

ATTENTION IN *DROSOPHILA*

Bruno van Swinderen

Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072 Australia

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As bluntly summarized by a psychologist over a century ago, everyone knows what attention is [James (1890). *The Principles of Psychology*]. Attention describes our capacity to focus perception on one or a group of related stimuli while filtering out irrelevant stimuli. The ease we have in recognizing this astounding capacity in ourselves is matched by a surprising difficulty in identifying it in others, and this is especially the case for measuring attention in other animals. Identifying and measuring attention-like processes in simple animals such as flies requires, to some extent, even more rigor than asking the same question for our closer animal relatives, such as apes and monkeys. This is because flies have completely different brains than humans do, so to study attention in these creatures one must rely purely on operational or behavioral measures rather than comparative neuroanatomy. There is a long history of using sophisticated behavioral paradigms to study visual responses in *Drosophila melanogaster*, and these studies have often provided early evidence of attention-like processes in flies. More recently, these fly paradigms have been applied to measuring visual attention directly, and the combination of electrophysiology with these preparations has provided insight into how a fly might pay attention. Together with more efficient methods for measuring some aspects of attention, such as stimulus suppression, these approaches should begin to uncover how visual attention might work in a small brain.

I. Introduction: the Problem of Attention in Nonhuman Animals

To study attention in a fly is somewhat analogous to studying attention in a human baby: without a verbal report, attention processes have to be inferred from behavioral or physiological clues. The human infant, however, has an advantage compared to flies: we know that attention-like processes must be operating in a child because they soon become able to describe them. For flies, and all other animals, we only have behavior and physiology to study attention. In larger animals such as monkeys, measured behaviors may involve button presses or eye movements, whereas in smaller animals such as mice and flies, entire body movements may be followed to infer attention states. In either case, behavior alone can never be entirely convincing about attention because it is difficult to know whether the behavior reflects a simple motor response to a stimulus or whether it is the outcome of a perceptual choice made in the brain, where an alternate choice was deliberately suppressed. For this reason, brain recordings are often used in animals to bolster the behavioral data, by showing neural correlates of attention associated with competing stimuli. Together, electrophysiology and behavior can be applied to satisfy the criteria required for identifying attention in an animal. Of course, combining these tools in animals gets more complicated as animals get smaller, culminating in fruit flies with a brain the size of a poppy seed. Identifying attention in a fly seems an impossible task, but in fact the genetic model *Drosophila melanogaster* has provided insight on fly attention for the past 30 years, and research in the past few years have yielded some of the most convincing evidence that flies do have a selective attention.

Attention is sometimes equated with consciousness. The problem of understanding the mystery of consciousness has therefore created some confusion surrounding the study of attention in animals, namely that attention requires some rudimentary consciousness. A subjective association between these phenomena in humans seems to require the same in other animals, but this need not be the case, and, indeed, a proper study of attention can be separated from the mystery of consciousness (van Swinderen, 2005). As a first step in this direction, researchers discuss two different kinds of attention: top-down and bottom-up (Itti and Koch, 2000; Treisman and Gelade, 1980). Both are concerned with the allocation of perceptual resources among competing stimuli, but in bottom-up processes this facility is initially achieved by a stimulus-driven process (loud noises or bright objects attract attention), whereas top-down processes are volitional and depend to some extent on past experience (Wolfe *et al.*, 2000). Bottom-up attention can be parallel, with feature salience determining whether objects are perceived, whereas top-down attention is a serial process where perceptual resources need to be divided among potential targets and distracters (Bichot and Desimone, 2006; Treue, 2003). Although somewhat artificial, this separation narrows the discussion

with regard to the problem of attention in animals: when we wonder whether animals have selective attention, we are really asking whether they have top-down processes, that is, whether they have an experience-dependent stimulus-selection/-suppression mechanism. However, it is easier to address bottom-up processes in simple animals such as insects. By thus framing the problem, we are then in a better position to devise experiments to study it.

Any study of attention in animals needs to demonstrate suppression of competing stimuli in addition to selection of the one object which might eventually lead to a behavioral response. This is actually harder to demonstrate than might be expected because it is not always obvious whether an animal has even registered a competing stimulus after it has made a behavioral choice. Brain recordings in restrained animals often come to the rescue here, by revealing neural responses that are suppressed during an attention task. Fine-grained behavioral paradigms, such as continuous tracking of optokinetic behavior (by tracking eye movements, for example), can deliver similar information about stimulus suppression (Rubinstejn and Abel, 2011). As an alternative to this rather complex approach, attention studies in animals have traditionally relied on measuring reaction latencies in distracter paradigms. This can be tested in working memory paradigms, such as the delayed match to sample task (DMST), where a choice must be made following a cue (Fig. 1A), usually for a reward. Distracters presented before or during the choice phase of the experiment can test the subject's allocation of attention to the stimuli. Another paradigm, the five-choice serial reaction time task (5CSRTT), is an operant chamber where rodents (for example) collect food pellets by responding to competing stimuli in a pattern set by the experimenter (Robbins, 2002). In either scenario, the problem is similar: distracters draw attentional resources and increase reaction times to target objects, but if they are bound in some way with targets then reaction time for a behavioral task can be decreased. The readout in all cases is whether an animal performs a task when distracters are present, and if so, how long it takes to do it. Finally, although experience or training can guide animal behavior in these tasks, it should not alter the fact that top-down attention is a serial process; increasing distracter numbers should increase the time it takes to find a target.

Studies of attention in animals are often implemented as part of a classical conditioning paradigm. Thus, behavioral choice in a DMST or 5CSRTT paradigm requires considerable prior training before animals are able to provide useful data for an attention experiment. Training for an attention experiment can be problematic, though. Top-down attention by definition implies certain flexibility in being able to choose what to attend to, but the extensive training often required to perform attention experiments in rats or monkeys may instead promote habit formation, especially when rewards are involved (Maunsell, 2004). Attention resources may then even be required to *override* a habit, so training presents a real conundrum for attention studies in animals, namely that the training procedure used to test performance in an attention paradigm may itself compromise attention

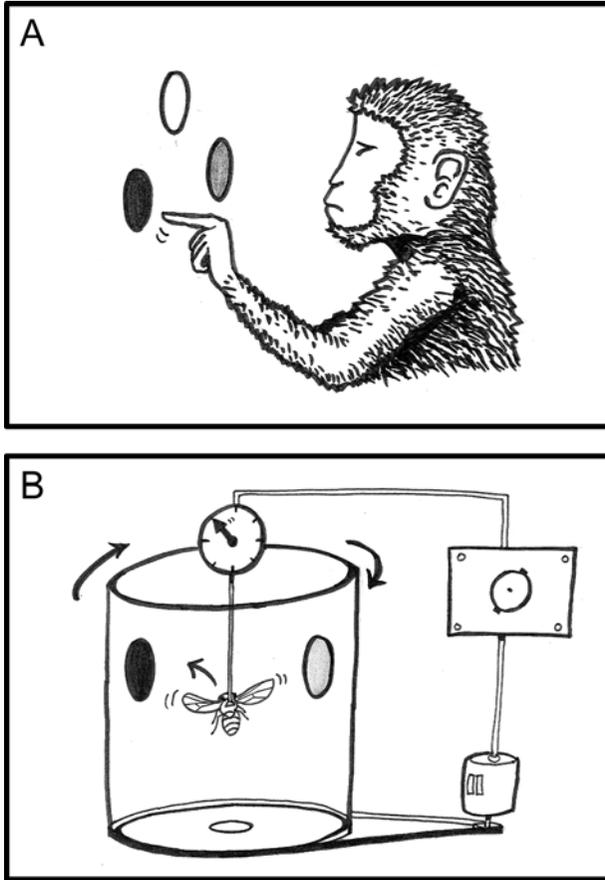


FIG. 1. Attention paradigms in monkeys and flies. (A) A delayed match to sample task (DMST), where choice is provided (black and gray circles) following a cue (white circle). (B) In the flight arena, tethered flies display choices by flying toward a visual object. A torque meter (dial) detects behavior, and a closed-loop circuit provides biofeedback for the fly (Heisenberg and Wolf, 1984).

processes. This problem applies to any animal studies, whether in a monkey, rat, or fly. The connection, if any, between attention processes and habit formation is unknown, just as it is not known whether top-down or bottom-up attention represents a continuum in the brain rather than discrete processes.

To summarize, there are a number of criteria that need to be satisfied for identifying attention-like processes in any animal, and it often appears that these criteria become stricter the further away from humans we explore for attention.

First, the original operational definition of attention put forth by Posner *et al.* (1980) still holds: it is the differentiation of objects of interest from other objects in the environment. To test this requires measuring responses to competing objects in space and time, such as targets and distracters. Ideally, some demonstration of stimulus suppression should be shown, and, finally, a thorough study of attention should also demonstrate neural correlates of these effects in the animal's brain. Research on attention in *D. melanogaster* has fulfilled all of these criteria, as will be reviewed here.

II. Classical Behavioral Approaches: Tethered Flight

Studies of attention in *Drosophila* have for the most part been an offshoot of studies of fly vision. This is for several reasons pertaining to the unique qualities of vision. First, the visual world is extremely cluttered, even for flies, and attention-like qualities would appear to be required for effective navigation through a barrage of competing cues. Early work on hoverfly flight, for example, showed how flies can track a singularity (e.g., another fly) while ignoring other motion cues (Collett, 1980). In more controlled experimental contexts, we find vision to be an ideal modality for the study of attention because visual stimuli can be partitioned in time and space more effectively than olfactory or auditory stimuli, for example. Such effective segregation of competing cues is crucial, as discussed above, for assigning behavioral responsiveness to one or another competing stimulus, and by the same token, for demonstrating suppression of certain stimuli. Several decades ago, flight arenas were engineered to study visual responses in tethered flies (Poggio and Reichardt, 1976; Gotz, 1980), and these could be easily adapted to questions relevant to visual attention, such as issues of perceptual load and response latency. Using these devices (Fig. 1B), it was soon evident that optomotor behavior did not always follow the predictions of a classic input–output system (i.e., the Reichardt detector for motion processing (Reichardt, 1969)). For example, flies typically respond in open loop to a horizontally moving bar by a torque displacement in the same direction, a classic optomotor response (Heisenberg and Wolf, 1984). Introducing a second moving bar, in the opposing hemi field for example, creates unpredictable behavior assigned to one or the other bar, rather than a summation of both effects. This suggested early on that flies were ignoring entire percepts rather than integrating the whole visual scene into a predictable output. A number of experiments performed by Reinhard Wolf and Martin Heisenberg in the early 1980s solidified this anecdotal observation into real data.

The first visual attention experiments in the tethered flight arena were aimed at quantifying the effect on the optomotor response of introducing a competing visual

stimulus. In the first set of experiments, the classic optomotor response was decreased by approximately 50% by introducing a static distracter (Wolf and Heisenberg, 1980). This suggested that the addition of a competing visual object increased perceptual load, and that half the time the fly's attention "spotlight" was not directed to the object that eventually moved. In a second similar set of experiments, opposing vertical bars (one to either side of the tethered fly), were oscillated front-to-back at different phases (Heisenberg and Wolf, 1984). Presented singly, each object would evoke compensatory optomotor responses in phase with the visual motion. Presented together, fly behavior was observed at times to track one or the other phase rather than a summation of both (Fig. 2A). This suggested

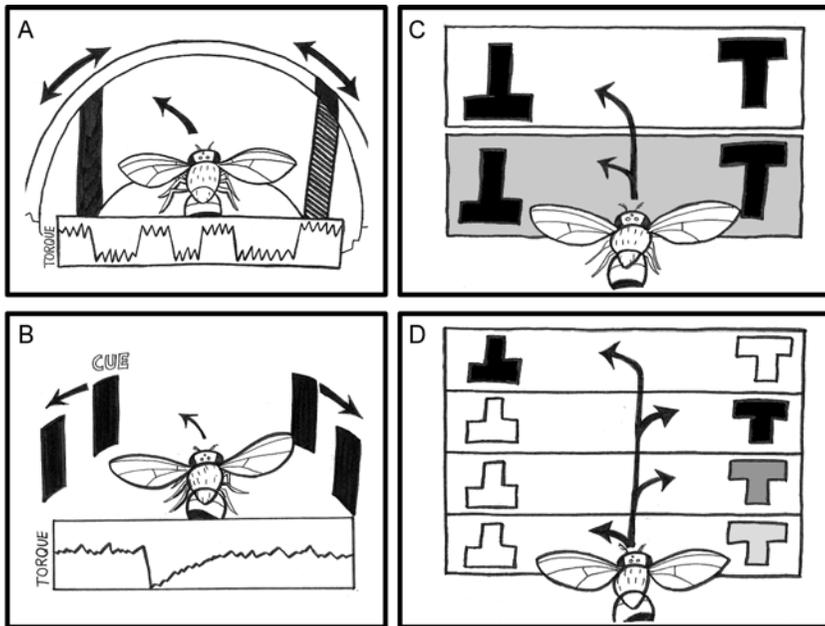


FIG. 2. Flight arena experiments. (A) Vertical bars oscillating out of phase in front of either eye can evoke responses (torque) locked to one or the other object in alternation (Wolf and Heisenberg, 1980). (B) A transient cue on the left (e.g., an oscillating bar) will bias fly responsiveness (torque) toward subsequent moving objects on the left, and suppress responses to simultaneous movement on the right (Sareen *et al.*, 2011). (C) Flies can be trained (by using heat as a negative re-enforcer) to discriminate visual objects (upper panel), and such learning is preserved through a change in background context (lower panel) (Liu *et al.*, 1999). (D) Flies can be trained to discriminate compound stimuli (Brembs and Heisenberg, 2001) (color and shape defines each object in the top panel), but will attend to individual features (e.g., color) when faced with contradictory cues (second panel). Attention will switch to the alternate feature (e.g., shape) at a defined threshold (Tang and Guo, 2001; Zhang *et al.*, 2007).

again that an attention spotlight was directed at only one percept while the other was suppressed. In addition to these stimulus-response experiments, closed-loop experiments (where the torque behavior of the fly actually controls the angular position of visual objects) showed that flies were able to fixate on targets while ignoring background motion (Wolf and Heisenberg, 1991).

These first forays into *Drosophila* attention-like behavior were performed on mechanical flight arenas, which involved motor-controlled drums rotating around the fly. When multiple objects were presented under separate control, as in the case of adding distracters or background motion, above, this meant adding successive layers of transparent drums. This arrangement was not ideal for fine control of visual stimuli, which is crucial for testing attention. For example, physical stimuli painted on rotating drums cannot be made to suddenly appear or disappear, so cuing or distraction effects are difficult to test. The development of virtual arenas using light emitting diodes (LEDs) solved this problem, but first considerable legwork was needed to show that optomotor responses to virtual motion using LEDs were comparable to previous experiments with physical objects (Dickinson *et al.*, 1993; Tammero *et al.*, 2004).

Thirty years after performing their seminal attention experiments in *Drosophila*, Wolf, Heisenberg, and colleagues have shown that responses of flies in the newly designed LED arenas correspond to behaviors conforming to an operational definition of attention (Sareen *et al.*, 2011). Taking advantage of the fine temporal control provided by the LEDs, the authors first tested a cueing effect on optomotor competition. This was done by oscillating one of two competing vertical bars (at 5 Hz) for 5 s prior to the displacement of either object. It was found that cueing significantly biased subsequent optomotor responses to the cued object (Fig. 2B). Importantly, the cueing effect could be dissociated in time (otherwise a direct interaction between the cue and test stimuli, such as mutual inhibition of central pattern generators, might be invoked). The study found that the salient cue attracted fly attention to its side even after a delay of 2 s, but not for longer delays such as 5 s. Further explorations using this paradigm found that the cue could attract attention to one side even if it appeared within a window 20° around the object, but not any larger, thereby defining a “window” size for the attention spotlight. Interestingly, the cueing was only effective in the lower visual field (LVF) of the fly, and this may be explained by the possibility that objects below the fly, such as landing and food sources, need to be attended more carefully. Finally, very salient distracters could override the system, as is to be expected for bottom-up attention effects. Together, this recent study provides the best behavioral evidence to date that fly attention is measurable, and that cueing effects within a narrow spatial and temporal range can modulate the choices made by a tethered fly.

In the interim between these two attention studies, the mechanical flight arena paradigm was nevertheless put to good use to study visual learning in *Drosophila* (Heisenberg *et al.*, 2001). Exploiting the closed-loop possibilities of the setup, the

same group and colleagues employed an operant conditioning paradigm (Wolf and Heisenberg, 1991) to unveil ever-increasing evidence of the sophistication of *Drosophila* visual behavior. First, it was found that flies could be trained to discriminate objects following classical conditioning with heat (the unconditioned stimulus), and that such closed-loop fixation was focused on the center of the object rather than its boundaries (where temperature changes occurred during training) (Wolf and Heisenberg, 1997). This implied that flies had indeed made a classical association between the unconditioned stimulus (US) and a visual object, and were able to discriminate among distinct objects by fixating preferentially on one object while ignoring the competing visual stimuli still within the fly's visual field.

By being able to measure fly choice following training, the flight arena next provided an obvious paradigm testing visual flexibility and perception in flies, by changing the stimuli after training. A first series of experiments asked whether learned objects were position-invariant, meaning that an object is perceived as the same even if it impacts a different latitude on the fly's retina (this is possible to test because the tethered flies cannot move up or down). If learning were retinotopic, then flies would not recognize a displaced object as being the same. Initial studies suggested that fly visual learning was retinotopic (Dill *et al.*, 1993), but subsequent studies found that flies did display learning even if the conditioned objects were displaced on their vertical or horizontal visual field (Tang *et al.*, 2004). It appears that these contradictory results stem from a peculiarity of fly vision: it is extremely sensitive to context (Brembs and Wiener, 2006). In the original retinotopic studies, modifying the visuals after training (actually exchanging physical sheets on the drum) probably provided substantial contextual contamination for the conditioned stimulus.

Flies can nevertheless extract features from their context. This was demonstrated in a study where visual training and testing for learning of distinct objects (upright and inverted Ts) was performed in different color backgrounds (blue or green). Wild-type flies could still learn following a change in context (Fig. 2C), suggesting that they can suppress the background and extract the feature to be learned (Liu *et al.*, 1999). This simple experiment has relevance for studies of attention: context generalization can be considered to be a form of selective attention because the animal has to suppress one visual stream while selecting another. Subsequent studies by Bjoern Brembs, a student of Heisenberg's, confirmed that context generalization is a feature rather than a failure of the fly brain (Brembs and Heisenberg, 2001; Brembs and Hempel de Ibarra, 2006; Brembs and Wiener, 2006). Indeed, the context need not be constrained to a background, but can be an aspect of the feature itself, such as its shape, position, or color. Not surprisingly, flies are thus able to perform some rather complex feats of visual learning, such as compound learning (Brembs and Heisenberg, 2001).

Following the success of contextual experiments in the arena, the path was set for increasing the sophistication of visual learning studies in flies, eventually

rivaling the kinds of experiments previously thought to be reserved for higher animals such as primates. These experiments were mostly conducted in the laboratory of Aike Guo, who also studied with Martin Heisenberg and Reinhard Wolf. By working with compound stimuli (upright or inverted Ts of either green or blue), a first study presented contradictory cues following training (upright green became blue) to test which feature of the stimulus (color or shape) was selected (color was). The color intensity was then gradually decreased in different experiments to determine the level when flies might switch to the shape feature (Fig. 2D). Interestingly, this switch happened within a rather narrow range, and the data were described by a sigmoidal function, which is often characteristic of attention-like processes in higher animals (Tang and Guo, 2001). The same conclusion, including the sigmoidal shape of decision making in flies, was reached in a later study combining color and center-of-gravity as the compound features (Zhang *et al.*, 2007). Again, although these were not attention studies *per se*, the work supported the notion that flies are able to select a feature of their visual environment while suppressing others, and that changes are subject to experience-dependent thresholds set in the fly brain. Further evidence of the “sigmoidal” nature of decision making in flies was found more recently using LED arenas, where within a single experiment, a visual could be gradually changed from vertical bar (which is attractive) to a square (which is repulsive): flies maintained fixation on a diminishing bar up to a point, whereupon they switched to antifixation, putting the now repulsive square behind them (Maimon *et al.*, 2008). The connection with this result and work from the Guo laboratory lies in the nonlinear dynamics of the behavior, which resembles attention-like effects in higher animals.

Saliency is a key feature of attention. This refers to the conspicuousness of a feature, such as how luminous it is, and typically salient objects attract more attention. Predictions relating object saliency to fly fixation behavior were tested in another study from the Guo laboratory (Xi *et al.*, 2008). In this study, the conspicuousness of a feature (a vertical bar) relative to background noise (contrast or spatial) was gradually decreased (in different experiments), showing that fixation behavior was correspondingly weakened. Olfactory distracters could similarly weaken fixation behavior on an object, as if perceptual resources were momentarily shifted to another modality. Finally, visual selection among three competing objects was biased toward the stronger (higher contrast) stimulus. These results were not necessarily surprising, except that ablation of an olfactory learning and memory center, the mushroom bodies, altered responsiveness thresholds for saliency. Indeed, the mushroom bodies were found to modulate various aspects of complex visual learning, from context generalization (Liu *et al.*, 1999) to resolving contradictory cues (Tang and Guo, 2001; Zhang *et al.*, 2007), to establishing saliency thresholds (Xi *et al.*, 2008). On the other hand, mushroom body neurons do not appear to be required for simple visual learning (Wolf *et al.*, 1998); instead, neurons of the central complex are required for elemental visual learning (Liu *et al.*, 2006;

Pan *et al.*, 2009). The neuroanatomy of visual learning, simple and nonelemental, will be revisited below in a discussion of possible attention systems in the fly brain.

There are limitations to purely behavioral approaches for studying attention, especially in animals lacking a behavioral report—but this applies to all nonhuman animals, and also some humans such as infants or paraplegic individuals. How does one separate bottom-up “reflexes” from top-down attention? Especially in flies, it is difficult to show that modulation of an optomotor reflex can be a form of attention rather than a failure (or inhibition) of a simple circuit response. If an animal responds to one stimulus and not another presented in competition, it is difficult to be certain that the animal has seen the competitor and is actually suppressing it. Ironically, the *Drosophila* flight arena presents one of the best paradigms for tackling this problem because the tethered fly cannot move away and cannot close its eyes; therefore, we are more confident about its tightly controlled visual world. In comparison, there is much less contextual control for rats or mice walking around a 5CSRTT chamber, the preferred apparatus for studying attention in rodents. Having taken fly behavior as far as possible for measuring various operational definitions of attention, it is clear that the next course needs to be insight into what is happening in the fly brain while the fly performs attention-like responses. In humans and other mammals, selective attention is associated with distinct neural correlates, such as γ -band (30–80 Hz) synchrony (Buzsaki, 2006). One might expect that if flies are really paying attention to their visual world, their brain activity might also display associated neural signatures.

III. Adaptations to Electrophysiology

Visual attention should be represented by neural correlates in the brain, for example, by increased activity of selected objects and/or decreased activity of ignored objects (Fries *et al.*, 2001). In mammalian preparations, brain activity (recorded either as field potentials of neuronal populations or as single neuron activity) informs the discussion of mechanisms of attention. For example, an ongoing debate in the field is whether attention involves increased gain of selected objects, suppression of ignored objects, or some mixture of both types of gain control (Maunsell and Treue, 2006). When multiple recording sites are considered, attention has often been found to be associated with neural synchrony or coherence within specific frequency domains. Clearly, the addition of brain recording electrodes to an attention experiment can provide substantial supporting evidence to determine whether a simple animal such as a fly is actually paying attention.

There is a long history of recording from the fly brain (Borst *et al.*, 2010). However, this has been primarily aimed at understanding visual processing in the

optic lobes of the brain. Early work on blowflies showed that large neurons in the lobula plate of blowflies spiked in response to moving visuals, and this preparation was soon taken up by a number of laboratories to investigate whether a neural code existed for integrating motion information across the fly retina (Egelhaaf *et al.*, 2002). Some lobula neurons only fired when objects moved front to back, whereas others fired when objects moved up and down. An ongoing debate has ensued around the question of whether visual information is processed as a temporal code (where the timing of every spike counts) or as a rate code (where the amount of spikes is what matters) (Nemenman *et al.*, 2008). More recently, novel recording techniques have moved the research questions to *Drosophila*, where genetic tools such as labeling or silencing of neurons can be used to sharpen the debate (Borst, 2009). Surprisingly, there have been few studies to date asking whether visual responses in the optic lobes can be modulated by arousal states. One study in bees found that motion-responsive neurons in the lobula became quiescent during sleep (even in the presence of motion), and that these could be “awakened” by stimulating the contralateral eye (Kaiser and Steiner-Kaiser, 1983). A series of studies in crayfish found that responses to flickering lights were altered in the central brain of the arthropod during sleep (Mendoza-Angeles *et al.*, 2007, 2010; Ramon *et al.*, 2004). That the suppression of stimuli, as expected during sleep, might also be measurable in attention experiments may seem obvious, but no studies specifically designed experiments around that question in insects until quite recently.

As the best evidence for visual selective attention in insects came from *Drosophila* in the flight arena, it is perhaps not surprising that this paradigm should have provided the obvious platform for probing neural correlates of attention in flies. This was actually more difficult than expected, perhaps due to the small size of the fly brain. A recent study modified the original tethered fly design to specifically test for attention-like effects in the fly brain, correlated to behavioral choices made by flies (Tang and Juusola, 2011). Three small wires were inserted into the fly brain, one in either optic lobe and a reference wire in the dorsal central brain. Two kinds of signals were recorded from the fly brain, depending on the sampling frequency: a local field potential (LFP), which typically reveals 1–100 Hz effects resulting from the summed activity of groups of neurons, and spiking activity, sampled at much higher frequencies (up to 25 KHz), to reveal action potentials of neurons close to the electrode. Both signals reflect neuronal activity, although not necessarily simultaneously. In mammalian preparations, spiking activity of individual neurons is often registered by recording intracellularly or from multiple nearby electrodes at the same time (e.g., tetrodes). This ensures that the same neuron (or “unit”) is identified through time, rather than a mixture of different spikes. In the current visual attention study, for technical reasons, spiking activity was most likely gathered from a mixture of nearby neurons.

Rather than using a circular drum around the fly, as in most previous flight arena experiments, the authors presented a continuously looping patterned strip to

either eye. This way, visual responses to the left or to the right eye could be evoked separately during the brain recordings. Presenting competing motion stimuli to either eye also allowed the authors to ask whether torque behavior in the fly alternated left and right, much like for Wolf and Heisenberg's attention experiments using competing oscillating stimuli in the arena (Wolf and Heisenberg, 1980). A form of stimulus rivalry was indeed seen in this novel preparation, with flies alternating flight directions when competing gratings moved front to back for either eye (Fig. 3A). These sustained behavioral choices were markedly different than the classic, much smaller optomotor responses to continuous field rotations, suggesting flies were selecting one flow and ignoring the other for periods of time. The behavioral responses were clearly not just input driven as they were not a summation of either stimulus, and onset of choice behavior was variable, sometimes delayed for up to a second. Brain recordings during these experiments

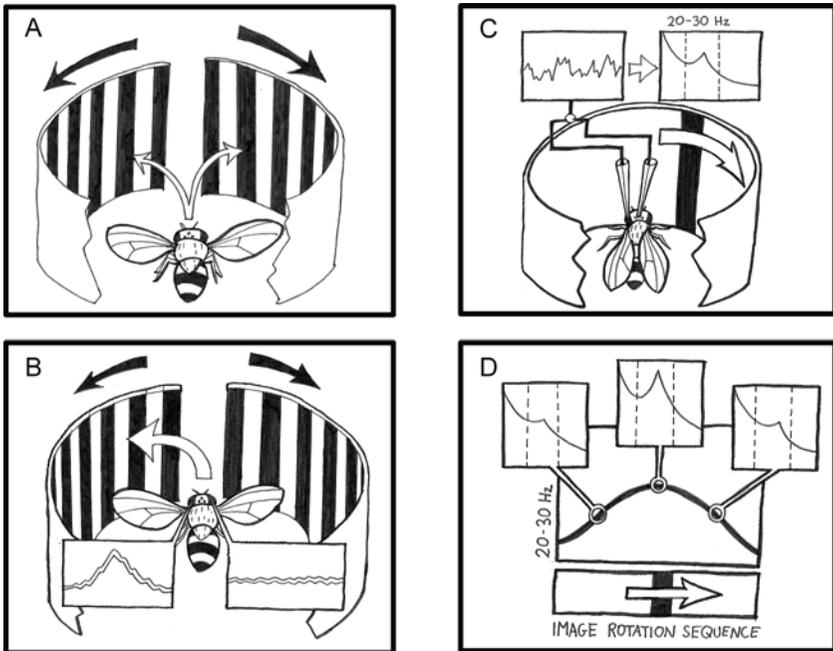


FIG. 3. Recording from the fly brain. (A) Competing moving gratings presented to either eye evoke alternating choice behavior (Tang and Juusola, 2011). (B) Recordings from either optic lobe reveal boosted brain activity (local field potential [LFP] amplitudes and spikes) on the behaviorally selected side (inset boxes) (Tang and Juusola, 2011). (C) Moving visual objects evoke 20–30 Hz LFP activity in the fly brain (van Swinderen and Greenspan, 2003). This is determined by a spectral analysis of the LFP signal (inset boxes). (D) The amplitude of the 20–30 Hz frequency band can be mapped onto the image rotation sequence, with the greatest amplitude occurring when the object sweeps in front of the fly.

revealed that the LFP was boosted on the side of the brain associated with eventual choice behavior (Fig. 3B). Indeed, the first neural responses (both LFP and spikes) started well before the behavioral response, at about 20 ms compared to an average of 300 ms for the behavior. The LFP was also anticipatory in nature, with a gradual shift in LFP amplitude between the left and right optic lobes predicting a subsequent shift in choice behavior (i.e., a left flight switching to a right flight). Finally, the boosted LFP on the side associated with behavioral choice occurred within a specific frequency range, between 20 and 50 Hz. A similar LFP effect was found in an earlier study: when flies initiate behavioral fixation (in closed loop) on a visual object, this is associated with a transient burst of 20–30 Hz activity (van Swinderen and Greenspan, 2003). In the more recent study, power in this range alternated between the optic lobes in correlation with the fly's behavior. Interestingly, boosted 20–50 Hz activity was even evident when the fly was not flying, but passively exposed to a moving grating only on one side. This raises the question of whether flies can attend to stimuli without actually performing a directed behavior, much like humans can attend to an object without necessarily walking toward it. The co-occurrence of 20–50 Hz LFP activity during passive exposure or active choice suggests that this may be the case.

The above work is consistent with the results from a series of earlier studies examining LFPs in tethered but nonflying *Drosophila* exposed to visual stimuli (van Swinderen, 2007a; van Swinderen and Brembs, 2010; van Swinderen and Greenspan, 2003; van Swinderen *et al.*, 2009). In these studies, LFPs were sampled at 300 Hz as a voltage differential between an electrode implanted in the left optic lobe and in the central brain. A recording preparation originally devised to measure neural correlates of sleep and wake in the fly brain (Nitz *et al.*, 2002) was adapted to visual experiments relevant to selective attention (van Swinderen and Greenspan, 2003). Visual salience could be evoked by a number of manipulations, such as heat, novelty, or odors, and these manipulations produced a transient increase in 20–30 Hz activity in the fly brain (Fig. 3C) which could be mapped onto the image position as it rotated around the fly (Fig. 3D). In general, the increase in salience did not last more than 20 s. The 20–30 Hz response could also be bound to another modality, such as odor. A repeating odor puff (a banana smell controlled by a valve driver) synchronized to the recurrence of a moving object (a vertical bar) specifically increased the salience of the visual, while the smell itself evoked responses in a different frequency range (70–80 Hz). Finally, this first study showed that classical conditioning (using heat as a US) could bias 20–30 Hz effects to either of two competing visual stimuli, and this selection/suppression effect could be detected even when the two objects were physically overlapping (by one object rotating around the fly twice as fast as the other).

The key to being able to assign attention-like effects in the fly brain to individual stimuli is to have them temporally tagged in some way. In human attention studies, competing visuals are often flickered at distinct frequencies, and the power

or coherence of these separable frequencies can be detected by electro-encephalography (EEG) or magneto-encephalography (MEG) approaches (Vialatte *et al.*, 2010). Typically, attended flickering objects are represented in the brain activity by increased amplitude or coherence effects, and it is thus in principle possible to infer what flickering stimulus a human subject is paying attention to by measuring which tag has greater power. In the preceding attention experiments, virtual objects in an LED arena were rotated around the fly, 180° apart, with a period of 3 s (Fig. 4A). This way, each object swept in front of the fly at a different time (for a different 1.5 s epoch); therefore, each object was tagged by its specific timing when it was in front of the fly. Importantly, 20–30 Hz responses to salient objects (e.g., by making them novel) was increased when these swept in front of the fly while responses to the competing object were suppressed—even though it also swept in front of the fly (Fig. 4B). Such temporal tagging of either object is one way of associating brain activity with competing stimuli; another way would be to flicker them at distinct frequencies or move them at different speeds around the fly.

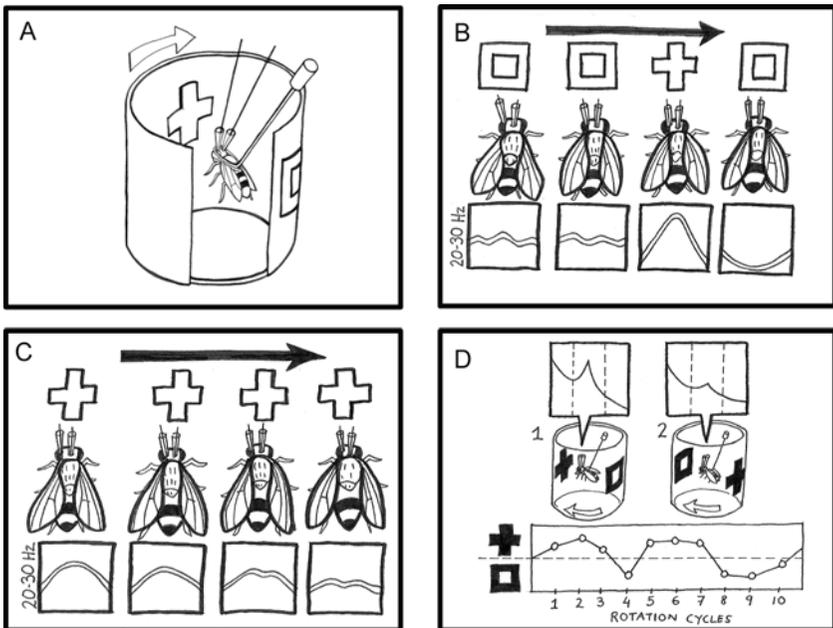


FIG. 4. Local field potential (LFP) recordings and visual attention (A) Two objects 180° apart rotate around the fly, once every 3 s, evoking 20–30 Hz responses at different times. (B) 20–30 Hz responsiveness is increased for visual novelty (the cross) and suppressed for the competing non-novel object (van Swinderen, 2007a; van Swinderen and Greenspan, 2003). (C) 20–30 Hz responsiveness to visual salience is abolished after three to four exposures of the stimulus (van Swinderen, 2007a, 2007b). (D) 20–30 Hz responsiveness for successive exposures of competing stimuli defines a time series, plotted as a 20–30 Hz ratio for successive image rotation cycles (van Swinderen, 2007b; van Swinderen and Brembs, 2010).

A recurrent argument in studies of attention, especially in smaller animals such as insects, is whether we are simply measuring habituation. Especially in the context of novelty conditioning, one could imagine that the transient responsiveness of the LFP to a novel object and subsequent attenuation might reflect desensitization effects in the eye. Behavioral observations, discussed earlier, show that flies can behave counter to expectations if habituation were the only mechanism involved here. For example, visual cuing can be separated from their targets by up to 2 s (Sareen *et al.*, 2011), visual learning can be position invariant (Tang *et al.*, 2004), or flies alternate their choice behavior to explore their visual environment, sometimes against optomotor expectations (Tang and Juusola, 2011). For electrophysiology, suppression effects argue against habituation, and novelty responses in the LFP have also been shown to be position invariant, arguing that this effect cannot be retinotopic (van Swinderen, 2007a). The argument could of course always be made that the LFP simply reflects habituation effect deeper in the brain, among neurons that code for the percepts (e.g., a cross or a square) rather than for the impacted photoreceptors. This argumentation, however, simply blurs the difference between attention and habituation; the dynamic properties of perceptual representations in the fly brain can be studied, regardless of what the phenomenon is called.

The study of visual learning and perception in *Drosophila* has provided valuable temporal information that is relevant to attention studies, although these data are often overlooked. We have seen in recent studies, discussed above, that fly choice behavior can be delayed by a second, following the presentation of competing visual stimuli (Tang and Juusola, 2011) and that a visual cue can remain in a fly's working memory for 2 s (Sareen *et al.*, 2011). In classical condition experiments, the performance index following training in the arena is usually represented as a single histogram describing choice bias over 2 min of tethered flight. Closer examination of this 2-min flight reveals that flies may require several seconds of exploration before "settling" on a decision, especially when nonelemental learning is involved, such as position invariance experiments (Tang *et al.*, 2004) or involving contradictory cues (Zhang *et al.*, 2007). These observations again argue against a habituation effect. The relevance of habit formation to visual learning has been studied specifically, with interesting implications for attention in flies. It appears that overtraining in the flight arena (by doubling the training time from 8 to 16 min) consolidates the motor component of learning (i.e., flying right is going to keep me from getting hot) while compromising the flexibility required to display fact learning (what object is not hot?) through alternate motor strategies (Brembs, 2009). On the other hand, short training sessions promoted the flexibility required for fact learning while preventing the rigidity of motor learning. These fascinating observations were possible by dissociating these different aspects of learning (motor from fact) in carefully designed flight arena experiments (Brembs, 2009). The implication for attention is that flexibility is required during early stages of fly

learning, whereas extensive training overrides attention systems to promote defined motor responses in the learned context. These results have clear implications even beyond fly work: most learning studies involve some overtraining of sorts.

Timing effects are also evident in LFP activity in the fly brain. In one study, the design of novelty salience experiments involved presentation of two identical objects (the training phase), whereupon one of these objects would change (this being an LED arena) to a competing choice (van Swinderen, 2007a). Following this change, the LFP was transiently increased for the novel object and simultaneously suppressed for the non-novel object (independent of position on the visual field, since the objects were continuously rotating around the fly). It was found that 50 s of training produced a selection/suppression effect, but 25 s of training did not. As this was a recurrent stimulus scenario (where training and test phases alternated for 10 cycles), this suggested that 25 s of training was not long enough to make a previously seen object salient again, thereby defining a possible window of working memory for the fly (<25 s). Interestingly, this window length was comparable with the duration of salience effects following training, also around 20 s (van Swinderen and Greenspan, 2003).

The observation that brain LFP activity could be transiently increased for one visual tag and suppressed for a competing visual raised the question: what is a fly's attention span in this paradigm? Is it really 20 s? The answer most likely has less to do with absolute time than with the number of repeated presentations of an object. In the LED arena, virtual objects were made to rotate around the fly with a period of 3 s. This means that following a salience manipulation such as classical conditioning with heat, competing choices swept in front of the fly every 3 s. A cycle-by-cycle analysis (tied to the image rotation period) of LFP effects revealed that selection/suppression in the 20–30 Hz range were significant for three to four successive presentations of the visual objects (Fig. 4C). After that, individual sweeps could be biased for one or the other object, but this varied among flies meaning that on average the attention-like effects disappeared after four successive presentations of a salient object (van Swinderen, 2007a; van Swinderen and Brembs, 2010; van Swinderen *et al.*, 2009).

Salience can be set by the experimenter, using heat, novelty, odors, and the like, but presumably salience is also a dynamic property that depends on an animal's recent attention history. For example, something that has not been attended to for a while might become salient again, whereas an attended object loses salience after repeated exposures. The same preparation to test for salience effects in the LFP was applied in subsequent studies to measure alternation dynamics in 20–30 Hz selection/suppression directed to two competing objects presented for extended periods (van Swinderen, 2007b; van Swinderen and Brembs, 2010). The way this was done was by calculating a ratio of 20–30 Hz power for each image rotation cycle, where the numerator was summed 20–30 Hz

power for one object (1.5 s of time when it was in front of the fly) and the denominator was summed 20–30 Hz power for the 1.5 s when the alternate object swept in front of the fly. This produced a time series of 20–30 Hz ratios (Fig. 4D) that could then be statistically compared with randomized data, using permutations of 20–30 Hz ratios from the same fly. Interestingly, this analysis revealed that even without experimenter-imposed salience, the LFP displayed a form of hysteresis where power would be biased for one or the other object for more cycles than expected by chance (van Swinderen, 2007b; van Swinderen and Brembs, 2010). This defined a possible “attention” span for flies, once again tied to image exposures rather than absolute time. In tethered flight, flies also alternate behavioral choices, and brain activity is correlated with these nonrandom alternations. Interestingly, changes in LFP activity in either optic lobe appeared to predict subsequent behavioral alternations (Tang and Juusola, 2011).

One of the implications of these and other visual attention studies in flies is that the animals have a flexible “spotlight” of attention that can cover a wide range of their visual field, even though the animals are fixed in space by a tether. We saw in the behavioral studies, discussed earlier, that cueing effects can occur 20° away from the visual target, and that the LVF is more sensitive to such cueing effects than the upper visual field (Sareen *et al.*, 2011). These and other experiments also showed that attention can be directed to one hemifield at a time, that is, the left eye versus the right eye (Tang and Juusola, 2011). The LFP recording experiments also provide some information regarding a possible attention spotlight in flies. First, the 20–30 Hz response appears to peak “in front” of the fly, meaning that LFP amplitude increases as the attended object sweeps by a $\sim 90^\circ$ wedge in front of the tethered animal. Importantly, when a competing nonsalient object sweeps by this same quadrant, 20–30 Hz responsiveness is suppressed, suggesting that the fly’s window of attention is either elsewhere or shut down when the nonsalient competing object sweeps by. It is interesting to speculate why, on average, the LFP response is greatest directly in front of the fly in this particular preparation. One possibility is that flies in general move forward, so it would be logical for a default window of attention to be centered, on average, in front of the animal. However, this positional response is malleable. Attaching salience to a rotating object in the LED arena shifts the 20–30 Hz response by 0.5 s, bringing it closer to its recurrent point of origin (van Swinderen and Greenspan, 2003). Thus, a salient object rotating clockwise around the fly will evoke a 20–30 Hz response further to the left. This suggests that flies may be anticipating the objects appearance, and correspondingly shifting their window of attention to an earlier portion of the image rotation sequence.

It is especially striking that LFP effects in the fly brain display attention-like qualities even in animals that are not actively behaving in a goal-directed manner. This suggests that attention can be uncoupled from behavior, even in insects. However, such studies are also problematic because they lack a behavioral

readout. Just as behavior without electrophysiology is never entirely convincing, the reverse is also true. Indeed, as for the study of sleep, behavioral tests remain the most convincing way of assessing whether attention exists in a simple animal. There have been very few studies combining electrophysiology and behavior in *Drosophila*, especially in the context of visual studies. One measured LFPs during fixation in the flight arena and another measured LFPs and spikes in a flight choice paradigm, both discussed above. The gold standard of electrophysiology is the patch-clamp recording, where information can be gained on how a single neuron's membrane potential is responding to a stimulus. Although this has been applied for a number of olfactory studies (Tanaka *et al.*, 2009) as well as a few visual studies (Joesch *et al.*, 2008; Schnell *et al.*, 2010), where the fly is often so restrained as to preclude any behavioral readout, only one study to date has succeeded in patching *Drosophila* brain neurons while animals are behaving, for example, in flight or walking. In this study (Maimon *et al.*, 2010), the authors show that the gain of visual interneurons (the well-studied VS cells, which respond to vertical motion) is doubled when animals are flying. Reduced membrane resistance in these neurons during flight suggests that synaptic drive from upstream motion-sensitive inputs is increased. This boosted visual response is consistent with the LFP results in the attention paradigm discussed above. Although the patch-clamp experiments were not designed as attention experiments *per se* (i.e., there was no “choice” in the flight behavior), it is worth speculating that the boosted intracellular activity reflects an attention-like process that gets activated once the fly starts flying; it seems logical that attention-like mechanisms need to be notched up in a flying animal, whereas a stationary animal may not require such active selection/suppression processes. Another study described a similar result, but employing instead optical imaging of visual neurons in walking flies (Seelig *et al.*, 2010). Together, these recent studies lend support to the idea that active behavior boosts attention processes in flies, although their viewpoint is that peripheral systems (e.g., neurons in the optic lobes) are directly controlled by behavioral state rather than by some central or brain-wide attention process.

IV. Related Behavioral Measures

Attention is a complex phenomenon, and to detect and measure attention in flies seems to therefore require complex devices. These have included, so far, tethered flight arenas (section II) and miniature brain recording paradigms (section III). The advantage of these sophisticated devices is that they can continuously track a signal (such as torques or LFP amplitudes), and in this way describe fine-grained changes that can be correlated with stimuli in attention experiments. This is in contrast, for

example, to olfactory learning paradigms where performance is measured as a single distribution of flies between two tubes (Tully and Quinn, 1985). The lack of access to behavioral processes in most *Drosophila* population assays, which measure fly distributions, makes it difficult to study attention, which is most concerned with processes through time. That said, not every laboratory has easy access to the sophisticated tools and devices that have been used to study visual learning and attention. Indeed, there are to date only half a dozen laboratories in the world doing flight arena work, and even fewer recording from the fly brain during visual experiments. What other approaches can be used to study visual attention in flies? This problem is especially relevant for eventually applying molecular genetic strategies to the question. The sophisticated preparations that have unveiled attention in *Drosophila* are not amenable to genetic screens, partly because they are laborious single-animal assays, but also because many mutants do not fly readily in the arena, and are therefore unable to “report” their attentional states that way. Simpler and high-throughput paradigms need to be developed to further the study of attention in *Drosophila*, but it is imperative that these actually measure phenotypes relevant to attention.

Attention is as much about stimulus suppression as it is about selection (Bichot and Desimone, 2006). An animal must be able to ignore a competing cue, even though it may be sometimes stronger than the attended cue. The expectation for simple animals such as flies is that they will always respond “reflexively” to salient stimuli, such as a bright light or a moving grating. This is in fact not the case. There is increasing evidence that experience can modulate simple reflexes in flies, not by habituation but by learning or by an internal motivational state. One way of studying attention indirectly is by using assays that measure suppression of a simple reflex. Some of these assays can be easily scaled up for genetic screening purposes, and subsequent focused experiments using the sophisticated attention paradigms discussed above might then be used to confirm or further characterize attention-like phenotypes in these mutants. Two assays of this sort are the aversive phototaxic suppression (APS) paradigm and the visual maze (Fig. 5).

Flies are attracted to light and if given a choice will overwhelmingly prefer to walk down a lit corridor than a dark one. Such observations have guided some of the first genetic studies in this model, such as Seymour Benzer’s countercurrent assays that identified the *photophobe* mutant (Ballinger and Benzer, 1988) and catalyzed similar designs for olfactory paradigms. Although phototaxis may appear to be a hard-wired reflex, it can be suppressed by training: associating a lit corridor with an aversive-tasting stimulus, such as quinine, will bias subsequent approaches (in retrials of the same fly) toward the alternate dark corridor, such that after 12–16 training sessions a wild-type will make the unnatural choice of choosing darkness more than half the time (Fig. 5A) (Le Bourg and Buecher, 2002; Seugnet *et al.*, 2009). To do this, flies must suppress their innate phototaxic tendency. It is hypothesized that this mechanism may be related to the suppression

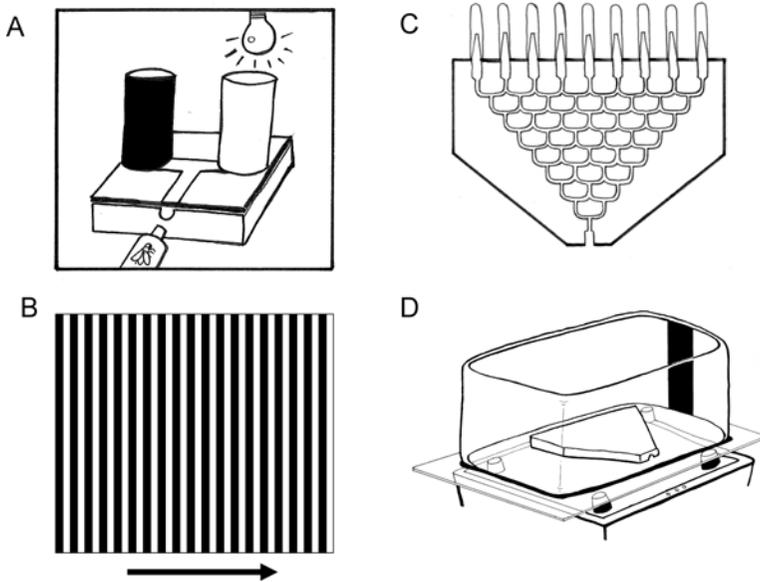


FIG. 5. Other behavioral paradigms 1. (A) The aversive phototaxis suppression (APS) assay measures suppression of a reflex to follow light, by associating a lit chamber with an aversive taste such as quinine (Le Bourg and Buecher, 2002). By 14–16 repeated trials, around half the flies will suppress phototaxis and enter the dark chamber instead (Seugnet *et al.*, 2009). (B) Flies display optomotor reflexes, by following the motion of a moving grating for example. (C) An eight-point choice maze placed over a moving grating will fractionate flies for visual behavior, yielding a score based on the distribution of flies among the nine collection tubes (van Swinderen and Flores, 2006). (D) Attention paradigms can be designed around the visual response, by for example providing visual distractors which suppress the optomotor reflex (van Swinderen, 2007a; van Swinderen and Flores, 2006).

involved in attention experiments, namely that central processes in the fly brain override input from the periphery to guide behavioral choices. Although the APS paradigm is quite laborious (single flies must be followed, collected, and re-run), it possible to imagine ways of multiplexing this paradigm to screen for visual suppression effects. Variations on this paradigm have also been applied to color choice behavior (Zhou *et al.*, 2010).

Flies have a tendency to follow motion (Fig. 5B). This reflex is termed an optomotor response (Gotz and Wenking, 1973; Gotz, 1980), and this behavior has been intensively studied in the flight arena as well as in tethered or freely walking paradigms. The expectation, as with phototaxis, is again that this is a hard-wired reflex. Although it is accurate to say that fly behavior is strongly dependant on the physical aspects of a motion stimulus (grating velocity, density, luminosity, and contrast have all been carefully studied with regard to their effect on the optomotor response (Borst *et al.*, 2010)), it is also true that individual flies do not

always respond according to optomotor expectations on a trial-by-trial basis, and flies have been shown to “ignore” optomotor cues, as in the attention experiments described above. An indirect way of studying attention might then be to measure optomotor suppression behavior, much like phototaxis suppression. This would require an efficient way of first measuring visual behavior in flies, preferably via a population paradigm in freely walking flies.

Population paradigms aimed at segregating *Drosophila* populations for optomotor traits have been developed decades ago by Gotz, Heisenberg, and colleagues (Gotz, 1970; Bausenwein *et al.*, 1986). These involved rather bulky contraptions, featuring rotating drums and choice corridors, and an experiment could last several hours. Nevertheless, the first optomotor mutant, *optomotor blind*, was isolated using such a device (Pflugfelder and Heisenberg, 1995). A different approach was introduced in 2006 (van Swinderen and Flores, 2006), exploiting a simple design used originally in geotaxis assays for flies (Toma *et al.*, 2002). In this novel paradigm, flies navigated an eight-point choice maze while they were exposed to moving visuals displayed on a computer monitor (Fig. 5C). At each choice point, flies can make either a left or a right turn, and this decision is influenced by the moving visuals flowing beneath them on the monitor. Each experiment, comprising about 30 flies running the maze, is completed in about 2 min, and a total of 4–8 maze runs yields sufficient data to quantify visual responsiveness in a strain. Upon completing the maze, flies end in one of nine collection tubes, and a weighted average of the distribution of flies among the nine tubes provides a visual response score for the strain. For an experiment, visuals are run in either direction (left to right and right to left) to balance any possible odor trails resulting from previous experiments or unaccounted-for asymmetries in the visual environment of the maze. Typically, wild-type flies will produce a visual response of 0.7 ± 0.1 to moving green/black gratings. The maze paradigm has recently been improved to a modular design, and the application of infra-red fly counters has allowed for the system to be fully automated, from introducing the flies to statistical analyses, thus providing a highly efficient way of assaying visual behavior in *Drosophila* (Evans *et al.*, 2011).

Experiments in the maze revealed that visual responsiveness in wild-type flies could be quite fickle, with the geometry of objects outside the transparent maze having a significant influence on the visual response. For example, one anecdotal observation was that visual responsiveness could be abolished by a person standing next to the maze during an experiment. This suggested that flies navigating the maze were partitioning their perceptual resources between the moving grating beneath their feet and surrounding objects around the maze. Although this called for careful control of the surrounding maze environment, it also implied that attention-like processes might be interfering with the optomotor response. Following this serendipitous observation, the maze paradigm was redesigned to allow for visual distracters to be presented to one or either side of the maze

(Fig. 5D), initially as a static black cardboard bars (van Swinderen and Flores, 2006). The addition of these distracters showed that (1) flies could see through the maze and were attracted to these objects, and (2) the presence of a competing visual distracter could abolish the visual response to moving gratings in the maze. Refinement of the distracter design, by placing it on liquid crystal display (LCD) panels to the sides of the maze, allowed for quantification of the distracter effect in subsequent studies (van Swinderen *et al.*, 2009).

The ability to completely abolish optomotor responsiveness in wild-type flies by a simple static visual placed to the side of the maze suggested that the response to moving gratings was not as hard-wired as once thought, at least in wild-type flies. Indeed, responsiveness to the static distracter suggested that wild-type flies could suppress their reflex to follow a moving grating, if they were instead paying attention to a distant object. This implied that flies were constantly engaged in optomotor suppression to some level, and that strains defective in visual attention would not be able to suppress the response to the moving grating as well. This hypothesis was first tested by a selective breeding scheme, much like the original geotaxis breeding experiments that isolated “hi” and “low” geotaxis lines (Toma *et al.*, 2002). Selective breeding of flies with increased visual responsiveness to a moving grating for over 20 generations produced individuals that were insensitive to the presence of a static distracter outside the maze (van Swinderen and Flores, 2006). Electrophysiology experiments on these genetic variants revealed that these flies indeed were characterized by an attention defect, using a novelty salience paradigm previously applied to wild-type flies. The simple maze design was thus validated as a useful approach to screen for *Drosophila* attention mutants, by paradoxically looking for strains with *increased* visual responsiveness to the grating. Increased responsiveness would thus be suggestive of a *failed* ability to suppress a prepotent optomotor reflex, much like failed phototaxis suppression would also signify a breakdown in fly cognitive processes (Seugnet *et al.*, 2008, 2009). These two assays, the optomotor maze and the APS paradigm, both reveal potential attention phenotypes by a failure to suppress an innate reflex. The same rationale, measuring increased visual reflexes, has also been used to study attention defects in humans (Rubinstein and Abel, 2011; Williams *et al.*, 2006). Results from first genetic screens in *Drosophila* using these assays will be discussed in the next section.

There are other *Drosophila* walking assays that can inform about visual selective attention, although these are mostly single-animal paradigms that measure selection of objects rather than suppression of alternate choices. Recent developments combining flexible visual displays with fly tracking software have created interesting possibilities for designing attention experiments in walking flies (Branson *et al.*, 2009). Most of the original open-field walking assays were based on Buridan’s paradigm, originally used by Karl Gotz and colleagues to describe the visual control of walking behavior in flies (Gotz, 1980) (Fig. 6). In this paradigm, flies’

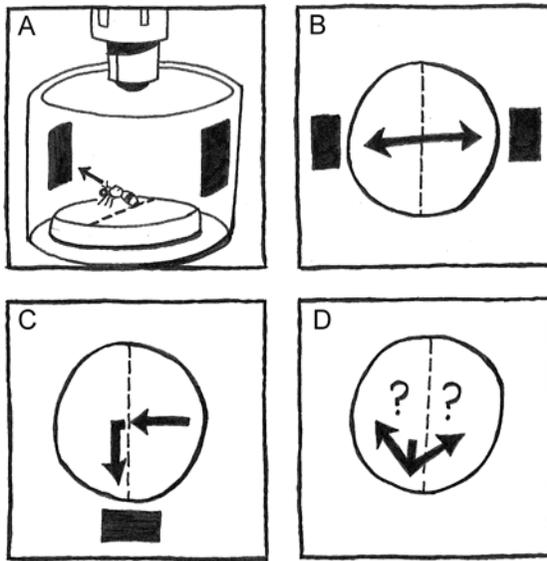


FIG. 6. Other behavioral paradigms 2. (A) In Buridan's paradigm, are placed in a chamber with competing visuals displayed on the wall, and walking choice behavior is tracked. (B) Flies will typically walk back and forth between the competing visuals (Gotz, 1980). (C) Changing the position of visual cues will re-orient fly locomotion. (D) Flies can still orient to temporarily invisible landmarks, based on their prior experience, thereby displaying a spatial memory (Neuser *et al.*, 2008).

wings are cut off and the following day they are placed in a small circular arena, usually surrounded by a moat of water (Fig. 6A). Visual objects are displayed on the walls of the arena, and the original observation by Gotz was that flies will continuously walk back and forth between two vertical bars positioned 180° apart on the arena (Fig. 6B, Buridan's ass, in Greek mythology, was similarly mesmerized by two choices). Numerous variations on this paradigm have revealed object preferences in flies, but it was not until the development of fly tracking and closed-loop feedback to LED arenas that experiments could be performed relating more specifically to visual selective attention. In a series of remarkable studies, Roland Strauss and colleagues exploited the fine temporal control of the LED system to change visual objects as the fly walked in the arena (Neuser *et al.*, 2008; Schuster *et al.*, 2002; Strauss and Pichler, 1998). For example, a visual target could disappear as the fly walked toward it, and a distracter could appear at another position on the drum (Fig. 6C). Using such a paradigm, it was shown that wild-type *Drosophila* have a spatial orientation memory for object positions in the arena, and will even walk toward *invisible* objects based upon their working memory of where the visual target used to be (Fig. 6D) (Neuser *et al.*, 2008). One of the hallmarks of top-down attention in humans is that it interacts with memory systems, and by

demonstrating a memory component to visual fixation in flies (without the object actually being present) effectively separates the visual response from an immediate visual input. The implication for attention in flies is tremendous, as this could be analogous to the fly attending to a memory. To understand the relationship between attention and memory is an obvious next step in the field, and demonstrations of both phenomena happening concurrently are crucial to moving the field forward.

V. Neural Substrates of Visual Attention in *Drosophila*

There is comparatively much less known about the molecular and neural underpinnings of vision in *Drosophila* than there is for olfaction. Consequently, even less is known about the neural substrates of visual attention in the fly. Nevertheless, a synthesis from genetic work done in the multiple paradigms discussed earlier paints a preliminary picture of neurons and molecules likely to modulate attention in the fly.

Olfactory learning in *Drosophila* is centered on the mushroom bodies (Fig. 7A) (Heisenberg *et al.*, 1985), which are paired structures in the central brain with direct input from the antennal lobes, which process odors (Keene and Waddell, 2007). Each mushroom body comprises ~2500 neurons, called Kenyon cells, and these are organized into distinct lobes that have been associated with different temporal phases of odor learning (Krushes *et al.*, 2007). Early on, the mushroom bodies were found not to be required for simple visual learning in the flight arena (Wolf *et al.*, 1998), and this was perhaps not surprising as there are no direct inputs from the optic lobes to the mushroom bodies in the fly (Heisenberg, 2003). What was surprising was that almost every subsequent study on visual learning in *Drosophila* has implicated the mushroom bodies for more complex forms of visual learning. The first indication of this was for context generalization: ablating the mushroom bodies (chemically or genetically) rendered flies unable to show learning in a different context, such as a different background color illumination (Liu *et al.*, 1999). Then, in a series of publications from the Guo laboratory, the mushroom bodies were also found to be required for resolving contradictory visual cues (Tang and Guo, 2001; Zhang *et al.*, 2007) and establishing salience levels for visual stimuli (Xi *et al.*, 2008). These results confirmed that the mushroom bodies are not just involved in olfactory processing. This had already been suggested by studies finding the mushroom bodies to be involved in motor control. Ablating the mushroom bodies appears to increase locomotor activity, suggesting that these neurons are somehow involved in suppressing motor output (Martin *et al.*, 1998). One question raised at this point is whether the mushroom bodies form part of a

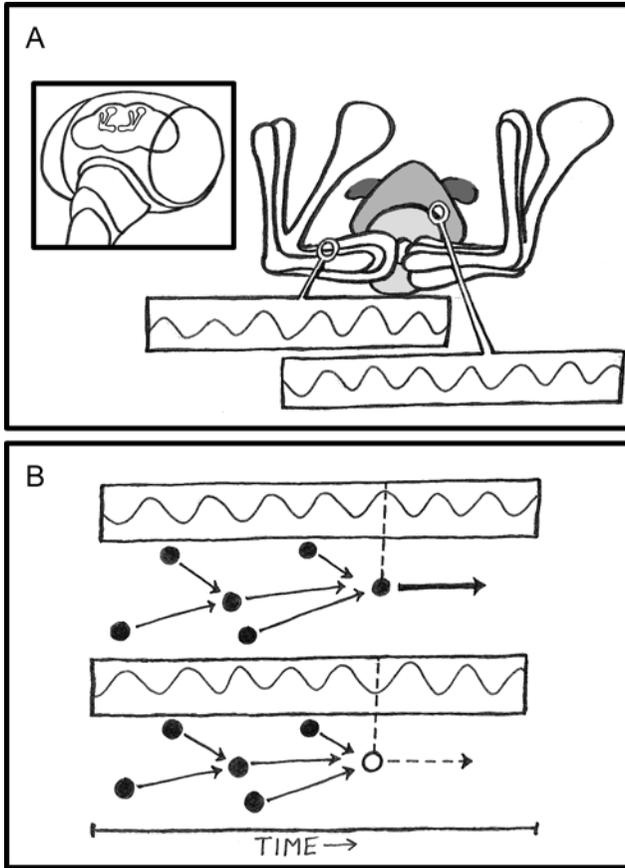


FIG. 7. A model of attention in the *Drosophila* brain. (A) The mushroom bodies (white) have been associated with olfactory memory and contextual visual learning. The central complex (gray), in particular the fan-shaped body (dark gray), has been associated with simple visual learning (Liu *et al.*, 2006). Both structures might be involved, together with neuromodulators such as dopamine, in generating patterns of oscillatory neural activity in the fly brain. Inset, a fly head with brain and mushroom bodies drawn. (B) Stimuli produce consequences (e.g., a behavior or a memory) via a polychronous network of precisely timed neuronal firing events (Izhikevich, 2006). Subthreshold oscillations could enable or suppress these polychronous groups depending on the phase of the background oscillation (black circle: firing neuron; open circle: nonfiring neuron).

broader suppression circuit because context generalization (for example) by definition implies suppression of nonpredictive cues, such as background color. Are these different forms of suppression related?

Recent work by Bjoern Brembs, using the flight arena, addressed this question in an indirect way. As discussed earlier, overtraining in the arena was found to

promote motor learning at the expense of behavioral flexibility (Brembs, 2009). This habit formation paradigm was found to be mushroom body–dependent; without the mushroom bodies, flies behaved as if they were overtrained even with the short training paradigm. This suggested that the mushroom bodies were required to promote behavioral flexibility, thereby allowing flies to display their learning via different motor programs. Without the mushroom bodies, motor learning took over and flies relied on just one skill (e.g., flying only left). Such lost flexibility may be useful in some scenarios, such as for behavioral responses that need not change, but will of course be maladaptive for behaviors where attention is involved. A synthesis with the preceding results involving the mushroom bodies in motor suppression, nonelemental learning, and setting salience thresholds would propose that these neurons are providing a gating mechanism for visual behavior: they suppress motor programs in order to promote a level of behavioral flexibility tied to the outcome of a selective attention contest among competing percepts in the brain. With increased training and habit formation, this circuit is somehow bypassed. The relevance of the mushroom bodies to visual selective attention has since been supported by electrophysiological studies where these structures have been silenced during salience paradigms (van Swinderen *et al.*, 2009).

Dopamine is a neurotransmitter that has been tightly associated with aversive olfactory conditioning in flies, but it now appears that this molecule may play a role in attention as well. Dopamine was originally proposed as a carrier for negative valence cues, such as electric shocks, whereas another neurotransmitter, octopamine, signaled rewarding cues such as sugar (Schwaerzel *et al.*, 2003). Dense dopaminergic innervations of the mushroom bodies supported this role in olfactory learning and memory, and convincing models have been proposed whereby coincident dopamine and olfactory input changes mushroom body neuron physiology, and that this memory trace biases subsequent odor presentations to guide the behavioral choices made by flies (Keene and Waddell, 2007). Nevertheless, dopamine has been found to affect several behaviors in addition to olfactory learning. Broadly, dopamine modulates general arousal levels: decreased dopamine promotes sleep, whereas increased dopamine promotes locomotion (Andretic *et al.*, 2005). Specifically, dopamine seems to regulate arousal thresholds for a variety of stimuli, from mechanical shocks to visual flashes or odors. Compromising dopamine signaling acutely, either by increasing or decreasing it, seems to have a common consequence: arousal thresholds are decreased (see (van Swinderen and Andretic, 2011) for a review of dopaminergic effects on fly behavior). As selective attention is also concerned with dynamic setting of arousal thresholds (to competing stimuli), it is possible that there exists a connection between dopamine's role in arousal and a potential role in attention.

The possible overlap between systems affecting olfactory learning and visual learning and attention extends beyond the mushroom body neurons and dopamine. Some olfactory learning and memory mutants are also visual learning and

attention mutants. This was first discovered by testing classical mutants such as *dunce*¹, *rutabaga*²⁰⁸⁰, and *amnesiac*¹ in Pavlovian conditioning paradigms associating colors with shaking in walking flies (Folkers, 1982). The suspicion that some of these mutations might be multimodal was confirmed by classical conditioning in the flight arena, for mutants that were capable of maintaining sustained flight (Gong *et al.*, 1998). Visual learning and memory deficits in *dunce*¹ and *rutabaga*²⁰⁸⁰ raised the possibility that these mutations were affecting more general aspects of memory formation that impact both sensory modalities. Although both of these genes, which are involved in cyclic AMP signaling in neurons (Davis *et al.*, 1995), are strongly expressed in the mushroom bodies (Nighorn *et al.*, 1991), they are also expressed in other structures, such as in neurons of the central complex—set of structures positioned between the mushroom bodies (Fig. 7A). The role of the central complex in simple visual learning was confirmed through genetic rescue of the *rutabaga*²⁰⁸⁰ visual learning defect (Liu *et al.*, 2006). Expression of a wild-type copy of *rutabaga* in the fan-shaped body of the central complex restored simple visual learning (associating a shape with heat), and finer-grained rescue experiments revealed that different layers of the fan-shaped body could rescue distinct learning of distinct visual features. Subsequent *rutabaga* rescue experiments demonstrated that the ellipsoid body, another central complex structure, is also involved in simple visual learning, and that an interaction between the fan-shaped body and the ellipsoid body is likely in the control of visual learning in flies (Pan *et al.*, 2009). These two structures show up again as necessary for visual performance in Buridan’s paradigm (Fig. 6), and the ellipsoid body specifically was also found to be crucial for spatial learning in walking flies (Neuser *et al.*, 2008).

There thus appears to be a separation between olfactory learning in the mushroom bodies and visual learning in the central complex. The fact that complex visual learning requires the mushroom bodies complicates this picture. One possibility is that the mushroom bodies are required for attention-like processes, as suggested earlier. Why would an “olfactory” neuropil be recruited for visual attention and nonelemental visual learning? One radical possibility is that a primary function for these neurons is stimulus gating, attention, and context generalization, but that simple odor learning assays were not designed to test for this, thereby yielding learning mutants that were really attention mutants. More likely is the possibility that the mushroom bodies are multimodal processing centers. Indeed, olfaction in insects may have predated mushroom body evolution (Strausfeld *et al.*, 2009). Although the mushroom bodies are clearly involved with olfactory discrimination and memory in flies, the neuronal architecture and connectivity of these structures may have been exploited for brain-wide attention processes as well. For example, olfactory input to the mushroom bodies has been shown in locusts to generate a 20–30 Hz oscillation, which can then be used as a timer of sorts to gate the occurrence of spiking activity in these neurons (Laurent, 2002). The 20–30 Hz oscillation is propagated by successive feed-forward and

feed-back systems to target neurons beyond the mushroom bodies (Cassenaer and Laurent, 2007), thus setting up an oscillation in the brain that could potentially be used to gate other modalities in addition to olfaction. The fact that such a brain-wide currency (e.g., a 20–30 Hz oscillation) was generated through olfactory input may be irrelevant; in other creatures such an oscillation may be generated through other modalities. Thus, one hypothesis for the relevance of the mushroom bodies (and perhaps dopamine) for visual attention is that these neurons are a necessary component for generating a brain-wide oscillation that gets used for dynamically selecting and suppressing stimuli from the environment (Fig. 7B). In support of a multimodal role for these neurons traditionally associated with olfaction, transiently silencing mushroom body neurons attenuates the 20–30 Hz response in the fly brain associated with visual salience (van Swinderen *et al.*, 2009), as does transiently silencing dopamine (Andreatic *et al.*, 2005).

The possibility of screening for visual attention mutants was introduced with the optomotor maze (Fig. 5), discussed earlier. It was suggested that attention-defective mutants might display *increased* visual responsiveness in the maze because they might be unable to suppress an optomotor reflex. As discussed above, silencing either the mushroom bodies or dopaminergic neurons increased visual responsiveness in this paradigm. A similar effect was seen in a screen of existing *Drosophila* mutants, with *dunce*¹ and *rutabaga*²⁰⁸⁰ displaying stronger responsiveness to a moving grating than any other mutant tested (van Swinderen, 2007a). Predictably, these learning mutants were also unresponsive to stationary distracters. Brain recordings showed that LFP correlates of visual attention were also compromised in these mutants: there was no detectable 20–30 Hz selection/suppression effect following novelty salience (van Swinderen, 2007a). *Dunce*¹ visual defects could be rescued by expressing the wild-type protein in mushroom body and central complex neurons (van Swinderen *et al.*, 2009), and it appears that *dunce* expression is required during the pupal stage of development; rescue only at the larval stage or only at the adult stage was not possible (van Swinderen, 2007a; van Swinderen *et al.*, 2009). This suggests that the *dunce* gene (phosphodiesterase II) is required during development to “wire” a circuit that is subsequently necessary for attention-like behavior in adult flies. This result contrasts with the role of these molecules in olfactory learning, which can be restricted to expression in adult neurons (McGuire *et al.*, 2003). Consequently, the role of *dunce* or *rutabaga* in attention processes is probably not direct: many molecules can disrupt wiring during development, and one prediction would be that many of these might produce attention-like defects (and consequently increased visual responsiveness in adult flies), for different reasons.

The connection between visual attention and memory mutants is further exemplified by the *radish*¹ mutation. *radish*¹, originally isolated as a mutant for anesthesia resistant memory (ARM) (Folkers *et al.*, 1993), is the sole member of a memory consolidation pathway that is proposed to be parallel to the *dunce* or *rutabaga* pathway (Isabel *et al.*, 2004). In olfactory memory assays, *radish*¹ flies are not able

to remember after they have been briefly anesthetized (by cooling), whereas wild-type flies are able to carry a learned response through this transient manipulation. The *radish* gene, with no close homology to human genes (Folkers *et al.*, 2006), is expressed strongly in the mushroom bodies, as are *dunce* and *rutabaga*. The connection between *radish*¹ and visual attention was uncovered using the same optomotor screening device that showed increased responsiveness in *dunce*¹ and *rutabaga*²⁰⁸⁰, except that *radish*¹ was found to be less responsive to moving gratings and more distractible than wild (van Swinderen and Brembs, 2010). Brain recordings and behavioral experiments in the flight arena confirmed that *radish*¹ is also an attention mutant, but different than *dunce*¹: *radish*¹ flies displayed a 1–2 Hz behavioral “jitter” when presented with competing visuals in the arena. Furthermore, 20–30 Hz responsiveness to novelty (in brain recordings) lasted only one exposure, compared to 3 or 4 in wild type (van Swinderen and Brembs, 2010). In other words, *radish*¹ was found to have a short attention span, possibly caused by an inherent 1–2 Hz oscillation that may periodically reset visual perception in the animal.

It is by now clear that learning and memory mutants originally isolated in olfactory paradigms are afflicted with other behavioral or brain defects when one looks more closely. Indeed, an optomotor screen of a panel of long-term memory mutants revealed yet another batch of candidates for visual attention studies (van Swinderen *et al.*, 2009). One open-ended question at this point is whether these attention-like effects cause the memory defects in any way. It is likely that attention and memory are intertwined and mutually causal: attention promotes memory formation, but memory also guides attention (Chun and Turk-Browne, 2007). Attention deficits are probably an important factor in eventual defective memory formation, consolidation, or retrieval. It is perhaps not so surprising that the same genes and neurons that have been central to understanding (olfactory) memory formation are being found to be relevant for attention. However, there is an inherent bias in this position: all of the visual learning or attention mutants were found by testing existing olfactory learning and memory mutants, many of which were uncovered in forward-genetic screens. To be truly convincing, visual attention and visual learning screens need to uncover their own mutants from scratch, as was done for olfactory paradigms. Fortunately, efficient visual paradigms now exist to do this (Evans *et al.*, 2011).

VI. Conclusion and Future Directions

Attention has traditionally been compared to a “spotlight” or a “zoom lens” (Eriksen and St James, 1986; LaBerge, 1983). To some extent, this is what attention feels like for us, especially as we consciously focus on a visual target. This

anthropocentric view may have distracted us from a broader definition of attention that may apply more readily to other animals. Attention is also concerned with suppressing incoming sensory information from multiple modalities, and this capacity may have evolved very early in animal evolution to provide experience-dependent flexibility in motile creatures. Rather than being simple stimulus-driven systems, animals became able to ignore the barrage of sensory information around them, to only process immediately relevant stimuli, and thus to learn. The emergence of experience-dependent stimulus-suppression mechanisms may have coincided with evolution of simple brains as well as with sleep—another stimulus-suppression mechanism. Whether stimulus selection and suppression involve different mechanisms is unclear, although some combined form of local gain control coupled to suppression of competing stimuli is likely in mammals (Moran and Desimone, 1985). The last 30 years of visual studies in *Drosophila* have provided good evidence for attention in insects. Future research will determine whether fly brains attend the same way we do.

There are two broad approaches to studying attention-like processes in *Drosophila*. One is to devise very sophisticated behavioral or brain recording devices to detect the difficult measures relevant to attention: stimulus selection/suppression, context, cueing, distraction. This has been the traditional approach in the field, namely to treat the fly as much as possible like a “mini-human.” This approach has clearly been successful and has been perhaps the best way to provide to level of confidence among the scientific community at large that simple animals such as flies may have a selective attention. Unfortunately, because the methods used to investigate attention in flies convincingly can be so difficult, they are less amenable to exploiting *Drosophila* in the way that it should: by high-throughput behavioral screens coupled to molecular genetics. It is by homing in on key behavioral measures that mysteries such as circadian rhythms or learning and memory were unraveled in the *Drosophila* model (Vosshall, 2007). To expect to do the same for attention using the sophisticated devices described in the first two sections of this review is perhaps unrealistic. Instead, it may be more productive to identify key measures that reflect attention-like processes in the fly. One of these is clearly the suppression of reflexes, and efficient screens can be designed around this problem, only then followed by the more sophisticated approaches in select strains of interest. This solution is comparable with the approach taken by *Drosophila* sleep researchers. To study sleep requires satisfying multiple criteria, from measuring arousal thresholds to homeostasis or brain recordings or conducting some pharmacology (Shaw and Franken, 2003). However, the *Drosophila* sleep community convinced themselves that one measure—5 min of continued quiescence (Shaw *et al.*, 2000)—was sufficient to proceed with sleep screens, before going back to check the other criteria in strains of interest. This insight has been crucial to moving the sleep field forward, even beyond *Drosophila*. Such a strategy for visual attention in *Drosophila* is likely to produce a similar outcome.

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