

Vision in *Drosophila*: Seeing the World Through a Model's Eyes

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Abstract

The fruit fly, *Drosophila melanogaster*, has been used for decades as a genetic model for unraveling mechanisms of development and behavior. In order to efficiently assign gene functions to cellular and behavioral processes, early measures were often necessarily simple. Much of what is known of developmental pathways was based on disrupting highly regular structures, such as patterns of cells in the eye. Similarly, reliable visual behaviors such as phototaxis and motion responses provided a solid foundation for dissecting vision. Researchers have recently begun to examine how this model organism responds to more complex or naturalistic stimuli by designing novel paradigms that more closely mimic visual behavior in the wild. Alongside these advances, the development of brain-recording strategies allied with novel genetic tools has brought about a new era of *Drosophila* vision research where neuronal activity can be related to behavior in the natural world.

INTRODUCTION

The visual world contains an extraordinarily complex combination of colors, contrast, motion, and patterns (**Figure 1**). How this barrage of visual information produces a picture in the brain has been a longstanding mystery, and it was only early in the past century that scientists began to describe possible mechanisms of visual processing, on the basis of careful examination of vertebrate and invertebrate retinas (13). The beautiful architecture of the compound eye of insects was particularly appealing for early studies of vision, perhaps because its crystalline regularity immediately suggested a mechanism for capturing a representation of the outside world onto a template. Neuroanatomical and physiological studies on a variety of insects, from beetles to dragonflies, have contributed to our understanding of how the structure of the compound eye captures and funnels the variety of visual information to the brain. Although most insect studies have focused on

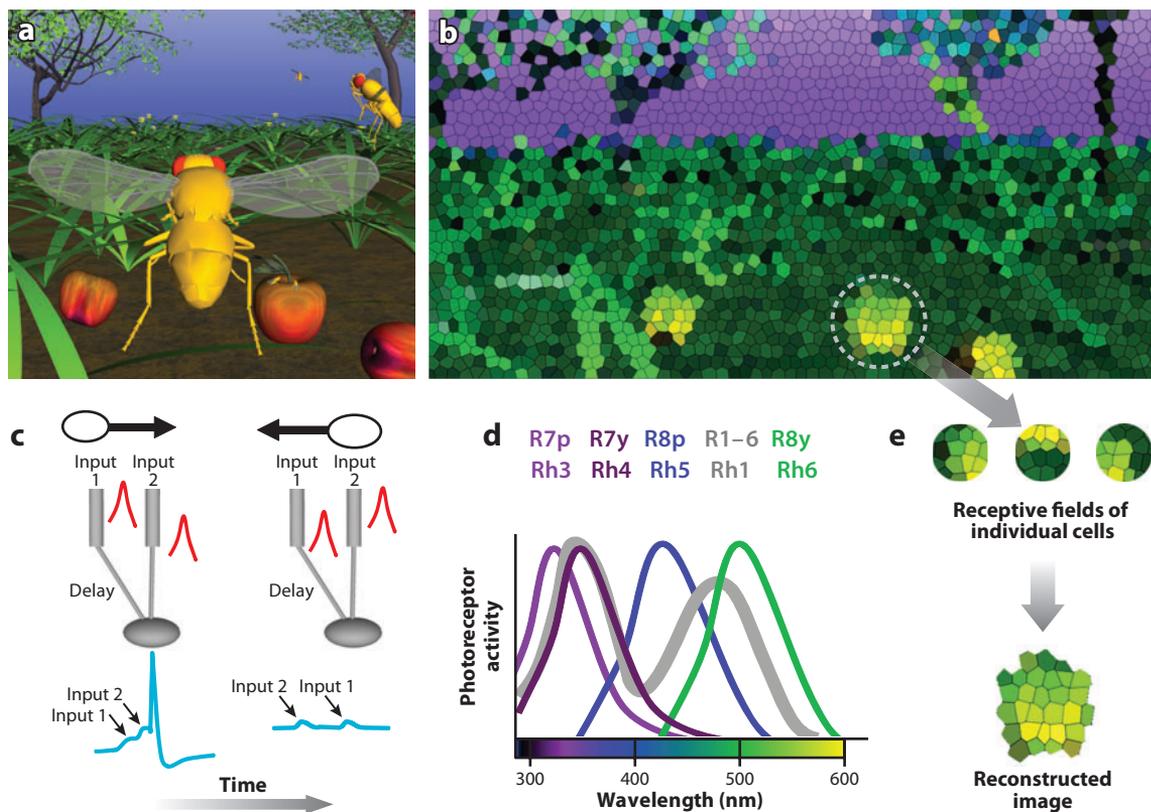


Figure 1

The fly's eye view of the world with three visual submodalities. (*a*) Flies moving through the environment are challenged with various visual inputs. (*b*) Their vision has lower resolution and a different set of color sensitivities than the vertebrate eye. (*c*) The flies experience motion, which can be modeled with the elementary motion detector (see text). Receptor potential changes (*red*) are triggered with the movement of an object (*arrow*), which when spaced in time can be summated (*blue, left*) or not summated (*blue, right*) to indicate the direction of motion. (*d*) Flies have true color vision, with different types of photoreceptor rhodopsins (Rh1, Rh3, Rh4, Rh5, Rh6) used to detect light that occupy different photoreceptors (R1–6, R7y, R8y, R7p, R8p) (adapted from Reference 102, with permission). Comparisons between these photoreceptor sensitivity curves allow the flies to detect specific wavelengths of light in the natural world. (*e*) Pattern vision is thought to involve reconstructing the image through comparing overlapping receptive fields of individual neurons. The individual neurons may be responsive to edges, light/dark, or other qualities of the image.

neuroanatomy or behavior, research on the fruit fly, *Drosophila melanogaster*, has proved extraordinary in that it has addressed the problem of vision from a variety of approaches, including insight into genes, molecules, development of neurons, circuits, and behavior. The success of *Drosophila* research in general, not only for vision, lies in the fact that it was chosen specifically as a genetic model. This meant that, early on, *Drosophila* development, physiology, and behaviors were studied in relation to changes in its genes. Thus, a common thread could be drawn between genes, brain anatomy, and behavior—by studying mutants, for example, in which all was held constant except for a mutation in one gene. To adequately dissect vision, the natural world in all its complexity was often put aside while differentiable components of vision, such as color or motion, were tackled separately. The power of this reductionist, one-thing-at-a-time approach to understanding a complex problem such as vision persists in *Drosophila* research today, although the genetic tools and the visual questions have become more sophisticated. In this review we describe how *Drosophila* research has contributed to our understanding of vision, what techniques are currently available to study visual processing, and how recent applications are finally bringing this genetic model back to the natural world to better understand how insects really see.

Photoreceptors: cells that respond to light through the phototransduction pathway, which converts photons to changes in cell excitability

R1–8: photoreceptor cells 1 through 8

MODULARITY OF THE FLY VISUAL SYSTEM

The compound eye of *D. melanogaster* is organized into modules, and this basic design affects most aspects of visual processing (**Figure 2**). The modularity begins in the retina, where 750 individual facets, called ommatidia, make up each compound eye. Each ommatidium is physically separated from its neighbor and contains one each of eight different photoreceptor (R) cells called R1–8 (37). The modular organization of the retina is maintained in the first optic brain region, the lamina, where R1–6 cells target to approximately 750 independent units, called cartridges, and form the first connections, or synapses, with downstream neurons involved in motion processing (27, 74). The second optic lobe region, the medulla, is composed of ~750 columns, and the first synapses for color vision (receptor cells R7 and R8) and the second synapses of the motion circuit assemble at distinct vertical positions in each column (27). The projections of photoreceptors and that of the majority of neurons in the optic lobe are retinotopic, meaning that the spatial relationship between neurons activated by the visual stimulus at the retina is preserved when this information is mapped onto the optic lobe. Neurons from the medulla project into the lobula and lobula plate and a retinotopic map is loosely maintained in these brain regions as well (74). One of the major challenges the fly visual system faces in terms of organization is in the specificity of the connections within a unit; even though compatible neurons are available close by (just 5–10 microns away) (27), connections with neurons in neighboring columns are prevented in order to preserve the modularity required for efficient visual processing. The mechanisms used to achieve these boundaries within and between columns have only recently begun to be revealed (63).

How does the compound eye process visual information? The ommatidia in the compound eye are analogous to pixels in a photograph; each contains unique bits of information that contribute to the overall image, but this information on its own is not instructive. In order to evoke a visual behavior, each pixel must be filtered, sampled, and integrated across the eye. Presumably, the neurons receiving this retinotopic map are wired to detect colored pixels, combinations of dark or light pixels, or whether pixels change over time. Visual responses can thus be divided into three different modalities, namely motion detection, color perception, and pattern recognition (**Figure 1**). Although these different modalities may be integrated to form a visual percept, *Drosophila* researchers have traditionally studied them separately to better uncover the genes and neuroanatomy subserving their function. In general, motion and color perception were

Optomotor

response: a visual behavior in which the animal turns in the direction of a moving visual scene

Rhodopsins:

light-absorbing pigments located in the photoreceptors that can be sensitive to different wavelengths of light

less complex problems than pattern recognition, so progress in the field began with uncovering mechanisms for the first two, and we shall follow that flow of understanding in this review as well.

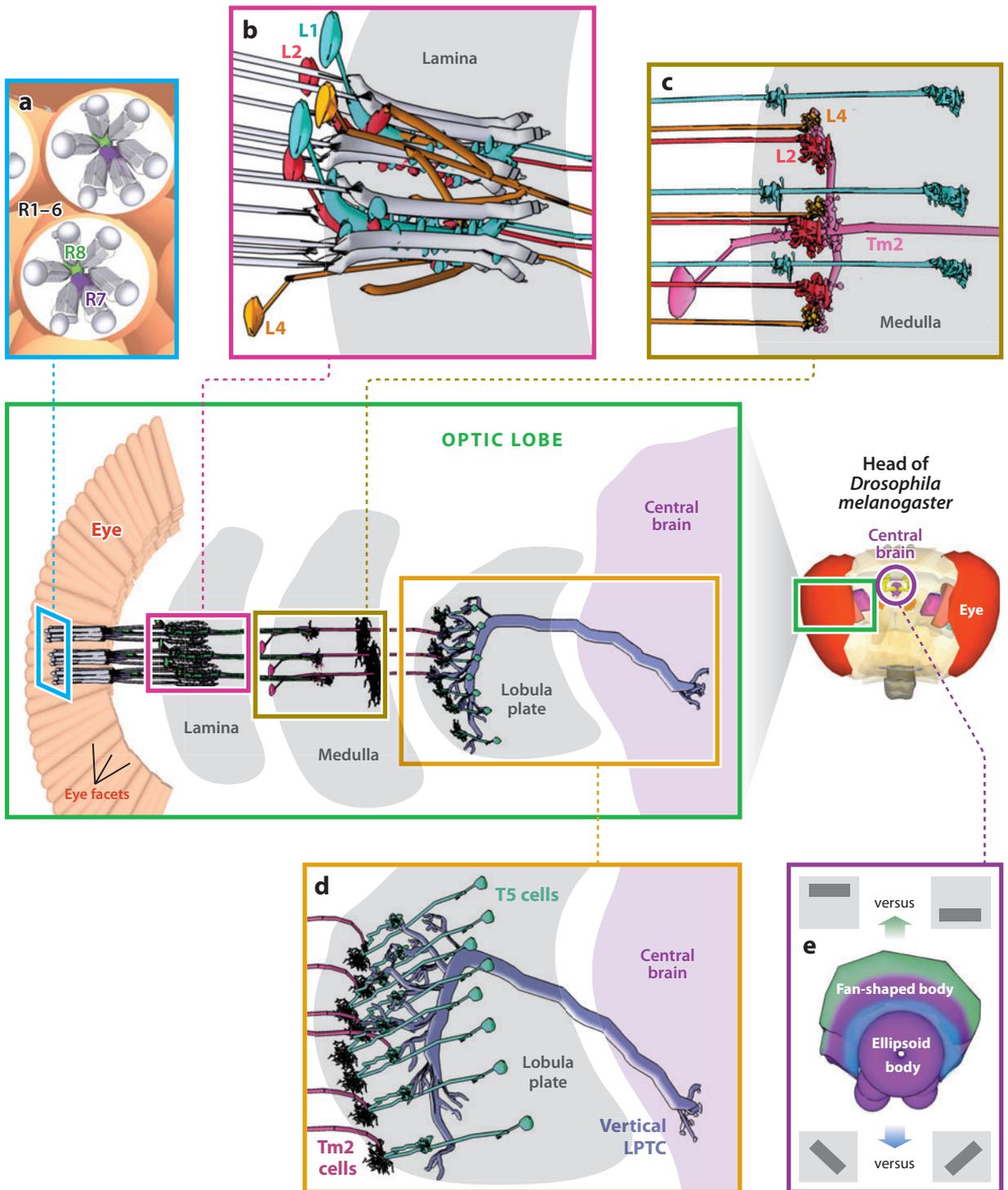
The precise geometric arrangement of facets on the fly compound eye already hints at a mechanism for motion perception: Any object sweeping by a fly eye affects different facets in succession at slightly different times. This suggests a fundamental quality of motion vision, already a clue for researchers investigating the underlying neuroanatomy: Motion vision is more concerned with contrast changes and the delays that describe a moving object than with specific spectral qualities or geometrical arrangements of objects (**Figure 1c**). Motion vision can be wide-field, as caused by optic flow as the fly moves through its environment, or small-field, as caused by another insect moving across the fly's visual field. In both cases, temporal sequences of contrast changes across the retina determine the starting signal for subsequent motion computations. Motion perception is relatively simple to conceptualize and therefore provides an obvious starting point to examine whether theory meets biology in the insect eye.

The generally accepted model of how motion vision operates is the Hassenstein-Reichardt correlation motion detector, originally proposed from observing a beetle responding to a moving, patterned drum (39). The behavior elicited in the beetle was to turn toward the direction of motion, termed an optomotor response. Optomotor, or optokinetic, behavior is observed in many animals (60) and is thought to provide a form of visual stability. If an animal does not turn with motion, it might erroneously perceive that it was moving in the opposite direction. In the Hassenstein-Reichardt model, motion is perceived by circuits measuring the temporal difference of light changes between two neighboring sampling units, such as photoreceptors in the insect eye (**Figure 1c**). This comparison can be made if somewhere in the visual circuitry the two sampling units communicate with one another, and if the circuits associated with these units only work in one direction. Hassenstein & Reichardt (39) hypothesized that a delay from the first unit would allow the signal in the second unit to be coincident and that these neighboring signals would be multiplied. To detect motion in the other direction, a similar, but separate, asymmetric circuit must exist. Together, these computations result in directional motion processing. This model has stood the test of time as a mechanism for motion detection (8) and is often called the elementary motion detector (**Figure 1c**). Yet, the neurons that make these rather complex computations have remained elusive. Recent genetic and electrophysiological approaches have proven crucial for validating aspects of this model (8).

Color vision contrasts with motion vision in that, instead of detecting a temporal sequence of photoreceptor stimulation, the spectral characteristics of the photoreceptors are compared (**Figure 1d**). A segregation of color channels is already evident in each ommatidium. The eight *Drosophila* photoreceptors in each ommatidium contain rhodopsins (light-absorbing pigments) that can detect ultraviolet (UV), blue, and green light (52). Specific photoreceptor neurons express

Figure 2

The anatomy of the fly visual system. The central image depicts a fly's head showing the position of the optic lobes and central brain, with the layout of the individual brain regions. The colored boxes correspond to the colors of the panels in *a–e*. (*a*) Visual information enters the eye via the retina, which contains photoreceptors R1–8 in individual ommatidia (two are cut away to reveal six of the eight photoreceptors in *white*, and R7 and R8 are *purple* and *green*). (*b*) The R1–6 photoreceptors input to the lamina. Lamina neurons L1, L2, and L4 also send output synapses of the motion circuit into the medulla. L4 receives input from neighboring cartridges, including L2 neurons, and outputs to multiple medulla neurons. (*c*) The medulla, like the lamina, is organized into reiterated units. Transmedulla (Tm2) neurons are postsynaptic to lamina neurons and presynaptic to cells in the lobula complex. (*d*) Motion information flows from the lobula to the lobula plate via T5 cells where vertical and horizontal lobula plate tangential cells (LPTCs) respond to specific motion stimuli. (*e*) Further information on patterns such as elevation (*top*) or orientation (*bottom*) is discriminated at different levels of the fan-shaped body (*green and blue layers*) in the central complex, a central brain structure.



Phototaxis: a visual behavior in which the animals approach a bright light

different rhodopsins that allow them to detect specific wavelengths of light (67, 75, 105). This chromatic information is conveyed to different regions of the optic lobe (65) (**Figure 2**), where it is presumably integrated to become a neural representation of the image. In contrast to the traditional view that there is a strict relationship between photoreceptor identity and spectral preference, recent studies have shown that all photoreceptors can contribute to color vision (102). These findings are supported by electron microscopic evidence that many of the photoreceptor pathways communicate with one another (33, 65). Therefore, for color vision, the sampling units are known, but there is no predominant model for how these are integrated, if at all, with other visual cues.

Finally, what neuroanatomical clues indicate that flies see different patterns? Unfortunately, unlike motion and color, there are none, neither imprinted on the compound eye's structure nor indicated in the quality or shape of neurons that pool visual information within the optic lobes. Pattern vision probably requires a complex series of computations wherein individual edges and other primitives (such as size and height) are coalesced into a cohesive whole by the brain (**Figure 1e**). Although pattern vision can be retinotopic, it can also be independent of what part of the retina is directly affected. This makes pattern vision a complex visual problem in the fly, because it most likely also integrates aspects of motion and color vision and may involve higher-order neurons beyond the optic lobes, perhaps linked to attention and memory systems.

FLY POPULATION PARADIGMS TO STUDY VISION

One striking aspect of *Drosophila* vision research is how simple the behavioral paradigms often are, compared with the complexity of the underlying circuits introduced above. Again, this is because initial approaches to vision were necessarily reductionist. These assays often involved fly populations walking down plastic tubes, thereby “voting” about their response to a stimulus. Indeed, one of the original attractions of using *D. melanogaster* to study behavior was that populations of flies could be screened, much like viruses or bacteria, to uncover mechanisms relevant to neural function, such as visual perception. Vision proved an ideal modality for early behavioral studies in *D. melanogaster* because flies display strong phototactic responses (61) (**Figure 3a**). To measure fly responsiveness to light, Benzer (3), who actually had a background in virus research, used an apparatus inspired from microbial selection studies. In this apparatus, a population of flies was iteratively partitioned into a group responding to a light source and a group not responding within a set time period (**Figure 3a**). This quantitative approach proved ideal for phenotyping mutant strains of *Drosophila*, to thus begin to understand the genes responsible for insect vision. For instance, blind mutants *norp* (no receptor potential) (46) produced random distributions in the assay (68). Mutations could be made that caused fly populations to walk away from light (e.g., *photophobe*) (2), suggesting that the reflex may be supported by a simple circuit.

The phototaxis assay also proved useful to understand color vision. *Drosophila* discriminates different colors in the range from green to UV (62, 79) (**Figure 1**), although flies have an innate preference for UV. This behavior is driven mainly by R7 photoreceptors, as flies lacking R7 (*sevenless* mutants) choose green over UV light (38). However, other R cells also have the capacity to detect UV as demonstrated by the reduction in UV preference in *rdg^{BKS222}* mutants with degenerate R1–6 cells (38). True color vision, however, is about not only responding to one wavelength of light or another, but also discriminating differences in simultaneously presented wavelengths (26). Recently, a simple T-maze providing color choice was used in population assays to identify the involvement of a medulla neuron, Dm8, in UV preference over green light, thereby illustrating that color circuits can be mapped beyond the fly retina (33).

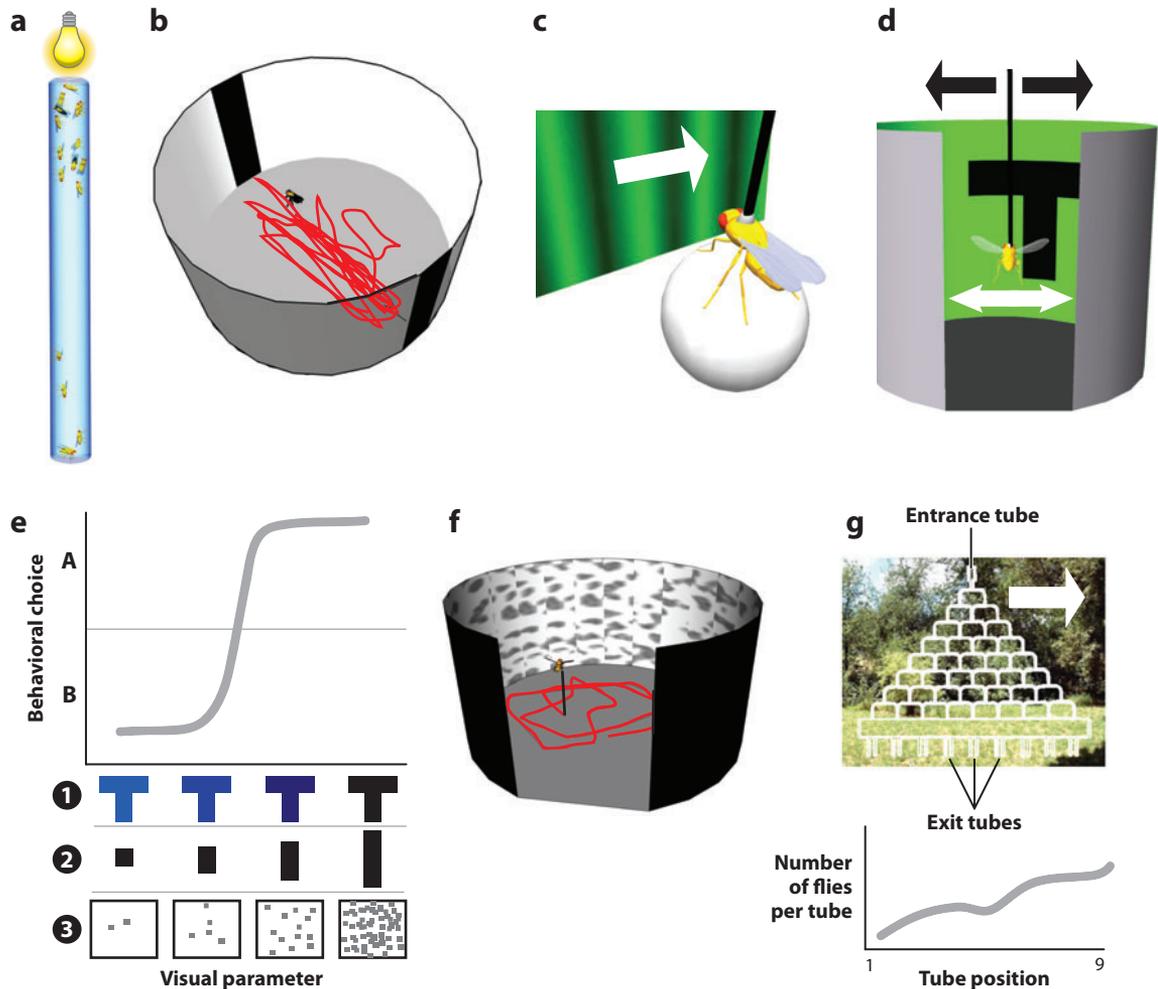


Figure 3

Visual behavioral paradigms. (a) Flies walk toward light in a phototaxis assay. (b) Flies will spontaneously walk between two dark vertical objects in a drum. (c) Flies can respond to moving grating patterns by attempting to turn left or right while walking on a ball, which can be measured through optical devices (81). (d) The flies can also fixate while flying using a closed loop system, in which their flight behavior positions the visual scene to the front of their field of view as detected with a torquemeter (arrows) (34). (e) Flies will exhibit a nonlinear (sigmoidal) behavioral switch in response to changing visual parameters, switching between ① color (B) and shape (A) parameters after training; ② fixating to a bar (B) or antifixating a dot (A); and ③ orienting against sparse moving dots (B) or with dense moving dots (A). (f) Flies in free flight execute a series of straight flights interspersed with turns as indicated by the flight trace below. Visual cues play an important role in these saccades. (g) Flies walking through an expanding choice maze introduced at the entrance tube follow the direction of motion (arrow) of the scene below the maze, such as a natural scene, resulting in an uneven distribution of flies in the exit tubes (below).

As simple as phototactic behavior can be, a surprising result is that responsiveness to color can depend on the fly's motivational state. Originally, researchers used two different approaches to understand color vision, namely the fast and slow phototaxis assays. The fast method involved agitating the flies to induce walking toward the light (47), whereas the slow method involved allowing the flies to walk freely in the tubes toward light (26, 43). Although both methods demonstrated that flies have an innate preference for UV light (26, 47), fast phototaxis is not impaired in *rdgB*

Fixation: when flies adjust their walking or flying to shift the visual to the front of its visual field in a closed loop tethered flight or walking arena

mutants, which have defective R1–6 cells, indicating R7 cells are involved in this behavior (47). On the other hand, the slow response to UV is impaired in mutants with defective R1–6 as well as R7 (*sevenless*) photoreceptors (26, 47), indicating different visual processes mediate the fast phototaxis behavior compared to slow phototaxis behavior, even though spectral stimuli may be identical for both.

Moving stimuli add a layer of complexity to vision studies. The study of *Drosophila* responses to motion required the development of devices that could fractionate fly populations responding to moving gratings. The first such devices again exploited walking behavior of flies in tubes, by surrounding them with a barber-pole cylinder that, when turned, would present optic flow in one direction (35). Subsequent maze-like designs allowed for screening for variable responses to motion in mutant strains (40), eventually resulting in isolation of a mutant, *optomotor blind* (*omb*) (40, 70). *omb* mutations result in failed development of the horizontal system (HS) and vertical system (VS) tangential cells in the *Drosophila* lobula plate (45, 70) (**Figure 2d**). HS and VS neurons sample photoreceptor input from across the retina, essentially pooling across different axes of the fly eye (7, 8), so their absence in *omb* mutants provided a clear connection between a gene, neuronal morphology, and a behavior. Similar to phototaxis, optomotor responsiveness appears to be modulated by motivational factors; for example, a study using fly populations required fly agitation immediately prior to testing to show reliable optomotor behavior (104). One conclusion from this and similar effects on phototaxis is that the behavioral context influences what were previously thought to be simple visual reflexes.

Whereas screening for phototaxis mutants was relatively straightforward and screening for optomotor mutants was laborious, screening for mutations affecting pattern vision proved impossible, without a single successful study published, to our knowledge. The study of pattern vision in *Drosophila* was more accessible in single-fly paradigms, where the context and behavioral choices of individuals responding to patterns could be carefully tracked through time.

INDIVIDUAL FLY PARADIGMS

In the approaches described in the preceding sections, vision in *Drosophila* was treated in a simplified manner in order to dissect the genetics and anatomy supporting motion or color processing. However, vision involves complex combinations of shape, color, and motion. An important step toward tackling more sophisticated visual questions in *Drosophila* is to design individual fly assays, in which, rather than having a population of flies vote about a visual stimulus, the behavior of single flies more accurately indicates their visual perception and choice. Individual fly paradigms to study vision come in two styles: freely behaving and tethered paradigms.

In principle, flies should be able to demonstrate their visual choices by walking (or flying) toward a selected visual object or pattern, as was shown for color perception. When flies' wings are cut off, to promote walking choice behavior, flies will reliably walk back and forth between two dark vertical stripes (82) (**Figure 3b**). This tendency to alternate fixation between competing visual objects has been exploited to study pattern preferences in *Drosophila*, especially more recently in which computer displays allow a level of flexibility to change the visual stimulus in real time to accommodate the changing perspective from a moving animal (80). This last consideration, the fly's own movement with regard to the visual stimulus, is probably the main reason why pattern discrimination has been difficult to study in walking flies: The object or pattern changes appearance depending on the fly's position in an experimental arena, so visual choices are always affected by positional or motion context, a likely source of inconsistency across trials—especially in population studies. Nevertheless, there were some early successful visual discrimination studies in walking flies. Experiments by Wehner (97) demonstrated spontaneous preferences for darker

objects, and Mimura (64) suggested that the R1–6 motion-processing channels were also used for pattern recognition by testing spontaneous responses to different backlit objects in some of the classical phototaxis mutants.

To better study visual discrimination, however, the implementation of a classical conditioning paradigm is often warranted to demonstrate that the flies can report an association between reward or punishment with a particular stimulus, as shown for light/dark associations with an aversive chemical, quinine (53), or color learning (28) in walking *Drosophila*. Such associative learning of patterns has only recently been shown in walking *Drosophila* by using aversive conditioning (to heat). These studies have revealed that flies learn to position themselves preferentially near patterns that have been associated with the absence of heat (30, 66). Even by associating patterns with punishment or reward, however, demonstration of pattern discrimination in freely walking *Drosophila* has been extremely difficult and results are often variable.

An obvious solution to reduce variability due to changes in the visual stimulus because of self-movement is to tether a walking or flying insect in place while presenting visual choices. The first optomotor studies used walking insects turning air-suspended balls (36, 72) to examine visual responses. Early studies showed that flies display predictable optomotor reflexes when confronted with moving gratings while walking on such devices (36) (**Figure 3c**). More recent adaptations to real-time visual displays and brain imaging suggest that this is the paradigm of choice to test strains of *Drosophila* that have been genetically manipulated in order to understand visual processing (15, 16, 81, 92). Until recently, however, almost all the *Drosophila* research on more sophisticated visual stimuli, such as pattern discrimination, has centered on the flight arena.

In the original flight arena concept, flies were tethered to a torque meter that measured the insect's left- or right-turn choices resulting from its flight behavior (34, 44) (**Figure 3d**). In the absence of visual stimuli, torque behavior appears semirandom (59). When visual stimuli, such as a moving grating, are introduced, flies display classic optomotor responses, turning in the same direction as the moving stimulus (101). A key development with this design was closing the loop between the fly's behavior and the movement of the stimulus. When negative feedback from the torque meter was provided to motors controlling the rotation of a display (a drum surrounding the fly), flies were able to control the angular position of objects by modulating their flight or fixation behavior (101). This was a crucial advance in the field, as it provided a way for the insect to get positive feedback from its visual choices while tethered. This finding opened up the possibility of monitoring the fly's preference for different visual cues (shape, size, color). An infrared beam of heat aimed at the fly when one pattern was in front introduced the possibility of training flies to avoid distinct visuals in order to study their visual discrimination capacities as well as their visual memory (12, 23).

Experiments on pattern recognition in the flight arena first exploited the tendency of *Drosophila* to fixate on novel objects when given a choice (22). These experiments suggested that *Drosophila* did not recognize shapes, but instead discriminated objects according to the extent of their overlap on the retina, as if the fly were simply summing pixels (21). For example, flies were unable to recognize an object as being the same if it was displaced to a different horizontal azimuth of their visual field. Although retinotopic matching appeared to be the primary mechanism for pattern recognition, there were some criteria, such as an object's center of gravity (i.e., whether it is top-heavy or bottom-heavy), that also played an important role in pattern recognition and that seemed independent of placement on the visual field. This observation engendered a series of visual learning studies using upright and inverted Ts as distinct objects that could be reliably discriminated by flies in the flight arena (41) (**Figure 3d**).

The robust T-pattern learning paradigm in the flight arena led to a number of psychophysical experiments that eventually overturned the view that pattern discrimination in flies was simply

Aversive conditioning: a form of training in which an unconditioned stimulus that induces a negative response is paired in time with a conditioned stimulus, such as a pattern or odor

Position-invariant:

when a visual response to an object does not depend on the location of the cue presented on the eye

retinotopic. By using aversive heat conditioning (where flies learn to avoid objects associated with heat) instead of novelty conditioning, visual learning was subsequently found to be position-invariant (91), showing that patterns do not need to overlap to be recognized as the same. In addition, flies can train to different colored shapes (12), arguing that color channels were involved in pattern recognition and thereby placing the requisite circuitry for such association in the medulla or deeper (where R1–6, R7, and R8 converge). Some evidence indicated that mushroom bodies, central neurons involved in olfactory memory (42), were required for discriminating compound patterns in which color and shape or pattern elevation were quantitatively altered (89, 103). Another study using the same flight arena paradigm to explore visual context (e.g., the background color around the objects) confirmed that these deep-brain structures were required for flies to disambiguate patterns from their background context (55).

Pattern recognition neurons in *Drosophila* were identified by first finding a mutant that was unable to display visual learning at all in the flight arena and then rescuing different aspects of visual learning piecemeal in this mutant (54). *rutabaga* mutants, which are defective in olfactory learning and memory (reviewed in Reference 96), were defective for aversive visual learning, as were a number of mutants affecting a central brain structure, the central complex (54). Rescue experiments involved expressing a wild-type version of the *rutabaga* gene in the mutant background, in specified brain neurons to see what neurons were required. Different neurons appeared to be required to rescue visual learning for different pattern parameters. Expression of wild-type *rutabaga* in different layers of the fan-shaped body of the central complex, an area associated with motor control (82), rescued elevation, size, or inclination specifically, suggesting that identified central neurons performed different roles in object pattern recognition. A subsequent study by the same group showed that neurons in another structure in the central complex, the ellipsoid body, most likely interacted with the fan-shaped body to regulate visual learning of objects (69) (**Figure 2e**).

One important conclusion from these individual fly paradigms is that visual responses to object parameters are most likely modulated by neurons far removed from the retina or optic lobes. Another conclusion is that *Drosophila* visual responses are indeed simple when flies are presented with simple stimuli in carefully controlled situations, but with more complex stimuli, motivational factors, and learning, flies can respond to visuals in more complex, less predictable ways. This is not surprising because flies evolved in the natural world with a plethora of visual cues of variable salience, suggesting that visual perception may be best understood by considering diverse, competing stimuli approximating more natural contexts.

DROSOPHILA IN THE NATURAL WORLD

When we think about the natural world and how it is different from the Ts and gratings used in most *Drosophila* vision research, we need to take into account the different temporal, spatial, and spectral dynamics that contrast a natural scene from simplified images (**Figure 1**). Natural scenes include complex patterns in combination with different types of motion and colors that also change when the fly moves through the environment. To this end, the complexity of the natural world presents a major problem for vision researchers: How can one determine how flies process and filter this information to produce appropriate behavioral responses? To study the fly's response to more natural visual stimuli, researchers have moved away from simple gratings and patterns to stimuli that incorporate multiple visual parameters. This can range from adding color to a pattern to displaying photographs of natural scenes to tethered flies. Although these approaches are still reductionist, they more closely approximate what happens in the real world and provide a way to study how this visual information gets integrated.

The tethered flight arena, although far from natural, can already provide some insight into more naturalistic situations, such as visual choice behavior in response to changing stimuli. In two related studies from the Guo laboratory (89, 103), flies were trained (using heat) to avoid compound stimuli combining color and shape characteristics. By gradually changing only one of the visual streams (e.g., color intensity) following classical conditioning with heat, the authors showed that flies would alter their fixation choice (e.g., to shape) depending on the quality of the parallel visual stream (e.g., color). How they did this was most interesting: Switches from color fixation to shape were best described by a sigmoidal function, meaning that small quantitative changes in a visual parameter led to major switches in behavior (**Figure 3e**). A similar nonlinear effect was found by Maimon et al. (57), who used an LED (light-emitting diode) flight arena where the shape or size of objects could be altered during the course of visual fixation experiments. *Drosophila* fixates on long, vertical dark objects, but antifixates (place behind it) small dark objects. When object shape was gradually changed from attractive (vertical bars) to repulsive (dots), the switch in behavior from fixation to antifixation was also sigmoidal, as in the aforementioned study combining shape and color. Together, these studies show that when the visual stimulus changes gradually, responsiveness does not necessarily also follow in a gradual fashion. Instead, there appear to be responsiveness thresholds. The central brain neurons (the mushroom bodies) that appear to be required for such visual decision making are the same as those required for context generalization (55, 103).

Another dynamic visual stimulus that evokes variable responses in the tethered flight arena is an expanding object. Expanding stimuli typically evoke a rapid escape response in the opposite direction (86), or a landing response if the expansion is frontal (88). One likely explanation for this visual behavior in tethered animals is that nonself-motivated expanding stimuli, resulting, for example, from a sudden gust of wind or an approaching predator, are potentially dangerous in the wild. However, tethered flies can also ignore expanding stimuli. When simultaneous rapid front-to-back movement is presented to either eye, which typically evokes a landing reflex, flies can choose to respond only to one side with an optomotor response instead (76). Visual cueing effects preceding the movement, such as a brief jiggle of the stimulus, can bias this attention-like behavior (76). Again, this shows that visual stimuli do not need to change much to completely alter behavioral responses.

One way to better examine *Drosophila* responses to more naturalistic stimuli is to track freely moving flies in confined arenas with high-speed, high-resolution filming technology (31, 83). When applied to the effect of looming discs on escape responses, such approaches can be quite revealing (14). In one study, flies were shown to have two distinct escape responses, which depended on the velocity of the approaching object. With high-speed approaches, flies would immediately jump without previous positional adjustments, whereas with lower-speed approaches, they would perform a series of directed positional adjustments before escaping (14). Therefore, a behavior that was previously thought to be simple and hard-wired (1) is actually more complicated and dependent on the visual context.

Filming free flight, within the confines of a patterned arena, can reveal stereotypical visual responses in *Drosophila*, such as flies' turning behavior as they approach the patterned arena walls (87) (**Figure 3f**). Flight turning depends on the pattern density of the arena walls, with flies turning less when the arena is completely white, for example. Processing of visual flow allows flies and other insects to avoid collisions with objects. To test how flies might adapt their flight behavior in more dynamic environments, direct manipulation of the visual flow field with virtual reality projector systems was recently used to induce flies to change their flight behavior in real time. Flies actively adapted to these dynamic stimuli in free flight, adjusting their flight altitude, turning, and speed to orient to edges and moving patterns (32, 84). These data suggest flies actively orient

Sigmoidal: a function that plateaus at lower and upper limits, with a linear relationship between the independent and dependent variables between these limits

LED: light-emitting diode

Course control: controlled adjustments to flight and walking paths to navigate through the environment

to selected visual cues while suppressing their responses to changes in the rest of the visual world around them.

One of the more complex visual scenes flies are likely to encounter is the presence of other flies—small moving dots that dart and loom, some requiring more attention than others. This is difficult to model in virtual reality but increasingly easy to track in real time. In a recent examination of how freely walking flies interacted, it appeared the flies did track one another and that they used a set of traffic rules (e.g., when to stop to allow another fly to pass) when interfacing with one another (11). On the basis of the identified behaviors, Branson et al. (11) developed a high-throughput automatic tracking system that could be used to distinguish males from females, because males would more often chase other flies, for example. Another study tracked fly responses to moving dots displayed on a computer screen (51). In that setup, *Drosophila* flies were shown to orient against the movement of a sparsely moving dot pattern but orient in the same direction of densely moving dot patterns (51) (**Figure 3e**). These opposite behaviors also appear to be nonlinearly related to the visual stimulus (25).

One drawback of video-tracking paradigms is that most still require considerable calibration to extract accurate descriptions of fly visual behavior. Bypassing video-tracking by using a population paradigm in which flies in a choice maze respond to moving stimuli displayed on a computer screen, Evans et al. (25) described motion responses in wild-type and mutant *Drosophila* flies exposed to a variety of visual parameters including moving dots and more complex naturalistic stimuli (**Figure 3g**). This approach is especially useful for high-throughput screening of potential visual mutants. In this study, a learning and memory mutant, *dunce*¹, responded more strongly than wild-type flies to wide-field motion under a variety of stimulus parameters (25). This finding again suggests that central brain mechanisms linked to learning and memory are modulating visual responsiveness levels, not just circuits in the eye.

Color, motion, and patterns are obvious aspects of vision to humans, but another form of visual information used by flies, especially in natural sunlight, is polarized light. Various insects use polarized light to navigate and move directionally through the environment; depending on the position of the sun, polarized light patterns across the sky are visible, even on cloudy days (17). Recent experiments using tethered flying flies in an outdoor arena demonstrated that *Drosophila* orients to polarized light in the environment (98). In this unusual experiment, flies both flew significantly “farther” in the presence of polarized light and oriented in the same relative direction to polarized light, suggesting they may use polarized light patterns in the sky as a distal landmark to navigate the environment. The detection of polarized light by *Drosophila* is made possible through specialized photoreceptors in dedicated parts of the retina, mostly on the top of the eye on the dorsal rim (99). However, there is some polarized light detection in randomly distributed facets of the ventral eye (99). What stimuli might flies detect using polarized light in the ventral portion of the eye? Plants and water sources also reflect polarized light, which may be patterned visual cues that could be recognized by flies. Therefore, polarized light detection may be used for both navigation using celestial cues affecting the dorsal rim and pattern detection from the ground affecting the rest of the eye.

INSIGHT FROM ELECTROPHYSIOLOGY AND BRAIN IMAGING

Since the advent of using whole-cell patch recording in *Drosophila*, researchers are ushering in a new era of recording from single cells in flies in combination with genetic tools to visualize and functionally control neurons (7, 95). Whole-cell patch involves recording from the cell bodies of single neurons with a sharpened glass capillary filled with saline. Although the technique has been used in neuroscience for decades, its application to neurons in the *Drosophila* brain is relatively

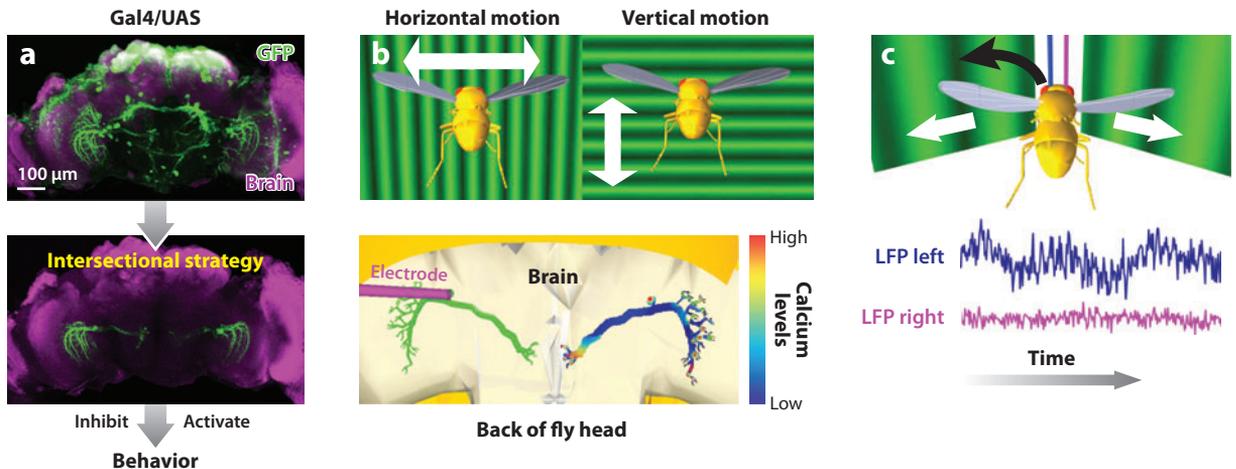


Figure 4

Investigating neural circuitry with genetics and electrophysiology. (a) Genetic techniques to narrow expression patterns in specific neurons. Populations of neurons can be targeted using Gal4/UAS (top panel). When intersectional strategies are used, Gal4 expression can be restricted to individual neurons and manipulated using responders that activate, inhibit, label, or report the activity of these neurons (bottom panel). Shown is a subset of lobula plate tangential cells (LPTCs). Green fluorescent protein (GFP) is green, and brain tissue is purple. (b) Vertical and horizontal motion can induce different behavioral responses (top panel), which can be correlated with activity recorded from cells in the brain (bottom panel). The Gal4/UAS system can be used to identify and record from these cells by using electrophysiology (left) or calcium imaging (right). (c) Behavioral assays can be combined with recording techniques to monitor the activity of neurons (top panel). Brain activity in the form of local field potentials (LFPs) correlates on the side the flies turn toward in a behavioral assay (bottom panel).

recent, with the first studies focused on olfactory processing in the antennal lobes (100). The Gal4/UAS system can be used to label neurons with fluorescent proteins, allowing for targeted recordings from specific types of neurons (Figure 4a) (see sidebar, Gal4/UAS Strategies to Dissect Visual Processing). Another technique to measure neuronal activity is calcium imaging, which involves genetically labeling neurons with calcium indicators such as GCaMP or TN-XXL (73, 81), which fluoresce with calcium influxes representative of neuronal activity (Figure 4b). These

Gal4/UAS STRATEGIES TO DISSECT VISUAL PROCESSING

Gal4 is a transcription factor that binds to upstream activating sequences (UASs) and induces transcription of genes (10, 19, 95). The two-component nature of the system makes it extremely versatile. Thousands of Gal4 strains of flies that express this transcription factor in subsets of cells have been established. When these strains are crossed to other flies that harbor UAS elements upstream of a gene of choice, this subset of Gal4-expressing cells can be engineered to express any protein. This system has been used to dissect visual system circuitry by implementing UAS effectors that highlight, activate, or silence particular neuron subtypes. In addition to manipulating neurons, the Gal4/UAS system has also been exploited to measure neuronal activity with genetically encoded calcium indicators (15, 73). Intersectional strategies can be used to increase specificity. Two different promoter elements that have overlapping expression patterns can be used to drive either half of the Gal4 molecule, resulting in functional expression only in overlapping cells (56), or a Gal4 inhibitor can be deleted in a subset of neurons by a recombination technique (6). This can be controlled spatially or temporally. Because single-cell resolution can be achieved by using these strategies, they are useful for dissecting visual circuits in *Drosophila melanogaster*.

EMD: elementary motion detector

new tools were recently applied to address a long-standing debate in fly vision: What is the neural circuit representing the elementary motion detector (EMD)?

It has been known for decades from work in blow flies that the lobula plate tangential cells (LPTCs) respond to directional motion (7–9). This was recently confirmed by cell-imaging and patch-recording techniques in *Drosophila* (48, 77) (**Figure 4b**). These studies also showed that *Drosophila* LPTCs form electrical connections with each other to support motion processing with high fidelity and at high speed. On the basis of extensive research in blow flies as well as *Drosophila*, LPTCs are now considered the likely output neurons of the EMD model of motion detection, which summate the total motion cues across the eye, likely via lamina and T4 and T5 neurons to signal front/back horizontal motion or up/down vertical motion (78) (**Figures 2e** and **4b**). Imaging and electrophysiological evidence has now confirmed that temporal summation of individual directionally sensitive elements occurs on the dendrites of the LPTCs (48, 77, 78), suggesting that the circuitry for the EMD model resides between the photoreceptors and the LPTC dendrites in the lamina and the medulla (**Figure 2c**).

Various researchers have confirmed that L1 and L2 neurons play a role in motion detection, because silencing synaptic transmission in L1 and L2 neurons blocks wide-field motion detection (16, 24, 49). These results were confirmed by electrophysiological recordings from LPTCs (49) (**Figure 4b**), calcium imaging of L1 and L2 neurons (73) (**Figure 4b**), and behavior in flies walking on an air-supported ball (16) (**Figure 3c**). One conclusion from these studies is that lamina neurons segregate into two types that are responsive to either increases (L1) or decreases (L2) in light. The L1 and L2 neurons are therefore obvious candidates for the input stages of the EMD circuit (**Figure 1c**). One major question presented by the EMD model is, What can account for the delay proposed to occur between the two units that receive the stimulus at slightly different times (**Figure 1c**)? The answer lies in the fact that the two inputs must be compared. Exciting new research has identified a medulla neuron, Tm2, as a candidate for performing this comparison, because it receives input from three neighboring cartridges. Tm2 is postsynaptic to both L2 and L4 neurons and integrates information across multiple modules (85) (**Figure 2c**). These neuroanatomical data are tantalizing but need to be tested by imaging, electrophysiology, and behavioral approaches.

Although responses to motion remain a complicated problem, simpler visual behaviors in the fly are supported by already known circuits and therefore are more amenable to functional studies relating stimulus parameters with behavioral outputs. The escape response induced by looming stimuli (14, 29) involves a well-studied set of neurons in the brain, which have been mapped from the visual input to the motor output (1). At the visual input level, de Vries & Clandinin (18) recorded from a looming sensitive neuron, called Foma-1, which branches extensively in the lobula plate and lobula. Genetically silencing either the lamina L2 neurons or Foma-1 abolished the behavioral response to looming stimuli. On the other hand, expressing channelrhodopsin in Foma-1 neurons (to transiently activate these neurons with blue light) elicited an escape response in otherwise blind flies (5, 18). The Foma-1 neurons send input to the giant fiber escape pathway, a series of electrically coupled neurons leading directly from the brain to leg motor neurons in the thorax to elicit escape behavior (a jump followed by flight) (14). Because escape behavior depends on the speed of the looming stimulus (14), a secondary escape circuit likely supports the alternate response eliciting postural adjustments that probably bypass the giant fiber system (29), enabling flies to perform multiple behaviors depending on context.

The connection between behavior and electrophysiological responses can be bidirectional, where behavioral states alter the physiological properties of neurons. A landmark study in honey bees, for example, showed that motion-sensitive neurons in the bee lobula are attenuated by sleep (50), so it is likely that the LPTCs also change their response properties in *Drosophila* during

different states of arousal. A recent study using whole-cell patch recording in tethered flying flies showed that the gain of the visual response of LPTCs in *Drosophila* increases significantly when tethered flies are flying (58). This study found that not only is the excitability of the LPTCs increased, but also their motion responses during flight are doubled compared to that of a nonflying animal. This effect seems to apply to walking flies as well: Similar results were found by using calcium imaging of the LPTCs for tethered flies walking on air-suspended balls (15).

The observation that neurons in the fly visual system increase their responsiveness as a consequence of walking or flying suggests that the gain of neurons is tuned to the behavioral requirements of the animal. As we have seen in the preceding sections, when a fly is moving through a natural environment, it is confronted with a multitude of competing visual cues, some of which need to be ignored whereas others need to be selected. Selective attention describes our ability to focus perceptual resources on one or a few relevant stimuli, while suppressing other simultaneous stimuli, in order to guide behavioral choices (71). Recent brain recordings from wild-type *Drosophila* in a flight arena have uncovered neural correlates of such attention-like behavior in flies (90). By implanting thin metal wires into the optic lobes of tethered flies, researchers recorded two kinds of brain activity as the animals chose to either turn right or left when confronted with competing gratings during flight (**Figure 4c**). Filtering the electrical brain signal can be used to detect local field potentials (LFPs), which represent the summed activity of populations of neurons around the electrode, thereby giving an idea of network activity as a whole (4). In this brain-recording study, the authors revealed that neuronal activity can increase only in one optic lobe, namely on the side associated with left- or right-turn behavior (90). This selectivity in the brain response to competing stimuli preceded the actual choice behavior.

Attention-like processes in *Drosophila* have been associated with oscillatory LFP activity in the *Drosophila* brain (93). LFP recordings from tethered flies presented with competing visual cues have revealed that visual salience is associated with increased LFP oscillations in the 20 to 30 Hz range (93), and the importance of this frequency range for visual attention-like behavior was also confirmed by the preceding flight choice study, albeit for a wider range (20–50 Hz) (90). Why is the fly brain buzzing at 20 to 50 Hz when the fly pays attention to a visual stimulus? Attention studies in other animals have also uncovered correlations between attention and oscillations in the so-called gamma frequency range (20–80 Hz) associated with stimulus selection behavior (20). The possibility that this may happen in the fly brain suggests that visual processing from either eye can be modulated by central mechanisms, perhaps tied to the learning and memory neurons such as the mushroom bodies and central complex (94).

Future studies of vision in *Drosophila* will probably need to consider global attention-like effects, as well as the local activity of single neurons, in order to understand how visual stimuli result in specific behavioral choices. As discussed in this review, when flies are exposed to more naturalistic stimuli, starting with basic visual competition, behavior can become much less predictable than suggested by the early EMD models. Just as visual paradigms have broadened to examine the complexity of naturalistic stimuli, brain-recording paradigms must be broadened to consider attention-like processes alongside single neuron responses. Determining how this might be done in such a small brain will depend on what tools are available, although a combination of global calcium imaging with electrophysiology seems promising.

SUMMARY POINTS

1. *Drosophila* genetic approaches have long been used to uncover the genes responsible for building the fly eye, but only relatively recently has the fly model been applied to understanding more complex visual behaviors.

Gain: the ability of neurons to increase or decrease the signal output

2. Vision can be divided into different subtypes, color vision, motion detection, and pattern vision; each may have distinct neural correlates in the *Drosophila* brain.
3. Population studies have been used successfully to study color and some aspects of motion vision, but they have been less successful to address how pattern vision operates owing to individual variation, context, and the complexity of pattern vision.
4. Experiments in individual flies have revealed that flies can discriminate and learn different patterns, even in combination with color or with changes in context, indicating that patterns may be represented as percepts in the insect brain.
5. Testing flies with more complex visual stimuli that approximate the natural world has shown that flies can produce complex, nonlinear responses, suggesting that visual behavior in *Drosophila* is modulated by arousal thresholds.
6. Targeted activation, inhibition, and expression of genes in defined neurons of the fly brain allow for visualization and functional control of visual circuits.
7. Application of genetic tools in combination with imaging and electrophysiology has been used to dissect the neural circuitry underlying simple visual behaviors, promising that the same techniques can now be applied to the more complex visual behaviors more likely to occur in nature.

DISCLOSURE STATEMENT

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