Genetic analysis of behavior in *Drosophila*

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1 Abstract

The main function of brains is to generate adaptive behavior. Far from being the stereotypical, robot-like insect, the fruit fly *Drosophila* exhibits astounding flexibility and choses different courses of actions even under identical external circumstances. Due to the power of genetics, we now are beginning to understand the neuronal mechanisms underlying this behavioral flexibility. Interestingly, the evidence from studies of disparate behaviors converges on common organizational principles common to many if not all behaviors, such as modified sensory processing, involvement of biogenic amines in network remodeling, ongoing activity and modulation by feedback. Seemingly foreseeing these recent insights, already the first research fields in *Drosophila* behavioral neurogenetics reflected this constant negotiation between internal and external demands on the animal as the common mechanism underlying adaptive behavioral choice in *Drosophila*.

2 Keywords

Insect, neurobiology, spontaneity, operant, neuromodulator

3 Introduction

Nervous systems accomplish adaptive behavioral choice by complex computations handling internal and external demands. The earliest genetic approaches to understanding behavior in the fruit fly *Drosophila* already reflect these two major factors. An excellent example of Seymour Benzer's many seminal works as the founder of *Drosophila* neurogenetics is that studying perhaps the most iconic of insect behaviors, the approach of a light source (positive phototaxis; (Benzer, 1967). In this publication, Benzer describes a simple apparatus to test a group of flies for their phototactic behavior. The flies make several decisions to walk or not to walk towards a light source and according to the sequence in which these decisions are made, the group of flies separates into different tubes. The distribution of the flies over the different tubes is a measure for the collective propensity of the group of flies to show phototaxis. Already before Benzer used this machine to screen for novel mutants defective in phototaxis (Hall, 1982), he described existing flightless mutants as being less phototactic, an observation that had already been described 49 years earlier (McEwen, 1918). McEwen had found that only startled flies would walk towards the light in his small tube. Sitting flies did not seem to find a light very attractive, suggesting that more than just light hitting the retina must be responsible for phototaxis to occur. He also observed that flies with clipped wings do not approach the light anymore, even when compared to walking, intact flies. These early results already indicated that several factors were influencing the flies' decision of whether or not to approach a light source. One of these factors was the light and its properties, and another appeared to be the state of the animal's wings. For the next almost 50 years, these observations received little attention.

4 Forward genetics

4.1 Learning and Memory

Instead, other early developments took off immediately, such as olfactory conditioning (Quinn et al., 1974). One could speculate that this difference is in part likely due to the mutants discovered in the wake of the experimental developments being more directly involved with central brain functions, while most phototaxis mutants were primarily affecting different aspects of vision (more on vision below). Classical olfactory conditioning is a great example of the power of forward genetics can kick-start a research field that is as vibrant and exciting now as it was more than 40 years ago. The experimental procedure is analogous to that Pavlov used with his dogs, except the conditioning uses aversive stimuli: a group of flies is first exposed to one odor while receiving electroshocks (or is allowed to ingest sucrose, in later experiments). Then a second odor is presented, this time without any electroshocks. After a varying number of such pairings, the animals are presented with a choice of both odors. Here, forward genetics implies that a mutagenesis is performed and each resulting mutant strain is tested for a lack of avoidance of the odor associated with the electroshocks. Such genetic screens revealed a whole host of mutants involved in various aspects of learning and memory (Heisenberg, 1989). Probably the set of genes with the most far-reaching effects were those involved in the cAMP pathway, such as *dunce* and *rutabaga*. This pathway, it turned out, is conserved among all bilaterian animals for every associative process that involves learning about relationships in the environment. Such processes are not only involved in the many forms of Pavlovian conditioning, but also in operant forms of learning, in particular those that train the animal to learn about the stimuli in its environment (Brembs, 2011; Colomb and Brembs, 2010). Ultimately, it was the combination of this research with discoveries in the marine snail *Aplysia* and genetically accessible organisms such as mice which demonstrated the fundamental role this pathway played in many learning situations that led to the Nobel Prize for Eric Kandel in 2000 (see also chapter 26 in this volume). In *Drosophila*, different subsets of the mushroom body Kenyon cells are modified by the cAMP and other cascades mediating learning and memory consolidation by altering synaptic plasticity in these neurons (Guven-Ozkan and Davis, 2014; Owald and Waddell, 2015; Wright, 2014).

4.2 Circadian rhythms

While phototaxis or classical olfactory conditioning mainly involved the processing of external events relevant for adaptive behavioral choice, the early forward genetics period also provided insights into internal processes critically involved in the control of behavior. Most prominently, among the first groups of genes discovered in the early mutant screens are those involved in circadian rhythmicity (Konopka and Benzer, 1971). Various mutants of the gene *period* were found to affect various rhythmic behaviors in flies such as the time of eclosion from the pupal case or diurnal activity patterns and sleep in the adult animals. Different alleles of the gene either rendered the mutants' rhythm shorter, or longer or made them arrhythmic. Importantly, these mutants were discovered in the absence of any external rhythmic stimuli, evincing some of the genetic basis for the neuronal mechanisms underlying the processing of internal demands on the animal. Today, also this research field still supports a vibrant community and not only many of the molecular components of the clock have been identified, but also how different environmental stimuli interact with it to reset and entrain it and which neurons in the brain are most important for which aspects of gene function (Allada and Chung, 2010; Hardin, 2011; Helfrich-Förster; Merbitz-Zahradnik and Wolf, 2015; Michel and Lyons, 2014; Ozkaya and Rosato, 2012; Yoshii et al., 2015). This field was among the first which started to unify the early accounts from different experiments, involving external and internal processing, respectively, but others were soon to follow.

4.3 Course control

One such field is that of visually guided behaviors, such as course control in flight or walking. Crucial for course control in flies is motion vision. The groundwork for understanding motion vision was laid by the early works of Götz, Reichardt and other colleagues in the tradition of biological cybernetics (Götz and Buchner, 1978; Götz, 1964, 1965, 1968, 1970, 1972, 1977, 1980; Götz et al., 1979; Poggio and Reichardt, 1973, 1976, 1973; Reichardt and Poggio, 1975, 1976; Reichardt, 1965; Wehrhahn and Reichardt, 1973). While there were some forward genetic approaches also to motion vision (Götz, 1970; Heisenberg and Götz, 1975), most early advances in this field were physiological, behavioral and anatomical in nature. The single most prominent mutant for motion vision, *optomotor-blind* (*omb*), was discovered to lack giant neurons in the lobula plate, already known to be motion-sensitive (Bausenwein et al., 1986; Heisenberg et al., 1978). The initial, very successful approach was to study the relationships between visual input and motor output thoroughly enough to be able to construct a control model which could predict the motor output of the fly for any, even yet untested visual input. One method of choice was the so-called open-loop experiment in which the tethered fly received visual motion input while its motor output was recorded. Importantly, the motor output was not allowed to interfere with the presentation of the stimuli, i.e., the feedback loop between the animal's behavior and its environment was open, and so the emphasis of this research phase was on the processing of external stimuli, in this case visual motion stimuli. The idea behind this approach was that in order to fly straight, flies would have to compensate for unintended displacement, for instance due to side-winds. This approach explicitly excluded voluntary turns initiated by the animal. Soon after these initial studies, it was discovered that there was a second factor involved in course control. First among these discoveries was the observation that even without any visual motion input, the flies would produce course control maneuvers on their own (Wolf and Heisenberg, 1980), implying that course control behavior was not dependent on visual motion input and that the earlier exclusion of voluntary movements was an oversimplification. Later, this time using forward genetics, it was found that even if there are visual motion stimuli available, their coupling to the behavior is better described in a feedback, rather than in a feed-forward fashion, as was previously thought. This second discovery was made with the double mutant *reduced optic lobes* (*rol*), *small optic lobes* (*sol*). Freely walking or flying wildtype flies in a visually structured rotating environment have a tendency to turn with the direction of the movement. The *rol sol* double mutant flies still show phototaxis (i.e., they are not blind and can orient with regard to visual cues), but are completely devoid of any such directed 'optomotor response'. The optomotor response was thought to be critical for stabilizing the animal's course in flight and thus *rol sol* flies were expected to lack the capacity to use visual motion stimuli for course control and thus should show unstable flight. However, in experiments with tethered flies in stationary flight where the feedback loop between attempted turning behavior (as measured by a torque meter recording the fly's yaw torque) and horizontal rotation of the environment was closed, *rol sol* mutant flies were able to stabilize their flight with respect to visual landmarks and fly straight (i.e., establish optomotor balance (Wolf and Heisenberg, 1986). The interpretation was that *rol sol* mutant flies are motion sensitive but lacked sensitivity to the direction of motion. This was demonstrated by performing a third, critical experiment. After inversion of the feedback loop between behavior and environment such that attempted left-turns lead to a left-turn of the environment and thus the visual impression of a right turn, *rol sol* mutants did not require any more time to stabilize their flight and fly straight than when then loop was closed 'correctly'. The conclusion that flies are actively initiating activity in order to 'try out' which motor output controls the environment was confirmed when wildtype flies were subjected to this "inverting goggles" experiment. Even wildtype flies, with their optomotor response intact, eventually learned to use turning maneuvers of the 'opposite' direction to control flight, i.e. left turning maneuvers for the

visual impression of right turns and *vice versa* (Brembs, 2009b; Heisenberg and Wolf, 1993; Wolf et al., 1992).

4.4 Courtship

A class of behaviors where the interactions between internal and external processing are more obvious is courtship behaviors. Clearly, sex-determination as well as a host of other internal factors will influence which kinds of external stimuli can and will be perceived and, once perceived, how they will be processed and evaluated. Among the first behavioral mutants in *Drosophila* was the courtship mutant *fruitless* (Gill, 1963). Male homozygous carriers of the mutation are affected both in their behavior towards females and other males. They rarely attempt copulation with females and never successfully copulate. In contrast, they vigorously court other males and stimulate courtship behaviors in wildtype and *fruitless* males. Female homozygous carriers, on the other hand do not show any mutant phenotype (Hall, 1978). In the original *fruitless* mutant, an inversion in the *fruitless* gene not only affects the mutants' behavior towards males, but presumably also leads to the production of female pheromones, stimulating other males to court the mutants (Gailey and Hall, 1989). The *fruitless* gene codes for a transcription factor with numerous sex-specific (and non sex specific) splice variants and is expressed in about 2000 adult neurons. Ultimately, sex-specific splicing establishes anatomical sexual dimorphisms in a small group of these neurons. These dimorphisms manifest themselves in the location of neurons as well as in the shape of their neurites (Cachero et al., 2010; Kimura et al., 2005, 2008). The resulting differences between male and female nervous systems not only lead to differences in the processing of, e.g. sex-specific odors or contact pheromones, but also to sex-specific circuitry in more central brain areas which control the production of sex-specific courtship behaviors (Villella and Hall, 2008; Yamamoto and Koganezawa, 2013).

Most of these later discoveries were made possible by the development of more sophisticated genetic tools allowing for the ever more fine-grained modification of neural components in a spatiotemporally controlled manner.

5 Bipartite expression systems

One of the main methodological drivers for innovation in *Drosophila* neurogenetics after the early forward genetic mutageneses, was the development of bipartite expression systems. The common operating principle of this method is the separation of where in the nervous system a modification is intended and what this modification should be. A third, temporal control dial can usually also be incorporated. The implementation takes advantage of a transcription factor and its binding site from an unrelated organism, such that the transcription factor has no endogenous binding site beyond the transgenic one – and the transgenic binding site will not be bound by any endogenous transcriptions factors. One fly strain carries a homozygous transgene with the transcription factor (driver line), another the binding site with the effector (effector line). Crossing these two lines together yields transheterozygote offspring where only the cells expressing the transcription factor also express the effector, by virtue of only the transgenic transcription factor (and none of the endogenous transcription factors) initiating expression on the effector.

The first such system and still the most widely used is the yeast GAL4/UAS system (Brand and Perrimon, 1993). In the absence of known sequences to drive transcription factor expression in targeted cells, a basal promotor is cloned in front of the coding region of the yeast GAL4 transcription factor and inserted in a random location in the fly genome. As the unique expression pattern for this insertion locus depends on the surrounding genomic landscape, this method has been called "enhancer trap" (O'Kane and Gehring, 1987). Later, specific promotors were cloned in front of the GAL4 open reading frame to direct the transcription factor expression to specific, known cells. In another step, the effects of the surrounding genomic landscape were minimized by developing specific target sites into which all transgenes were specifically inserted (Groth et al.,

2004). These methods keep evolving generating tens of thousands of fly lines with unique expression patterns allowing researchers to target virtually any single cell or combination of cells in *Drosophila*.

Only the imagination of the scientists is the limit for which effectors could be cloned behind the Upstream Activation Sequence (UAS). Among the first were effectors which would allow staining of the target cells, e.g. β-galactosidase or green fluorescent protein. Other options are effectors which interfere with neuronal functioning such as electrical or synaptic activity. Among the latest effectors are those whose activity can be switched via light or temperature, allowing for temporal control at any timescale.

6 Reverse genetics

Because the traditional forward genetics approach looked for the genes underlying a behavioral phenotype, the approach of looking for the behavioral phenotypes underlying a genetic modification was dubbed 'reverse genetics'. In reverse genetics, the structure or expression of known genes is modified in order to analyze the associated phenotypes.

6.1 Phototaxis

These new genetic tools quickly boosted the mechanistic analysis of the already existing fields of behavioral study in *Drosophila*. For instance, the long-forgotten flexibility in phototaxis came under renewed scrutiny (Gorostiza et al., 2015). It was discovered that rather than just affecting the approach of a light source, the flies' ability to fly affected their light/dark preference across several different behavioral tests, none of which tested phototaxis, but forced the flies to choose between more or less bright stimuli. If flying ability was compromised only temporarily by expressing a temperature-sensitive depolarizing channel in the flight muscles, the flies' photopreference reversed concomitantly. Neuronal activity in circuits expressing dopamine and octopamine, respectively, doubly dissociated in this case of behavioral flexibility (Gorostiza et al., 2015). This tight control of neuronal activity was also accomplished by expressing temperature-sensitive variants of gene products involved in neuronal function: expressing the *shibire*^{ts} allele of dynamin quickly blocks vesicle recycling in the presynapse at the restrictive temperature, while expressing the trpA1 channel depolarizes the cell at high temperatures. The involvement of these two biogenic amines octopamine and dopamine suggests that valuation of stimuli may play a role in the flies' shifts in photopreference. Apparently, flies somehow monitor their ability to fly, and the outcome of this evaluation exerts a fundamental effect on action selection. This work suggests that even innate preferences which appear simple and hard-wired, such as those expressed in classic phototaxis experiments, comprise a value-driven decision-making stage, negotiating external and internal demands, before an action is selected. This endows the animal with the possibility to decide, for example, when it is better to move towards the light or hide in the shadows. At the time of this writing, it is not yet clear if this decision is made by comparing the current sensory input with the internal state, or if the outcome of an evaluation of flying ability influences the decision of how to valuate bright or dark stimuli (i.e., a kind of alliesthesia (Cabanac, 1971) before these stimuli are actually encountered. Whatever the actual mechanism, the fact that flies adapt their photopreference in accordance with their flying ability raises the tantalizing possibility that flies may have the cognitive tools required to evaluate the capability to perform an action and to let that evaluation impact other actions - an observation reminiscent of meta-cognition (Brembs, 2016; Gorostiza et al., 2015).

6.2 Course Control

A particularly attractive application of forward genetic techniques is their replacement of or combination with traditional methods of recording neuronal activity. In the study of course control,

both have been applied to discover more mechanisms of how internal processing affects the processing of external stimuli. For instance, expressing green fluorescent protein as a marker in the motion-sensitive giant lobula plate neurons (the ones which are missing in the *omb* mutants), allows them to be recorded from using a patch electrode (Maimon et al., 2010). With this technique, is was discovered that the gain of these neurons was increased in flying flies, compared to flies at rest. These observations are corroborated using genetically encoded calcium sensors expressed in these lobula plate cells. Using the differential fluorescence of these sensors in the calcium bound vs. unbound state, one can record the activity of these neurons via the calcium concentration inside the cells. Compared to flies at rest, flies walking on a Styrofoam ball showed stronger calcium transients in response to visual motion stimuli (Chiappe et al., 2010). The interpretation of these observations is that the change in the behavioral state of the animal determines the mode of sensory processing and hence how internal and external demands are negotiated to generate behavior. These genetic studies were further corroborated by behavioral and electrophysiological experiments without genetic manipulations (Haag et al., 2010; Rosner et al., 2010; Tang and Juusola, 2010). Interestingly, the biogenic amine octopamine appears to be involved in these processes (van Breugel et al., 2014; Longden and Krapp, 2009; Suver et al., 2012; Tuthill et al., 2014).

The conceptual consequences of these discoveries are far-ranging. After Werner Reichardt had proposed a model for elementary motion perception in the beetle *Chlorophanus* in the 1950s (Hassenstein and Reichardt, 1956; Reichardt and Varju, 1959), the "Reichardt movement detector" had become an entity to be discovered. Briefly, this algorithmic model for motion detection consists of two mirror-symmetrical subunits. Each subunit monitors the luminance values measured in two adjacent photoreceptors and multiplies them after one has been delayed by a low-pass filter. The output values of both subunits are subtracted to arrive at a speed and direction-sensitive motion detector. Famously, most of his proposed structure later turned out to indeed be biologically implemented (for a review see (Borst et al., 2010) and chapter 13 in this volume). However, the anatomical structure and general working principle are probably the only two aspects in which the mathematical model still holds. Already the forward genetic works using optomotor-blind flies (*rol sol* and *omb* mutants) showed that the early open-loop experiments only captured the processing of external inputs, omitting the rich internal processing required for active course control (see above). The recent discoveries entailed that even the processing of external events is dependent on internal processes to an extent that justifies the description of what initially was a singular, all-purpose movement detector for all circumstances as a 'beetle-walking-only' movement detector. The parameters and settings of the neurons implementing the Reichardt model are constantly tuned to the internal demands of the animal, such that there likely are many different movement detector settings: the literature already knows about the rest-movement-detector and the flight-movement-detector, besides the walking-movement-detector. It is conceivable that there are yet more settings to be discovered for states such as aggression, mating or hunger, all, perhaps, modulated by biogenic amines according to the demands of the organism. The flexibility that this complexity and richness enables already for a single sensory input channel evinces the connectedness and interdependence of the neural processes negotiating external and internal demands on the animal. The most recent research in this area appears to confirm this speculative extrapolation by showing that also odor-driven octopamine release modulates the gain of some of the motion-sensitive neurons in the lobula plate (Wasserman et al., 2015). Sensory systems are far from neutral information providers upon whose command an adaptive reaction is computed. Instead, the case of *Drosophila* course control is a prime example of how internal processing influences decision-making already at early sensory stages and how inter-dependent the processing of external stimuli is among different sensory modalities. The nervous system subjugates its assessment of the current situation to a range of modulators in order to accomplish an adaptive degree of autonomy from external demands. Work in the only adult animal from which the connectome is known, the nematode *C. elegans*, suggests that large parts of the nervous system are dedicated to reduce the immediate effects sensory input has on the ongoing neuronal activity and accomplish such autonomy (Gordus et al., 2015). Analogously, work in *Drosophila* course control suggests analogous mechanisms are providing the

animals with the capacity to increase or decrease the effects of sensory inputs, depending on internal processing.

6.3 Learning and Memory

Drosophila course control was also the starting point for advances in learned behavior which would come to complement the advances in olfactory classical conditioning. Modern reverse genetic tools have allowed to pinpoint which components of an olfactory appetitive or aversive memory are processed in which neurons at what stage during or after classical conditioning (for recent reviews see, e.g. (Guven-Ozkan and Davis, 2014; Owald and Waddell, 2015; Wright, 2014). However, while these recent advances deepen our understanding of the mechanisms by which initially neutral stimuli come to acquire valence, a deeper understanding of behavior came from studies involving the conditioning of behaviors, rather than stimuli.

The early observation that even without any visual motion input, flies tethered to a torque meter would produce course control maneuvers on their own (Wolf and Heisenberg, 1980), together with the discovery that flies can overrule their optomotor response (Wolf and Heisenberg, 1986), prompted the hypothesis that the flies are spontaneously generating different kinds of behavior in order to 'try out' which of these behaviors would control sensory input, i.e., a case of operant behavior. Making a non-directional infrared heat-beam contingent on such spontaneous left or right turning maneuvers of tethered flies provides instantaneous punishment without any other stimuli being contingent on the behavior (Wolf and Heisenberg, 1991). This feedback differs from visual feedback in that there can be no inborn link between the behavior and the stimulus – the animal has to discover the link on its own. Indeed, within seconds, the fly learns (by trial and error) that its turning attempts control the unpleasant heat. Moreover, after only eight minutes of such training, it continues to bias its spontaneous decisions towards the previously unpunished turning maneuvers, even if the heat is now permanently switched off. This experiment constituted the first operant conditioning experiment where all other external stimuli except the heat had been removed.

The significance of this development remained largely unnoticed, until traditional and modern neurogenetics were combined to compare the underlying genetics of this with other learning experiments. Using the classic learning mutant *rutabaga*, affecting a type I adenylyl cyclase and discovered in the early screens for classical learning mutants, it was discovered that the canonical, cAMP-dependent plasticity pathway was not involved in this type of operant learning. Instead, using the GAL4/UAS system to manipulate protein kinase C (PKC) signaling by expressing an inhibitory peptide of PKC, PKCi, abolished learning in this paradigm completely (Brembs and Plendl, 2008). These results reverse in an experiment which is almost identical, albeit with one small difference. The small change in this experiment is that whenever the direction of turning maneuvers changes, the entire visual field of the fly instantaneously turns from one color (say, green) to another (e.g., blue). Because now the colors change both with the yaw torque and the heat, the fly has the option to learn that one of the colors signals heat (in addition to learning that its behavior is controlling the heat). 'Contaminating' an otherwise 'pure' operant learning experiment with a single stimulus that can be learned, reversed the entire genetic basis of the experiment. This now composite learning task now requires the cAMP cascade and is independent of any PKC signaling. Importantly, the operant nature of the experiment was not altered: the flies' yaw torque remained in control of all stimuli during the entire experiment.

To solve this composite situation, it is sufficient for the flies to learn that one of the colors is associated with the heat and then use whatever means necessary to avoid this color. In the most Pavlovian sense, the flies learn the color-heat contingency independently of the behavior with which it was acquired, they only learn about the world around them, without leaving an observable trace that the behavioral decision-making circuitry itself has been altered: if the flies are asked to avoid the previously punished color with an orthogonal behavior to the one used during training, they manage to do so (Brembs, 2009a). In contrast to the composite situation, to solve the purely operant

experiment devised by Wolf and Heisenberg in 1991, the flies need to modify their behavioral output directly. It is perhaps not surprising, then, that one of the areas of the nervous system where this PKC-based plasticity is required appears to be motorneurons (Colomb and Brembs, 2016). Therefore, the mechanism mediating learning about external stimuli was termed 'world-learning' and the one mediating learning about the animal's own behavior 'self-learning' (Brembs, 2011; Colomb and Brembs, 2010). This distinction is conceptually similar to those between episodic and procedural learning or allocentric and egocentric strategies in other fields of the behavioral sciences and may even be biologically identical.

Once the crucial elements had been identified, it was straightforward to compare these results with other instances of such pure operant learning. Vocal learning, be it human language or birdsong, follows the same purely operant trial-and-error principle as *Drosophila* self-learning at the torque meter: Spontaneous, variable behavior is generated to find out which behavior controls the reafferent feedback. While in the fly case, the feedback stimulus was heat, in vocal learning, the feedback can be both auditory and social. However, the outcome is analogous in both cases: a modified behavioral system which reliably biases behavioral output towards previously rewarded behaviors. One prominent gene which has been discovered studying the biological basis of vocal learning is the gene FOXP2 (Fisher and Scharff, 2009; Lai et al., 2001). Every member of a now prominent family who suffers from severe verbal dyspraxia carries a mutated FOXP2 allele (Lai et al., 2001). If FOXP2 is knocked down in the basal ganglia of zebrafinches, they fail to learn their song properly (Haesler et al., 2007). *Drosophila* and other invertebrate genomes also contain a *FoxP* gene (Santos et al., 2011). A mutation or RNAi-mediated knockdown of *FoxP* in *Drosophila* yields a phenocopy of the PKC manipulations described above, i.e., impaired self-learning and unaffected world-learning (Mendoza et al., 2014). Also in mice, transgenic FoxP2 manipulations revealed self-learning phenotypes (Schreiweis et al., 2014) and PKC is known to be involved in vocal learning in birds (Sakaguchi and Yamaguchi, 1997; Yoshida et al., 2003) as well as in *Aplysia* operant self-learning (Lorenzetti et al., 2008). These converging lines of evidence from across taxa support the interpretation that, similar to cAMP-dependent world-learning, PKC-dependent self-learning evolved at the base of the bilaterian branch (Brembs, 2016). However, in contrast to world-learning, the effect of self-learning on behavior is direct and not via a modulation of the processing of external stimuli.

One may wonder why contaminating a purely operant self-learning experiment with only one predictive stimulus can come to have such far-reaching consequences? Clearly, if flies had eye-lids and closed their eyes during the composite experiment, they would exclude the predictive colors and force themselves to learn only about their own behavior – converting the composite world-learning task into a self-learning task. Apparently, when faced with such a choice, the flies preferentially learn about the world surrounding them and inhibit direct modifications to their behavioral control system. A prominent structure in the insect brain, the mushroom bodies (MBs, *corpora pedunculata*) mediate this inhibition. Inactivating synaptic output from these structures by expressing tetanus toxin light chain in the MB-intrinsic Kenyon cells allows the animals to show a yaw torque preference after training them with the colors present, even when the colors are removed during the test phase. Control flies do not show such a preference after the standard training period of eight minutes. However, after 16 minutes of composite color/torque training, in a test without colors, also wildtype flies show a conditioned preference for the unpunished turning direction (Brembs, 2009a). This preference was abolished in *FoxP* mutant flies (Mendoza et al., 2014), indicating that the process mediating such self-learning in 16 minutes of training with colors is the same one that mediates self-learning without colors in 8 minutes. It is straightforward to hypothesize that the MBs slow down acquisition of self-learning whenever world-learning is engaged, such that self-learning only takes place after an extended training period. Mimicking the formation of habits or skills in vertebrate animals, the extended training has overcome the inhibition of self-learning by world-learning such that self-learning could take place and modify the fly's decision-making circuits. The flexible, goal-directed actions controlling the heat have become stereotyped,

habitual responses: not unlike habit-interference, testing wild type flies after the extended training for color preference, reveals they have lost the flexibility to avoid the previously punished color with an orthogonal behavior (Brembs, 2011, 2016).

These experiments extend the insights from the course control experiments detailed above. Also in these learning experiments, negotiating internal and external demands provides the animal with the autonomy to behave differently in the face of the same stimuli: depending on which behavior is required to avoid the punished color, the animal is in principle able to choose the most useful one (Brembs, 2009a). However, the observation that this flexibility becomes reduced after extended training suggests a higher level organization of behavior. For animals to survive, the goal justifies the means. If stereotypic responses to external stimuli will get the animal to its goal faster or more efficiently than its competitors, than these responses will evolve. If flexible, exploratory behaviors will provide the animal with resources its competitors cannot reach, flexibility will evolve. In most, if not all cases, no individual can be born with the appropriate set of behaviors for its lifetime. Moreover, surviving in a semi-predictable environment requires efficiency and speed as much as it requires flexibility and creative problem-solving. In order to be able to constantly find the 'sweet spot', the Goldilocks zone where efficiency and flexibility are traded off optimally, animals have to be flexible with their flexibility. If flexibility is required, animals need to deploy it, to the detriment of speed and efficiency. If efficiency is required, they need to be able to deploy it, to the detriment of their flexibility and with the risk of becoming predictable and hence exploitable (Brembs, 2016; Catania, 2008, 2009, 2010; Corcoran et al., 2009; Jablonski and Strausfeld, 2001; Jabloński and Strausfeld, 2000; Miller, 1997; Mitra et al., 2009).

The biological mechanisms of behavioral flexibility are being discovered in more and more of the classic fields of *Drosophila* behavior, but also in fields that did not exist in the forward genetics time.

6.4 Courtship

By merely observing *Drosophila* courtship, its constituent behaviors may seem stereotypic and innately choreographed in both sexes. However, there is marked flexibility also in this behavior. Probably one of the most drastic changes in courtship behavior is that of females after their first copulation. While virgin females entice males to court them by various behaviors from initial running, later stopping and finally opening their genital plates for copulation, once mated, females reject courting males (e.g., by extending their ovipositor) and focus on egg-laying instead (Connolly and Cook, 1973).

As an aside: there is flexibility also in male courtship behavior, which is dependent on the female postmating switch in receptivity. While males will vigorously court any mated females in their first encounter, extended experience with the rejection by a mated female will drastically reduce courtship in this male even when he encounters a virgin for several hours. Early forward genetics approaches found that this effect is dependent on learning genes discovered in classical olfactory conditioning such as *amnesiac* and *dunce* (Siegel and Hall, 1979) and was dubbed 'courtship conditioning'. Presumably owing to the male learning primarily about sensory cues that predict the rejection (i.e., world-learning), it appears that courtship conditioning shares many genetic and cellular mechanisms with classical olfactory conditioning (Griffith and Ejima, 2009), suggesting that sensory processing is modified such that initially attractive female stimuli lose attractiveness after rejection such that courtship is no longer the behavior of choice when a female is encountered.

For the female, after mating, none of the stimuli which otherwise reliably promote courtship are now able to trigger female receptivity until she has run out of sperm to continue laying fertilized eggs about 6-9 days post-mating. This switch is brought about by the transfer of a specific male peptide with the sperm during copulation. The peptide is produced by the male accessory glands and binds to a receptor expressed in the female genital tract. Specifically, the sex peptide receptor expresses in neurons co-expressing the *fruitless* gene. A small subset of sensory neurons innervates

the female reproductive tract and projects to the central nervous system. These neurons are necessary and sufficient to bring about the post-mating switch in *Drosophila* females (Chapman et al., 2003; Chen et al., 1988; Häsemeyer et al., 2009; Liu and Kubli, 2003; Walker et al., 2015; Yapici et al., 2008). Binding of the sex peptide silences the activity in the sex peptide receptor neurons, which synapse onto second-order neurons in the abdominal ganglion. These second order neurons project into the dorsal protocerebrum where the behavioral switch is accomplished by so far unknown mechanisms (Feng et al., 2014). Analogous mechanisms may take place in photopreference, where the state of the animal also shifted the preference for external stimuli. It appears, thus, that the genetic analysis of behavior in *Drosophila* may be converging on general principles by discovering similar neuronal mechanisms for how an animal's current situation is accounted for in order to reach its goals. These principles can be found also in a class of behaviors not among the classic forward genetic fields: feeding behavior.

6.5 Feeding behavior

Obviously, together with courtship, feeding counts among the most directly evolutionary relevant behaviors. Not surprisingly, perhaps, there are strong links between the two classes. One of the most prominent ones is a post-mating switch in feeding behavior in females. All animals need to constantly balance their food intake according to nutrient level and so feeding behavior in general is already a prime example of how nervous systems negotiate internal and external demands, specifically here with regard to nutrient status and availability (Itskov and Ribeiro, 2013). However, the post-mating switch in *Drosophila* female feeding behavior goes beyond general demand-driven feedback cycles controlling behavior. Not only is the animal feeding more after mating it also reverses its preference to prefer protein over carbohydrates and increases its preference for salt. Importantly, the sex peptide receptor neurons apparently modify the female nervous system to change the fly's behavior even if there is no change in nutrient demand, i.e., in a feed-forward or centrifugal fashion (Carvalho et al., 2006; Ribeiro and Dickson, 2010; Vargas et al., 2010; Walker et al., 2015). As in so many other instances where modulation of behavioral preferences have been observed, also here, biogenic amines (serotonin, octopamine) seem to play a crucial role in orchestrating the shift in some of these preferences (Rezával et al., 2014; Vargas et al., 2010). There is evidence that shifts in preference may be caused by a shift in how taste stimuli are processed and evaluated (Walker et al., 2015), reminiscent of how motion-sensitive neurons adjust their gain when the behavioral status of the animal changes (see above).

7 Conclusions

Already in Seymour Benzer's time, the simplistic notion of behavior in general, but insect behavior in particular being best characterized as a sensorimotor transformation was belied by the numerous fields of study that he and his contemporaries initiated, most of which support vibrant research communities to this day. Rather than tying specific behaviors to identifiable stimuli, sensory information – if at all relevant – interacts with ongoing neural activity to instruct the organism which type of action to generate. The mechanisms underlying these interactions are being studied with the help of a growing arsenal of neurogenetic manipulations of the fly's nervous system. The short presentations above, of a few, highly selective examples within a much larger and encompassing *Drosophila* behavior research enterprise, provide converging evidence that adaptive behavioral choice entails constant processing of numerous factors of which external stimuli are but one aspect. Moreover, the sensory situation is not relayed neutrally to the rest of the nervous system, but tweaked, bent, colored and focused according to the ongoing internal processes dealing with the other factors. It is straightforward to hypothesize that the constant interaction between ongoing neural activity and the incoming sensory stream allows the organism to balance behavioral

flexibility with efficiency to accomplish adaptive behavioral choice in an often only semi-predictably changing environment.

The recurring common themes of modified sensory processing, involvement of biogenic amines in the network remodeling, ongoing activity and modulation by feedback in the examples described above supports the hypothesis that there may be common principles underlying adaptive behavioral choice that are either universally implemented across behaviors in *Drosophila*, or repeatedly implemented in disparate behavioral circuits. Comparing these principles underlying adaptive behavioral choice in *Drosophila* with results from studies in other organisms, the impression emerges that this organization may have evolved at the base of the bilateria and remained conserved in the ensuing approx. 500 million years (Brembs, 2016). In this case, invertebrate organisms, with their accessible nervous systems, are the prime models for the discovery of the general neural principles underlying our most central brain function: to generate behavior.

8 References

Allada, R., and Chung, B. Y. (2010). Circadian organization of behavior and physiology in Drosophila. *Annu. Rev. Physiol.* 72, 605–24. doi:10.1146/annurev-physiol-021909-135815.

Bausenwein, B., Wolf, R., and Heisenberg, M. (1986). Genetic dissection of optomotor behavior in Drosophila .Studies on wild-type and the mutant optomotor-blindH31. *J. Neurogenet.*, 87–109.

Benzer, S. (1967). Behavioral mutants of Drosophila isolated by countercurrent distribution. *Proc. Natl. Acad. Sci. U. S. A.* 58, 1112–1119. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16578662.

Borst, A., Haag, J., and Reiff, D. F. (2010). Fly Motion Vision. *Annu. Rev. Neurosci.* 33, 49–70. doi:10.1146/annurev-neuro-060909-153155.

Brand, A. H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. 118, 401–415. Available at: http://www.cob.org.uk/Development/118/02/dev2110.html.

Brembs, B. (2009a). Mushroom bodies regulate habit formation in Drosophila. *Curr. Biol.* 19, 1351–5. doi:10.1016/j.cub.2009.06.014.

Brembs, B. (2009b). The importance of being active. *J. Neurogenet.* 23, 120–6. doi:10.1080/01677060802471643.

Brembs, B. (2011). Spontaneous decisions and operant conditioning in fruit flies. *Behav. Processes* 87, 157–64. doi:10.1016/j.beproc.2011.02.005.

Brembs, B. (2016). "Operant Behavior in Model Systems," in *Learning and Memory: A Comprehensive Reference*, ed. J. H. Byrne (Elsevier Science B. V.), in press.

Brembs, B., and Plendl, W. (2008). Double dissociation of pkc and ac manipulations on operant and classical learning in drosophila. *Curr. Biol.* 18, 1168–1171. doi:10.1016/j.cub.2008.07.041.

van Breugel, F., Suver, M. P., and Dickinson, M. H. (2014). Octopaminergic modulation of the visual flight speed regulator of Drosophila. *J. Exp. Biol.* 217, 1737–44. doi:10.1242/jeb.098665.

Cabanac, M. (1971). Physiological Role of Pleasure. *Science (80-.).* 173, 1103–1107. doi:10.1126/science.173.4002.1103.

Cachero, S., Ostrovsky, A. D., Yu, J. Y., Dickson, B. J., and Jefferis, G. S. X. E. (2010). Sexual Dimorphism in the Fly Brain. *Curr. Biol.* 20, 1589–1601. doi:10.1016/j.cub.2010.07.045.

Carvalho, G. B., Kapahi, P., Anderson, D. J., and Benzer, S. (2006). Allocrine Modulation of Feeding Behavior by the Sex Peptide of Drosophila. *Curr. Biol.* 16, 692–696. doi:10.1016/j.cub.2006.02.064.

Catania, K. C. (2008). Worm Grunting, Fiddling, and Charming—Humans Unknowingly Mimic a Predator to Harvest Bait. *PLoS One* 3, e3472. doi:10.1371/journal.pone.0003472.

Catania, K. C. (2009). Tentacled snakes turn C-starts to their advantage and predict future prey behavior. *Proc. Natl. Acad. Sci. U. S. A.* 106, 11183–7. doi:10.1073/pnas.0905183106.

Catania, K. C. (2010). Born Knowing: Tentacled Snakes Innately Predict Future Prey Behavior. *PLoS One* 5, e10953. doi:10.1371/journal.pone.0010953.

Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., et al. (2003). The sex peptide of Drosophila melanogaster: female post-mating responses analyzed by using RNA interference. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9923–8. doi:10.1073/pnas.1631635100.

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., and Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female D. melanogaster. *Cell* 54, 291–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3135120.

Chiappe, M. E., Seelig, J. D., Reiser, M. B., and Jayaraman, V. (2010). Walking modulates speed sensitivity in Drosophila motion vision. *Curr. Biol.* 20, 1470–5. doi:10.1016/j.cub.2010.06.072.

Colomb, J., and Brembs, B. (2010). The biology of psychology: “Simple” conditioning? *Commun. Integr. Biol.* 3, 142–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2889970&tool=pmcentrez&rendertype=abstract [Accessed August 26, 2010].

Colomb, J., and Brembs, B. (2016). PKC in motorneurons underlies self-learning, a form of motor learning in Drosophila. *PeerJ* 4, e1971. doi:10.7717/peerj.1971.

Connolly, K., and Cook, R. (1973). Rejection Responses By Female Drosophila Melanogaster : Their Ontogeny, Causality and Effects Upon the Behaviour of the Courting Male. *Behaviour* 44, 142–165. doi:10.1163/156853973X00364.

Corcoran, A. J., Barber, J. R., and Conner, W. E. (2009). Tiger Moth Jams Bat Sonar. *Science (80-.).* 325, 325–327. doi:10.1126/science.1174096.

Feng, K., Palfreyman, M. T., Häsemeyer, M., Talsma, A., and Dickson, B. J. (2014). Ascending SAG neurons control sexual receptivity of Drosophila females. *Neuron* 83, 135–48. doi:10.1016/j.neuron.2014.05.017.

Fisher, S. E., and Scharff, C. (2009). FOXP2 as a molecular window into speech and language. *Trends Genet.* 25, 166–77. doi:10.1016/j.tig.2009.03.002.

Gailey, D. A., and Hall, J. C. (1989). Behavior and cytogenetics of fruitless in Drosophila melanogaster: different courtship defects caused by separate, closely linked lesions. *Genetics* 121, 773–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2542123.

Gill, K. S. (1963). A mutation causing abnormal mating behavior. *Drosoph. Inf. Serv.* 38, 33.

Gordus, A., Pokala, N., Levy, S., Flavell, S. W., and Bargmann, C. I. (2015). Feedback from Network States Generates Variability in a Probabilistic Olfactory Circuit. *Cell* 161, 215–227. doi:10.1016/j.cell.2015.02.018.

Gorostiza, E. A., Colomb, J., and Brembs, B. (2015). A value-based behavioural choice underlies phototaxis in Drosophila. *BioArxiv*. doi:10.1101/023846.

Götz, K. (1972). Principles of optomotor reactions in insects. 82, 251–259.

Götz, K. G. (1964). Optomotorische Untersuchung des visuellen Systems einiger Augenmutanten der Fruchtfliege Drosophila. 2, 77–92.

Götz, K. G. (1965). Die optischen Ubertragungseigenschaften der Komplexaugen von Drosophila. 2, 215–221.

Götz, K. G. (1968). Flight control in Drosophila by visual perception of motion. 4, 199–208.

Götz, K. G. (1970). Fractionation of Drosophila populations according to optomotor traits. *J. Exp. Biol.*, 419–436.

Götz, K. G. (1977). Normale Entwicklung der Fliege Drosophila in Niederfrequenten Magnetfeldern. 125–132.

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

Götz, K. G., and Buchner, E. (1978). Evidence for one-way movement detection in the visual system of Drosophila. 31, 243–248.

Götz, K. G., Hengstenberg, B., and Biesinger, R. (1979). Optomotor control of wingbeat and body posture in Drosophila. 35, 101–112.

Griffith, L. C., and Ejima, A. (2009). Courtship learning in Drosophila melanogaster: diverse plasticity of a reproductive behavior. *Learn. Mem.* 16, 743–50. doi:10.1101/lm.956309.

Groth, A. C., Fish, M., Nusse, R., and Calos, M. P. (2004). Construction of transgenic Drosophila by using the site-specific integrase from phage phiC31. *Genetics* 166, 1775–82.

Guven-Ozkan, T., and Davis, R. L. (2014). Functional neuroanatomy of Drosophila olfactory memory formation. *Learn. Mem.* 21, 519–26. doi:10.1101/lm.034363.114.

Haag, J., Wertz, A., and Borst, A. (2010). Central gating of fly optomotor response. *Proc. Natl. Acad. Sci. U. S. A.* 107, 20104–9. doi:10.1073/pnas.1009381107.

Haesler, S., Rochefort, C., Georgi, B., Licznerski, P., Osten, P., and Scharff, C. (2007). Incomplete and Inaccurate Vocal Imitation after Knockdown of FoxP2 in Songbird Basal Ganglia Nucleus Area X. *PLoS Biol.* 5, 12. doi:10.1371/journal.pbio.0050321.

Hall, J. C. (1978). Courtship among males due to a male-sterile mutation inDrosophila melanogaster. *Behav. Genet.* 8, 125–141. doi:10.1007/BF01066870.

Hall, J. C. (1982). Genetics of the nervous system in Drosophila. *Q. Rev. Biophys.* 15, 223–479.

Hardin, P. E. (2011). Molecular genetic analysis of circadian timekeeping in Drosophila. *Adv. Genet.* 74, 141–73. doi:10.1016/B978-0-12-387690-4.00005-2.

Häsemeyer, M., Yapici, N., Heberlein, U., and Dickson, B. J. (2009). Sensory neurons in the Drosophila genital tract regulate female reproductive behavior. *Neuron* 61, 511–8. doi:10.1016/j.neuron.2009.01.009.

Hassenstein, B., and Reichardt, W. (1956). Systemtheoretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers Chlorophanus. *Zeitschrift für Naturforsch. B* 11. doi:10.1515/znb-1956-9-1004.

Heisenberg, M. (1989). “Genetic approaches to learning and memory (mnemogenetics) in Drosophila melanogaster.,” in *Fundamentals of Memory Formation: Neuronal Plasticity and Brain Function*, ed. G. Rahmann (Stuttgart/New York: Fischer Verlag), 3–45.

Heisenberg, M., and Götz, K. G. (1975). The use of mutations for the partial degradation of vision inDrosophila melanogaster. *J. Comp. Physiol. ? A* 98, 217–241. doi:10.1007/BF00656971.

Heisenberg, M., and Wolf, R. (1993). The sensory-motor link in motion-dependent flight control of flies. *Vis. Motion its Role Stab. Gaze*, 265–283.

Heisenberg, M., Wonneberg, R., and Wolf, R. (1978). Optomotor-blind-h-31 a drosophila mutant of the lobula plate giant neurons. 124, 287–296.

Helfrich-Förster, C. From neurogenetic studies in the fly brain to a concept in circadian biology. *J. Neurogenet.* 28, 329–47. doi:10.3109/01677063.2014.905556.

Itskov, P. M., and Ribeiro, C. (2013). The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in Drosophila. *Front. Neurosci.* 7, 12. doi:10.3389/fnins.2013.00012.

Jablonski, P. G., and Strausfeld, N. J. (2001). Exploitation of an ancient escape circuit by an avian predator: relationships between taxon-specific prey escape circuits and the sensitivity to visual cues from the predator. *Brain Behav. Evol.* 58, 218–240.

Jabloński, P. G., and Strausfeld, N. J. (2000). Exploitation of an ancient escape circuit by an avian predator: prey sensitivity to model predator display in the field. *Brain. Behav. Evol.* 56, 94–106. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11111136 [Accessed August 24, 2010].

Kimura, K., Hachiya, T., Koganezawa, M., Tazawa, T., and Yamamoto, D. (2008). Fruitless and Doublesex Coordinate to Generate Male-Specific Neurons that Can Initiate Courtship. *Neuron* 59, 759–769. doi:10.1016/j.neuron.2008.06.007.

Kimura, K.-I., Ote, M., Tazawa, T., and Yamamoto, D. (2005). Fruitless specifies sexually dimorphic neural circuitry in the Drosophila brain. *Nature* 438, 229–233. doi:10.1038/nature04229.

Konopka, R. J., and Benzer, S. (1971). Clock mutants of Drosophila melanogaster. *Proc. Natl. Acad. Sci. U. S. A.* 68, 2112–6.

Lai, C. S., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F., and Monaco, A. P. (2001). A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 413, 519–23. doi:10.1038/35097076.

Liu, H., and Kubli, E. (2003). Sex-peptide is the molecular basis of the sperm effect in Drosophila melanogaster. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9929–33. doi:10.1073/pnas.1631700100.

Longden, K. D., and Krapp, H. G. (2009). State-dependent performance of optic-flow processing interneurons. *J. Neurophysiol.* 102, 3606–18. doi:10.1152/jn.00395.2009.

Lorenzetti, F. D., Baxter, D. A., and Byrne, J. H. (2008). Molecular mechanisms underlying a cellular analog of operant reward learning. *Neuron* 59, 815–28. doi:10.1016/j.neuron.2008.07.019.

Maimon, G., Straw, A. D., and Dickinson, M. H. (2010). Active flight increases the gain of visual motion processing in Drosophila. *Nat. Neurosci.* 13, 393–9. doi:10.1038/nn.2492.

McEwen, R. S. (1918). The reactions to light and to gravity in Drosophila and its mutants. *J. Exp. Zool.* 25, 49–106. doi:10.1002/jez.1400250103.

Mendoza, E., Colomb, J., Rybak, J., Pflüger, H.-J., Zars, T., Scharff, C., et al. (2014). Drosophila FoxP mutants are deficient in operant self-learning. *PLoS One* 9, e100648. doi:10.1371/journal.pone.0100648.

Merbitz-Zahradnik, T., and Wolf, E. (2015). How is the inner circadian clock controlled by interactive clock proteins?: Structural analysis of clock proteins elucidates their physiological role. *FEBS Lett.* 589, 1516–29. doi:10.1016/j.febslet.2015.05.024.

Michel, M., and Lyons, L. C. (2014). Unraveling the complexities of circadian and sleep interactions with memory formation through invertebrate research. *Front. Syst. Neurosci.* 8, 133.

doi:10.3389/fnsys.2014.00133.

Miller, G. F. (1997). “Protean Primates: The Evolution of Adaptive Unpredictability in Competition and Courtship,” in *Machiavellian Intelligence II: Extensions and evaluations*, eds. A. Whiten and R. W. Byrne (Cambridge, Ma.: Cambridge University Press), 312–340.

Mitra, O., Callaham, M. ., Smith, M. ., and Yack, J. . (2009). Grunting for worms: seismic vibrations cause Diplocardia earthworms to emerge from the soil. *Biol. Lett.* 5, 16–19. doi:10.1098/rsbl.2008.0456.

O’Kane, C. J., and Gehring, W. J. (1987). Detection in situ of genomic regulatory elements in Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* 84, 9123–7.

Owald, D., and Waddell, S. (2015). Olfactory learning skews mushroom body output pathways to steer behavioral choice in Drosophila. *Curr. Opin. Neurobiol.* 35, 178–84. doi:10.1016/j.conb.2015.10.002.

Ozkaya, O., and Rosato, E. (2012). The circadian clock of the fly: a neurogenetics journey through time. *Adv. Genet.* 77, 79–123. doi:10.1016/B978-0-12-387687-4.00004-0.

Poggio, T., and Reichardt, W. (1973). Considerations on models of movement detection. *Kybernetik* 13, 223–227.

Poggio, T., and Reichardt, W. (1976). Visual control of orientation behaviour in the fly. Part II. Towards the underlying neural interactions. *Q. Rev. Biophys.* 9, 377–438.

Quinn, W. G., Harris, W. A., and Benzer, S. (1974). Conditioned behavior in Drosophila melanogaster. *Proc. Natl. Acad. Sci. U. S. A.* 71, 708–12. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=388082&tool=pmcentrez&rendertype=abstract [Accessed August 26, 2010].

Reichardt, W. E. (1965). Quantum sensitivity of light receptors in the compound eye of the fly Musca. *Cold Spring Harb. Symp. Quant. Biol.* 30, 505–515.

Reichardt, W., and Poggio, T. (1975). A theory of the pattern induced flight orientation of the fly Musca domestica II. *Biol. Cybern.* 18, 69–80.

Reichardt, W., and Poggio, T. (1976). Visual control of orientation behaviour in the fly. Part I. A quantitative analysis. *Q. Rev. Biophys.* 9, 311–375,428–438.

Reichardt, W., and Varju, D. (1959). Übertragungseigenschaften im Auswertesystem für das Bewegungssehen. *Zeitschrift für Naturforsch. B* 14. doi:10.1515/znb-1959-1008.

Rezával, C., Nojima, T., Neville, M. C., Lin, A. C., and Goodwin, S. F. (2014). Sexually dimorphic octopaminergic neurons modulate female postmating behaviors in Drosophila. *Curr. Biol.* 24, 725–30. doi:10.1016/j.cub.2013.12.051.

Ribeiro, C., and Dickson, B. J. (2010). Sex peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in Drosophila. *Curr. Biol.* 20, 1000–5. doi:10.1016/j.cub.2010.03.061.

Rosner, R., Egelhaaf, M., and Warzecha, A.-K. (2010). Behavioural state affects motion-sensitive neurones in the fly visual system. *J. Exp. Biol.* 213, 331–8. doi:10.1242/jeb.035386.

Sakaguchi, H., and Yamaguchi, A. (1997). Early song-deprivation affects the expression of protein kinase C in the song control nuclei of the zebra finch during a sensitive period of song learning. *Neuroreport* 8, 2645–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9295093 [Accessed December 7, 2011].

Santos, M. E., Athanasiadis, A., Leitão, A. B., DuPasquier, L., and Sucena, E. (2011). Alternative splicing and gene duplication in the evolution of the FoxP gene subfamily. *Mol. Biol. Evol.* 28, 237–47. doi:10.1093/molbev/msq182.

Schreiweis, C., Bornschein, U., Burguière, E., Kerimoglu, C., Schreiter, S., Dannemann, M., et al. (2014). Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance. *Proc. Natl. Acad. Sci. U. S. A.* 111, 14253–8. doi:10.1073/pnas.1414542111.

Siegel, R. W., and Hall, J. C. (1979). Conditioned responses in courtship behavior of normal and mutant Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* 76, 3430–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16592682.

Suver, M. P., Mamiya, A., and Dickinson, M. H. (2012). Octopamine neurons mediate flight-induced modulation of visual processing in Drosophila. *Curr. Biol.* 22, 2294–302. doi:10.1016/j.cub.2012.10.034.

Tang, S., and Juusola, M. (2010). Intrinsic Activity in the Fly Brain Gates Visual Information during Behavioral Choices. *PLoS One* 5, e14455. doi:10.1371/journal.pone.0014455.

Tuthill, J. C., Nern, A., Rubin, G. M., and Reiser, M. B. (2014). Wide-field feedback neurons dynamically tune early visual processing. *Neuron* 82, 887–95. doi:10.1016/j.neuron.2014.04.023.

Vargas, M. A., Luo, N., Yamaguchi, A., and Kapahi, P. (2010). A Role for S6 Kinase and Serotonin in Postmating Dietary Switch and Balance of Nutrients in D. melanogaster. *Curr. Biol.* 20, 1006–1011. doi:10.1016/j.cub.2010.04.009.

Villella, A., and Hall, J. C. (2008). "Chapter 3 Neurogenetics of Courtship and Mating in Drosophila," in *Advances in genetics, Volume 62* (Elsevier Science B. V.), 67–184. doi:10.1016/S0065-2660(08)00603-2.

Walker, S. J., Corrales-Carvajal, V. M., and Ribeiro, C. (2015). Postmating Circuitry Modulates Salt Taste Processing to Increase Reproductive Output in Drosophila. *Curr. Biol.* 25, 2621–2630. doi:10.1016/j.cub.2015.08.043.

Wasserman, S. M., Aptekar, J. W., Lu, P., Nguyen, J., Wang, A. L., Keles, M. F., et al. (2015). Olfactory Neuromodulation of Motion Vision Circuitry in Drosophila. *Curr. Biol.* 25, 467–472. doi:10.1016/j.cub.2014.12.012.

Wehrhahn, C., and Reichardt, W. (1973). Visual orientation of the fly Musca domestica towards a horizontal stripe. *Naturwissenschaften* 60, 203–204.

Wolf, R., and Heisenberg, M. (1980). On the fine structure of yaw torque in visual flight orientation ofDrosophila melanogaster. II. A temporally and spatially variable weighting function for the visual field ("visual attention"). *J. Comp. Physiol. ? A* 140, 69–80. doi:10.1007/BF00613749.

Wolf, R., and Heisenberg, M. (1986). Visual orientation in motion-blind flies is an operant behavior. *Nature* 323, 154–156.

Wolf, R., and Heisenberg, M. (1991). Basic organization of operant behavior as revealed in Drosophila flight orientation. *J. Comp. Physiol. A.* 169, 699–705. doi:10.1007/BF00194898.

Wolf, R., Voss, a., Hein, S., Heisenberg, M., and Sullivan, G. D. (1992). Can a Fly Ride a Bicycle? [and Discussion]. *Philos. Trans. R. Soc. B Biol. Sci.* 337, 261–269. doi:10.1098/rstb.1992.0104.

Wright, N. J. D. (2014). Evolution of the techniques used in studying associative olfactory learning and memory in adult Drosophila in vivo: a historical and technical perspective. *Invert. Neurosci.* 14, 1–11. doi:10.1007/s10158-013-0163-z.

Yamamoto, D., and Koganezawa, M. (2013). Genes and circuits of courtship behaviour in Drosophila males. *Nat. Rev. Neurosci.* 14, 681–692. doi:10.1038/nrn3567.

Yapici, N., Kim, Y.-J., Ribeiro, C., and Dickson, B. J. (2008). A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. *Nature* 451, 33–7. doi:10.1038/nature06483.

Yoshida, Y., Yamada, T., and Sakaguchi, H. (2003). Activation of protein kinase C by the error signal from a basal ganglia-forebrain circuit in the zebra finch song control nuclei. *Neuroreport* 14, 645–9. doi:10.1097/01.wnr.0000059995.72968.e0.

Yoshii, T., Hermann-Luibl, C., and Helfrich-Förster, C. (2015). Circadian light-input pathways in Drosophila. *Commun. Integr. Biol.* 9, e1102805. doi:10.1080/19420889.2015.1102805.