Chapter 1
Biological investigation of neural circuits in the insect brain

Abstract Watching insects thoughtfully one cannot but adore their behavioural capabilities. They have developed amazing reproductive, foraging and orientation strategies and at the same time they followed the evolutionary path of miniaturization and sparseness. Both features together turn them into a role model for autonomous robots. Despite their tiny brains, fruit flies (*Drosophila*) can orient, walk on uneven terrain, in any orientation to gravity, can fly in adverse winds, find partners, places for egg laying, food and shelter. *Drosophila melanogaster* is *the* model animal for geneticists and cutting-edge tools are being continuously developed to study the underpinnings of their behavioural capabilities. This provided novel insight into the wiring and the working of central brain structures like the mushroom bodies and the central complex. Plasticity of the nervous system underlies adaptive behaviour. *Drosophila* flies show various memories from a 4-seconds working memory for orientation to a life-long body-size memory. Here we will discuss some of the functions and brain structures underlying fitness and role model function of insects for autonomously roving robots.

1.1 Introduction

On closer inspection one cannot but adore the behavioural capabilities of insects. First and foremost one might think of social insects like bees and ants with their ability to communicate and organize states, however, the solitary species have developed amazing reproductive, foraging or orientation strategies as well. While mankind evolved one of the largest brains in the animal kingdom, insects followed the path of miniaturization and splendid sparseness. Those features turn them into role models for autonomous robots. Among insects the fruit fly *Drosophila melanogaster* is the most intensely studied species owing to the unrivalled genetic tools available. Despite their tiny brains, fruit flies can orient with respect to outside stimuli - and remember previous encounters with such stimuli, can walk on uneven terrain - and this in any orientation to gravity, can fly in adverse winds, can find partners, places for egg laying, food and shelter. In order to achieve these goals, *Drosophila melanogaster* can build various memories starting from a 4-seconds visual working memory [36, 52] and a place memory [53], to a minutes- to 3-hours-long idiothetic working memory [55, 62, 63], a short-term and long-term olfactory memory (over days; review: [57]), a courtship-suppression memory [71], all the way to a life-long body-size memory [34]. Flies can also learn at what time during the day certain information is important [10, 11].

1.2 Memories in orientation

Place learning (cold spot on a hot plate). The paradigm adapts Morris water maze in which swimming rodents remember the position of an invisible platform underneath the water surface of a circular tank with milky water with the help of visual landmarks in the room [50]. The insect adaptation of the paradigm uses a hot plate on which a cold spot with pleasant temperature can be found and remembered by visual cues on the walls of the arena. The insect paradigm was first described by Mizunami et al. [48, 49] and used to study orientation in cockroaches. The recent adaptation for *Drosophila* by Ofstad et al. [53] uses a tiled floor of Peltier elements and a circular LED arena to show a 360° panorama of 120° vertical, 120° horizontal and 120° oblique stripes. Several flies can be video-tracked at the same time. The cold spot and the pattern can be switched congruently to alternative positions almost instantaneously after the flies have found the cold spot. A heat barrier and clipped wings keep the flies within the arena and the time until the flies find the cold spot and their track lengths are measured. Flies use visual information to memorize the pleasant place.

Place learning (heat box). The heat-box paradigm was developed by Wustmann et al. [94] to device a large-scale mutant screening for operant conditioning. Flies learn to avoid one half of a long, narrow chamber, because it heats up on entry. Flies learn from their own actions and later stay in the pleasant half even when the heat is turned off. Two different memory components were identified in immediate retention tests, a spatial preference for one side of the chamber and a "stay-where-you-are-effect" [62]. Intermittent training is shown to give higher retention scores than continuous training and strengthens the latter effect. When flies were trained in one chamber and tested in a second one after a brief reminder training, an aftereffect of the training can still be observed 2h later. The longest memory of 3h is achieved at aversive temperatures of 41°C [55].The various memory effects are independent of the mushroom bodies. Since training and test occur in the dark, and chambers can be switched, the paradigm tests for idiothetic orientation.

Visual orientation memory. In this paradigm single flies with clipped wings walk on a circular platform towards a landmark on a cylindrical LED screen beyond a water-filled moat [52]. This landmark disappears while at the same instance an indistinguishable distracter landmark appears under 90° to the first-seen. One second after the fly has changed its heading towards the distracter, it disappears as well so that the fly is left without visual orientation cues. Normal flies nevertheless return to their original heading and continue the approach of the first-chosen, now invisible landmark. The paradigm tests for a 4-seconds visual working memory important to bridge phases of interrupted visibility in a cluttered environment. It has been used to identify the seat of the memory in the brain and the underlying biochemistry [36].

Olfactory memory. In the olfactory conditioning paradigm of Tully and Quinn [83] up to 50 flies in a test-tube like structure are confronted with an odour while

at the same time electric shocks are given to their feet. Thereafter, a second odour is presented without foot-shocks. Then, the flies are gently pushed into an elevator and brought to a choice point, from where they can walk into two test-tube like chambers facing each other. One chamber presents the previously punished odour, the other the neutral odour. This so-called one-cycle training leads to the formation of a labile memory that can be detected for up to 7h. Learning is tested within 2 min after training, short-term memory between 30 min and 1h after training, and middle-term memory 3h after training. But flies can also acquire olfactory long-term memory that lasts up to one week if they are repeatedly trained with pauses between the training cycles (spaced training). Gene regulation and protein synthesis are inevitable for long-term memory. If the same overall training time as in spaced training is given without pauses, this is called massed training. Massed training leads to the formation of anaesthesia-resistant memory and is assessed one day after training [29, 84]. Appetitive olfactory memory is achieved when sugar reward instead of electric shocks are applied [57, 67]. To allow for a direct comparison between olfactory and visual conditioning, Vogt et al. [89] have developed a four-quadrant arena with transparent electric grid that can be used to administer electric shocks in visual and olfactory experiments.

Courtship suppression. Mated female flies reject courting male flies as long as sperm remains in their spermatheca. Males are actively kicked away and the ovipositor is protruded [71]. While courting, naïve male flies learn to avoid mated females in an aversive conditioning procedure. The taste receptor GR32a functions as pheromone receptor and conveys courtship suppression towards mated females [47]. Delay time to courting virgin females can serve as indictor for learning.

Body size memory. Certain behavioural decisions depend on body reach, in vertebrates represented in the brain as peripersonal space. E.g. in flies, the decision to overcome a chasm in the walkway by gap-climbing depends on body size, which, in turn, is determined by genetic and environmental factors like temperature and quality of food during larval stages. Therefore body size is learned after hatching using a multisensory integration strategy. Visual input is correlated with tactile experience or corollary discharge for walking. During acquisition of the own body size, the fly generates parallax motion by walking in a structured environment. The average step size, which is proportional to leg lengths and therefore to body size, creates an average parallax motion on the eyes that allows to calibrate behavioural decisions [34]. Later decisions to climb a gap are instructed by the parallax motion generated on the eyes by edges of the opposite side [60, 81].

Time-of-day memory. In a study by Chouhan et al. [11] starved flies were trained in the morning to associate a particular odour with sucrose reward. A respective training was repeated in the afternoon but with a different odour. The procedure was repeated the next day, and time-dependent odour preference was tested in the morning and afternoon of the third day. *Drosophila* chose the previously rewarded odour dependent on the time of the test whenever the two different training events on day 1 and 2 had been separated by more than 4h. Flies can form

time-odour associations in constant darkness as well as in daily light-dark cycles, but not under constant light-on conditions. The study demonstrates that flies can utilize temporal information as an additional cue in appetitive learning. Indeed, key genes of the circadian clock were essential for time-odour learning in flies. Circadian clock mutants, *period*^{01} and *clock*^{AR}, learned to associate sucrose reward with a certain odour but were unable to form time-odour associations. Those require a *period*- and *clock*-dependent endogenous mechanism. In a follow-up study by Chouhan et al. [10] the extent of starvation was shown to be correlated with the fly's ability to establish time-odour memories. Prolonged starvation promotes time-odour associations after just one single cycle of training. Starvation is required for acquisition but not for retrieval of time-odour memory.

1.3 The *Drosophila* genetic construction kit

Drosophila is *the* model animal for geneticists and cutting-edge tools are available to manipulate the nervous system of the fly and study the underpinnings of its behavioural capabilities. Over the years the fruit fly has been literally turned into a genetic construction kit.

First, so called *driver lines* express a xenogeneic transcription factor like GAL4 just in parts of the nervous system [4, 59]. GAL4 has been borrowed from baker's yeast and is *per se* functionless in *Drosophila*. Thousands of driver lines are available from stock centres, each with a specific expression pattern in the nervous system. E.g., expression can be restricted to all olfactory receptor neurons, to all serotonergic neurons (i.e. neurons using serotonin as their neurotransmitter), to all neurons within a particular brain centre or all neurons using a specific molecule; the lines are catalogued and the list of expression patterns is almost endless.

Second, xenogeneic transcription factors like GAL4 are functionless without their specific binding site, which does not exist in *Drosophila*. The binding site for GAL4 in baker's yeast is upstream activating sequence or UAS. Upon binding of GAL4 to UAS the gene behind UAS is transcribed and expressed. Instead of the genuine yeast gene any gene from any organism can be placed behind UAS and the construct be integrated in the *Drosophila* genome. This constitutes an *effector line* which is ideally functionless without GAL4. Again, thousands of effector lines can be found in stock centres. The term construction kit signifies the fact that parental flies from a driver line and an effector line can be crossed with each other so that the offspring possesses driver and effector elements in one fly. In those individuals, and only in neurons within the expression pattern of the GAL4 line, GAL4 binds to UAS and the effector is expressed. Effectors can be reporter genes like green fluorescent protein, or GFP, taken from a bioluminescent jellyfish, so that the GAL4 expression pattern can be visualized [9]. They can be genes encoding molecules for inactivating the neurons in the expression pattern, like tetanus toxin light chain taken from the tetanus bacterium [77]. In this case the chemical synapses cease to function. Effectors can be genes for additional proteins, but also

genes for knocking down the RNA building plan for particular molecules that normally would be expressed in the neuron. RNA interference, as the latter technique is called (review: [14]), is the method of choice for probing into the function of biochemical cascades.

Third, effective methods for controlling the *time of expression of an effector* have been developed. E.g., $GAL80^{ts}$ is a repressor of GAL4-controlled expression in yeast. At 18°C and lower temperatures it binds to GAL4 (that itself is bound to UAS) and represses transcription of the effector gene behind UAS. At 29°C and higher temperatures $GAL80^{ts}$ detaches from GAL4 and transcription will start [46]. Time-controlled expression of effectors allows for discerning between developmental and acute functional defects caused by the effector. Other methods of expression control have been developed, e.g. a hormone-based system. Expression starts when a particular hormone is administered by special fly food.

In recent years effectors have been developed that by themselves can be controlled by temperature or light. *Shibire^{ts}* encodes for a dominant negative dynamin needed for recycling of vesicles at the rim of chemical presynapses. Upon shifting the ambient temperature to 32°C the neurons in the expression pattern are silenced within seconds, because existing vesicles are used up and no new vesicles can be formed [33]. Within reasonable limits, the block is reversible by lowering the temperature. Functional analysis requires also artificial activation of neurons and potent methods have been developed to this end. TrpA1 is a temperature-sensitive cation channel naturally occurring in human and animal cells. Expressed under GAL4 control it allows activating neurons by shifting the temperature [19]. Channels are closed at 21°C and firing gradually increases with temperature up to a maximum at 29°C. A third example for controllable effectors are light-activated cation channels (e.g. ReaChR; [28]).

1.4 Mushroom bodies – structure and function

Structure. Mushroom bodies are a paired structure of the *Drosophila* brain; they are found in similar form in all insects. Mushroom bodies develop from four neuroblasts per hemisphere, each of which can produce three different intrinsic neuron types called Kenyon cells. The Kenyon cells developing from *Drosophila* embryo up to and including the second larval stage are γ neurons. Within the third larval stage exclusively α'-/β'-mushroom body neurons are formed. All Kenyon cells born in the subsequent pupal stage are α-/β-neurons [37]. The three Kenyon-cell types refer to the specific axonal projection patterns in the adult stage. All Kenyon cells, about 2000 per hemisphere, receive their main and predominantly olfactory input in the calyx of their brain side. From there they project through the peduncle into one of five mushroom-body lobes. α-/β-Kenyon cells and α'-/β'-Kenyon cells bifurcate at the anterior ventral end of the peduncle and project into the α-/β-lobes (1000 cells; [6]) or α'-/β'-lobes (370 cells), respectively. About 670 γ-Kenyon cells project without bifurcation just into the γ-lobes [6, 12]. α- and α'-

lobes are also called vertical lobes, whereas β, β' and γ lobes are referred to as medial lobes (Fig. 1.1).

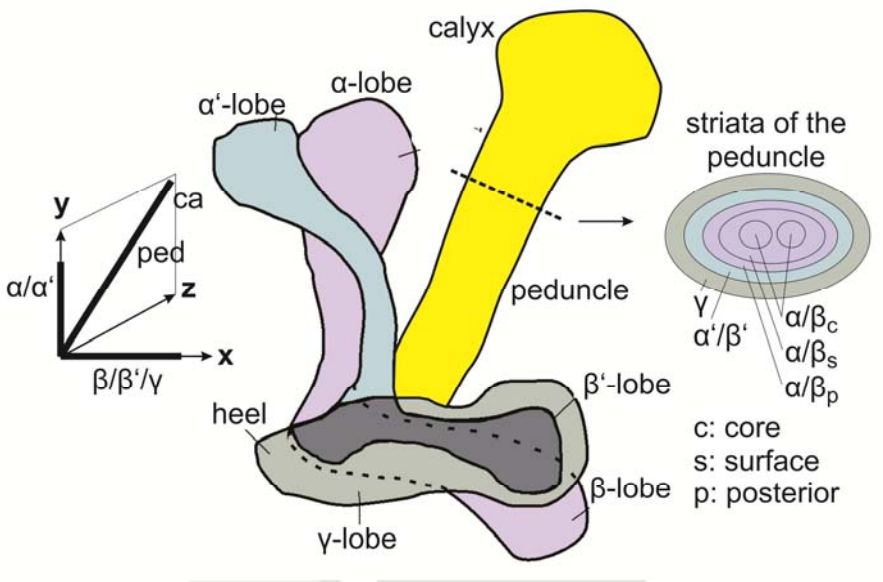

Fig. 1.1: Left mushroom body of *Drosophila melanogaster*. Frontal view. About 2000 Kenyon cells project from the calyx through the peduncle to the five lobes. They either bifurcate into α- and β-lobes, or α'- and β'-lobes, or project without bifurcation into the γ-lobe. The most anterior γ-lobe is shown in grey. The β'-lobe is slightly dorsal to the β-lobe. The α'-lobe winds around the α-lobe, their ends are in the same plane. Inset right: schematic cross section of the peduncle at the dashed line showing the strata of the peduncle (after [78]).

Laminar organization of the Kenyon cell axons divides the peduncle into at least five concentric strata (Fig. 1.1; [78]). The α-, β-, α'- and β'-lobes are each divided into three strata, whereas the γ lobe appears homogeneous. Specifically the outermost stratum of the α-/β-lobes is connected with the accessory calyx, a protruded sub-region of the calyx, which does not receive direct olfactory input [78].

Noteworthy, three cell types have been identified, which are mushroom-body intrinsic but non-Keyon-cell neurons. One pair of MB-DPM neurons (DPM refers to the position of the cell bodies in the dorsal posterior medial area of the brain), one pair of MB-APL neurons (APL: anterior paired lateral), and about 50 MB-AIM neurons (AIM: anterior inferior medial; [78]). The arborisation of the single DPM neuron per hemisphere covers all lobes and the anterior part of the peduncle. The arborisation of the single APL neuron per hemisphere is even wider and comprises, in addition to all lobes, also the calyx and the entire peduncle. In contrast, the 25 AIM neurons per hemisphere arborize in small areas of the γ- and β'-lobes [78].

All Kenyon cells of one brain hemisphere converge onto 34 mushroom body output neurons (MBONs) of which 21 types have been discriminated [1]. Judged by their roles in several associative learning tasks and in sleep regulation, some distinct single MBON output channels and a multi-layered MBON network have been identified.

Besides the olfactory input to the calyx region by projection neurons, 17 other types of mushroom body extrinsic neurons (MBENs) were identified by Tanaka et al. [78]. Those arborize in the calyx, lobes, and peduncle. Lobe-associated MBENs arborize in specific areas of the lobes along their longitudinal axes. This fact defines three longitudinal segments in the α- and three in the α'-lobes, two segments in the β- and two in the β'-lobes, and five segments in the γ-lobes. Taken together, the laminar arrangement of the Kenyon cell axons in the lobes and the segmented organization of the MBENs divide the lobes into smaller synaptic units, possibly facilitating characteristic interaction between intrinsic and extrinsic neurons in each unit for different functional activities (schematic maps are given e.g. in [27, 78]).

Olfactory learning. The foremost function of mushroom bodies is found in olfactory learning and memory. Flies naturally learn and can be trained to be attracted by or to avoid particular odours. Odour perception and processing is essential for *Drosophila*, because flies detect food and analyse sex partners using this sense. Olfactory receptors reside on the antennae and the maxillary bulbs; they are localized in the sensory dendrites of olfactory receptor neurons (ORN) that are housed in hair-like, chemosensory sensilla. Each ORN expresses only one type of olfactory receptor, together with the co-receptor Or83b; 62 different primary olfactory receptors are known in *Drosophila*. ORN axons project to the paired antennal lobes, each receptor type determines a particular target glomerulus. In most respects antennal lobes resemble the olfactory bulbs of vertebrates, which are the first stage of olfactory processing, as well [90, 91]. Within their glomerulus ORNs become connected to projection neurons (PNs) and local interneurons. About 150 cholinergic PNs convey the processed information to the mushroom bodies and the lateral horn [42, 78]. By mushroom-body inactivation experiments it has been determined that the direct influence of the lateral horn is sufficient to particularly release inborn behaviours [22], whereas the mushroom body path serves plasticity of behaviour. More recent studies characterized an additional olfactory path that uses inhibitory PNs projecting exclusively to the lateral horn thereby conveying hedonic value and intensity of odours [76].

Mushroom bodies build associative memories for natural odours. The associated experience may be "good" or "bad" and is used to control adaptive behaviour. A high selectivity for bouquets of odours is achieved, because every Kenyon cell receives input from a randomly developed set of, on average, three PNs [6] and thus serves as a coincidence detector. A high odour selectivity and at the same time low population activity of the mushroom body Kenyon cells is achieved by GABAergic attenuation. "Sparse coding" is fostered by a pair of GABAergic anterior paired lateral neurons (APLs), which innervate the entire mushroom body.

The Kenyon cells, in turn, possess $GABA_A$ receptors which allow for chloride anion influx when opened [38]. Altogether, the system allows discrimination between and storage of experience with natural odours for a wide range of primary odorant combinations.

Input from other sensory modalities is anatomically not obvious in *Drosophila* but mushroom-body dependent tasks related to vision are nevertheless described for flies (e.g. [41, 64, 79]). The mushroom bodies of honeybees receive, additional to olfactory information, prominent visual [18], gustatory, and mechanosensory [66] inputs. These connections likely provide mixed-modality signals that lead to experience-dependent structural changes documented in freely behaving bees (e.g. [15]). In flies and bees the lobe regions receive information on sugar reward and electric foot shock. Whereas dopaminergic neurons undisputedly convey negative reinforcement information to Kenyon cells, octopaminergic neurons have long been considered to convey reward to Kenyon cells in insects (e.g. [20, 67]). Burke et al. [5] showed in *Drosophila* that only short-term appetitive memory is reinforced by octopaminergic neurons and that these neurons signal on a specific subset of dopaminergic neurons, which in turn synapse on Kenyon cells. More specifically, hedonic information (sweet taste) goes to the β'_2- and γ_4-regions of the mushroom body lobes and can establish just a short-term memory. Appetitive long-term memory is nutrient-dependent and requires different dopaminergic neurons that project to the γ_{5b}-lobe region, and it can be artificially reinforced by dopaminergic neurons projecting to the β-lobe and adjacent α_1-lobe region [27].

Thus, during associative learning dopaminergic neurons convey the reinforcing effect of the unconditioned stimuli of either valence to the axons of *Drosophila* mushroom body Kenyon cells for normal olfactory learning. Cervantes-Santoval et al. [8] show that Kenyon cells and dopaminergic neurons form axo-axonic reciprocal synapses. The dopaminergic neurons receive cholinergic input from the Kenyon cells via nicotinic acetylcholine receptors. The neurogenetic knock-down of these receptors in dopaminergic neurons impaired olfactory learning. When the synaptic output of the Kenyon cells was blocked during olfactory conditioning, this reduced the presynaptic calcium transients in the dopaminergic neurons, a finding consistent with reciprocal communication and positive feedback. The blocking also reduced the normal chronic activity of the dopaminergic neurons.

The different mushroom body lobes serve distinct functions in the establishment of olfactory memory. The γ-lobes play the central role in short-term memory, whereas the α'-/β'-lobes are indistinguishable for memory consolidation after training. The longer the time lag between training and memory retrieval gets, the more important will be the α-/β-lobes; they are important for the long-term memory [7, 80]. Shortly later came the finding that, within a given Kenyon cell type, particular sub-groups of neurons might be responsible for the engrams of different durability (shell- and core units; [58]).

Using optogenetic artificial activation of MBONs Aso et al. [1] could induce repulsion or attraction in flies. The behavioural effects of MBON activation was combinatorial, suggesting that the MBON ensemble represents valence collective-

ly. Aso et al. propose that local, stimulus-specific dopaminergic modulation selectively alters the balance within the MBON network for those stimuli in order to bias memory-based action selection.

As pointed out in chapt.1.2, long-term olfactory memory requires spaced training and relies on new protein synthesis in order to stabilize learning-induced changes of synapses. Associations ought to be reproducible, before a long-term memory should form. Wu et al. [93] describe a sequence of events in the mushroom bodies that lead to stable aversive long-term memory from earlier labile phases. In a neurogenetic survey through all 21 distinct types of MBONs Wu and colleagues show that sequential synthesis of learning-induced proteins occurs within just three types of MBONs. Down-regulation of protein synthesis in any of these three MBON types impaired long-term memory. Moreover, synaptic outputs from all three MBON types are all required for memory retrieval. The requirement for protein synthesis during long-term memory consolidation is sequential; first it is needed in MBON-α3 (0h to 12h), then in MBON-γ3,γ3β′1, and finally in MBON-β′2mp. The time shift in the requirement for protein synthesis in the second MBON is 2h and in the third another 2h.

But even consolidated long-term memory can be extinct if the learned prediction is later inaccurate. If still correct, it will be maintained by re-consolidation. Felsenberg et al. [16] identified neural operations underlying the re-evaluation of appetitive olfactory memory in *Drosophila*. Both, extinction or re-consolidation activate specific parts of the mushroom body output network and specific subsets of reinforcing dopaminergic neurons. Extinction requires MBONs with dendrites in the α- and α′-lobes of the mushroom body, which drive negatively reinforcing dopaminergic neurons that innervate neighbouring compartments of the α- and α′-lobes. The aversive valence of this extinction memory neutralizes the previously learned odour preference. Memory re-consolidation, in turn, requires $\gamma_2\alpha'_1$-MBONs. This pathway recruits negatively reinforcing dopaminergic neurons innervating the same γ-lobe compartment and re-engages positively reinforcing dopaminergic neurons to re-consolidate the original reward memory. Thus, a recurrent and hierarchical connectivity between MBONs and dopaminergic neurons enables memory re-evaluation depending on reward-prediction accuracy.

Other functions. But mushroom bodies serve also other, more general and non-olfactory functions. It has been noticed that flies with ablated or inactivated mushroom bodies enhance their spontaneous walking activity as measured over several hours [44]. Within the first 15 minutes, however, the walking activity is reduced in comparison to intact flies, because their initial arousal phase is missing without mushroom bodies [70]. Mushroom bodies regulate activity with regard to internal stimuli like hunger and outside stimuli indicating, for instance, food [64]. Repeated inescapable stress reduces behavioural activity with all signs of a depression, whereas repeated reward can reactivate flies and make them resilient to stress ([64]; see chapt. 1.6). When courting, mushroom-body-less flies show deficits in detecting female pheromones [65]; they can neither form short-term nor long-term courtship-suppression memory [43]. If unforeseeable obstacles occur,

mushroom-body-less flies have problems switching to a new type of behaviour. For instance, at a water-filled moat they keep trying to reach the landmark beyond the water barrier, sometimes for minutes, despite the fact that their wings are clipped [51]. In flight-simulator experiments, decisions upon contradictory visual stimuli mirror the average between the valences of the stimuli, whereas intact flies take clear-cut decisions for one or the other stimulus [79]. Again in tethered flight, it was found that context generalization requires mushroom bodies [41]. Mushroom-body-less flies learned the heat-punished object and the colour of the background whereas intact flies abstracted from the background and learned the dangerous object as such. Mushroom bodies are the centre of sleep control [96]. Motor learning requires the mushroom bodies [31]. This was shown in a study of gap climbing in flies. Reiterative training can lead to a long-term motor memory. According to electrophysiological experiments, Kenyon cells respond to olfactory, visual, tactile and gustatory stimuli. It has been proposed that mushroom bodies are a multimodal sensory integration centre [12]. This has been confirmed in behavioural experiments (e.g. [64, 89]). With their four-quadrant apparatus Vogt et al. [89] showed that both visual and olfactory stimuli are modulated in the same subset of dopaminergic neurons for positive associative memories. Another subset of dopaminergic neurons was found to drive aversive memories of both the visual and olfactory stimuli. With regard to mushroom-body intrinsic neurons, distinct but partially overlapping subsets are involved in visual and olfactory memories. The work of Vogt et al. shows that associative memories are processed by a centralized circuit that receives both visual and olfactory inputs, thus reducing the number of memory circuits needed for such memories.

1.5 Central complex– structure and function

Structure. The central complex resides at the midline of the protocerebral hemispheres of insects. In *Drosophila* it is composed of four neuropilar regions, the largest of which is the fan-shaped body. Within an anterior depression resides the ellipsoid body, a perfectly toroid-shaped neuropil. Ventral to the ellipsoid body are the paired noduli and posterior to the fan-shaped body a neuropil with the shape of a bicycle's handle bar is found called protocerebral bridge [21]. The central complex neuropils are composed of columnar elements, which interconnect the four neuropils and are often output elements of the central complex, and of tangential cells, which mostly provide input to all or several columnar elements within one neuropil. A third type of neurons stays intrinsically and connects homologous elements within neuropils. The conspicuous columnar organization is seen as 18 glomeruli (9 per hemisphere) in the protocerebral bridge, 16 sectors within the ellipsoid body, and 8 fans of the fan-shaped body. E.g. Wolff et al. [92] provide a comprehensive catalogue of 17 cell types arborizing in the protocerebral bridge and insights into the anatomical structure of the four components of the central complex and its accessory neuropils. Revised wiring diagrams take into

account that the bridge comprises 18 instead of 16 glomeruli as previously published. Most recent information on the wiring of the fan-shaped body can be found in [3], and on the ellipsoid-body wiring in [54].

The conspicuous tangential elements of the ellipsoid body are called ring neurons; each ring neuron innervates all 16 sectors of the ellipsoid body. Five ring systems are known to date (R1 to R5; R4 is further subdivided into R4m for medial and R4d for dorsal) [45, 54]. The average number of ring neurons per set is 20 to 25 per hemisphere and the total number amounts to 200 to 250 cells [97, supplement]. According to [45] 37±4 ring neurons of the adult brain are not GABAergic. Ring neurons are postsynaptic at the bulb (formerly called lateral triangle; [30] and predominantly presynaptic within the ring. Novel coincident synapses have been found in the ellipsoid-body ring; these are precise arrays of two independent active zones over the same postsynaptic dendritic domain [45]. Such arrays are compatible with a role as coincidence detectors and represent about 8% of all ellipsoid-body synapses in *Drosophila*.

The pathways for visual input from the optic lobes, through the anterior optic tubercles to the bulbs have been recently described in *Drosophila* [54]. Starting from the development of the ellipsoid body, Omoto et al. describe that all ring neurons are formed by a single neuroblast lineage. Two other lineages give rise to the neurons that connect the anterior optic tubercles with the dendrites of the ring neurons in the bulb and to neurons that connect the medullae of the optic lobes with the anterior optic tubercles. This anterior visual pathway conveys information on the polarization pattern of the sky (sky-compass) in locusts and other insects [26]. The two lineages form two parallel pathways; DALcl1 neurons connect the peripheral ring neurons and DALcl2 neurons the central ring neurons with the anterior optic tubercles. All DALc1/2 neurons respond predominantly to bright objects. Whereas DALcl1 neurons show small and retinotopically ordered receptive fields on the ipsilateral eye, the DALcl2 neurons possess one large excitatory receptive field on the contralateral eye that they share, and one large inhibitory field on the ipsilateral side. When a bright object enters the ipsilateral field, they become inhibited and they respond with extra excitation when such an object leaves the ipsilateral receptive field. A second visual pathway is currently known just from larger insects. The protocerebral bridge is connected to the posterior optic tubercles, which in turn receive input from the accessory medullae [26].

Columnar sets of neurons interconnect the central-complex neuropils and accessory areas [21, 39, 92]. They usually come in sets in a multiple of eight.

Functions. Fan-shaped body. Learning experiments at the flight simulator revealed visual memory functions of the fan-shaped body in *Drosophila* [40]. Flies recognize previously heat-punished visual objects by parameters such as size, colour or contour orientation, and store respective parameter values for later avoidance. The features are stored independently of the retinal position of the objects on the eyes during learning for later visual pattern recognition. Short-term memory traces of the pattern parameters ‘elevation in the panorama’ and ‘contour orientation’ were localized in two different layers of the fan-shaped body. At the time of

the study the fan-shaped body was thought to be six-layered, meanwhile nine layers are distinguished [92]. Layers are perpendicular to the vertical columns in horizontal planes of the fan-shaped body.

Protocerebral bridge. The protocerebral bridge is involved in step-size control for speed increase and directional control of walking and gap-climbing [61, 82]. Structural mutants of the protocerebral bridge are walking slowly because their step size remains at a basic level [75]. In contrast, wild-type flies increase their step size with stepping frequency, thereby reaching almost double the maximum speed of bridge-defective flies [73, 74]. Flies turn by keeping step size on the inside of the curve at the basic level, whereas strides on the outside are increased. Stepping frequency is invariant between inside and outside; a model of how the bridge differentially influences step size is provided in [75]. Flies with clipped wings overcome chasms in the walkway by gap climbing, provided the gap is of surmountable width [60]. The distance to the opposite side is determined by the amount of parallax motion distal edges create on the retina during approach. The fly's own body size, which can vary by 15% within a given genetic background, is taken into account (see chapt. 1.2). Energetically costly climbing is thereby restricted to surmountable gaps. It has been observed that bridge-defective mutant flies are losing direction when initiating climbing. At the end of the proximal side of the catwalk their climbing attempts point in all directions, whereas intact flies stay in a small angular corridor that targets the distal side of the gap [82]. In locusts, the protocerebral bridge is shown to hold a map-like representation of the polarized light information of the sky compass [25].

Ellipsoid body. The ellipsoid body plays essential roles in visual pattern memory [56], orientation memory [35, 36, 52] and place learning [53], and thus it is considered to be a centre of visual learning and memory. Pan and colleagues [56] demonstrated in a follow-up study to Liu et al. [40] at the flight simulator that not just neurons in the fan-shaped body, but also a small set of neurons in the ellipsoid body are involved in visual pattern memory. Localized expression of a learning gene in the respective learning mutant revealed, that the rescue of the memory defect could be achieved in either of the central complex neuropils. Knock-down experiments in either structure demonstrated that both were required for visual pattern memory. A test of different visual pattern parameters, such as size of the retinal image, contour orientation, and vertical compactness revealed differential roles of the fan-shaped body and the ellipsoid body for visual pattern memory.

A short-lived visual orientation memory helps flies to bridge phases of an object approach, during which the chosen target gets temporarily out of sight. Even after a detour to a distracter landmark, flies can return to their previously planned path, given that the distracting landmark is seen for just a short time [52]. The working memory resides in the R3-ring neurons of the ellipsoid body [35]. The 16 sectors are considered to represent azimuth sections in the outside world. Two gaseous neurotransmitters are found, which can explain the volatility of the memory that lasts for about 4s. One of them, nitric oxide, was known to act as ret-

rograde neurotransmitter from post- to pre-synapses; in orientation memory it acts within R3-ring neurons in a signalling cascade that leads to the production of the second messenger cGMP and likely to the opening of cyclic nucleotide gated cation channels [36]. A second gaseous messenger, hydrogen sulfide, was known to act synergistically with nitric oxide in smooth muscle relaxation, but it was surprising to find the same interaction of the two gases in neurons. The memory trace is modelled as elevated cGMP levels in single sectors of R3 neurons (Figure 1.2); levels are jointly regulated by the production of nitric oxide, which activates cGMP-producing guanylyl cyclase, and hydrogen sulfide, which inhibits phosphodiesterase 6 that would otherwise degrade cGMP. In accord with the outcome of behavioural experiments, the model assumes that NO and cGMP are formed in the ellipsoid body sector that represents the azimuth sector of the visual field holding the target landmark. When this landmark disappears and a distracter appears at a different azimuth position, the respective sector in the ellipsoid body starts to generate nitric oxide and cGMP, whereas in the original sector the signal starts to degrade. If the distractor is shown for longer than 4s, it will become the actual target, because its cGMP signal is now stronger than that of the original target landmark (Figure 1.2). The memory is read out only, if no visual cues are present; it is a back-up system that helps flies to stay on track during short phases of missing visual input. Idiothetic input (e.g. corollary discharge from turning commands) helps to update the orientation memory during such phases by path integration. R2- and/or R4-ring neurons are suitable to provide map-like visual input [68]. The R3-ring neurons provide an off-signal, when the target disappears from sight [54]. Several types of neurons are known [92] that connect the ellipsoid body with the protocerebral bridge for a read-out that differentially influences step size and thus walking direction.

The place memory for a cool spot on a hot surface [53] uses visual cues in the surrounding to re-identify the pleasant place. R1-neurons of the ellipsoid body are required for proper function of the place memory. Of note, the visual information in this paradigm is always available, whereas in the case of the visual orientation memory R3-neuron activity is required only after no visual cues are left. Thus different ring-neuron systems have distinct orientation functions.

In what form does visual information enter the central complex? To this end Seelig and Jayaraman [68] performed two-photon calcium imaging experiments on behaving flies walking stationary on an airstream-supported sphere. They describe R2- and R4d-ring neurons to be visually responsive. Their dendrites in the accessory area called bulb [30] are retinotopically arranged. Their receptive fields comprise excitatory as well as inhibitory subfields and, according to Seelig and Jayaraman [68], resemble properties of simple cells in the mammalian primary visual cortex V1. Authors found a strong orientation tuning in the R2- and R4d-ring neurons which in some cases was direction-selective. Vertically oriented visual objects evoked particularly strong responses.

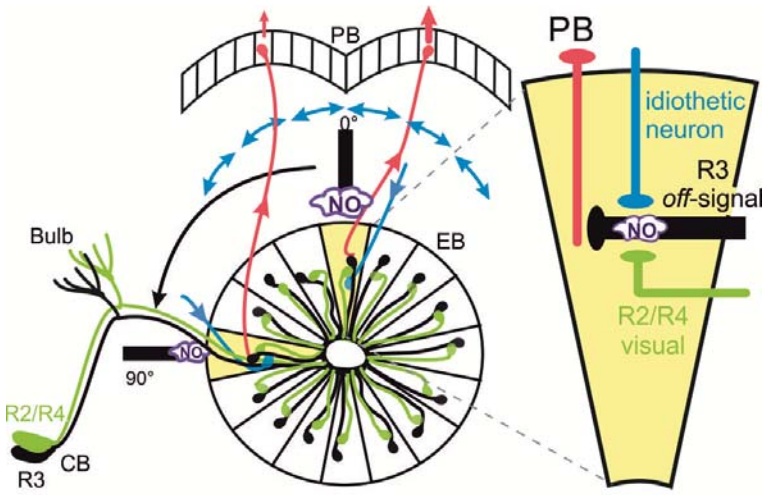

Fig. 1.2: Proposed biological model of visual working memory storage and retrieval. Depicted is the connectivity and flow of information in *Drosophila* in one of the 16 segments of the ellipsoid body (EB) and between the EB and the protocerebral bridge (PB). Ring neurons R3 (black) receive visual input from the R2- or R4-system (green) and simultaneously from neurons conveying information on the fly's self-motion (idiothetic; blue). Landmark approach stimulates NO production in one axonal branch of R3 neurons, and elevated levels of NO induce cGMP production. cGMP-mediated opening of cyclic-nucleotide-gated ion channels (CNGs; violet) results in Calcium influx, representing the memory trace of the landmark. When the distractor appears instead of the original target, the described processes are repeated in an EB segment representing the respective azimuth angle. When no object is visible (off signal), a columnar neuron (red) projecting from the EB to the PB will be activated by the R3 neurons. Differential activation of the EB-PB neurons in the segments is then used to steer the fly. Note that a longer-shown distractor will become the main target as its NO/cGMP signal rises, whereas that of the original landmark volatilizes (adapted from [36]).

In their follow-up study Seelig and Jyaraman [69] demonstrate that visual landmark orientation and angular path integration are combined in the population responses of columnar ellipsoid body neurons whose dendrites tile the ellipsoid body. Authors point out that the responses of these cells are reminiscent of mammalian head direction cells that represent the direction of heading relative to a landmark and update their directional tuning by path integration if the lights are turned off. Calcium peaks were seen to rotate within the ellipsoid body together with the landmark on a virtual-reality panorama. The angular relation between the landmark in the visual world and the position of the calcium peak varied from trial-to-trial, it is therefore relative. However, within a given measuring episode it faithfully represents the angular azimuth motion of the landmark. Moreover, in the absence of visual cues, its rotation represents self-motion of the fly. Several features of the population dynamics of these neurons and their circular anatomical ar-

rangement are suggestive of ring attractors, network structures that have been proposed to support the function of navigational brain circuits. This idea has been elaborated by Kim et al. [32]. Noteworthy, the calcium-imaging experiments showed, that for several landmarks in the environment only one bump, apparently for the chosen landmark, was visible [69].

Turner-Evans et al. [85] and Green et al. [17] present the working of the internal compass orientation within the ellipsoid body of *Drosophila*. In addition to the head-direction cells [85], which project from the ellipsoid body to the protocerebral bridge and an accessory region called gall [30], shifting cells are described. Head-direction cells are systematically termed EBw.s-PBg.b-gall.b neurons by [92], have previously been referred to as eb-pb-vbo neurons by [21], E-PG neurons by [17], EIP neurons by [39], and "wedge neurons" in a review by [23]. They may be homologous to CL1a neurons in the locust [25] and the butterfly [24]. Shifting neurons or P-EN neurons [17] are systematically termed PBG2–9.s-EBt.b-NO1.b neurons [92], correspond to PEN neurons of [39] and tile neurons of [23]. These columnar neurons of the central complex project from the protocerebral bridge to the ellipsoid body and the noduli. While head-direction cells represent the heading in relation to a landmark (and two of them signal from one sector of the ellipsoid body to two individual glomeruli on either side of the protocerebral bridge), the shifting cells become active in conjunction with body rotations of the fly. Two sets of shifting cells are projecting from the identical glomerulus of the bridge to a sector adjacent to where the currently active head-direction cells emerge. The set of shifting cells emerging from the left-hemisphere glomeruli of the bridge is offset by one sector of the ellipsoid body to the right and is responsible for right turns of the body and the calcium signal. The set of shifting cells emerging on the right side of the bridge is offset by one sector to the left and is responsible for left-shifts and counter-clockwise body turns [23].

Last not least it should be noted that the ellipsoid body is involved in certain memory phases of olfactory aversive memory. This came as a surprise, as the mushroom bodies seemed sufficient for a long time to explain olfactory memory. It seems to be a modulation function of the ellipsoid body more than an actual memory trace that is required for aversive olfactory middle-term memory in *Drosophila* [98]. The artificial activation of R2 and R4m neurons did not affect learning with regard to middle-term memory, but eliminated a particular labile component, the anaesthesia-sensitive memory. The majority of the activated ring neurons is inhibitory, thus it seems that they actively suppress the anaesthesia-sensitive memory. Evidence is provided, that ellipsoid body ring neurons and mushroom body medial lobes may be synaptically connected.

1.6 Towards long-term autonomy – more things to be learned from insects

Autonomy in roving robots is currently mostly restricted to autonomous orientation and locomotion in rather short-term goals defined by humans. Admittedly, autonomous vacuum cleaners and lawn mowers show a first sign of a "long-term survival strategy", when they reach for their power station upon their batteries going low. But on Mars, robots will need more of such long-term strategies. Just two thoughts on that.

Robots might have to find new resources and that calls for curiosity behaviour, whenever the robot is in a relaxed and saturated state. Insects show novelty choice behaviour, which can be seen as one component of curiosity. It requires memory of what is already familiar in order to identify new objects in the environment. Those objects are consequently explored. In flight-simulator experiments involving tethered-flying fruit flies, Dill and Heisenberg [13] were the first to show that flies preferentially oriented for objects with a new size or a new visual contrast with regard to the background. van Swinderen and colleagues argue that novelty comprises an aspect of visual salience in *Drosophila*. In local field potentials derived from the fly's central brain, novelty of a stimulus increases endogenous 20-to-30-Hz local field potential activity [86, 87, 88]. According to Solanki et al. [72], three functional components underlie novelty choice at the flight simulator, visual azimuth orientation, a working memory, and the ability for pattern discrimination. In an attempt to map novelty choice in the fly brain, they find that both the central complex and the mushroom bodies are involved. The latter are specifically needed when it comes to a comparison of patterns of different sizes. Within the central complex particularly the ellipsoid body and its ring-neuron systems are involved.

A compact system for activity control is another feature we might learn from insects. Ries et al. [64] recently found an activity control system in *Drosophila*. It resides in the mushroom bodies and uses serotonin as neurotransmitter and neurohormone. Hungry flies become more active and even more, if food odour is provided. Long-term stress, on the other hand, in the study consisting of inescapable sequences of adverse vibrations that were unforeseeably repeated over days, causes a depletion of serotonin (5-HT) in the mushroom bodies; the depletion correlates with behavioural inactivity with all signs of depression. Sugar reward, in turn, replenishes 5-HT in the mushroom bodies and leads to normal activity. The bipartite system requires tightly balanced serotonergic signalling to two different lobes of the mushroom bodies to generate adequate behavioural activity. Activation of α-lobes (relevant Kenyon cells express 5-HT-1A receptors) enhances behavioural activity, whereas activation of γ-lobes (relevant Kenyon cells express 5-HT-1B receptors) reduces such activity. Modern antidepressants suppress reuptake of 5-HT from the synaptic cleft so that 5-HT molecules activate postsynaptic receptors more intensely; they ameliorate the depression-like state of *Drosophila*, too. The relevant next questions for basic research are now, how a simple nervous

system adds up stress information and how it identifies reward. Short episodes of stress activate rather than depress the fly. It is the long-term repetition of stress, and the fact that it is inescapable, that leads to activity reduction (for learned helplessness in flies see [2, 95]). A similar integration happens with reward: the normal amount of sugars in the fly food has no antidepressant effect. A short sugar rush, however, given after each day of vibration stress and before the normal food overnight, turns flies resilient to stress. While the signalling path of externally-administered reward is understood to some extent, we need to find out how a simple nervous system can deduce reward-like signals from successful own actions as opposed to punishment-like signals from unsuccessful own attempts.

1.7 Conclusions

The tremendous progress in neurogenetic tools has advanced our understanding of *Drosophila* brain functions. Mushroom bodies and central complex are in the centre of interest of basic research and precise information on the actual wiring of behavioural control circuits is coming up. Calcium imaging is the method of choice when it comes to watching the brain at work, but the dynamics the method can mirror is restricted. Electrophysiological studies are needed to complement imaging. Modelling can guide basic researchers to their next experiments, but it can also be used to develop bio-inspired control of autonomously roving robots. This book wants to show what we can learn from *Drosophila* and other insects in order to achieve such astounding adaptive behaviour that flies control with just about 150000 central neurons.

References

1. Aso, Y., Sitaraman, D., Ichinose, T., Kaun, K.R., Vogt, K., Belliart-Guérin, G., Plaçais, P.Y., Robie, A.A., Yamagata, N., Schnaitmann, C., Rowell, W.J., Johnston, R.M., Ngo, T.T., Chen, N., Korff, W., Nitabach, M.N., Heberlein, U., Preat, T., Branson, K.M., Tanimoto, H., Rubin, G.M.: Mushroom body output neurons encode valence and guide memory-based action selection in *Drosophila*. eLife, **3**, e04580 (2014)
2. Batsching, S., Wolf, R., Heisenberg, M.: Inescapable stress changes walking behavior in flies - learned helplessness revisited. PLoS ONE **11**(11), e0167066 (2016)
3. Boyan, G., Liu, Y., Khalsa, S.K., Hartenstein V.: A conserved plan for wiring up the fan-shaped body in the grasshopper and *Drosophila*. Development, Genes and Evolution **227**(4), 253-269 (2017)
4. Brand, A.H., Perrimon, N.: Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development **118**(2), 401-415 (1993)
5. Burke, C.J., Huetteroth, W., Owald, D., Perisse, E., Krashes, M.J., Das, G., Gohl, D., Silies, M., Certel, S., Waddell, S.: Layered reward signaling through octopamine and dopamine in *Drosophila*. Nature **492**(7429), 433–437 (2012)

6. Caron, S.J.C., Ruta, V., Abbott, L.F., Axel, R.: Random convergence of olfactory inputs in the *Drosophila* mushroom body. Nature **497**(7447), 113–117 (2013)
7. Cervantes-Sandoval, I., Martin-Pena, A., Bery, J., Davis, R.: System-like consolidation of olfactory memories in *Drosophila*. Journal of Neuroscience **33**(23), 9846-9854 (2013)
8. Cervantes-Sandoval, I., Phan, A., Chakraborty, M., Davis, R.L.: Reciprocal synapses between mushroom body and dopamine neurons form a positive feedback loop required for learning. eLife **10**, e23789 (2017)
9. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., Prasher, D.C.: Green fluorescent protein as a marker for gene expression. Science **263**(5148), 802-805 (1994)
10. Chouhan, N.S., Wolf, R., Heisenberg, M.: Starvation promotes odor/feeding-time associations in flies. Learning & Memory **24**(7), 318-321 (2017)
11. Chouhan, N.S., Wolf, R., Helfrich-Förster, C., Heisenberg, M.: Flies remember the time of day. Current Biology **25**(12), 1619-1624 (2015)
12. Crittenden, J., Skoulakis, E., Han, K., Kalderon, D., Davis, R.: Tripartite mushroom body architecture revealed by antigenic markers. Learning & Memory **5**(1-2), 38-51 (1998)
13. Dill, M., Heisenberg, M.: Visual pattern memory without shape recognition. Philosophical Transactions of the Royal Society London B **349**, 143-152 (1995)
14. Duffy, J.B.: GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. Genesis **34**(1-2), 1-15 (2002)
15. Farris, S.M., Robinson, G.E., Fahrbach, S.E.: Experience-and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. Journal of Neuroscience **21**(16), 6395-6404 (2001)
16. Felsenberg, J., Barnstedt, O., Cognigni, P., Lin, S., Waddell, S.: Re-evaluation of learned information in *Drosophila*. Nature **544**, 240–244 (2017)
17. Green, J., Adachi, A., Shah, K.K., Hirokawa, J.D., Magani, P.S., Maimon, G.: A neural circuit architecture for angular integration in *Drosophila*. Nature **546**, 101-106 (2017)
18. Gronenberg, W., López-Riquelme, G.O.: Multisensory convergence in the mushroom bodies of ants and bees. Acta Biologica Hungarica **55**(1), 31–37 (2004)
19. Hamada, F.N., Rosenzweig, M., Kang, K., Pulver, S., Ghezzi, A., Jegla, T.J., Garrity, P.A.: An internal thermal sensor controlling temperature preference in *Drosophila*. Nature **454**, 217–220 (2008)
20. Hammer, M., Menzel, R.: Multiple sites of associative odour learning as revealed by local brain microinjections of octopamine in honeybees. Learning & Memory **5**, 146–156 (1998)
21. Hanesch, U., Fischbach, K.-F., Heisenberg, M.: Neuronal architecture of the central complex in *Drosophila melanogaster*. Cell Tissue Research **257**, 343-366 (1989)
22. Heimbeck, G., Bugnon, V., Gendre, N., Keller, A., Stocker, R.: A central neural circuit for experience-independent olfactory and courtship behavior in *Drosophila melanogaster*. Proceedings of the National Academy of Science USA **98**(26), 15336-15341 (2001)
23. Heinze, S.: Neural coding: bumps on the move. Current Biology **27**, R410–R412 (2017)

24. Heinze, S., Reppert, S.M.: Sun compass integration of skylight cues in migratory monarch butterflies. Neuron **69**, 345-358 (2011)
25. Heinze, S., Gotthardt, S., Homberg, U.: Transformation of polarized light information in the central complex of the locust. Journal of Neuroscience **29**, 11783–11793 (2009)
26. Homberg, U.: Sky compass orientation in desert locusts - evidence from field and laboratory studies. Frontiers in Behavioral Neuroscience **9**, 346 (2015)
27. Huetteroth, W., Perisse, E., Lin, S., Klappenbach, M., Burke, C., Waddell, S.: Sweet taste and nutrient value subdivide rewarding dopaminergic neurons in *Drosophila*. Current Biology **25**(6), 751-758 (2015)
28. Inagaki, H.K., Jung, Y., Hoopfer, E.D., Wong, A.M., Mishra, N., Lin, J.Y., Tsien, R.Y., Anderson, D.J.: Optogenetic control of freely behaving adult *Drosophila* using a red-shifted channelrhodopsin. Nature Methods **11**(3), 325–332 (2014)
29. Isabel, G., Pascual, A., Preat, T.: Exclusive consolidated memory phases in *Drosophila*. Science **304**, 1024-1027 (2004)
30. Ito, K., Shinomiya, K., Ito, M., Armstrong, D., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A., Keshishian, H., Restifo, L.L., Rössler, W., Simpson, J.H., Strausfeld, N.J., Strauss, R., Vosshall, L.B.: A systematic nomenclature of the insect brain. Neuron **81**, 755-765 (2014)
31. Kienitz, B.: Motorisches Lernen in Drosophila melanogaster. Shaker Verlag, Aachen (2010)
32. Kim, S.S., Rouault, H., Druckmann, S., Jayaraman, V.: Ring attractor dynamics in the *Drosophila* central brain. Science **356**(6340), 849-853 (2017)
33. Kitamoto, T.: Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive *shibire* allele in defined neurons. Journal of Neurobiology **47**, 81–92 (2001)
34. Krause, T., Strauss, R.: Body reach learning from parallax motion in *Drosophila* requires PKA/CREB. Journal of Neurogenetics, **26** Suppl. 1, 48 (2012)
35. Kuntz, S., Poeck, B., Sokolowski, M.B., Strauss, R.: The visual orientation memory of *Drosophila* requires Foraging (PKG) upstream of Ignorant (RSK2) in ring neurons of the central complex. Learning & Memory **19**, 337-340 (2012)
36. Kuntz, S., Poeck, B., Strauss, R.: Visual working memory requires permissive and instructive NO/cGMP signaling at presynapses in the *Drosophila* central brain. Current Biology **27**(5), 613-623 (2017)
37. Lee, T., Lee, A., Lou, L.: Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons form a neuroblast. Development **126**(18), 4065-4076 (1999)
38. Lei, Z., Chen, K., Li, H., Liu, H., Guo, A.: The GABA system regulates the sparse coding of odors in the mushroom bodies of *Drosophila*. Biochemical and Biophysical Research Communications **436**(1), 35-40 (2013)
39. Lin, C.-Y., Chuang, C.-C., Hua, T.-E., Chen, C.-C., Dickson, B. J., Greenspan, R. J., Chiang, A.-S.: A comprehensive wiring diagram of the protocerebral bridge for visual information processing in the *Drosophila* brain. Cell Reports **3**, 1739-1753 (2013)

40. Liu, G., Seiler, H., Wen, A., Zars, T., Ito, K., Wolf, R., Heisenberg, M., Liu, L.: Distinct memory traces for two visual features in the *Drosophila* brain. Nature **439**(7076), 551-556 (2006)
41. Liu, L., Wolf, R., Ernst, R., Heisenberg, M.: Context generalization in *Drosophila* visual learning requires the mushroom bodies. Nature **400**(6746), 753-756 (1999)
42. Masse, N., Turner, G., Jefferis, G.: Olfactory information processing in *Drosophila.* Current Biology **19**(16), R700-R713 (2009)
43. McBride, S., Giuliani, G., Choi, C., Krause, P., Correale, D., Watson, K., Baker, G., Siwicki, K.: Mushroom body ablation impairs short-term memory and long-term memory of courtship conditioning in *Drosophila melanogaster*. Neuron **24**(4), 967-977 (1999)
44. Martin, J., Ernst, R., Heisenberg, M.: Mushroom bodies suppress locomotor activity in *Drosophila melanogaster*. Learning & Memory **5**(1), 179-191 (1998)
45. Martin-Pena, A., Acebes, A., Rodriguez, J.-R., Chevalier, V., Triphan, T., Strauss, R., Ferrus, A.: Cell types and coincident synapses in the ellipsoid body of *Drosophila*. European Journal of Neuroscience **39**(10), 1586-1601 (2014)
46. McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., Davis, R.L.: Spatiotemporal rescue of memory dysfunction in *Drosophila*. Science **302**(5651), 1765-1768 (2003)
47. Miyamoto, T., Amrein, H.: Suppression of male courtship by a *Drosophila* pheromone receptor. Nature Neuroscience **11**(8), 874-876 (2008)
48. Mizunami, M., Weibrecht, J., Strausfeld, N.: A new role for the insect mushroom bodies: place memory and motor control. In: *Biological neural networks in invertebrate neuroethology and robotics,* R. Beer, Ed., Academic Press, Cambridge, UK, pp. 199–225 (1993)
49. Mizunami, M., Weibrecht, J., Strausfeld, N.: Mushroom bodies of the cockroach: their participation in place memory. Journal of Comparative Neurology **402**, 520–537 (1998)
50. Morris, R.: Spatial localization does not require the presence of local cues. Learning and Motivation **12**, 239–260 (1981)
51. Mronz, M., Strauss, R.: Proper retreat from attractive but inaccessible landmarks requires the mushroom bodies. 9th European Symposium on Drosophila Neurobiology, Neurofly Dijon (Abstract) (2002)
52. Neuser, K, Triphan, T. Mronz, M., Poeck, B, Strauss, R.: Analysis of a spatial orientation memory in *Drosophila.* Nature **453**(7199), 1244-1247 (2008)
53. Ofstad, T.A., Zuker, C.S., Reiser, M.B.: Visual place learning in *Drosophila melanogaster*. Nature **474**(7350), 204-207 (2011)
54. Omoto, J.J., Keleş, M.F., Nguyen, B.-C.M., Bolanos, C., Lovick, J.K., Frye, M.A., Hartenstein, V.: Visual input to the *Drosophila* central complex by developmentally and functionally distinct neuronal populations. Current Biology **27**(8), 1098-1110 (2017)
55. Ostrowski, D., Kahsai, L., Kramer, E.F., Knutson, P., Zars, T.: Place memory retention in *Drosophila*. Neurobiology of Learning and Memory **123**, 217-224 (2015)
56. Pan, Y., Zhou, Y., Guo, C., Gong, H., Gong, Z., Liu, L.: Differential roles of the fan-shaped body and the ellipsoid body in *Drosophila* visual pattern memory. Learning & Memory **16**, 289-295 (2009)

57. Perisse, E., Burke, C., Huetteroth, W., Waddell, S.: Shocking revelations and saccharin sweetness in the study of *Drosophila* olfactory memory. Current Biology **23**(17), R752-R763 (2013)
58. Perisse, E., Yin, Y., Lin, A., Lin, S., Hütteroth, W., Waddell, S.: Different Kenyon cell populations drive learned approach and avoidance in *Drosophila*. Neuron **79**(5), 945-956 (2013)
59. Pfeiffer, B.D., Jenett, A., Hammonds, A.S., Ngo, T.-T.B., Misra, S., Murphy, C., Scully, A., Carlson, J.W., Wan, K.H., Laverty, T.R., Mungall, C., Svirskas, R., Kadonaga, J.T., Doe, C.Q., Eisen, M.B., Celniker, S.E., Rubin, G.M.: Tools for neuroanatomy and neurogenetics in *Drosophila.* Proceedings of the National Academy of Science USA **105**, 9715–9720 (2008)
60. Pick, S., Strauss, R.: Goal-driven behavioral adaptations in gap-climbing *Drosophila*. Current Biology **15**, 1473-1478 (2005)
61. Poeck, B., Triphan, T., Neuser, K., Strauss, R.: Locomotor control by the central complex in *Drosophila* – an analysis of the *tay bridge* mutant. Developmental Neurobiology **68**, 1046-1058 (2008)
62. Putz, G., Heisenberg, M.: Memories in *Drosophila* heat-box learning. Learning & Memory **9**(5), 349-359 (2002)
63. Putz, G., Bertolucci, F., Raabe, T., Zars, T., Heisenberg, M.; The *S6KII* (*rsk*) gene of *Drosophila melanogaster* differentially affects an operant and a classical learning task. Journal of Neuroscience **24**(44), 9745-9751 (2004)
64. Ries, A.-S., Hermanns, T., Poeck, B., Strauss, R.: Serotonin modulates a depression-like state in *Drosophila* responsive to lithium treatment. Nature Communications **8**, 15738 (2017)
65. Sakai, T., Kitamoto, T.: Differential roles of two major brain structures, mushroom bodies and central complex, for *Drosophila* male courtship behavior. Journal of Neurobiology **66**(8), 821-834 (2006)
66. Schröter, U., Menzel, R.: A new ascending sensory tract to the calyces of the honeybee mushroom body, the subesophageal-calycal tract. Journal of Comparative Neurology **465**(2), 168–178 (2003)
67. Schwärzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S., Heisenberg, M.: Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. Journal of Neuroscience **23**, 10495-10502 (2003)
68. Seelig, J.D., Jayaraman, V.: Feature detection and orientation tuning in the *Drosophila* central complex. Nature **503**, 262-266 (2013)
69. Seelig, J.D., Jayaraman, V.: Neural dynamics for landmark orientation and angular path integration. Nature **521**, 186-191 (2015)
70. Serway, C., Kaufman, R., Strauss, R., de Belle, J.: Mushroom bodies enhance initial motor activity in *Drosophila*. Journal of Neurogenetics **23**(1-2), 173-184 (2009)
71. Siegel, R.W., Hall, J.C.: Conditioned responses in courtship behavior of normal and mutant *Drosophila*. Proceedings of the National Academy of Science USA **76**, 3430–3434 (1979)
72. Solanki, N., Wolf, R., Heisenberg, M.: Central complex and mushroom bodies mediate novelty choice behavior in *Drosophila*. Journal of Neurogenetics **29**(1), 30-37 (2015)

73. Strauss, R., Hanesch, U., Kinkelin, M., Wolf, R., Heisenberg, M.: *no bridge* of *Drosophila melanogaster*: portrait of a structural mutant of the central complex. Journal of Neurogenetics **8**, 125-155 (1992)
74. Strauss, R., Heisenberg, M.: Coordination of legs during straight walking and turning in *Drosophila melanogaster*. Journal of Comparative Physiology A, **167**, 403-412 (1990)
75. Strauss, R., Krause, T., Berg, C., Zäpf, B.: Higher brain centers for intelligent motor control in insects. In *ICIRA 2011, Part II, LNAI 7102*, Jeschke, S., Liu, H. Schilberg, D. eds., Springer, Berlin, Heidelberg, pp. 56–64 (2011)
76. Strutz, A., Soelter, J., Baschwitz, A., Farhan, A., Grabe, V., Rybak, J., Knade, M., Schmucker, M., Hansson, B., Sachse, S., Decoding odor quality and intensity in the *Drosophila* brain. eLife **3**, e04147 (2014)
77. Sweeney, S.T., Broadie, K., Keane, J., Niemann, H., O'Kane, C.J.: Targeted expression of tetanus toxin light chain in *Drosophila* specifically eliminates synaptic transmission and causes behavioral defects. Neuron **14**, 341-351 (1995)
78. Tanaka, N.K., Tanimoto, H., Ito, K.: Neuronal assemblies of the *Drosophila* mushroom body. Journal of Computational Neuroscience **508**, 711–755 (2008)
79. Tang, S., Guo, A.: Choice behavior of *Drosophila* facing contradictory visual cues. Science **294**(5546), 1543-1547 (2001)
80. Trannoy, S., Redt-Clouet, C., Dura, J., Preat, T.: Parallel processing of appetitive short- and long-term memories in *Drosophila*. Current Biology **21**(19), 1647-1653 (2011)
81. Triphan, T., Nern, A., Roberts, S.F., Korff, W., Naiman, D.Q., Strauss, R.: A screen for constituents of motor control and decision making in *Drosophila* reveals visual distance-estimation neurons. Scientific Reports **6**, 27000 (2016)
82. Triphan, T., Poeck, B., Neuser, K., Strauss, R.: Visual targeting of motor actions in climbing *Drosophila*. Current Biology **20**, 663-668 (2010)
83. Tully, T., Quinn, W.G.: Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. Journal of Comparative Physiology A **157**(2), 263-277 (1985)
84. Tully, T., Preat, T., Bonyton, S.C., Del Vecchio, M.: Genetic dissection of consolidated memory in *Drosophila*. Cell, **79**, 35-47 (1994)
85. Turner-Evans, D., Wegener, S., Rouault, H., Franconville, R., Wolff, T., Seelig, J.D., Druckmann, S., Jayaraman, V.: Angular velocity integration in a fly heading circuit. eLife **6**, e23496 (2017)
86. van Swinderen, B.: Attention-like processes in *Drosophila* require short-term memory genes. Science **315**, 1590–1593 (2007)
87. van Swinderen, B., Greenspan, R.J.: Salience modulates 20-30 Hz brain activity in *Drosophila*. Nature Neuroscience **6**, 579–586 (2003)
88. van Swinderen, B., McCartney, A., Kauffman, S., Flores, K., Agrawal, K., Wagner, J., Paulk, A.: Shared visual attention and memory systems in the *Drosophila* brain. PLoS ONE **4**, e5989 (2009)
89. Vogt, K., Schnaitmann, C., Dylla, K.V., Knapek, S., Aso, Y., Rubin, G.M., Tanimoto, H.: Shared mushroom body circuits underlie visual and olfactory memories in *Drosophila*. eLife **3**, e02395 (2014)
90. Vosshall, L., Stocker, R.: Molecular architecture of smell and taste in *Drosophila*. Annual Reviews of Neuroscience **30**, 505-533 (2007)

91. Vosshall, L., Wong, A., Axel, R.: An olfactory sensory map in the fly brain. Cell **102**(2), 147-159 (2000)
92. Wolff, T., Iyer, N.A., Rubin, G.M.: Neuroarchitecture and neuroanatomy of the *Drosophila* central complex: a GAL4-based dissection of protocerebral bridge neurons and circuits. Journal of Comparative Neurology **523**, 997–1037 (2015)
93. Wu, J.-K., Tai, C.-Y.. Feng, K.-L., Chen, S.-L., Chen, C.-C., Chiang, A.-S.: Long-term memory requires sequential protein synthesis in three subsets of mushroom body output neurons in *Drosophila*. Scientific Reports **7**, 7112 (2017)
94. Wustmann, G., Rein, K., Wolf, R., Heisenberg, M.: A new paradigm for operant conditioning of *Drosophila melanogaster*. Journal of Comparative Physiology A **179**(3), 429-436 (1996)
95. Yang, Z., Bertolucci, F., Wolf, R., Heisenberg, M.: Flies cope with uncontrollable stress by learned helplessness. Current Biology **23**(9), 799–803 (2013)
96. Yi, W., Zhang, Y., Tian, Y., Guo, J., Li, Y., Guo, A.: A subset of cholinergic mushroom body neurons requires go signaling to regulate sleep in *Drosophila*. Sleep **36**(12), 1809-1821 (2013)
97. Young, J.M., Armstrong, J.D.: Structure of the adult central complex in *Drosophila*: Organization of distinct neuronal subsets. Journal of Comparative Neurology **518**, 1500-1524 (2010)
98. Zhang, Z., Li, X., Guo, J., Li, Y., Guo, A.: Two clusters of GABAergic ellipsoid body neurons modulate olfactory labile memory in *Drosophila*. Journal of Neuroscience **33**(12), 5175-5181 (2013)