gap and the probability for a successful transition. *climbing sisyphus* flies initiate climbing behaviour even at clearly insurmountable gap widths. The climbing success itself is not improved in comparison to the wild-type siblings. The mutant *climbing sisyphus* is a rare example of a hyperactive mutant besides many mutants that show a reduced activity. Basic capabilities in vision have been tested in an optomotor and a distance-estimation paradigm. Since they are not affected, a defect in decision making is most probably the cause of this behavioural aberration.

A second module of climbing control is keeping up orientation towards the opposite side of the gap during the execution of climbing behaviour. Mutants with a structural defect in the protocerebral bridge show abnormal climbing behaviour. During the climbing attempt, the longitudinal body axis does not necessarily point into the direction of the opposite side. Instead, many climbing events are initiated at the side edge of the walking block into the void and have no chance to ever succeed. The analysed mutants are not blind. In one of the mutants, *tay bridge*^{1} (*tay*^{1}) a partial rescue attempt used to map the function in the brain succeeded such that the state of the bridge was restored. That way, a visual targeting mechanism has been activated, allowing the flies to target the opposite side. When the visibility of the opposing side was reduced, the rescued flies went back to a *tay*^{1} level of directional scatter. The results are in accord with the idea that the bridge is a central constituent of the visual targeting mechanism.

The *tay*^{1} mutant was also analysed in other behavioural paradigms. A reduction in walking speed and walking activity in this mutant could be rescued by the expression of UAS-*tay* under the control of the 007Y-GAL4 driver line, which concomitantly restores the structure of the protocerebral bridge.

5. Summary

The separation of bridge functions from functions of other parts of the brain of *tay*^{1} was accomplished by rescuing the reduced optomotor compensation in *tay*^{1} by the mb247-GAL4>UAS-*tay* driver. While still having a *tay*^{1}-like protocerebral bridge, mb247-GAL4 rescue flies are able to compensate at wild-type levels. An intact compensation is not depended on the *tay* expression in the mushroom bodies, as mushroom body ablated flies with a *tay*^{1} background and expression of UAS-*tay* under the control of mb247-GAL4 show wild-type behaviour as well. The most likely substrate for the function are currently unidentified neurons in the fan-shaped body, that can be stained with 007Y-GAL4 and mb247-GAL4 as well.

6. Zusammenfassung

In der vorliegenden Arbeit wurde eine Verhaltensanalyse verschiedener Mutanten der Fruchtfliege *Drosophila melanogaster* durchgeführt. Dazu wurde primär das Lücken-überwindungsparadigma (Pick & Strauss, 2005) herangezogen, das sich auf besondere Weise zur Erforschung von Entscheidungsfindung und adaptivem Verhalten anbietet. Weiterhin zeigt sich hier, wie einfache motorische Aktionen zu einem komplexen motorischen Verhalten zusammengefügt werden können. Dank der Möglichkeiten der Gentechnik bietet sich *Drosophila* hier als Studienobjekt an.

Zwei Module der Kletterkontrolle wurden genauer untersucht. Im Bezug auf die Entscheidungsfindung wurde die Mutante *climbing sisyphus* getestet. Während der Wildtyp sein Kletterverhalten sehr genau an die Lückenbreite und die Wahrscheinlichkeit einer erfolgreichen Überquerung anpasst (Pick & Strauss, 2005), werden bei *climbing sisyphus* auch bei einer unmöglich zu überquerenden Lücke noch Kletteraktionen initiiert. Der Klettererfolg selbst ist im Vergleich zum Wildtyp nicht verbessert. Die Mutante *climbing sisyphus* ist ein seltenes Beispiel einer hyperaktiven Mutante neben vielen Mutanten die eine reduzierte Aktivität zeigen. Grundlegende Fähigkeiten im visuellen Bereich wurden in der Optomotorik und im Entfernungsschätzen getestet und sind in *climbing sisyphus* nicht beeinträchtigt, ein Defekt in der Entscheidungsfindung ist wahrscheinlich Ursache des gestörten Verhaltens.

Ein zweites Modul der Kletterkontrolle betrifft die Aufrechterhaltung der Orientierung hin zur gegenüberliegenden Seite der Lücke. Mutanten mit einem Strukturdefekt in der Protozerebralbrücke des Zentralkomplexes zeigen ein abnormes Kletterverhalten. Die Körperlängsachse zeigt während des Klettervorgangs nicht in die Richtung der gegenüberliegenden Seite. Stattdessen werden oft Klettervorgänge am seitlichen Rand des Klettersteges initiiert, die keinerlei Aussicht auf Erfolg haben. Die untersuchten mutanten Fliegen sind nicht blind. In einem der Stämme, *tay bridge*^{1} (*tay*^{1}), gelang zur funktionellen Kartierung eine partielle Rettung dieses Verhaltens durch die Expression des wildtypischen Gens in einem kleinen Teil des Nervensystems. Das Wiederherstellen der wildtypischen Brückenstruktur in *tay*^{1} aktiviert einen visuellen Zielmechanismus, der eine Ausrichtung der Fliegen auf die gegenüberliegende Seite ermöglicht. Wenn die Sichtbarkeit der gegenüberliegenden Seite reduziert wird, geht dieser Rettungseffekt verloren. Die Brücke ist nach diesen Befunden ein zentraler Bestandteil der visuell gesteuerten Zielmotorik.

Die *tay*^{1} Mutante wurde auch in weiteren Verhaltensexperimenten untersucht. So konnte eine in dieser Mutante vorliegende Reduktion der Laufgeschwindigkeit und Laufaktivität durch die

Expression von UAS-*tay* unter der Kontrolle des Treibers 007Y-GAL4 zusammen mit der Struktur der Brücke gerettet werden.

Eine Rettung der reduzierten Kompensation für optomotorische Stimuli in tay^1 durch den Treiber mb247-GAL4 erlaubte eine Trennung von tay^1 Defekten in der Brücke von Defekten in anderen Teilen des Gehirns. Trotz einer tay^1-typischen unterbrochenen Brücke sind mit mb247-GAL4>UAS-*tay* gerettete Fliegen in der Lage eine Stimulation mit optomotorischen Reizen auf wildtypischem Niveau zu kompensieren. Diese Kompensation hängt nicht von den Pilzkörpern ab, da auf chemischen Wege pilzkörperablatierte Fliegen mit einer Expression von UAS-*tay* unter der Kontrolle von mb247-GAL4 sich trotz tay^1 Hintergrund ebenfalls wildtypisch verhalten. Die wahrscheinlichsten Träger für diese Rettung sind noch nicht identifizierte Neurone im Fächerförmigen Körper des Zentralkomplexes, die mit 007Y-GAL4 und mb247-GAL4 angefärbt werden können.

7. Abbreviations

α/β/γ	α-/β-/γ-lobes of the mushroom bodies
ca	Mushroom body calyx
cDNA	Complementary DNA
cM	Centimorgan
CS	Canton Special
eb	Ellipsoid body
ebc	Ellipsoid body channel
EMS	Ethyl methanesulfonate
fb	Fan-shaped body
HU	Hydroxy urea
LED	light emitting diode
MB	Mushroom bodies
mb	Median bundle
N	Number of animals of a certain genotype used in one experiment
n	Number of single experiments by the total number of flies of one genotype
no	Noduli
pb	Protocerebral bridge
pe	Mushroom body peduncle
PI	Performance index
SEM	Standard error of mean
t-test	Student’s t-test
TNT	Tetanus toxin
U-test	Mann-Whitney U test
WTB	Wild-type Berlin

8. References

The FlyBase Consortium (2003). The FlyBase database of the *Drosophila* genome projects and community literature. *Nucleic Acids Research* 31, 172-175.

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., George, R.A., Lewis, S.E., Richards, S., Ashburner, M., Henderson, S.N., Sutton, G.G., Wortman, J.R., Yandell, M.D., Zhang, Q., Chen, L.X., Brandon, R.C., Rogers, Y.H., Blazej, R.G., Champe, M., Pfeiffer, B.D., Wan, K.H., Doyle, C., Baxter, E.G., Helt, G., Nelson, C.R., Gabor, G.L., Abril, J.F., Agbayani, A., An, H.J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R.M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E.M., Beeson, K.Y., Benos, P.V., Berman, B.P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M.R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K.C., Busam, D.A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J.M., Cawley, S., Dahlke, C., Davenport, L.B., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A.D., Dew, I., Dietz, S.M., Dodson, K., Doup, L.E., Downes, M., Dugan-Rocha, S., Dunkov, B.C., Dunn, P., Durbin, K.J., Evangelista, C.C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A.E., Garg, N.S., Gelbart, W.M., Glasser, K., Glodek, A., Gong, F., Gorrell, J.H., Gu, Z., Guan, P., Harris, M., Harris, N.L., Harvey, D., Heiman, T.J., Hernandez, J.R., Houck, J., Hostin, D., Houston, K.A., Howland, T.J., Wei, M.H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G.H., Ke, Z., Kennison, J.A., Ketchum, K.A., Kimmel, B.E., Kodira, C.D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z., Lasko, P., Lei, Y., Levitsky, A.A., Li, J., Li, Z., Liang, Y., Lin, X., Liu, X., Mattei, B., McIntosh, T.C., McLeod, M.P., McPherson, D., Merkulov, G., Milshina, N.V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S.M., Moy, M., Murphy, B., Murphy, L., Muzny, D.M., Nelson, D.L., Nelson, D.R., Nelson, K.A., Nixon, K., Nusskern, D.R., Pacleb, J.M., Palazzolo, M., Pittman, G.S., Pan, S., Pollard, J., Puri, V., Reese, M.G., Reinert, K., Remington, K., Saunders, R.D., Scheeler, F., Shen, H., Shue, B.C., Siden-Kiamos, I., Simpson, M., Skupski, M.P., Smith, T., Spier, E., Spradling, A.C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A.H., Wang, X., Wang, Z.Y., Wassarman, D.A., Weinstock, G.M., Weissenbach, J., Williams, S.M., WoodageT, Worley, K.C., Wu, D., Yang, S., Yao, Q.A., Ye, J., Yeh, R.F., Zaveri, J.S., Zhan, M., Zhang, G., Zhao, Q., Zheng, L., Zheng, X.H., Zhong, F.N., Zhong, W., Zhou, X., Zhu, S., Zhu, X., Smith, H.O., Gibbs, R.A., Myers, E.W., Rubin, G.M., and Venter, J.C. (2000). The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185-2195.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* 215, 403-410.

Ashburner, M., and Drysdale, R. (1994). FlyBase--the *Drosophila* genetic database. *Development* 120, 2077-2079.

8. References

Ashburner, M., Golic, K.G., and Hawley, R.S. (2005). *Drosophila*: A Laboratory Handbook, Second Edition Edition, (New York: Cold Spring Harbor Laboratory Press).

Aso, Y., Grübel, K., Busch, S., Friedrich, A.B., Siwanowicz, I., and Tanimoto, H. (2009). The mushroom body of adult *Drosophila* characterized by GAL4 drivers. *Journal of Neurogenetics* 23, 156-172.

Bedichek, S., and Patterson, J.T. (1934). New mutants. *Drosophila Information Service* 2, 9.

Benzer, S. (1967). Behavioral mutants of *Drosophila* isolated by countercurrent distribution. *Proceedings of the National Academy of Sciences of the United States of America* 58, 1112-1119.

Bläsing, B., and Cruse, H. (2004a). Mechanisms of stick insect locomotion in a gap-crossing paradigm. *Journal of Comparative Physiology [A] Neuroethology, Sensory, Neural, and Behavioral Physiology* 190, 173-183.

Bläsing, B., and Cruse, H. (2004b). Stick insect locomotion in a complex environment: climbing over large gaps. *The Journal of Experimental Biology* 207, 1273-1286.

Bläsing, B. (2006). Crossing Large Gaps: A Simulation Study of Stick Insect Behavior. *Adaptive Behavior* 14, 265-286.

Boutros, M., Kiger, A.A., Armknecht, S., Kerr, K., Hild, M., Koch, B., Haas, S.A., Paro, R., and Perrimon, N. (2004). Genome-wide RNAi analysis of growth and viability in *Drosophila* cells. *Science* 303, 832-835.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401-415.

Bullock, T., and Horridge, G. (1965). Structure and function in the nervous systems of invertebrates, (San Francisco: Freeman).

Callaerts, P., Leng, S., Clements, J., Benassayag, C., Cribbs, D., Kang, Y.Y., Walldorf, U., Fischbach, K.F., and Strauss, R. (2001). *Drosophila* Pax-6/eyeless is essential for normal adult brain structure and function. *Journal of Neurobiology* 46, 73-88.

Collett, T.S. (2002). Insect vision: controlling actions through optic flow. *Current Biology* 12, R615-617.

Cruse, H. (1976). The control of body position in the stick insect Carausius morosus, when walking over uneven surfaces. *Biological Cybernetics* 24, 25–33.

Cruse, H. (1979). A New Model Describing the Coordination Pattern of the Legs of a Walking Stick Insect. *Biological Cybernetics* 32, 107-113.

de Belle, J.S., and Heisenberg, M. (1994). Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science* 263, 692-695.

8. References

Diegelmann, S., Fiala, A., Leibold, C., Spall, T., and Buchner, E. (2002). Transgenic flies expressing the fluorescence calcium sensor Cameleon 2.1 under UAS control. *Genesis* 34, 95-98.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., Couto, A., Marra, V., Keleman, K., and Dickson, B.J. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* 448, 151-156.

Duffy, J.B. (2002). GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. *Genesis* 34, 1-15.

Durr, V. (2001). Stereotypic leg searching movements in the stick insect: kinematic analysis, behavioural context and simulation. *Journal of Experimental Biology* 204, 1589-1604.

Eyding, D. (1993). Lernen und Kurzzeitgedächtnis beim operanten Konditionieren auf visuelle Muster bei strukturellen und biochemischen Mutanten von *Drosophila melanogaster*. Diploma thesis. (Würzburg: Bayerische Julius-Maximilians-Universität).

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 391, 806-811.

Götz, K.G. (1980). Visual guidance in *Drosophila*. *Basic Life Science* 16, 391-407.

Hanesch, U., Fischbach, K.-F., and Heisenberg, M. (1989). Neuronal architecture of the central complex in *Drosophila melanogaster*. *Cell and Tissue Research* 257, 343-366.

Heinze, S., and Homberg, U. (2007). Maplike representation of celestial E-vector orientations in the brain of an insect. *Science* 315, 995-997.

Heisenberg, M., and Böhl, K. (1979). Isolation of anatomical brain mutants of *Drosophila* by histological means. *Zeitschrift für Naturforschung* 34c, 143-147.

Heisenberg, M. (1994). Central brain functions in insects: genetic studies on the mushroom bodies and central complex in *Drosophila*. In Neural Basis of Behavioural Adaptations, Volume 39, Schildberger, K. and Elsner, N., eds. (Stuttgart: G. Fischer), pp. 61-79.

Hirth, F., Therianos, S., Loop, T., Gehring, W.J., Reichert, H., and Furukubo-Tokunaga, K. (1995). Developmental defects in brain segmentation caused by mutations of the homeobox genes orthodenticle and empty spiracles in *Drosophila*. *Neuron* 15, 769-778.

Homberg, U. (1987). Structure and functions of the central complex in insects. In Arthropod Brain:Its Evolution, Development, Structure and Functions., Gupta, A.P., ed. (New York: Wiley), p. p. 347±367.

Homberg, U. (1994). Flight-correlated activity changes in neurons of the late accessory lobes in the brain of the locust Schistocerca gregaria. *Journal of Comparative Physiology [A] Neuroethology, Sensory, Neural, and Behavioral Physiology* 175, 597-610.

Hotta, Y., and Benzer, S. (1972). Mapping of behaviour in *Drosophila* mosaics. *Nature* 240, 527-535.

Ilius, M., Wolf, R., and Heisenberg, M. (1994). The central complex of *Drosophila melanogaster* is involved in flight control: studies on mutants and mosaics of the gene ellipsoid body open. *Journal of Neurogenetics* 9, 189-206.

Katzen, A. (1990). Proto-oncogenes in *Drosophila*: Molecular and genetic analysis. Volume Dissertation. (San Francisco: University of California).

Kitamoto, T. (2001). Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive shibire allele in defined neurons. *Journal of Neurobiology* 47, 81-92.

Konopka, R.J., and Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* 68, 2112-2116.

Kretzschmar, D., Tschape, J., Bettencourt Da Cruz, A., Asan, E., Poeck, B., Strauss, R., and Pflugfelder, G.O. (2005). Glial and neuronal expression of polyglutamine proteins induce behavioral changes and aggregate formation in *Drosophila*. *Glia* 49, 59-72.

Lai, S.L., and Lee, T. (2006). Genetic mosaic with dual binary transcriptional systems in *Drosophila*. *Nature Neuroscience* 9, 703-709.

Leng, S., and Strauss, R. (1999). Instable courtship and altered spontaneous behavior in three genetically independent *Drosophila* mutant strains with protocerebral bridge defects. *Journal of Neurogenetics* 13, 41-42.

Liu, G., Seiler, H., Wen, A., Zars, T., Ito, K., Wolf, R., Heisenberg, M., and Liu, L. (2006). Distinct memory traces for two visual features in the *Drosophila* brain. *Nature* 439, 551-556.

Luo, L., Liao, Y.J., Jan, L.Y., and Jan, Y.N. (1994). Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes and Development* 8, 1787-1802.

Martini, S.R., and Davis, R.L. (2005). The dachshund gene is required for the proper guidance and branching of mushroom body axons in *Drosophila melanogaster*. *Journal of Neurobiology* 64, 133-144.

McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., and Davis, R.L. (2003). Spatiotemporal rescue of memory dysfunction in *Drosophila*. *Science* 302, 1765-1768.

8. References

Milani, R. (1941). Two new eye-shape mutant alleles in *Drosophila melanogaster*. *Drosophila Information Service*

Morgan, T.H. (1911). The Origin of Five Mutations in Eye Color in *Drosophila* and Their Modes of Inheritance. *Science* 33, 534-537.

Mronz, M. (2004). Die visuell motivierte Objektwahl laufender Taufliegen (*Drosophila melanogaster*) – Verhaltensphysiologie, Modellbildung und Implementierung in einem Roboter In Department of Genetics and Neurobiology, Dissertation. (Würzburg: Bayerische Julius-Maximilians-Universität).

Neuser, K., Triphan, T., Mronz, M., Poeck, B., and Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. *Nature* 453, 1244-1247.

Nusslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287, 795-801.

O'Dell, K.M., Armstrong, J.D., Yang, M.Y., and Kaiser, K. (1995). Functional dissection of the *Drosophila* mushroom bodies by selective feminization of genetically defined subcompartments. *Neuron* 15, 55-61.

Pearson, K.G., and Franklin, R. (1984). Charactersitics of leg movements and patterns of coordination in locusts walking on rough terrain. *International Journal of Robotics Research* 3, 101-112.

Pick, S., and Strauss, R. (2005). Goal-driven behavioral adaptations in gap-climbing *Drosophila*. *Current Biology* 15, 1473-1478.

Pielage, J., Steffes, G., Lau, D.C., Parente, B.A., Crews, S.T., Strauss, R., and Klambt, C. (2002). Novel behavioral and developmental defects associated with *Drosophila* single-minded. *Developmental Biology* 249, 283-299.

Poeck, B., Triphan, T., Neuser, K., and Strauss, R. (2008). Locomotor control by the central complex in *Drosophila* - An analysis of the tay bridge mutant. *Developmental Neurobiology* 68, 1046-1058.

Power, M.E. (1943). The Brain of *Drosophila melanogaster*. *Journal of Morphology* 72, 517-559.

Putz, G., Bertolucci, F., Raabe, T., Zars, T., and Heisenberg, M. (2004). The S6KII (rsk) gene of *Drosophila melanogaster* differentially affects an operant and a classical learning task. *Journal of Neuroscience* 24, 9745-9751.

Renn, S.C., Armstrong, J.D., Yang, M., Wang, Z., An, X., Kaiser, K., and Taghert, P.H. (1999). Genetic analysis of the *Drosophila* ellipsoid body neuropil: organization and development of the central complex. *Journal of Neurobiology* 41, 189-207.

Robinow, S., and White, K. (1991). Characterization and spatial distribution of the ELAV protein during *Drosophila melanogaster* development. *Journal of Neurobiology* 22, 443-461.

Rubin, G.M., and Spradling, A.C. (1982). Genetic transformation of *Drosophila* with transposable element vectors. *Science* 218, 348-353.

Ryder, E., Blows, F., Ashburner, M., Bautista-Llacer, R., Coulson, D., Drummond, J., Webster, J., Gubb, D., Gunton, N., Johnson, G., O'Kane, C.J., Huen, D., Sharma, P., Asztalos, Z., Baisch, H., Schulze, J., Kube, M., Kittlaus, K., Reuter, G., Maroy, P., Szidonya, J., Rasmuson-Lestander, A., Ekstrom, K., Dickson, B., Hugentobler, C., Stocker, H., Hafen, E., Lepesant, J.A., Pflugfelder, G., Heisenberg, M., Mechler, B., Serras, F., Corominas, M., Schneuwly, S., Preat, T., Roote, J., and Russell, S. (2004). The DrosDel collection: a set of P-element insertions for generating custom chromosomal aberrations in *Drosophila melanogaster*. *Genetics* 167, 797-813.

Scholz, H., Ramond, J., Singh, C.M., and Heberlein, U. (2000). Functional ethanol tolerance in *Drosophila*. *Neuron* 28, 261-271.

Schuster, S., Strauss, R., and Gotz, K.G. (2002). Virtual-reality techniques resolve the visual cues used by fruit flies to evaluate object distances. *Current Biology* 12, 1591-1594.

Spradling, A.C., and Rubin, G.M. (1982). Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* 218, 341-347.

Stapleton, M., Carlson, J., Brokstein, P., Yu, C., Champe, M., George, R., Guarin, H., Kronmiller, B., Pacleb, J., Park, S., Wan, K., Rubin, G.M., and Celniker, S.E. (2002). A *Drosophila* full-length cDNA resource. *Genome Biology* 3, RESEARCH0080.

Strauss, R., and Heisenberg, M. (1990). Coordination of legs during straight walking and turning in *Drosophila melanogaster*. *Journal of Comparative Physiology [A] Neuroethology, Sensory, Neural, and Behavioral Physiology* 167, 403-412.

Strauss, R., Hanesch, U., Kinkelin, M., Wolf, R., and Heisenberg, M. (1992). No-bridge of *Drosophila melanogaster*: portrait of a structural brain mutant of the central complex. *Journal of Neurogenetics* 8, 125-155.

Strauss, R., and Heisenberg, M. (1993). A higher control center of locomotor behavior in the *Drosophila* brain. *Journal of Neuroscience* 13, 1852-1861.

Strauss, R. (1995). A screen for EMS-induced X-linked locomotor mutants in *Drosophila melanogaster*. *Journal of Neurogenetics* 10, 53-54.

Strauss, R., Schuster, S., and Götz, K.G. (1997). Processing of artificial visual feedback in the walking fruit fly *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1281-1296.

Strauss, R., and Pichler, J. (1998). Persistence of orientation toward a temporarily invisible landmark in *Drosophila melanogaster*. *Journal of Comparative Physiology [A] Neuroethology, Sensory, Neural, and Behavioral Physiology* 182, 411-423.

Strauss, R. (2002a). The central complex and the genetic dissection of locomotor behaviour. *Current Opinion in Neurobiology* 12, 633-638.

Strauss, R. (2002b). Die übergeordnete Steuerung des Laufverhaltens durch das Insektengehirn, studiert mit Methoden der *Drosophila*-Neurogenetik. Habilitation Thesis. (Würzburg: Bayerische Julius-Maximilians-Universität).

Sweeney, S.T., Broadie, K., Keane, J., Niemann, H., and O'Kane, C.J. (1995). Targeted expression of tetanus toxin light chain in *Drosophila* specifically eliminates synaptic transmission and causes behavioral defects. *Neuron* 14, 341-351.

Tettamanti, M., Armstrong, J., Endo, K., Yang, M., Furukubo-Tokunaga, K., Kaiser, K., and Reichert, H. (1997). Early development of the *Drosophila* mushroom bodies, brain centres for associative learning and memory. *Development Genes and Evolution* 207, 242-252.

Vitzthum, H., Müller, M., and Homberg, U. (2002). Neurons of the central complex of the locust Schistocerca gregaria are sensitive to polarized light. *Journal of Neuroscience* 22, 1114-1125.

Watson, J.T., Ritzmann, R.E., Zill, S.N., and Pollack, A.J. (2002a). Control of obstacle climbing in the cockroach, Blaberus discoidalis. I. Kinematics. *Journal of Comparative Physiology [A] Neuroethology, Sensory, Neural, and Behavioral Physiology* 188, 39-53.

Watson, J.T., Ritzmann, R.E., and Pollack, A.J. (2002b). Control of climbing behavior in the cockroach, Blaberus discoidalis. II. Motor activities associated with joint movement. *Journal of Comparative Physiology [A] Neuroethology, Sensory, Neural, and Behavioral Physiology* 188, 55-69.

Wehner, R. (1976). Polarized-light navigation by insects. *Scientific American* 235, 106-115.

Wehner, R. (2001). Polarization vision--a uniform sensory capacity? *Journal of Experimental Biology* 204, 2589-2596.

Yang, M.Y., Armstrong, J.D., Vilinsky, I., Strausfeld, N.J., and Kaiser, K. (1995). Subdivision of the Drosophila mushroom bodies by enhancer-trap expression patterns. *Neuron* 15, 45-54.

Yeh, E., Gustafson, K., and Boulianne, G.L. (1995). Green fluorescent protein as a vital marker and reporter of gene expression in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* 92, 7036-7040.

Yellman, C., Tao, H., He, B., and Hirsh, J. (1997). Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* 94, 4131-4136.

Zars, T., Fischer, M., Schulz, R., and Heisenberg, M. (2000). Localization of a short-term memory in *Drosophila*. *Science* 288, 672-675.

9. Curriculum Vitae

Tilman Martin Triphan

Geburtsdatum: 18.12.1980

Geburtsort: Werneck

Schulische Laufbahn:

1986-1990	Grundschule in Oberschwarzach und Schallfeld
1991-2000	Gymnasium in Wiesentheid

Biologiestudium:

2000-2005	Biologiestudium an der Julius-Maximilians-Universität Würzburg, Hauptfach Genetik, Nebenfächer Bioinformatik und Biotechnologie
Juni 2004 -März 2005	Diplomarbeit am Lehrstuhl für Genetik und Neurobiologie mit dem Titel: “Verwendung von GAL80 zur Veränderung von Expressionsmustern in GAL4-Linien” unter der Betreuung von Prof. Dr. Martin Heisenberg

Promotionsstudium:

Juni 2005- August 2009	Promotion im Fach Biologie an der Julius-Maximilians-Universität Würzburg am Lehrstuhl für Genetik und Neurobiologie unter der Betreuung von Prof. Dr. Roland Strauß. Die Arbeit mit dem Titel „The Central Control of Gap Climbing Behaviour in *Drosophila melanogaster*“ wurde im Rahmen des Internationalen Graduiertenkollegs 1156 der DFG „Von der synaptischen Plastizität zur Verhaltensmodulation in genetischen Modellorganismen“ angefertigt.
August 2009-	Student Researcher am Howard Hughes Medical Institute, Janelia Farm Research Campus in Ashburn, Virginia, U.S.A.

10. Publications and Congress Contributions

Publications

Neuser K, **Triphan T**, Mronz M, Poeck B, Strauss R (2008) Analysis of a spatial orientation memory in *Drosophila*. Nature 2008 Jun 26;453(7199):1244-7

Poeck B, **Triphan T**, Neuser K, Strauss R (2008) Locomotor control by the central complex in *Drosophila* - An analysis of the *tay bridge* mutant. Dev Neurobiol. 2008 Jul;68(8):1046-58.

Carmine-Simmen K, Proctor T, Tschäpe J, Poeck B, **Triphan T**, Strauss R, Kretzschmar D (2009) Neurotoxic effects induced by the *Drosophila* amyloid-beta peptide suggest a conserved toxic function. Neurobiol Dis. 2009 Feb;33(2):274-81

Triphan T, Poeck B, Strauss R (2009) Lost in Orientation: Visual targeting of motor actions in *Drosophila* (*eingereicht*)

Congress Contributions

Triphan T, Strauss R (2006) Towards the Neuronal Basis of Decision Making and Adaptive Reaching Behaviour in the Climbing Fruit Fly *Drosophila*. 11th European *Drosophila* Neurobiology Conference, Neurofly 2006 Leuven, Belgium.

Triphan T, Strauss R (2007) The Control of Gap Climbing in *Drosophila melanogaster*. Proc. 7th Goettingen Meeting of the German Neuroscience Society, 31st Goettingen Neurobiology Conference, Germany.

Strauss R, **Triphan T**, Neuser K and Poeck B (2007) Modules of Locomotor Control in the Central Complex of the Fruit Fly - an Analysis of the *tay bridge* Mutant. Proc. 7th Goettingen Meeting of the German Neuroscience Society, 31st Goettingen Neurobiology Conference, Germany.

Triphan T, Kienitz B, Strauss R (2008) Higher-Level Motor Control in *Drosophila melanogaster*. Functional Anatomy of the Arthropod Central Complex & Motor System Conference, HHMI Janelia Farm Research Campus, Ashburn, Virginia.

Triphan T, Strauss R (2008) The Modular Structure of Climbing Control in *Drosophila*. 12th European *Drosophila* Neurobiology Conference, Neurofly 2008 Wuerzburg, Germany.

Schnaitmann C, **Triphan T**, Triphan S, Heisenberg M, Tanimoto H (2008) Behavioural Assay For Appetitive Visual Memory. 12th European *Drosophila* Neurobiology Conference, Neurofly 2008 Wuerzburg, Germany.

Triphan T, Strauss R (2009) Targeting Modules in the Gap-climbing Control of *Drosophila melanogaster*. Proc. 8th Goettingen Meeting of the German Neuroscience Society, 32nd Goettingen Neurobiology Conference, Germany.

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Erklärung

gemäß § 4 Absatz 3 der Promotionsordnung der Fakultät für Biologie der Bayerischen Julius-Maximilians-Universität zu Würzburg vom 15. März 1999:

Hiermit erkläre ich, die vorliegende Dissertation selbständig angefertigt zu haben und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben. Alle aus der Literatur entnommenen Stellen sind als solche kenntlich gemacht.
Des Weiteren erkläre ich, dass die vorliegende Arbeit weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.
Zuvor habe ich keine akademischen Grade erworben oder zu erwerben versucht.

Ashburn, den 23.10.2009

Tilman Triphan