Object directed behavior of walking blowflies

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Erklärung

Ich versichere, dass ich diese Arbeit selbständig und ohne unzulässige Hilfe verfasst habe, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und Zitate kenntlich gemacht habe.

José Monteagudo

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General summary

The small size of flies, and thus of their nervous systems, makes them highly interesting for those trying to find out how a small autonomous system could deal with the challenges of vision-based behavior. Despite being mostly known for their acrobatic flight, as well as being a nuisance, flies also regularly land and explore their environments by walking, often selecting an object in their surroundings and approaching it. However, compared to flight, performing such a task while walking poses certain challenges.

In contrast to flying flies, walking ones are bound to a surface, and if said surface is not horizontal the walking apparatus and the head attempt to compensate for this to some degree. Flies can use multiple cues, such as gravity, the orientation of the horizon, or surrounding visual cues, to gain information about their orientation relative to the surroundings and try to orient their gaze accordingly. However, how the animal reacts when these cues convey contradictory information is still largely unknown.

One of the most important cues used by insects to obtain spatial information is the shift of retinal images resulting from its own motion, known as optic flow. The optic flow produced during translation can be used to both estimate distance and detect camouflaged objects through their motion relative to the background since closer objects will move faster thus reflecting spatial information. The optic flow produced by rotations, however, does not contain spatial information because all objects move equally fast. Flying flies account for this by splitting their movements into quick turns and segments where the gaze is kept straight, which is known as saccadic flight strategy. While walking the walking apparatus of flies imposes rotations and translations of the body tied to the stride cycle of the animal, which remain largely uncompensated by head motion. As a consequence, it is currently unclear if walking blowflies can make use of optic flow to obtain spatial information about their environment like flying flies do, and it is similarly unknown if they shape their walking behavior to make easier use of optic flow like they do during flight.

In addition to these constraints imposed by the walking mode of locomotion, there is another important question that has been largely unanswered so far: how do walking blowflies choose a goal when multiple objects are available? Some work has been done to address which features of an object may result in an animal approaching a particular object more often. Two types of mechanisms have been proposed to explain how flies select one potential goal over another: 1) that the animal "sums up" the turning response induced by all present objects, resulting in a net turn towards one of them and 2) that one of the objects is ignored for at least some time while the other

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object induces a turning response leading to its fixation. Thus, how goal selection takes place has been an unsolved issue until now.

In my first project, I ask how animals integrate multiple and potentially contradictory cues about their body and head orientation in space. I use high-speed video recordings to analyze the head and body orientation of flies approaching an object while both the orientation of the ground as well as visual cues that might be used by the animal to assess its orientation in space, such as surrounding visual features or the orientation of its goal, were manipulated in a dedicated way. I show that the orientation of the blowfly's head, and thus its gaze, is mostly determined by the orientation of the walking surface, though both gravity and visual cues also contribute. On the other hand, the orientation of the body is mostly bound to the ground orientation and largely independent of visual cues. Modeling the orientation of the animal's head and body as the weighted sum of the orientations suggested by different environmental cues results in fairly accurate predictions of the animal's head and body orientations and furthermore suggests that when less visual cues are available the animal gives more weight to gravity when determining its gaze orientation.

In my second project, I address whether walking blowflies make use of optic flow to estimate distance and detect camouflaged objects as well as potentially shape their behavior in order to be able to obtain spatial information from optic flow despite stride-coupled oscillations. I recorded flies as they walked while surrounded by multiple objects, which could differ in their distance and be camouflaged to address to what degree optic flow was employed while walking. Blowflies walked between objects offering a clear contrast against the background without any preference based on the distance of the objects but made use of optic flow to detect and approach camouflaged objects. An analysis of the walking style revealed that blowflies do not change the way they walk when approaching camouflaged objects compared to conspicuous objects, suggesting that walking blowflies are able to make use of optic flow to detect camouflaged objects despite stride coupled oscillations.

In my third project, I ask which factors and mechanisms determine the object that is selected when choosing between two potential goals and which mechanisms may underlay this choice. I allowed walking blowflies to choose between two visually identical objects that come into view at different azimuthal positions to investigate how blowflies chose between the available objects. Blowflies preferentially approach initially frontal objects over lateral ones and respond to objects by turning towards them with different turning speeds depending on their azimuthal positions. A modeling

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approach reveals that, in order to account for the behavior observed in walking blowflies, it is necessary to incorporate an attention mechanism that allows the animal to ignore one of the potential goals for at least some time. In addition, I could show that a simple model of attention processes is able to emergently generate multiple features of walk behavior.

General Introduction

1. Flies as neuroethological models for vision based behavior

Insects are usually small. Consequently, their brains are small. While the human brain contains around 86 billion neurons (Azevedo et al. 2009), the brain of a fruit fly, for instance, is made of only around 100.000 (Simpson 2009). Despite this much smaller number of nerve cells insects are able to act autonomously, reacting to visual stimuli often even faster than we humans are capable of, as anyone trying to swat a flying fly can attest. This combination of a small neuron number with quick and efficient interactions with the environment suggest that the computations underlying the behavior are very efficient, making flies and other insects interesting for those aiming to develop autonomous robots. For this reason, insects have long been important model organisms for the study of vision-based behavior and its underlying mechanisms: they are literally living examples of how a small and efficient autonomous system solves a particular task or addresses a specific problem.

Flies, in particular, have been heavily employed to address how vision can be used to control behavior (Egelhaaf and Kern 2002, Nordström and Carrol 2009, Borst et al. 2010, Borst 2014, Dyakova and Nordström 2017), and a behavior that has been employed specially often in this research is the tendency of flies to fixate a vertical object (Horn and Wehner 1975). The reason for this is that fixation behavior can be exploited to address a variety of questions: By letting a flying fly fixate a vertical bar and then expanding it, one can simulate an approach to an object and study how the animal decides to avoid or land on the object (Maimon et al. 2008). By presenting multiple objects at different distances and analyzing which are approached one can obtain evidence of whether the animal uses distance estimation information for object fixation (Schuster et al. 2002). By presenting an object with the same texture as the background, but moving at a different speed relative to it, one can show that flies are able to detect an object through relative motion information (Reichardt et al 1983, Egelhaaf 1985). This behavior can also be exploited to analyze specific mechanisms for example showing that flies are able to track objects both by detecting the location of contrast edges and by tracking the objects motion (Virsik and Reichardt 1976, Bahl et al 2013, Aptekar et al 2012).

Being animals known mostly for their flight behavior most research has been performed on flying flies. However, flies will also land and explore their environment by walking. Walking flies, just like flying flies, tend to fixate objects, which they eventually approach (Bülthoff et al 1982, Schuster et al. 2002). However, compared to flight, walking behavior imposes several specific

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constraints that can affect visual behavior. Any autonomous walking system could easily be affected by the very same constraints.

2. Constraints and limitations of walking behavior

When addressing vision-based behaviors there are several features that can affect the way visual input is received. In this section I will elaborate on two of these features that will be addressed in this dissertation.

2.1. The constraints of surface attachment

Most animals try to keep their gaze horizontal. Clear attempts to compensate deviations of the gaze from a horizontal orientation are observed in humans (Guitton et al 1986), rabbits (Baarsma and Collewijn 1974), guinea-pigs (Escudero et al. 1993), chinchillas (Merwin et al. 1989), frogs, (Dieringer and Precht 1982), lizards (Gioanni et al 1993), and birds (Wallman and Letelier 1993). Flies are no different and, during flight, strong reflexes stabilize their heads very robustly. Being animals with immobile eyes, this also stabilizes their gaze (Hateren and Schilstra 1999) in a horizontal orientation. However, during flight a fly is suspended in air and thus its body orientation is not constrained by its surroundings as it is when walking, because the fly is in the latter case physically attached to a surface. The orientation of the walking surface thus determines the animal's gaze orientation, unless head and leg movements alter it. Thus, walking blowflies attempt to compensate for orientations of the surface deviating from the horizontal orientation, such as irregularities in the terrain, mostly by adjusting their walking apparatus but also through head movements in an attempt to keep the head horizontal (Kress and Egelhaaf 2012). In a similar vein, flies walking on tilted surfaces try to compensate for the tilt of the surface (Horn and Lang 1978) using gravity as a cue. There are multiple other cues that could also contribute to the head orientation, having been shown to play a role during flight (Hengstenberg 1993), such as the orientation of edges in the animal's surroundings or the location of a light source. However, it has still not been clarified what role those cues play while walking.

Further complicating this issue is the fact that the multiple cues available to the animal to align its gaze could indicate contradictory orientations of their environment. For example, let us imagine a fly walking on a wall, a fairly common occurrence. It has been suggested that flies try to align their head to the contrast edge between the surface they stand on and the usually brighter area above it (Horn and Knapp 1984). In this scenario, this cue would be aligned with the wall and thus perpendicularly opposed to gravity. The way these potential cues interact to control gaze orientation

as a walking fly approaches an object has been largely unknown. This problem could similarly affect to some degree any autonomous walking system implemented as a hexapod walking robot, such as the HECTOR walking robot (Meyer et al. 2016), when walking on tilted surfaces.

2.2. The issue of stride cycle coupled movements

The walking mode of locomotion involves for most legged animals the alternating coordinated motion of its legs. In the case of flies walking involves holding the front and hind legs of one side of the animal and the mid leg of the other side on the ground, forming a tripod, and moving the remaining legs. When those legs touch the ground, forming a new tripod, the other three legs are moved. This way an alternating tripod is produced (Strauss and Heisenberg 1990). This cycle of leg motions, known as stride cycle, produces the movement of a walking fly. Its walking speed oscillates according to the stride cycle. But these motions not only produce forward motion of the fly's body. Even during straight walks the stride cycle produces side-wise movements and rotations around the vertical axis of the animal (Kress and Egelhaaf 2012), whose speed also oscillates with the stride cycle.

The reason these translational movements and rotations are relevant for vision-based behavior relates to the optic flow that is induced on the eyes during locomotion. The optic flow is the shift of retinal images projected onto the eyes of an animal while it moves. Optic flow is a very important visual cue because during translational movements far objects appear to move more slowly than closer objects. Hence, the translational optic flow contains spatial information, which can be used, for example, to estimate distances (David 1979, Kern et al. 2012, van Breugel et al. 2014) or to detect otherwise hidden objects through their motion relative to the background (Reichardt et al 1983, Egelhaaf 1985, Dittmar et al. 2010). This is not the case with optic flow produced by rotations, because during a rotation all objects move at the same speed irrespective of distance (Koenderink 1986). Since the stride cycle introduces both rotations and translations of the body of the animal, and flies do not compensate for the rotations of the body by head movements (Kress and Egelhaaf 2014), it may be a problem for the animal to obtain spatial information from optic flow.

Having to deal with rotational optic flow is not a problem exclusive to walking flies. Flying flies address it by shaping their behavior: they structure their flight in sharp turns (saccades) and straight segments (intersaccades) (Schilstra and van Hateren 1999, Egelhaaf et al 2009, Kern et al. 2012), thus restricting rotational optic flow to brief segments and thus facilitating the use of optic flow for distance estimation (Lindemann et al. 2008). Could walking blowflies also shape their behavior to

limit the amount of rotational optic flow experienced and thus make it easier to obtain spatial information from visual cues? That question remains unanswered.

Addressing these issues could be of great interest in the development of distance estimation modules for autonomous walking platforms (Bertrand et al. 2015), such as the HECTOR walking robot (Meyer et al. 2016)

3. The process of decision and choice

In addition to orienting its gaze and detecting objects a walking fly about to approach an object has to perform a task that may easily be overlooked as obvious: it has to turn towards its goal. This may seem simple, but should multiple objects be present the fly has to somehow orient itself to one of them, which inevitably involves not fixating the other object. How does this decision come about? There are certain features of objects that make them more attractive to flies such as color (Fukushi 1989) or the dimensions of the object (Wehner 1972), but flies also have to choose when objects are visually identical. Research using virtual reality settings has suggested that in such cases walking fruitflies are more likely to approach the more frontal of the objects (Mronz 2004). This however still leaves open the question of what mechanisms underlie this choice. Investigating fixation behavior has suggested that, when faced with two objects, flies simply respond to both of them, with one of them eliciting a stronger response which eventually leads to a fixation (Reichardt and Poggio 1976). On the other hand, it has also been revealed in different paradigms that a fly confronted with multiple visual stimuli will react to one of them while ignoring the other (Wolf and Heisenberg 1980). Thus, it has still been not been clarified what mechanisms or strategies blowflies employ to choose between two available goals. A simple mechanism to perform this choice would be of great interest for the development of autonomous systems.

4. Thesis outline

In the first chapter of the thesis I ask how walking blowflies integrate multiple cues to determine gaze orientation while approaching an object. I analyze the head and body orientation of blowflies as they approach a bar while the different cues were in conflict with each other. I reveal that the orientation of the surface is a major determinant of gaze orientation, but that visual and gravitation cues also play an important role. I show that the integration of cues to control both head and body orientation can be modeled with reasonable accuracy by a weighted sum of the orientation suggested by the different cues. The weights involved are shown to be adaptable depending on the

available cues such that animal will give more weight to gravity when in presence of less visual cues.

In the second chapter I record blowflies as they approach objects that differ in their distance, and thus in the optic flow generated. Optic flow information is not only required when it comes to estimating the distance, but also to detect an object that is camouflaged by the same texture as its background. Therefore, I tested both black and white objects that differed from the background by their brightness and contrast as well as camouflaged objects. I show that walking blowflies are able to detect camouflaged objects through the use of motion cues but that they do not show a preference for objects based on their distance alone. I also analyze and compare the walking style of flies as they approach conspicuous and camouflaged objects and show that the behavior of flies remains largely constant, suggesting that walking blowflies can use motion cues despite the rotations imposed by the stride cycle.

In the third chapter I allowed flies to freely choose and approach objects which differed only in the azimuth position at which they were seen first. I find that approach probability is tied to the azimuth position at which an object is first seen. A model based on my behavioral experiments then reveals that in order to account for the flies' behavior it is necessary to model an attention process. In addition, I show that a relatively simple mechanism of modeling attention is able to generate multiple features of the fly behavior emergently.

References

Aptekar, J. W., Shoemaker, P. A., & Frye, M. A. (2012). Figure tracking by flies is supported by parallel visual streams. *Current biology*, *22*(6), 482-487.

Azevedo, F. A., Carvalho, L. R., Grinberg, L. T., Farfel, J. M., Ferretti, R. E., Leite, R. E., ... & Herculano-Houzel, S. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *Journal of Comparative Neurology*, *513*(5), 532-541.

Baarsma, E. A., & Collewijn, H. (1974). Vestibulo-ocular and optokinetic reactions to rotation and their interaction in the rabbit. *The Journal of physiology*, *238*(3), 603-625.

Bahl, A., Ammer, G., Schilling, T., & Borst, A. (2013). Object tracking in motion-blind flies. *Nature neuroscience*, *16*(6), 730.

Bertrand, O. J., Lindemann, J. P., & Egelhaaf, M. (2015). A bio-inspired collision avoidance model based on spatial information derived from motion detectors leads to common routes. *PLoS computational biology*, *11*(11), e1004339.

De Bivort, B. L., & Van Swinderen, B. (2016). Evidence for selective attention in the insect brain. *Current opinion in insect science*, *15*, 9-15.

Borst, A., Haag, J., & Reiff, D. F. (2010). Fly motion vision. *Annual review of neuroscience*, *33*, 49-70.

Borst, A. (2014). Fly visual course control: behaviour, algorithms and circuits. *Nature Reviews Neuroscience*, *15*(9), 590-599.

Van Breugel, F., Morgansen, K., & Dickinson, M. H. (2014). Monocular distance estimation from optic flow during active landing maneuvers. *Bioinspiration & biomimetics*, *9*(2), 025002.

Bülthoff, H., Götz, K. G., & Herre, M. (1982). Recurrent inversion of visual orientation in the walking fly, Drosophila melanogaster. *Journal of comparative physiology*, *148*(4), 471-481.

David, C. T. (1979). Optomotor control of speed and height by free-flying Drosophila. *Journal of Experimental Biology*, *82*(1), 389-392.

Dittmar, L., Stürzl, W., Baird, E., Boeddeker, N., & Egelhaaf, M. (2010). Goal seeking in honeybees: matching of optic flow snapshots?. *Journal of Experimental Biology*, *213*(17), 2913-2923.

Dieringer, N., & Precht, W. (1982). Compensatory head and eye movements in the frog and their contribution to stabilization of gaze. *Experimental brain research*, *47*(3), 394-406.

Dyakova, O., & Nordström, K. (2017). Image statistics and their processing in insect vision. *Current opinion in insect science*, *24*, 7-14.

Egelhaaf, M. (1985). On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. *Biological Cybernetics*, *52*(2), 123-140.

Egelhaaf, M., & Kern, R. (2002). Vision in flying insects. *Current opinion in neurobiology*, *12*(6), 699-706.

Egelhaaf, M., Kern, R., Lindemann, J. P., Braun, E., & Geurten, B. (2009). Active vision in blowflies: strategies and mechanisms of spatial orientation. In *Flying Insects and Robots* (pp. 51-61). Springer, Berlin, Heidelberg.

Escudero, M., De Waele, C., Vibert, N., Berthoz, A., & Vidal, P. P. (1993). Saccadic eye movements and the horizontal vestibulo-ocular and vestibulo-collic reflexes in the intact guinea-pig. *Experimental brain research*, *97*(2), 254-262.

Fukushi, T. (1989). Learning and discrimination of coloured papers in the walking blowfly, Lucilia cuprina. *Journal of Comparative Physiology A*, *166*(1), 57-64.

Gioanni, H., Bennis, M., & Sansonetti, A. (1993). Visual and vestibular reflexes that stabilize gaze in the chameleon. *Visual neuroscience*, *10*(5), 947-956.

Guitton, D., Kearney, R. E., Wereley, N., & Peterson, B. W. (1986). Visual, vestibular and voluntary contributions to human head stabilization. *Experimental brain research*, *64*(1), 59-69.

Hateren, J. H. V., & Schilstra, C. (1999). Blowfly flight and optic flow. II. Head movements during flight. *Journal of Experimental Biology*, *202*(11), 1491-1500.

Hengstenberg, R. (1993). Multisensory control in insect oculomotor systems. *Visual Motion and its Role in the Stabilization of Gaze*, *5*, 285-298.

Horn, E., & Lang, H. G. (1978). Positional head reflexes and the role of the prosternal organ in the walking fly, Calliphora erythrocephala. *Journal of comparative physiology*, *126*(2), 137-146.

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Horn, E., & Wehner, R. (1975). The mechanism of visual pattern fixation in the walking fly, Drosophila melanogaster. *Journal of comparative physiology*, *101*(1), 39-56.

Horn, E., & Knapp, A. (1984). On the invariance of visual stimulus efficacy with respect to variable spatial positions. *Journal of Comparative Physiology A*, *154*(4), 555-567.

Kern, R., Boeddeker, N., Dittmar, L., & Egelhaaf, M. (2012). Blowfly flight characteristics are shaped by environmental features and controlled by optic flow information. *Journal of Experimental Biology*, *215*(14), 2501-2514.

Koenderink, J. J. (1986). Optic flow. *Vision research*, *26*(1), 161-179.

Kral, K., & Poteser, M. (1997). Motion parallax as a source of distance information in locusts and mantids. *Journal of insect behavior*, *10*(1), 145-163.

Kress, D., & Egelhaaf, M. (2012). Head and body stabilization in blowflies walking on differently structured substrates. *Journal of Experimental Biology*, *215*(9), 1523-1532.

Kress, D., & Egelhaaf, M. (2014). Gaze characteristics of freely walking blowflies Calliphora vicina in a goal-directed task. *Journal of Experimental Biology*, *217*(18), 3209-3220.

Lindemann, J. P., Weiss, H., Möller, R., & Egelhaaf, M. (2008). Saccadic flight strategy facilitates collision avoidance: closed-loop performance of a cyberfly. *Biological cybernetics*, *98*(3), 213

Maimon, G., Straw, A. D., & Dickinson, M. H. (2008). A simple vision-based algorithm for decision making in flying Drosophila. *Current Biology*, *18*(6), 464-470.

Merwin, W. H., Wawll, C., & Tomko, D. L. (1989). The chinchilla's vestibulo-ocular reflex. *Acta oto-laryngologica*, *108*(3-4), 161-167.

Meyer, H. G., Bertrand, O. J., Paskarbeit, J., Lindemann, J. P., Schneider, A., & Egelhaaf, M. (2016, July). A bio-inspired model for visual collision avoidance on a hexapod walking robot. In *Conference on Biomimetic and Biohybrid Systems* (pp. 167-178). Springer, Cham.

Mronz, M. (2004). Die visuell motivierte Objektwahl laufender Taufliegen (Drosophila melanogaster)-Verhaltensphysiologie, Modellbildung und Implementierung in einem Roboter.

Nordström, K., & O'Carroll, D. C. (2009). Feature detection and the hypercomplex property in insects. *Trends in neurosciences*, *32*(7), 383-391.

Osorio, D., Srinivasan, M. V., & Pinter, R. B. (1990). What causes edge fixation in walking flies?. *Journal of Experimental Biology*, *149*(1), 281-292.

Paulk, A. C., Stacey, J. A., Pearson, T. W., Taylor, G. J., Moore, R. J., Srinivasan, M. V., & Van Swinderen, B. (2014). Selective attention in the honeybee optic lobes precedes behavioral choices. *Proceedings of the National Academy of Sciences*, *111*(13), 5006-5011.

Reichardt, W., & Poggio, T. (1975). A theory of the pattern induced flight orientation of the fly Musca domestica II. *Biological cybernetics*, *18*(2), 69-80.

Reichardt, W., & Poggio, T. (1976). Visual control of orientation behaviour in the fly: Part I. A quantitative analysis. *Quarterly reviews of biophysics*, *9*(3), 311-375.

Reichardt, W., Poggio, T., & Hausen, K. (1983). Figure-ground discrimination by relative movement in the visual system of the fly. *Biological Cybernetics*, *46*(1), 1-30.

Schilstra, C., & Hateren, J. H. V. (1999). Blowfly flight and optic flow. I. Thorax kinematics and flight dynamics. *Journal of experimental biology*, *202*(11), 1481-1490.

Schuster, S., Strauss, R., & Götz, K. G. (2002). Virtual-reality techniques resolve the visual cues used by fruit flies to evaluate object distances. *Current Biology*, *12*(18), 1591-1594.

Simpson, J. H. (2009). Mapping and manipulating neural circuits in the fly brain. *Advances in genetics*, *65*, 79-143.

Strauss, R., & Heisenberg, M. (1990). Coordination of legs during straight walking and turning in Drosophila melanogaster. *Journal of comparative physiology. A, Sensory, neural, and behavioral physiology*, *167*(3), 403-412.

Varju, D. (1975). Stationary and dynamic responses during visual edge fixation by walking insects. *Nature*, *255*(5506), 330.

Viollet, S., & Zeil, J. (2013). Feed-forward and visual feedback control of head roll orientation in wasps (Polistes humilis, Vespidae, Hymenoptera). *Journal of Experimental Biology*, *216*(7), 1280-1291.

Virsik, R. P., & Reichardt, W. (1976). Detection and tracking of moving objects by the fly Musca domestica. *Biological Cybernetics*, *23*(2), 83-98.

Wallman, J., & Letelier, J. C. (1993). Eye movements, head movements, and gaze stabilization in birds.

Wehner, R. (1972). Spontaneous pattern preferences of Drosophila melanogaster to black areas in various parts of the visual field. *Journal of insect physiology*, *18*(8), 1531-1543.

Wolf, R., & Heisenberg, M. (1980). On the fine structure of yaw torque in visual flight orientation of Drosophila melanogaster. *Journal of comparative physiology*, *140*(1), 69-80.

Head orientation of walking blowflies is controlled by visual and mechanical cues

Abstract

During locomotion animals employ visual and mechanical cues in order to establish the orientation of their head, which reflects the orientation of the visual coordinate system. However, in certain situations, contradictory cues may suggest different orientations relative to the environment. We recorded blowflies walking on a horizontal or tilted surface surrounded by visual cues suggesting a variety of orientations. We found that the different orientations relative to gravity of visual cues and walking surface were integrated, with the orientation of the surface being the major contributor to head orientation, while visual cues and gravity also play an important role. In contrast, visual cues did not affect body orientation much. Cue integration was modeled as the weighted sum of orientations suggested by the different cues. Our model suggests that in case of lacking visual cues more weight is given to gravity.

Introduction

During locomotion many animals aim to keep their visual systems aligned with an external frame of reference. Insects, having immobile eyes in their head capsule, achieve this by choosing and maintaining an appropriate head orientation. The frame of reference can be defined by a variety of sensory cues. The gravity vector, pointing downwards, offers a clear and constant reference, but visual structures in natural surroundings can also be used to align the visual system, as they contain a preponderance of horizontal and vertical edges (Baddeley and Hancock, 1991; Coppola et al., 1998; Hancock et al., 1992; Keil and Cristóbal, 2000; Schwegmann et al., 2014; Switkes et al., 1978), even if the horizon is not visible (Hansen and Essock, 2004). Moreover, the overall

asymmetry in brightness between the upper and the lower parts of the environment can be used for alignment of the visual system, given that the sky is usually brighter than the ground. This manifests itself in a behavior known as dorsal light response (Hengstenberg, 1993; Meyer and Bullock, 1977), which results in the animal keeping its dorsal towards the light source. In walking animals the orientation of the walking surface, perceived through proprioception, can also act as a reference. Consequently, a variety of cues can be used to establish the frame of reference for an animal and, thus, the orientation of its visual system.

Humans have been shown to use visual, gravitational, and proprioceptive input to control and maintain body posture (Chiba et al., 2016; Day et al., 1993; Massion, 1994; Previc et al.,1993; Straube et al., 1994; Winter, 1995) and compensate for body rotations (Guitton et al., 1986). Fish reach a compromise between gravitational and light position to determine the orientation of their body (von Holst, 1935; Kasumyan, 2004; Watanabe et al., 1989). Insects employ visual cues (Goulard et al., 2015; Horn and Knapp, 1984; Srinivasan, 1977; Viollet and Zeil, 2013) as well as the position of the light source (Hengstenberg, 1993; Mittelstaedt, 1950; Mittelstaedt, 1997; Tomioka and Yamaguchi, 1980) to control body orientation. Gravity is also used, though this has only been shown in crickets (Horn and Bischof, 1983) and walking dipterans (Horn and Lang, 1978; Horn, 1982). How these cues may interact to determine the final head and gaze orientation largely remains an open question.

To the best of our knowledge, all work published until now has considered the visual system to be consistently providing a single cue. In reality, an animal may encounter conditions in which different visual cues may suggest different frames of reference for the visual system. For example, an animal walking on a tilted surface is expected to see a horizon defined by the surface it is standing on. But it may also see plants which, growing in alignment with gravity, generate vertical features that may not be perpendicular to the visual horizon. As a result, two visual frames of reference oppose each other. This is particularly relevant for blowflies, which often land on irregular, tilted or outright vertical surfaces without a clear axis of reference.

In this study, we aim to understand how visual and mechanical cues are integrated to determine the frame of reference for the alignment of the visual system in freely walking blowflies. This is achieved by letting blowflies walk on a tilted surface, while presenting multiple visual cues potentially contradicting both each other and the mechanical cues, i.e. orientation of the gravity vector and of the walking surface. We analyzed the contribution of the different cues to the resulting orientation of the head orientation and, thus, of the visual coordinate system. Furthermore,

we addressed how changes in body orientation contribute to head alignment, an issue which has not been addressed in walking blowflies either.

Methods

Animals and animal preparation

Female blowflies (*Lucilia cuprina,* Wiedemann 1830) bred in our laboratory were prepared 1-3 days after hatching. The animals were briefly anesthetized with CO_2, and a drop of beeswax was placed on the wing joints to prevent flight.

We placed markers on the head and thorax that could be tracked semi-automatically (see Video analysis) to allow the reconstruction of head and body orientation. The markers consisted of dots of white acrylic paint (Revell 36301, REVELL GmbH, Bünde, Germany): two on the head between the ocelli and the antennae, and two on the thorax, approximately at the level of the wing joints. Reflections on the wings and the cuticle which could interfere with the automatic tracking of the markers were prevented by painting the thorax, the wings, and the head area surrounding the proboscis and located directly below the antennae with matt black acrylic paint (Revell 36108) prior to placing the markers. To allow individual identification without disturbing the tracking process a number was painted with matt bronze green acrylic paint (Revell 36165) on the animal. No noticeable changes in the animals´ behavior were observed by these manipulations. The animals were kept in a cage with free access to water and sugar.

Experimental setup

Blowflies were released into a cylindrical PVC arena (diameter of 60 cm) through an entry hole (diameter of 2.5 cm) in the center of the ground (Fig. 1). The ground of the arena was covered with black paper to allow an easy tracking of the markers (see Video analysis). The walls were covered with white paper and could additionally be outfitted with 3 red stripes (4 cm wide, located 8 cm from the ground and at the same distance from one another) either placed horizontally or in a tilted position with an angle of 30°. A vertical bar made of red cardboard (6.5cm x 58 cm) and of the same height as the arena walls was attached to the wall as a target for the blowflies, as walking flies have previously been shown to be attracted by dark vertical bars (Bülthoff et al., 1982; Kress and Egelhaaf, 2014). Both the stripes and the bar provide high-contrast cues, as blowfly photoreceptors are insensitive in the long-wavelength range (Bernard and Stavenga, 1979).
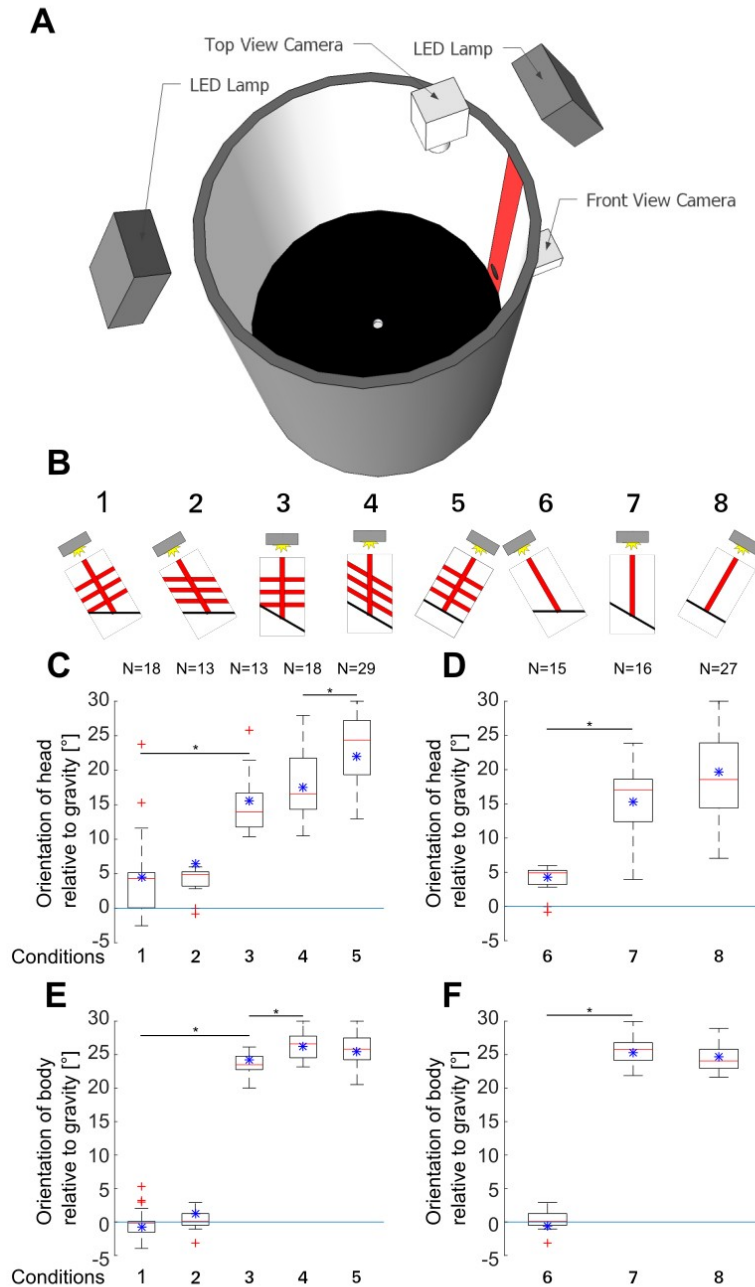
Figure 1. A. The experimental setup. **B.** Sketch of the experimental arena cut at the entrance hole. The black thick line indicates orientation of the ground surface, the rectangle indicates the drum orientation. Diagrams 1 to 8 show the different experimental conditions as encountered by an animal in the different experimental situations when the fly walks towards the target. **C-F.** Orientation of head (C&D) and body (E&F) relative to the horizontal determined by gravity with various cues indicating different frames of reference and orientation predicted by the weighted sum of the suggested orientations. Box and whisker plots indicate median (red line) and first and third quartiles with outliers (red plus). The symbol * indicates significant differences (Wilcoxon rank sum test, p<0.05). Blue asterisk indicates the value predicted by the weighted sum of the cues.

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The animals were recorded by 2 synchronized cameras (Basler ac A2040 – 90um, Basler AG, Ahrensburg, Germany) controlled by a custom program based on the Pylon 4 software suite (Basler, Basler AG, Ahrensburg, Germany). One camera was placed for a top-view of the arena and equipped with a Kowa TV Zoom Lens 12.5-75 mm f/1.8 (Kowa Company Ltd., Nagoya, Aichi, Japan), the other one recorded a frontal view of the fly approaching the bar through a hole (diameter of 5 cm) in the arena wall. The latter was equipped with a Pentax TV Lens C20616TH 6.5 mm f/1.8 (C.R. Kennedy & Co., Port Melbourne, Australia). Recordings were made at 90 frames/s and 4 mega-pixel resolution. The arena was illuminated with white light by 2 lamps (GSVITEC Marathon MultiLED, GS Vitec GmbH, Gelnhausen, Germany), one pointing to the target bar and the other illuminating the opposite side of the arena. The lamps were fixed to the walls of the arena and tilted with it (see below).

The ground of the arena and the cylindrical walls could be tilted independently by up to 30° along the axis connecting the entrance hole and the base of the red bar. By tilting the ground, we manipulated the mechanosensory input to the legs, and by tilting the walls of the arena along the path to the bar, we manipulated the orientation of the visual input which could further be modified by additional bars on the walls. As the animals approach the target along the rotation axis of the ground and of the visual cues, this setup allowed us to estimate the alignment of the roll angle of the body and head, respectively, according to different possible frames of reference.

Experimental procedure

The recording process started when a fly was released into the arena. Once the walking fly had reached the red bar, recorded data was stored for analysis. If the animal did not approach the bar, the recording was rejected and the run restarted. For each animal 5 approaches of the bar were recorded.

To calibrate marker positions every animal was recorded in the arena with both the ground and the walls being horizontal (reference condition). Then the animals were recorded while approaching the target bar under different arena settings (experimental conditions).

Animals were allowed to rest overnight between tests under the reference and the eight different experimental conditions (Fig. 1B): The floor was either horizontal with respect to gravity (1), (2), and (6) or tilted by 30° (3), (4), (5), (7), and (8). It should be noted that the floor provides mechanical cues as well as a visual horizon line. This was combined with different visual conditions: The orientation of the walls, which was always aligned with light direction and target orientation, was either parallel to gravity (3), (4), and (7), or tilted by 30° (1), (2), (5), (6), and (8).

Horizontal stripes on the wall, if present (1)-(5), were either parallel to the floor (2), (4), and (5) or perpendicular to the target orientation (1) and (3).

Video analysis

The video recordings were analyzed frame by frame using the open source software ivTrace (https://opensource.cit-ec.de/projects/ivtools). With this software it was possible to automatically track the markers on head and body using a simple binarization of the brighter spots in the image, in this case the markers. Using custom-written Matlab scripts and Jean-Yves Bouguet's Matlab Camera Calibration Toolbox (https://www.vision.caltech.edu/bouguetj/calib_doc/), we triangulated the 3-dimensional positions of the markers from our two camera views. We then calculated the roll angle of head and body relative to the orientation of the ground for each time point. Only those recordings from which the roll could be reconstructed over the full recording time were used for further analysis. Data from animals contributing less than 3 recordings were discarded.

To compensate for any individual offsets caused by the position of the markers we calculated the average head and body roll for each animal for the reference condition and, while assuming an upright head and body orientation, used the resulting values as calibration values for the experimental conditions. Hence, the reference head and body roll is 0° by definition.

To assess the accuracy of our reconstruction we calculated the distance between the two markers placed on the head and assessed their variability. The mean distance between head markers is 0.8973 mm, with a mean standard deviation of 0.0194 mm over a run, showing that the reconstruction is sufficiently reliable and unlikely to affect the determined orientations in a significant way.

Head and body orientation as a function of cue orientation

To identify the contribution of each cue to the frame of reference for head and body orientation of the flies we developed a simple model by describing the orientation as the weighted sum of the different cues available to the animal.

We use a weighted sum to combine the expected orientations for all cues:

$$O_p = w_g O_g + w_f O_f + w_l O_l + w_s O_s \quad (1),$$

with O_p being the prediction for the resulting orientation, O_g, O_f, O_l and O_s the expected orientations indicated by *g*ravity, the *f*loor, the *l*ights and target bar, and the *s*tripes on the walls, respectively, and w_g, w_f, w_l, and w_s the corresponding normalized weights (i.e. $w_g + w_f + w_l + w_s = 1$).

The optimal weights were estimated by random variation minimizing the mean square difference between the orientation predicted and the median orientation measured under each of our experimental conditions.

For those conditions in which the stripes on the walls were absent we applied the same approach to predict an orientation from the cues available only (i.e. $w_s=0$). We tested the robustness of our model by 20 times randomly selecting two thirds of the data set and recalculating the predicted values. The predicted values were very robust.

Results and Discussion

Different cues are integrated to modulate head orientation

A strong role of gravity on head orientation of walking flies can be detected if all other cues oppose the frame of reference indicated by gravity. In such cases the head deviates slightly from the other cues in favor of gravity (Fig. 1C condition 5, Fig. 1D condition 8). Gravity perception in walking insects has previously been described as a consequence of a proprioceptive mechanism measuring the differential load on the legs (Horn and Lang, 1978; Horn, 1982). Though, with tethered flies walking on an unsupported ball, the experimental paradigm differed from our free walk paradigm, the flies in both setups most likely experienced similar mechanosensory leg stimulation. Indeed, we observed a 5-15° roll towards gravity when the walking surface was tilted by 30°, similar to the one they observed (Fig. 1C condition 5, Fig. 1D condition 8 and Horn and Lang, 1978). Our results show that the reflexes described by Horn and Lang (1978) can also be observed during free walk under the influence of gravity.

The overall direction of the light sources and the orientation of the target bar also have a significant influence on head orientation, as they align the head with both cues (Fig. 1C conditions 4 & 5, p< 0,005). There is only limited research on the role of vertical bars on head orientation (Hengstenberg, 1993), and previous investigations on the role of dorsal light in blowflies have not quantified the head rotation elicited by a light source held at any particular orientation relative to the head (Hengstenberg, 1993; Schuppe and Hengstenberg, 1993). In other insects, such as crickets (Tomioka and Yamaguchi, 1980), where head roll was quantified, a light source rotated by 30° with respect to the dorsal position has been shown to elicit a head reorientation of similar magnitude as observed here, i.e. a roll of around 5° relative to the remaining cues (see Fig. 1D condition 6). Since the possible impact of a target on head orientation is unclear and the effect of a static light source

has been described, but not quantified, we believe that the effect observed on head orientation is - at least partially - caused by the dorsal light response with a possible contribution of the orientation of the target.

The effect of floor orientation differs significantly from that of the target bar and the light sources. When comparing situations in which only the floor or only the bar and light sources oppose the remaining cues, the floor causes a much bigger head roll of around 15° relative to gravity (Fig. 1 C conditions 1 & 3, $p<0.001$). The surface contains two separate cues: the visual horizon, as a contrast edge between the dark ground and the white walls of the arena, and the physical orientation of the ground, which affects head orientation at least by restricting body orientation. The effect of a visual horizon was addressed by Horn and Knapp (1984). His work revealed that a horizon rotated by 30° around the roll axis elicited a head roll of 10°. Thus, we conclude that, in addition to the influence of the visual horizon, the orientation of the head is controlled or constrained by the orientation of the body.

Stripes on the vertical walls (Fig. 1C) do not lead to a statistically significant difference in the flies' head orientation, irrespective of stripe orientation with respect to the ground (Fig. 1C, conditions 1 & 2, $p=0.435$, and conditions 3 & 4, $p=0.082$). As stripes seem to play little role in affecting head orientation, we also recorded head orientation in the absence of the stripes, thus providing the animal with less visual cues in the surroundings (Fig. 1 D & F). We find significantly more alignment with the floor than with the light source and the target bar (Fig. 1D, conditions 6 & 7, $p<0.001$), confirming the greater importance of the floor. No significant change in head orientation is found when changing the orientation of the target bar and light sources alone (Fig. 1D, conditions 7 & 8, $p=0.105$). In the absence of the stripes a change in the orientation of the light source and the target failed to elicit a significant change in head orientation (Fig 1D, conditions 7 and 8) in contrast to the situation with stripes (Fig 1C, conditions 4 and 5). Hence, the availability of particular visual cues affects the integration of other cues.

Body orientation is mainly controlled by gravity and ground orientation

Body orientation is almost unaffected by the orientation of the light sources relative to the animal and the orientation of the target bar (see Fig. 1 E & F, conditions 1 and 6, compare conditions 4 & 5, $p=0.412$, conditions 7 & 8, $p=0.105$.).

By contrast, the orientation of the walking surface has a big impact on body orientation, aligning itself much more towards the same than towards the target bar and the light sources (Fig. 1 E & F, conditions 1 & 3, $p<0.001$, conditions 6 & 7, $p<0.001$). Although we cannot exclude the influence

of the visual horizon on body orientation, we consider the strong alignment of the body with the orientation of the surface to be likely due to the constraints of walking, i.e. limits in the leg posture restricting body roll.

A contribution of gravity is also observed, but its effect is small compared to the one of the walking surface (Fig. 1E condition 5, Fig. 1F condition 8). It has been shown that locomotion along a tilted surface can cause roll of the body as a consequence of the change of the gravity vector relative to the surface (Diederich et al., 2002). Thus, although a mechanism to compensate for gravity cannot be excluded, we consider the small effect of gravity observed in our experiments most likely to be due to the physical constraints of walking along a tilted surface.

The orientation of visual cues presented in the form of stripes in the surroundings of the animal appears to have only a minor effect on body orientation. They do not cause a significant effect when walking on level surfaces (Fig 1E conditions 1 & 2, Wilcoxon rank sum test, p=0.238), but seem to cause a significant change when the fly walks on tilted surfaces (Fig 1E conditions 3 and 4 p=0.001), though small in magnitude.

Linearity and Adaptability of Integration

To analyze the relative contribution of the different cues to head orientation we performed a simple linear fit and estimated the relative weights of the different cues we manipulated for both the experimental conditions with stripes and without any stripes. Our model allows us to account for head and body orientation based on the orientation suggested by the cues available to the animals (Fig. 1 C-F).

In the presence of stripes gravity ($w_g=0.27$) and the floor orientation ($w_f=0.52$) are the major factors controlling head orientation, but the orientation of the light sources and target bar ($w_l=0.15$) as well as the stripes ($w_s=0.07$) still contribute to head orientation, with the stripes playing the smallest role. In the absence of stripes (fixed setting of $w_s=0$), we find that the relative weights of light sources and target bar ($w_l=0.14$) and of the floor ($w_f=0.51$) are almost unchanged, while a more substantial change occurs in the relative weight of gravity ($w_g=0.34$). This suggests that in the absence of additional visual cues gravity gains a bigger impact on head orientation.

For the body, in the presence of stripes, the orientation is mostly controlled by the orientation of the surface ($w_f=0.81$) and gravity ($w_g=0.15$), while orientation of light sources and target bar ($w_l=-0.03$) as well as orientation of the stripes ($w_s=0.07$) only contribute weakly. Body orientation in the absence of stripes confirms that the orientation of the surface ($w_f=0.85$) and gravity ($w_g=0.18$) are

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major contributors, while the contribution of the orientation of light sources and target bar (w_l=-0.02) is negligible.

Previous research has shown that the integration of visual and gravitational cues is linear to some extent (Horn and Knapp, 1984). This is confirmed by our linear model which is able to account for head and body orientation with a reasonable accuracy (Fig. 1 C - F).

The integration shows some signs of adaptability. In the absence of stripes, the normalized weights of the light source, the target stripe, and the walking surface remain almost unchanged (w_l=0.15 to 0.14 and w_f=0.52 to 0.51, respectively). Instead, only the weight of gravity increases (w_g=0.27 to 0.34). This may explain the lack of any significant effect of the light sources and the target bar in absence of stripes in the surroundings (Fig. 1D condition 7 and 8), as the change may become too small to be functionally significant. This finding suggests that in some way the animals evaluate the availability of visual cues and compensate for a lack of them by giving more importance to gravity.

Open questions

Walking blowflies evaluate the availability of different types of visual cues, such as the direction of light sources or the orientation of environmental features. If such cues are lacking, the relative role of gravity for head orientation increases. How this is accomplished, however, is still an open question. We also remain in the dark on how the different cues are integrated, though both previous results (Horn and Knapp, 1984) and the accuracy of our model suggest at least some degree of linearity. It is also unknown where the integration takes place in the nervous system, but research on the neck motor neurons (Strausfeld and Seyan, 1985) has revealed connections between cervical neurons and visual neurons at the level of the prothoracic ganglion suggesting some degree of integration to take place there.

We can only speculate about why the animals integrate the orientation of different cues into a final head orientation instead of choosing to align themselves according to a specific cue. One possibility is that instead of using a more computationally complex procedure, blowflies might just perform a simple weighted sum of the different orientations detected and accept the possible deviations from the optimal orientation as a trade-off. It is worth mentioning that, despite rotating their head, and thus their eyes, as a compromise between the orientation of the walking surface and a target bar, walking blowflies are able to detect and approach their targets. This indicates that the mechanisms by which this behavior is achieved are robust with regard to rotations of the visual system.

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Author contributions

References

Baddeley, R. and Hancock, P. (1991). A Statistical Analysis of Natural Images Matches Psychophysically Derived Orientation Tuning Curves. Proceedings of the Royal Society of London B: Biological Sciences 246, 219-223.

Bernard, G.D. and Stavenga, D.G. (1979). Spectral sensitivities of retinular cells measured in intact, living flies by an optical method. Journal of Comparative Physiology 134, 95-107

Bülthoff, H., Götz, K.G. and Herre, M. (1982). Recurrent inversion of visual orientation in the walking fly, *Drosophila melanogaster*. Journal of Comparative Physiology 148, 471-481

Chiba, R., Takakusaki, K., Ota, J., Yozu, A. and Haga, N. (2016). Human upright posture control models based on multisensory inputs; in fast and slow dynamics. Neuroscience Research 104, 96-104.

Coppola, D., Purves, H., McCoy, A. and Purves, D. (1998).The distribution of oriented contours in the real world. Proceedings of the National Academy of Sciences 95, 4002-4006.

Day, B.L., Steiger, M.J., Thompson, P.D. and Marsden, C.D. (1993). Effect of vision and stance width on human body motion when standing: implications for afferent control of lateral sway. The Journal of Physiology 469, 479-499.

Diederich, B., Schumm, M. and Cruse, H. (2002). Stick Insects Walking Along Inclined Surfaces. Integrative and Comparative Biology 42, 165-173.

Goulard, R., Julien-Laferriere, A., Fleuriet, J., Vercher, J.L. and Viollet, S. (2015). Behavioural evidence for a visual and proprioceptive control of head roll in hoverflies (*Episyrphus*

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balteatus). Journal of Experimental Biology 218, 3777-3787.

Guitton, D., Kearney, R.E., Wereley, N. and Peterson, B.W. (1986). Visual, vestibular and voluntary contributions to human head stabilization. Experimental Brain Research 64, 59-69.

Hancock, P., Baddeley, R. and Smith, L.S. (1992). The principal components of natural images. Network: Computation in Neural Systems 3, 61-70.

Hansen, B.C. and Essock, E. (2004). A horizontal bias in human visual processing of orientation and its correspondence to the structural components of natural scenes. Journal of Vision 4: 5.

Hengstenberg, R. (1993). Multisensory control in insect oculomotor systems. Reviews of Oculomotor Research 5, 285-298.

Holst, E. von (1935). Über den Lichtrückenreflex bei Fischen. Pubblicazioni Della Stazione Zoologica di Napoli 15, 143-158.

Horn, E. (1982). Gravity reception in the walking fly, *Calliphora erythrocephala*: Tonic and modulatory influences of leg afferents on the head position. Journal of Insect Physiology 28, 713-721.

Horn, E. and Bischof, H.J. (1983). Gravity reception in crickets: the influence of cereal and antennal afferences on the head position. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 150, 93-98.

Horn, E. and Knapp A. (1984). On the invariance of visual stimulus efficacy with respect to variable spatial positions. Journal of Comparative Physiology A 154, 555-567

Horn, E. and Lang, H.G. (1978). Positional head reflexes and the role of the prosternal organ in the walking fly, *Calliphora erythrocephala.* Journal of Comparative Physiology 126, 137-146.

Kasumyan, A.O. (2004). The vestibular system and sense of equilibrium in fish. Journal of Ichthyology 44, 224-268.

Keil, M.S. and Cristóbal, G. (2000). Separating the chaff from the wheat: possible origins of the oblique effect. Journal of the Optical Society of America 17, 697-710.

Kress, D. and Egelhaaf, M. (2014).Gaze characteristics of freely walking blowflies Calliphora vicina in a goal-directed task. Journal of Experimental Biology 217, 3209-3220.

Massion, J. (1994). Postural control system. Current Opinion in Neurobiology 4, 877-887.

Meyer, D. and Bullock, T. (1977). The hypothesis of sense-organ-dependent tonus mechanisms: history of a concept, Annals of the New York Academy of Sciences 290, 3-17.

Mittelstaedt, H. (1950). Physiologie des Gleichgewichtssinnes bei fliegenden Libellen. Zeitschrift für vergleichende Physiologie 32, 422-463.

Mittelstaedt, H. (1997). Interaction of eye-, head-, and trunk-bound information in spatial perception and control. Journal of Vestibular Research 7, 283-302.

Previc, F.H., Kenyon, R., Boer, E.R. and Johnson, B. (1993). The effects of background visual roll stimulation on postural and manual control and self-motion perception. Perception & Psychophysics 54, 93-107.

Schuppe, H. and Hengstenberg, R. (1993). Optical properties of the ocelli of *Calliphora erythrocephala* and their role in the dorsal light response. Journal of Comparative Physiology A 173, 143-149.

Schwegmann, A., Lindemann, J.P. and Egelhaaf, M. (2014). Depth information in natural environments derived from optic flow by insect motion detection system: a model analysis. Frontiers in Computational Neuroscience 8:83.

Srinivasan, M. (1977). A visually-evoked roll response in the housefly. Journal of Comparative Physiology 119, 1-14.

Straube, A., Krafczyk, S., Paulus, W. and Brandt, T. (1994). Dependence of visual stabilization of postural sway on the cortical magnification factor of restricted visual fields. Experimental Brain

27

Research 99, 501-506.

Strausfeld, N.J. and Seyan, H.S. (1985). Convergence of visual, haltere, and prosternai inputs at neck motor neurons of *Calliphora erythrocephala.* Cell and Tissue Research 240, 601-615.

Switkes, E., Mayer, M.J. and Sloan, J.A. (1978). Spatial frequency analysis of the visual environment: Anisotropy and the carpentered environment hypothesis. Vision Research 18, 1393-1399.

Tomioka, K. and Yamaguchi, T. (1980). Steering responses of adult and nymphal crickets to light, with special reference to the head rolling movement. Journal of Insect Physiology 26, 4751-4957.

Viollet, S. and Zeil, J. (2013). Feed-forward and visual feedback control of head roll orientation in wasps (*Polistes humilis, Vespidae, Hymenoptera*). Journal of Experimental Biology 216, 1280-1291.

Watanabe, S., Takabayashi, A., Takagi, S., von Baumgarten, R. and Wetzig, J. (1989). Dorsal light response and changes of its responses under varying acceleration conditions. Advances in Space Research 9, 231-240.

Winter, D.A. (1995). Human balance and posture control during standing and walking. Gait & Posture 3, 193-214.

Walking blowflies and optic flow: uses and behavioral adaptations

Introduction

In their day-to-day live many insects rely on visual cues to obtain spatial information about their surroundings. A key visual cue in this context is the motion of images across the retina, known as optic flow. During translational motion objects further away will move more slowly across the retina than object closer, thus allowing animals to derive spatial information from optic flow. This cue is employed by a multitude of insects: locust, mantids, and dragonflies use it to judge the distance to prey (Sobel 1990, Kral and Poteser 1997, Olberg et al 2005), fruit flies use it to control their flight height (David 1979), honey bees and flies use it to regulate their distance to walls (Kirchner and Srinivasan 1989, Kern et al. 2012), bumblebees use it to judge the width of gaps (Ravi et al. 2019), and flies (Reichardt et al 1983, Egelhaaf 1985) as well as honeybees (Dittmar et al. 2010) can use it to detect camouflaged objects and learn spatial information (Lehrer et al. 1988, Kimmerle et al 1996) through their motion compared to the movement of the background.

However, it is important to note that only optic flow generated by translatory locomotion contains spatial information, as during rotations all features of the environment rotate at an identical speed (Koenderink 1986). To account for this issue many flying insects employ a saccadic flight strategy, in which the flight is segregated into quick turns called saccades and straight segments called intersaccades. This behavior has been described in bees (Boeddeker et al 2010, Braun et al. 2012) and flies (Land 1973, Schilstra and van Hateren 1999, van Hateren and Schilstra 1999, Braun et al. 2010), and can facilitate the use of optic flow (Egelhaaf et al. 2012), for example during collision avoidance, as shown by modeling work (Lindemann et al 2008, Bertrand et al. 2015).

However, many insects walk in addition to flying, and the walking mode of locomotion poses significant challenges to the use of optic flow for spatial tasks. While walking blowflies experience rotations coupled to the stride cycle of the animal, i.e. the succession of leg movements performed during locomotion (Kress and Egelhaaf, 2012; Kress and Egelhaaf, 2014). These rotations introduce rotational optic flow that might hinder the extraction of distance information. Despite this limitation, there is evidence that walking flies use optic flow to estimate distance since they prefer to approach closer objects in a virtual reality setup (Schuster et al. 2002), though later work suggested that this preference might be the consequence of the faster object motion irrespective of how this motion relates to distance (Mronz 2004).

Thus, we address the question of whether walking blowflies distinguish objects based on their distance and potentially make use of optic flow. We developed a free walk paradigm that allowed flies to approach objects at different distances. The detectability of the objects was varied by covering them and their background with different textures. In particular, we asked whether blowflies approach closer objects preferentially and whether they may use optic flow to detect even camouflaged objects and, thus, approach objects otherwise indistinguishable from the background. Furthermore, we analyzed if walking blowflies change their walking style to better detect camouflaged objects, which might reveal a strategy to optimize the use of optic flow for this particular task.

Methods

Experimental animals

We made use of female blowflies (*Lucilia cuprina*) bred in our lab stock. 1-3 days after hatching animals were captured, briefly anesthetized with CO_2 and prevented from flying by immobilizing their wings with a drop of wax placed on the wing joints. Until recording the blowflies were kept in a cage with free access to sugar and water.

Experimental setup

The experimental setup consisted of a walking platform surrounded by a cylinder of 70 cm diameter and 70 cm height, which formed the walls of the overall walking arena. The walls were covered either with a random cloud pattern with spatial frequencies f characterized by a 1/f spectrum, which ensures that the spatial frequency of the pattern remains largely the same when viewed from different distances, or with a homogeneously gray paper sheet of the same average brightness as the cloud pattern.

The walking platform was a 1 cm thin PVC plate with a 2 cm diameter hole in its center, which allowed to introduce animals into the setup. The area 15 cm around the center of the entry hole is called the recording area, which is separated from the remaining arena floor by a 2,5 cm wide and 0,5 cm deep moat. The moat was filled with water and to prevent the animals from escaping the recording area. The arena floor, except for the moat and the recording area, was covered with the same random cloud pattern as the one used to cover the walls.

Three objects were designed to be placed equally spaced around the center of the entry hole at different distances while conserving the same apparent size when viewed from the center. Thus, in one set of experiments the objects, which were placed at a distance of 20 cm, 25 cm, and 30 cm to the entry hole, had a height and diameter of 12 cm and 3.5 cm, 15 cm and 4.4 cm, and 18 cm and 5.2 cm, respectively. The objects were covered with either black or white paper or the same cloud pattern as used for the floor and walls of the arena. In another set of experiments, all objects were placed at the same distance of 20 cm from the entry hole; their height and diameter were 12 cm and 3,5 cm, respectively.
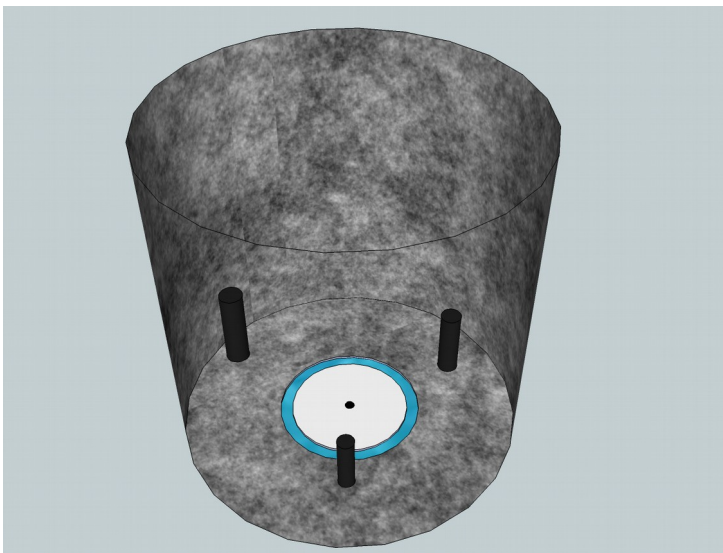
Figure 1. 3D model of the arena. Visible are the entry hole in the center of the arena, the white recording area, the water moat (in blue) and the remaining floor and walls, for this example covered in the random cloud pattern (see Methods). The objects are placed at 20 cm, 25 cm, and 30 cm from the entry hole as an example (See Methods).

The setup was illuminated by a 3 white LED lamps (GSVITEC Marathon MultiLED, GS Vitec GmbH, Gelnhausen, Germany) placed on tripods surrounding the arena and arranged to stand opposite of one object each, thus shining directly on them and hiding shadows behind the objects. This allowed us both to camouflage the objects, if they had the same texture as the background, and to provide enough illumination for a high-speed tracking of the animal.

The direct illumination with LED lamps does, however, produce sharp shadows of the animal that, while unproblematic to track the position of the fly, make it very difficult to adequately obtain the animal's orientation (see Analysis). Thus in the experiments with the 3 objects at the same distance, a diffuser was placed over the setup, with a hole to allow recording inside the arena. This produced a softer light that hid the shadows of the objects and simultaneously allowed for better tracking of the animals' orientation.

The animals were recorded at 90 frames/second with a camera (Basler ac A2040 – 90um, Basler 112 AG, Ahrensburg, Germany) placed above the arena and a custom-made program based on the Pylon 4 software suite (Basler, Basler AG, Ahrensburg, Germany).

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Experimental procedure

Flies were captured from the cage and introduced into the arena through the hole in the center of the walking platform. The animals were recorded in two sets of experiments: with the 3 objects at different distances, or with the 3 objects at the same distance.

When the 3 objects were placed at different distances, the animals were recorded for 2,5 minutes or, as sometimes happened until they escaped the recording area by crossing the water moat. The objects located at different distances were exchanged in a pseudo-random sequence. We recorded flies under four conditions: 1) black objects with the arena wall covered by a random cloud patterns (N=50), 2) white objects with the wall covered by a random cloud patterns (N=30), 3) objects covered by the random cloud pattern with the wall covered by the same patterns (N=30), and 4) objects covered with the random cloud pattern with the walls beings homogeneously gray (N=30). Each animal was recorded only once to avoid potential experience effects.

When the 3 objects were placed at the same distance, the animals were recorded for 20 seconds or until they reached the moat for the first time. We recorded flies in two conditions: 1) with black objects and the walls covered by a random cloud pattern, and 2) with objects covered by the random cloud pattern and the walls covered with the same random cloud pattern. 30 flies were recorded, with individual animals being recorded in both conditions. Half of the flies were recorded when facing black objects first and the other half when facing textured objects first. The position of the objects was varied in a pseudo-random manner to control for any possible effect of the layout of the arena. Only recordings in which the flies moved towards the objects were further analyzed in this condition.

Analysis

We tracked the animals using the open-source software ivTrace (https://opensource.cit-ec.de/projects/ivtools). This software allowed us to track the center of mass of the body of walking flies. When we recorded animals in the presence of 3 objects at the same distance we also tracked the animals' orientation by automatically fitting an ellipse to the body using ivTrace. The results of tracking were manually reviewed to verify they adequately reproduce the position and orientation of the blowflies.

The optic flow generated by objects on the eyes of the animals was calculated using the open-source OpticFlow Toolbox for Matlab available as part of ivTools (https://opensource.cit-ec.de/projects/ivtools).

Results

To address if blowflies can discriminate objects based on their distance and distinctness from the background we recorded their behavior in a free-walking object choice paradigm and then compared their behavior when facing different objects to assess if they adapted their walking style to facilitate the detection of objects.

Black objects are approached independent of distance, but white objects are not attractive

To address whether blowflies showed a preference for objects based on their distance we recorded blowflies as they walked in an arena surrounded by clearly visible objects at different distances. We released individual blowflies into an arena surrounded by 3 objects at different distances (20 cm, 25 cm, and 30 cm from the entry hole). The objects were either black or white. Either way, the objects offered a clear contrast against the background.
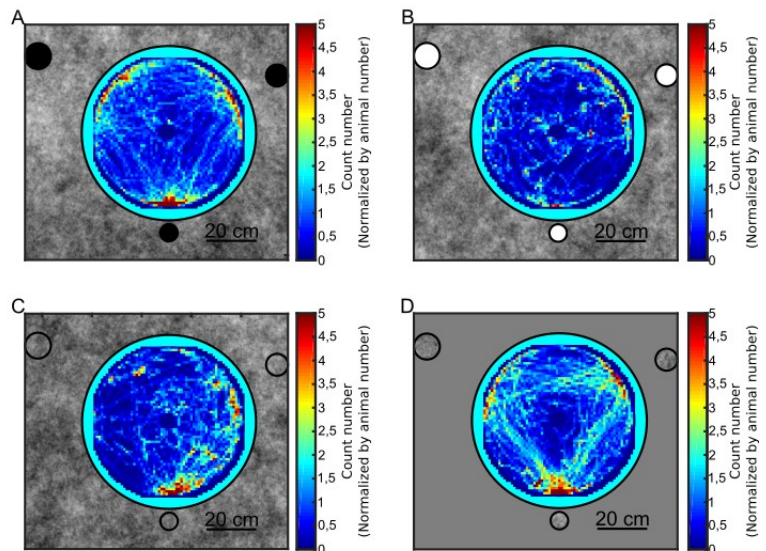
Upon entering the arena, blowflies confronted with black objects usually walk along fairly straight paths towards one of the objects and then proceed to walk between all three objects, again on relatively direct paths (Figure 2 A). When the objects are white, the flies instead move towards the edge of the walking arena and then usually follow its border, occasionally walking along straight paths until they encounter the border again (Figure 2 B). To assess if they walked
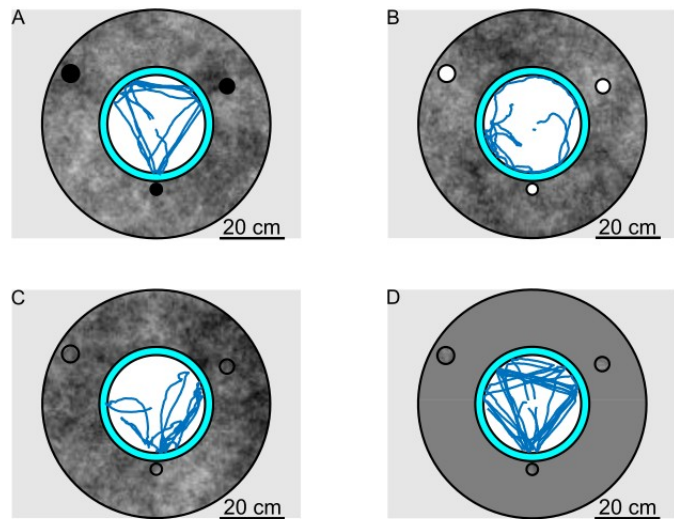
Figure 2. Examples of behavior of flies facing different objects. Depicted is the behavior of a single fly when facing 3 objects at 20 cm, 25 cm, and 30 cm from the entry hole while A) the background is covered in a random cloud pattern and the objects are black B) the background is covered in a random cloud pattern and the obejcts are white C) the background is covered in a random cloud pattern and the objects are covered in the same pattern D) the background is homogeneously gray and the objects are covered in a random cloud pattern.

towards one of the black objects more often than to the others, we determined the relative frequency of presence at the different locations of the arena across all trajectories recorded. Since we were

interested in where the animals were walking, we removed all stop phases from this analysis. We visualized this analysis in the form of a heat map. We find that the flies visit all three objects in an obvious manner when the objects are black, as evidenced by the 3 'hot spots' visible in front of all 3 objects (Figure 3 A), but not when the objects are white (Figure 3 B).

Though we removed stop phases from the previous analysis, the locations at which the animals stop might also reveal information about which objects they preferred, for example by stopping preferentially in front of one object. Thus, we determined the relative frequency of stops at the different locations of the arena across all

Figure 3. Heatmap of the position of walking flies when confronted with different objects. The objects are located at 20 cm, 25 cm, and 30 cm from the entry hole and A) the background is covered in a random cloud pattern and the objects are black B) the background is covered in a random cloud pattern and the obejcts are white C) the background is covered in a random cloud pattern and the objects are covered in the same pattern D) the background is homogeneously gray and the objects are covered in a random cloud pattern.
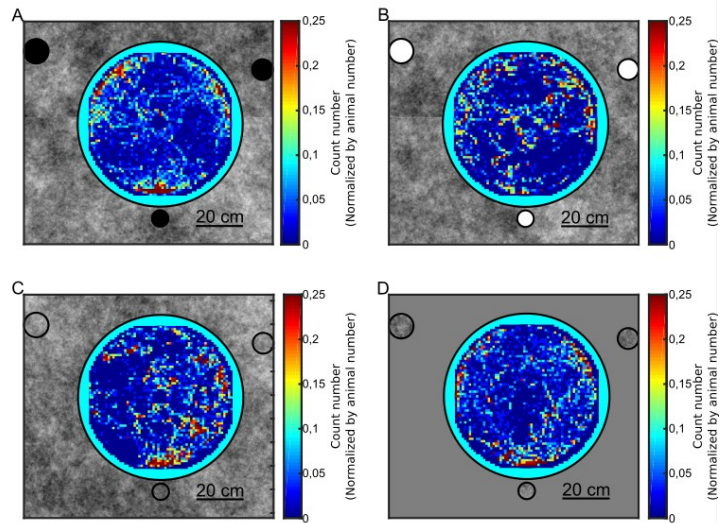
recorded flies and visualized this frequency in the form of a heat map. Blowflies stopped mostly in front of the objects when they are black (figure 4 A), but seem to stop at variable positions widely distributed in the arena when the objects are white. To check whether blowflies visited all black objects similarly often, we divided the arena into 3 sectors, defined around the entry hole and one of the objects and then calculated the overall proportion of time spent in each sector. Blowflies spent almost identical amounts of time in each of the 3 sectors (Figure 5 A). Thus, we conclude that blowflies approach black objects equally often independent of distance, suggesting that they either do not estimate distance in this experimental paradigm or the distance differences are not sufficiently large to generate a change in preference. White objects, despite being contrasted against the background, do not seem to be attractive to walking blowflies. This lack of interest of walking blowflies for white objects has been reported before for the first walk in an arena (Osorio et al, 1990), and our results confirm that this effect persists even if the animals are allowed to roam freely in the arena, substantiating the evidence that white objects are not attractive to walking blowflies.

Camouflaged objects can be detected if they are away from the background

To address if walking blowflies were able to detect camouflaged objects we recorded blowflies as they walk in an arena surrounded by 3 objects at different distances (20 cm, 25 cm, and 30 cm) from the entry hole which were covered by the same texture as the ground and walls of the arena; thus, they should be only detectable by relative motion. We also recorded animals while walking in an arena surrounded by 3 objects identical to the ones described before but with the walls covered by a homogeneously gray background, so that we can observe any preference that may be derived from the texture of the objects rather than the objects being camouflaged against the background.

When the objects are camouflaged the flies tend to walk towards one of the two closest objects and then back and forth between them along fairly straight trajectories (Figure 2C). When the textured objects are presented in front of homogeneously gray background, blowflies approach all of them (Figure 2D), just as when the objects where black and the background had a random cloud pattern (Figure 2 A) and along

Figure 4. Heatmap of the location of stop phases when flies are facing different objects. The objects are located at 20 cm, 25 cm, and 30 cm from the entry hole and A) the background is covered in a random cloud pattern and the objects are black B) the background is covered in a random cloud pattern and the obejcts are white C) the background is covered in a random cloud pattern and the objects are covered in the same pattern D) the background is homogeneously gray and the objects are covered in a random cloud pattern.

a

similarly straight paths. To asses where the flies were preferentially walking, we determined the relative frequency of presence in the arena across all conditions, excluding stop phases, and visualized the frequency distribution as a heat map. When the objects are camouflaged the blowflies did indeed walk preferentially close to the two closest objects and between them, but they rarely stayed at the most distant object (Figure 3C). When the objects were textured, but the background was gray the flies approached and tended to walk close to all of them (Figure 4D). To make sure we didn't miss a preference stemming from the flies preferably stopping in front of the more distant objects we also determined the relative frequency of the locations of stop phases. The corresponding heat maps confirm our previous observations: when the objects were camouflaged blowflies stopped mostly in front of the two closer objects (Figure 4C) but when the objects were

textured and presented against a gray background the blowflies stopped in front of all three objects (Figure 4 D). To quantify whether the blowflies approached any of the objects more than the others we divided the arena into 3 sectors, defined around the entry hole and centered on each of the objects and calculated the overall proportion of time spent by the flies in each sector. As expected, blowflies spent less time walking in the sector containing the farthest of the camouflaged objects (figure 5 B) but spent a similar amount of time in each sector when the same textured objects were presented against a gray background (Figure 5 C). Thus we conclude that when the objects have the same texture as the background blowflies can detect and approach them when they have at least some distance from the background and that this effect is not due to the texture of the objects since objects with the same texture presented against a gray background are approached as if they were clearly visible, like black objects against a textured background.
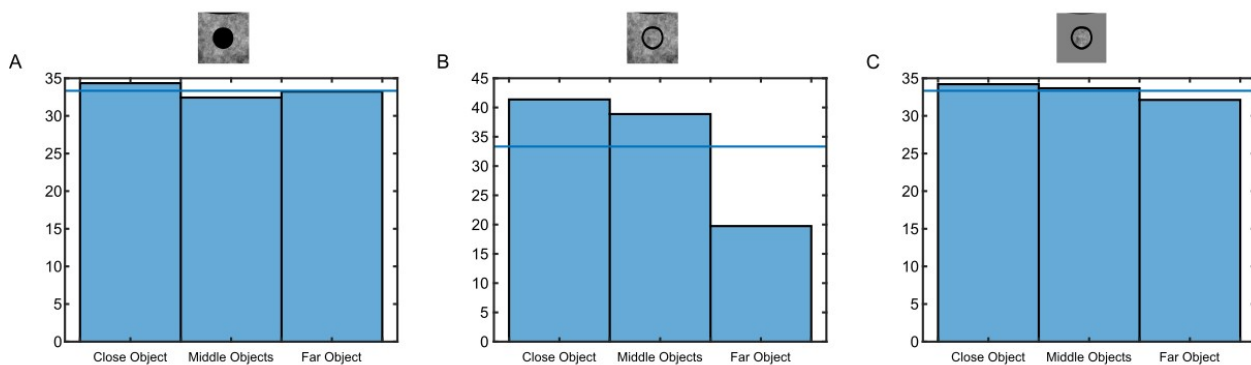
Figure 5. Proportion of walking time spent in the thirds of the arena containing each object. A)When the background is covered in a random cloud pattern and the objects are black B) When the background is covered in a random cloud pattern and the objects are covered in the same pattern C)When the background is homogeneously gray and the objects are covered in a random cloud pattern.

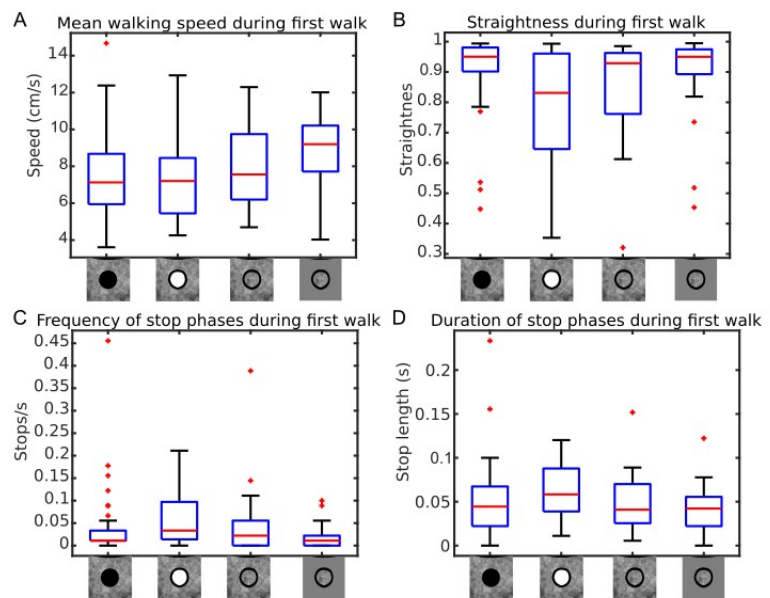## Walking style does not change when confronted with different objects

To address if walking blowflies changed their behavior to better make use of motion cues we analyzed several walking parameters while the animals walked after entering the arena until they reached the moat. We did this analysis across all conditions as described before and calculated the walking speed, since it directly relates to the optic flow generated, the straightness, since a change in it could be indicative of a more sinuous walk style, and the number and length of stop phases, since they have been described in the context of object approach (Kress & Egelhaaf 2014).

The walking speed remains largely unchanged when the objects are camouflaged compared to when they are clearly visible (Figure 7A). The straightness of the first walk also remains high in all recordings in which objects were directly approached irrespective of their texture (i.e. for black objects, camouflaged objects, and textured objects against a grey background) (Figure 7B),

suggesting that flies walk along fairly straight paths even when objects are camouflaged and are concluded to be detected by relative motion cues. Flies appear to walk along more curved trajectories only when the objects are not approached. Lastly, the number and duration of stop phases (Figure 7 C and D) remain constant across all conditions tested, suggesting that they are a mostly constant feature of the walk behavior of blowflies.

To further assess whether blowflies changed their walking style when approaching camouflaged objects, we performed additional recordings to analyze fine details of their walking style. We recorded 30 blowflies in two scenarios: 1) while facing 3 black objects at a 20 cm distance from the entry hole against a background textured with a random cloud pattern and 2) while facing 3 objects at 20 cm


Figure 6. Comparison of parameters of the first walk bout until reaching the moat. The objects are located at 20 cm, 25 cm, and 30 cm from the entry hole. Depicted are the parameters calculated when the background is covered in a random cloud pattern while the objects are black, when the background is covered in a random cloud pattern while the obejcts are white, when the background is covered in a random cloud pattern while the objects are covered in the same pattern, and when the background is homogeneously gray and the objects are covered in a random cloud pattern, respectively. Parameter depicted are: A) Boxplot of mean walking speed B)Boxplot of straightness C) Boxplot of the n° of stop phases per second D) Boxplot of the duration of stop phases

from the entry hole camouflaged with the same cloud pattern as the background. Only traces in which an object was approached were further analyzed (N=17 for black objects, N=14 for camouflaged objects). We determined the side-wise speed and the yaw speed of the animal over the entire trajectory. If the animal changed its behavior to increase relative motion between the camouflaged object and its equally textured background we expect them to reduce the amount of yaw speed oscillations, since they introduce rotational optic flow, and/or to increase its side-wise speed, since that would increase the amount of translational optic flow. Since both of these parameters oscillate with the stride cycle (Figure 7A, Kress & Egelhaaf 2014), we calculated the interquartile range (IQR) of the yaw speed and sidewise speed of each fly as a measure of the amplitude of their oscillations. Neither the sideways speed (Figure 7 B) nor the yaw speed (Figure 7 C) changed their magnitude during the stride-coupled oscillations in any obvious way, indicating

that yaw speed, as well as side speed oscillations, remain the same when approaching a camouflaged object or a conspicuous object, that can be detected without relative motion cues.

To verify whether the optic flow generated during an object approach does not change we calculated the optic flow generated by each of the edges of the object and by the background in the immediate vicinity of the objects' edges. Just by visual inspection it is difficult to distinguish the optic flow generated by the background from that generated by the object (Figure 8 A). Therefore, we determined both the retinal velocities generated by the edges of the object as well as that in the adjacent regions of the background and then calculated the difference between both measures (Figure 8 B) as a proxy of the relative motion between object and background. Since both the optic

flow induced by the object and the background and, accordingly, their difference oscillate with the stride cycle (Figure 8 A) we calculated the IQR of both measures for each fly as a measure of these oscillations. If the animal shapes its behavior to facilitate the detection of the camouflaged objects, the optic flow oscillations are expected to be bigger when the object is camouflaged than when it can be easily detected without relative motion cues. We find however that neither the optic flow generated by the object nor its motion relative to the background differ clearly. The optic flow generated by the edges of the objects is largely the same for both edges irrespective of the object being camouflaged or immediately salient (Figure 8 C &D). Also, the relative motion between the edges and their background does not differ systematically for both black and camouflaged objects (Figure 8D). This finding suggests that the optic
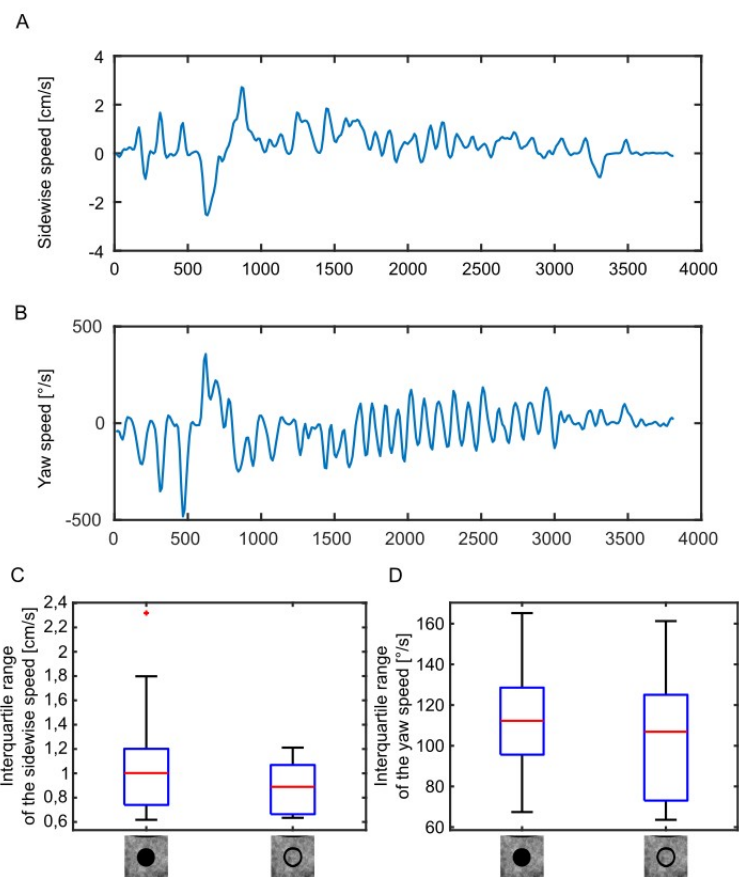
Figure 7. Analysis of the yaw speed and sidewise speed when approaching black and camouflaged objects. The background was covered in random cloud pattern. The camouflaged object is covered in the same pattern. A) Example of the sidewards speed over a trajectory. Observe the oscillations along the trajectory. B) Example of the yaw speed over a trajectory. Observe the oscillations along the trajectory. C) Boxplot of the inter-quartile range of the sidewise speed when facing black and camouflaged objects. D) Boxplot of the inter-quartile range of the yaw speed when facing black and camouflaged objects. D

flow generated by the object as a result of the stride-coupled body and head fluctuations is largely the same when approaching camouflaged objects as when approaching objects that can easily be detected without motion cues and that even relatively small velocity difference between object and background seem to be sufficient to break the camouflage.
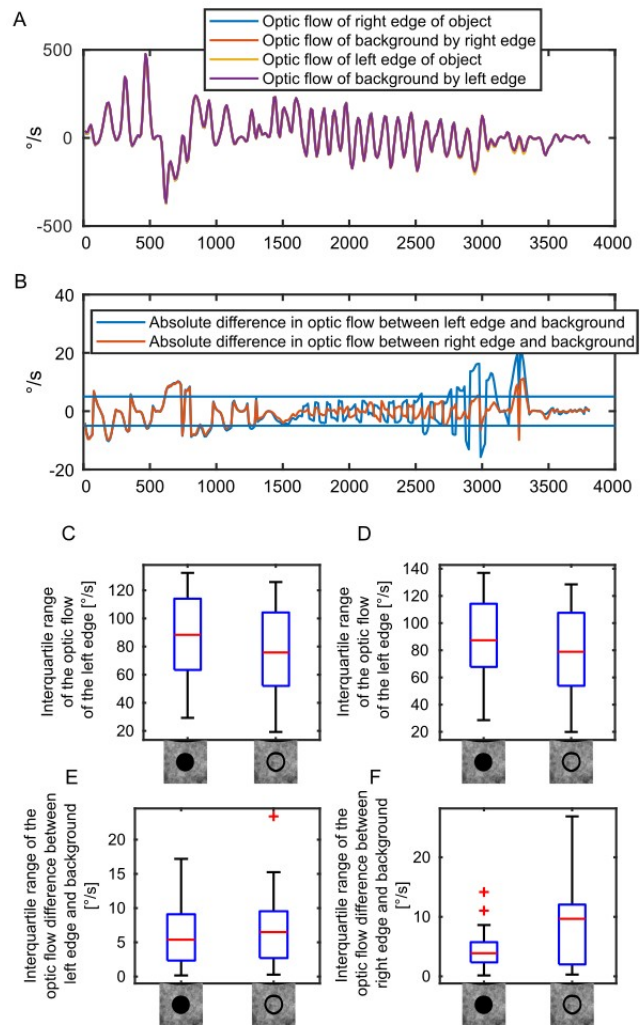
Discussion

We recorded blowflies as they walked towards and between differently distant objects and revealed that walking blowflies don't show a preference based on distance but seem to use motion cues to detect camouflaged objects. We further analyzed their walking style and found that it remains largely unchanged when approaching camouflaged objects.

Blowflies employ motion cues to detect camouflaged objects

When objects are camouflaged by covering them with the same texture as their background, blowflies are still able to detect and approach them (Figure 2 C, 3 C), provided the object has a sufficient distance to the background (Figure 3 C, Figure 5 B). Since this phenomenon is not observed when objects with the same texture are

Figure 8. Analysis of the optic flow generated during approach to black and camouflaged objects. The background was covered in random cloud pattern. The camouflaged object is covered in the same pattern. A) Example of the horizontal optic flow of both edges of the object and of the background immediately to its side. Notice that for most of the trajectory they overlap, and that they oscillate regularly along the trajectory. B) Example of the difference between the optic flow of the edge and of the background immediately to its side. Observe the oscillations along the trajectory. C) Boxplot of the inter-quartile range of the optic flow when facing black and camouflaged objects. D) Boxplot of the inter-quartile range of the difference in optic flow between edge and background when facing black and camouflaged objects. D

presented against a gray background, it is most likely that this lack of approach to the more distant object is due to its camouflage. Our results highlight both the fact that our behavioral paradigm

allows us to camouflage an object and that walking blowflies can break this camouflage if the distance between object and background is large enough.

One should take into account the limitations of our experimental setup when drawing conclusions from this finding. While the cloud patterns we employed to camouflage objects result is similar patterns even when viewed from different distances and the cylindrical shape of the objects makes sure that the view does not change drastically depending on the angle the object is viewed from, there is still some distortion of the pattern at the edges of the object that could provide cues to the animal about the presence of an object. Furthermore, despite our efforts to prevent shadows from revealing the presence of objects, there is still the possibility that some remain. However, since the far object is indeed not approached, it is reasonable to assume that the objects are well camouflaged and that the closer objects are detected by relative motion cues. The use of motion cues has already been described in many insects like mantids, dragonflies, and locusts (Sobel 1990, Kral and Poteser 1997, Olberg et al 2005), but also flies (Kirchner and Srinivasan 1989, Kern et al. 2012), even specifically for the task of detecting objects otherwise undetectable (Reichardt et al 1983, Lehrer et al. 1988, Kimmerle et al 1996). With this experiment, we confirm the ability of flies to use motion cues to detect camouflaged objects also during walking.

While the results with camouflaged objects indicate that flies make use of motion cues, the results using objects with an obvious contrast against the background suggests that the animals do not obtain an estimate of the magnitude of optic flow generated by the objects.

Motion cues do not affect distance preferences of walking blowflies within short ranges

Walking blowflies confronted with clearly conspicuous objects, such as black objects presented against a background with a random cloud pattern and objects textured with a random cloud pattern presented against a homogeneously gray background, initially walk towards the objects and then back and forth between them (Figure 2 A & D) without staying close to any of them more than close to the others in any obvious manner (Figure 5 A&C). This finding suggests that, at least in the range of distances analyzed in this project (20 cm – 45 cm), blowflies do not discriminate between their goals based on their distance, as long as they are clearly contrasted against the background. Thus, one might conclude that while walking blowflies can detect the difference in optic flow between an object and its background they are not able to estimate distances from the magnitude of the optic flow. This conclusion, however, would contrast with the conclusion from virtual reality experiments on walking fruit flies (Schuster et al. 2002) that fruit flies can estimate distance through

motion cues. However, animals in the experimental setup of that study display a preference for objects that can be attributed to motion irrespective of distance (Mronz 2004), casting some doubt on the conclusion that the flies estimate distance. The use of motion cues to estimate distance has been shown in multiple insects and paradigms (Sobel 1990, Kral and Poteser 1997, Olberg et al 2005, David 1979; Lehrer et al. 1988; Kimmerle et al. 1996), and as such it might be surprising not to observe it in our experimental paradigm. One possible explanation is that the distance range is not behaviorally relevant since all objects are reachable within short time windows. To address this last issue we performed preliminary experiments in a setup like the one employed in chapter 3, where objects could be presented at much bigger distances than in the walking arena used in this project. The results of these experiments were however not conclusive in this regard.

Walking style of blowflies remains largely constant even when detecting camouflaged objects

The walking style of blowflies is remarkably stereotypical independent of the characteristics of the environment surrounding them and the behavioral context (Figure 6). Even in the face of camouflaged objects, the walking speed, straightness, and duration, as well as the number of stop phases, remain largely unchanged. Even walking characteristics directly tied to the amount of rotational and translational optic flow generated, like the yaw speed and side-wise speed, are remarkably similar when approaching a camouflaged object compared to when approaching a clearly visible black object (Figure 7), which even results in very similar amounts of optic flow being induced on the fly's eye by the goal (Figure 8 C&D).

These findings are surprising given that like honeybees (Kirchner and Srinivasan 1989) flies do shape their flight behavior depending on the environment (David 1979; Kern et al. 2012) during flight, and that honeybees shape their behavior to detect camouflaged objects (Dittmar et al. 2010; Braun et al 2012). Under the assumption that the animal is not detecting the camouflaged objects through other mechanisms, these results suggest that the translational motion generated by the regular walk style of the animal is enough to be sufficiently large to detect camouflaged objects and that the rotational motion introduced by the stride cycle is not enough to hinder the process, though it might not be enough to obtain an estimate of distance, given the flies lack of preference for conspicuous closer objects.

References

Bertrand, O. J., Lindemann, J. P., & Egelhaaf, M. (2015). A bio-inspired collision avoidance model based on spatial information derived from motion detectors leads to common routes. *PLoS computational biology*, *11*(11), e1004339.

Boeddeker, N., Kern, R., & Egelhaaf, M. (2003). Chasing a dummy target: smooth pursuit and velocity control in male blowflies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1513), 393-399.

Boeddeker, N., Dittmar, L., Stürzl, W., & Egelhaaf, M. (2010). The fine structure of honeybee head and body yaw movements in a homing task. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1689), 1899-1906.

Braun, E., Geurten, B., & Egelhaaf, M. (2010). Identifying prototypical components in behaviour using clustering algorithms. *PloS one*, *5*(2), e9361.

Braun, E., Dittmar, L., Boeddeker, N., & Egelhaaf, M. (2012). Prototypical components of honeybee homing flight behavior depend on the visual appearance of objects surrounding the goal. *Frontiers in behavioral neuroscience*, *6*, 1.

David, C. T. (1979). Optomotor control of speed and height by free-flying Drosophila. *Journal of Experimental Biology*, *82*(1), 389-392.

Dittmar, L., Stürzl, W., Baird, E., Boeddeker, N., & Egelhaaf, M. (2010). Goal seeking in honeybees: matching of optic flow snapshots?. *Journal of Experimental Biology*, *213*(17), 2913-2923.

Egelhaaf, M. (1985). On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. *Biological Cybernetics*, *52*(2), 123-140.

Egelhaaf, M., Boeddeker, N., Kern, R., Kurtz, R., & Lindemann, J. P. (2012). Spatial vision in insects is facilitated by shaping the dynamics of visual input through behavioral action. *Frontiers in neural circuits*, *6*, 108.

Ewing, A. W. (1983). Functional aspects of Drosophila courtship. *Biological Reviews*, *58*(2), 275-292.

Hateren, J. H. V., & Schilstra, C. (1999). Blowfly flight and optic flow. II. Head movements during flight. *Journal of Experimental Biology*, *202*(11), 1491-1500.

Kern, R., Boeddeker, N., Dittmar, L., & Egelhaaf, M. (2012). Blowfly flight characteristics are shaped by environmental features and controlled by optic flow information. *Journal of Experimental Biology*, *215*(14), 2501-2514.

Kral, K., & Poteser, M. (1997). Motion parallax as a source of distance information in locusts and mantids. *Journal of insect behavior*, *10*(1), 145-163.

Kimmerle, B., Egelhaaf, M., & Srinivasan, M. V. (1996). Object detection by relative motion in freely flying flies. *Naturwissenschaften*, *83*(8), 380-381.

Kirchner, W. H., & Srinivasan, M. V. (1989). Freely flying honeybees use image motion to estimate object distance. *Naturwissenschaften*, *76*(6), 281-282.

Koenderink, J. J. (1986). Optic flow. *Vision research*, *26*(1), 161-179.

Kress, D., & Egelhaaf, M. (2012). Head and body stabilization in blowflies walking on differently structured substrates. *Journal of Experimental Biology*, *215*(9), 1523-1532.

Kress, D., & Egelhaaf, M. (2014). Gaze characteristics of freely walking blowflies Calliphora vicina in a goal-directed task. *Journal of Experimental Biology*, *217*(18), 3209-3220.

Land, M. F. (1973). Head movement of flies during visually guided flight. *Nature*, *243*(5405), 299-300.

Lehrer, M., Srinivasan, M. V., Zhang, S. W., & Horridge, G. A. (1988). Motion cues provide the bee's visual world with a third dimension. *Nature*, *332*(6162), 356-357.

Lindemann, J. P., Weiss, H., Möller, R., & Egelhaaf, M. (2008). Saccadic flight strategy facilitates collision avoidance: closed-loop performance of a cyberfly. *Biological cybernetics*, *98*(3), 213.

Mronz, M. (2004). Die visuell motivierte Objektwahl laufender Taufliegen (Drosophila melanogaster)-Verhaltensphysiologie, Modellbildung und Implementierung in einem Roboter.

Olberg, R. M., Worthington, A. H., Fox, J. L., Bessette, C. E., & Loosemore, M. P. (2005). Prey size selection and distance estimation in foraging adult dragonflies. *Journal of comparative physiology A*, *191*(9), 791-797.

Osorio, D., Srinivasan, M. V., & Pinter, R. B. (1990). What causes edge fixation in walking flies?. *Journal of Experimental Biology*, *149*(1), 281-292.

Ravi, S., Bertrand, O., Siesenop, T., Manz, L. S., Doussot, C., Fisher, A., & Egelhaaf, M. (2019). Gap perception in bumblebees. *Journal of Experimental Biology*, *222*(2), jeb184135.

Reichardt, W., Poggio, T., & Hausen, K. (1983). Figure-ground discrimination by relative movement in the visual system of the fly. *Biological Cybernetics*, *46*(1), 1-30.

Schilstra, C., & Hateren, J. H. V. (1999). Blowfly flight and optic flow. I. Thorax kinematics and flight dynamics. *Journal of experimental biology*, *202*(11), 1481-1490.

Schuster, S., Strauss, R., & Götz, K. G. (2002). Virtual-reality techniques resolve the visual cues used by fruit flies to evaluate object distances. *Current Biology*, *12*(18), 1591-1594.

Sobel, E. C. (1990). The locust's use of motion parallax to measure distance. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *167*(5), 579-588.

Virsik, R. P., & Reichardt, W. (1976). Detection and tracking of moving objects by the fly Musca domestica. *Biological Cybernetics*, *23*(2), 83-98.

Attention is required to explain quick choices in free walking blowflies

Introduction

Despite being known mostly for their flight behavior, flies often land and explore their environment by walking. During such explorations, flies have frequently been observed to approach distinct dark objects on a bright background (Wehner 1972). The mechanisms flies use to orient themselves towards such objects have been mostly studied in tethered flight, where it has been revealed that, on average, they tend to fixate vertical bars in the frontal visual field by generating a torque that depends on the bar's azimuthal position. In blowflies, the torque is always directed towards the side on which the bar is seen and increases until the bar is 30° away from the front, at which point the torque starts dropping until it is zero when the object is at 0°, i.e. frontal (Reichardt 1973). This characteristic tuning curve results in moving the object into the frontal visual field of the animal and to stabilizing it in front of the animal. A similar mechanism was proposed for walking *Drosophila* (Horn and Wehner 1975, Horn 1978).

When a blowfly is confronted with two objects in a closed-loop tethered flight scenario it fixates one of the objects unless the objects were close to each other; then, on average, it fixates the midpoint between them (Reichardt 1973, Reichardt and Poggio 1975). Based on this behavior it was proposed that, if confronted with two objects, the flies reacted to both objects simultaneously, resulting in an average torque as predicted by the summation of the torque responses associated with the two objects when presented in isolation. This was derived from the observation that, when the objects are separated by 60° or more (i.e. the distance between the angles of maximum torque), one objects could be fixated frontally despite the torque generated by the remaining object. If the objects were separated less however, both objects end up generating similar torques as the animal was moved towards the mid-line between the two objects (Reichardt 1973, Reichardt and Poggio 1976). Similarly, the behavioral data obtained from walking *Drosophila* facing two or three objects could be explained by adding the expected responses elicited by the objects when presented individually (Horn and Wehner 1975).

However, later work on tethered *Drosophila* revealed that, when scrutinizing the time-dependent behavior in an open-loop two-object paradigm where one bar oscillated synchronously and in anti-phase in each half of the visual field, individual flies did not behave according to the summation of the responses induced by each object alone. Instead, they responded as if only one bar was present,

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apparently ignoring the other for long time windows before switching to respond to the other bar (Wolf and Heisenberg 1980). This behavior has been interpreted as a consequence of selective attention and attention switches between the objects.

These analyses were performed under tethered flight conditions, where the animal could not approach the objects, essentially simulating a condition where the objects were at infinite distance, a situation occurring in a fly's real-life only under very special conditions. To overcome this limitation, we performed behavioral experiments on free-walking flies in a specially designed object choice paradigm. The use of a walking paradigm made it possible to allow the animal to approach objects while making it much easier to contain the animals within a limited area than if they were flying. Some work has been done to address which object parameters appear to be attractive to the flies resulting in frequent approaches. For instance, it has been established that the object preference of walking *Drosophila* depends on how high and broad a bar was (Wehner 1972). The flies also seemed to prefer closer objects in a virtual reality closed-loop paradigm, where the distance of bars was simulated on a screen by the relative motion coupled to the animal's self-motion, which led to the hypothesis that flies use relative motion to estimate distances (Schuster et al. 2002). However, further work (Mronz 2004) revealed that flies were even attracted by bars if the relative motion of the bars was inversely coupled to the movement of the animal generating a highly unnatural stimulus at odds with the notion of distance, suggesting that fruit flies were attracted by faster-moving bars in general. Despite knowing several factors that account for object preferences and fixation of walking flies, a comprehensive concept of how individual flies select a goal to approach among available objects is still lacking.

To address this issue, we developed a behavioral paradigm in which we recorded the object selection behavior of freely walking blowflies (*Lucilia cuprina*). We analyzed whether blowflies show any preference based on the object's azimuth position, like other flies do, and tried to predict the preference of the animal using simple object parameters, such as which object is seen first, or which object is more frontal. We then derived a behavior-based model for object choice that combines the simultaneous summed response to individual objects with the ability to occasionally 'ignore' one of them. Our model can reproduce the choice behavior of walking flies and their preference for frontal over lateral objects. It further reveals that apparent choice behavior can be explained without the need of a specific decision module.

Methods

Animals and animal preparation

For our experimental analysis, we employed female blowflies (*Lucilia cuprina*) bred in our laboratory. Animals were captured 1-3 days after hatching, briefly anesthetized with CO_2, and prevented from flying by placing a drop of wax on the wing joints. The prepared animals were kept in a cage with free access to sugar and water.
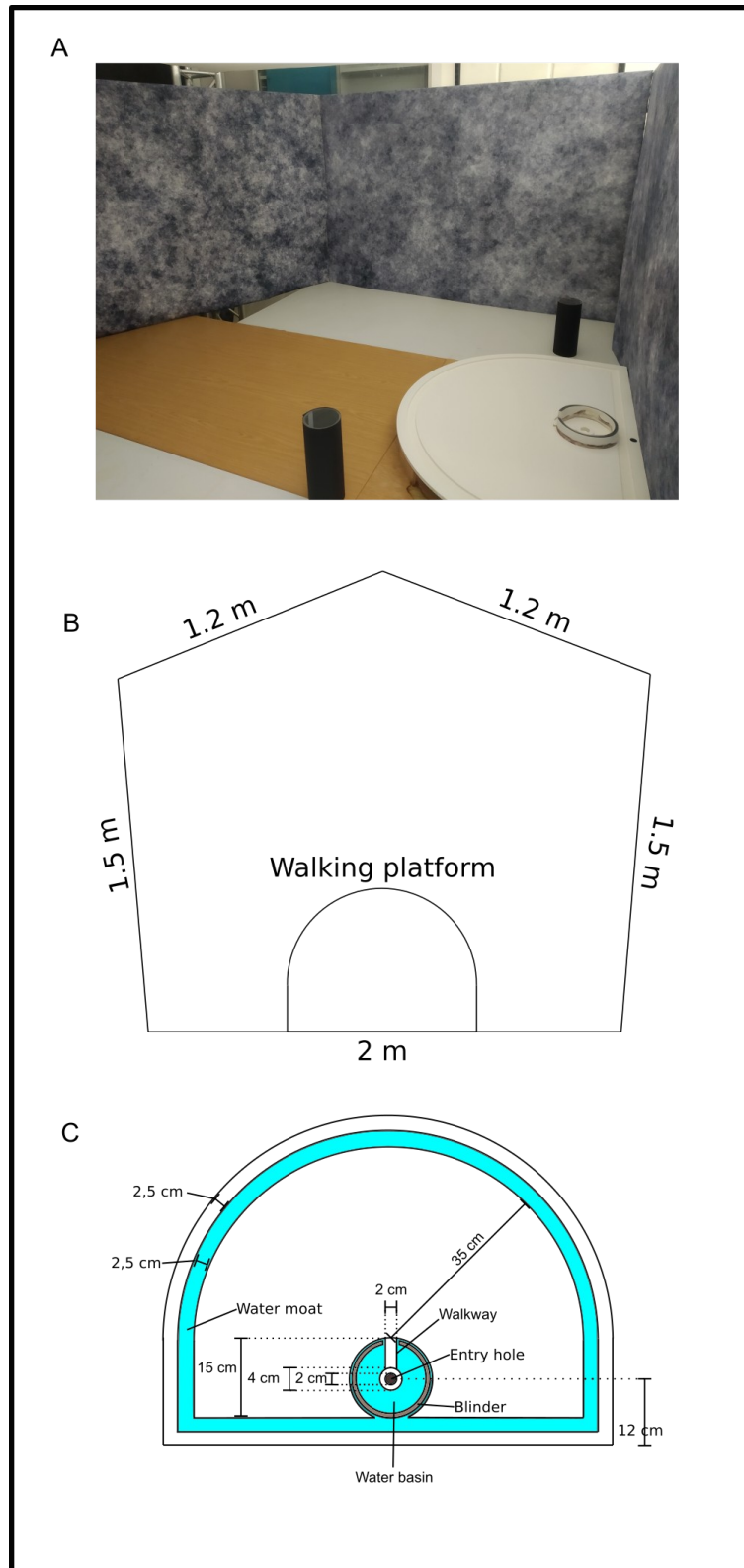
Experimental setup

Our experimental setup (Figure 1 A) consisted of a pentagonal arena (for dimensions see Figure 1 B), constructed of blank canvases. The walls of the arena were covered with a random cloud pattern with spatial frequencies f characterized by 1/f spectrum.

A walking platform was placed within the arena, centered against the 2 m long wall (See Figure 1 B). The walking platform was a 1 cm thick plate of white PVC (for dimensions see Figure 1 B) with an entry hole drilled through which an animal could be introduced into the setup. Upon entry, the animal is surrounded by a water basin, and the view into the test arena is initially occluded by a blinder surrounding it, except for a walkway connecting the entry hole to the test arena. In the actual test arena, the animal is prevented from leaving the walking platform by a water moat surrounding its edges. Both the water moat and the basin are connected and are 0.5 cm deep. The walking platform was elevated on wood blocks of 5.5 cm height to prevent the flies from seeing the lower borders of the walls and the table surface with the walls placed on it, allowing us to better control what the fly was seeing.

Cylinders of 8.2 cm diameter and 20 cm height were placed at previously marked positions at a distance of 60 cm from the end of the walkway, i.e. the point where the center of the walkway connected with the end of the moat around the entry hole. The cylinders were placed at three different angular positions, with 0° being in front of the end of the walkway and 90° being to its side. If more than one object was placed in the arena, one was placed in the right half of the arena and the other in the left half.

This setup allowed us to release an animal into an arena where its view on the objects was initially occluded. When the blowflies walk along the walkway the blinders eventually stop occluding their view. Since the fly has to walk roughly parallel to the walkway this allows us to effectively control

Figure 1. Sketch of experimental setup. A) Photograph of the experimental setup, with three of the five walls of the arena visible as well as the walking platform, elevated on 5,5 cm high blocks (not visible), and two objects. Objects visible are at 90° and 37° positions. B) Sketch of a top view of the arena walls and position of the walking platform. Arena walls are 1 m high C) Sketch of the walking platform. The platform is 1 cm thick. The moat is 0.5 cm deep. The blinder is 0,7 cm thick, 3 cm high, and leaves 0,5 cm to both sides of the walkway. Blue areas mark the moat and basing filled with water during recordings.

the orientation of the animal when objects become visible and thus at which azimuthal position the objects first appear.

The arena was illuminated by 6 white LED lamps (GSVITEC Marathon MultiLED, GS Vitec GmbH, Gelnhausen, Germany) placed above the arena and shining through a diffusion cloth that covered the entire arena. The resulting softer light reduced shadows and allowed for easier tracking of the walking flies.

The behavior of walking blowflies was recorded at 90 frames/second using a camera (Basler ac A2040 – 90um, Basler 112 AG, Ahrensburg, Germany) placed above the arena and a custom-made program based on the Pylon 4 software suite (Basler, Basler AG, Ahrensburg, Germany). Animals were tracked on the video footage using the open-source software ivTrace (https://opensource.cit-ec.de/projects/ivtools), which allowed us to track the center of mass of the body of a walking blowfly. The animal's orientation was also automatically determined by fitting an ellipse to the body and using the orientation of its long axis as the animal's orientation. The results of tracking were manually reviewed and found to adequately fit the position and orientation of the animals.


Experimental procedure

Individual blowflies were taken from the cage and released from below through the entry hole into the experimental setup. Flies were recorded until they reached the borders of the walking platform. If an animal attempted to take off or failed to reach the borders of the platform because it refused to walk, it was captured and released again. Each animal was recorded under a given stimulus condition until it reached the borders of the platform 10 times. We recorded blowflies walking under seven conditions: in the absence of objects, in presence of one object at 37°, at 60°, or at 90°, and in the presence of two objects at 37° and 60°, at 37° and 90°, or at 60° and 90°. For each condition, we recorded 10 different flies. For both the one- and the two-object condition, the object constellation was mirrored along the symmetry axis of the walking arena according to a pseudo-random sequence, to control for any potential asymmetry that might have escaped our notice.

Modeling: Object Response Addition Model (ORAM)

We attempted to account for the experimental data with a model that was inspired by previous work (Poggio and Reichardt 1973, Horn and Wehner 1975), which proposed that the fixation behavior of flies is due to the sum of two behavioral components: (1) object-induced turning behavior, with the torque generated depending on the azimuthal position of the object and (2) a spontaneous turning

tendency characterized by filtered white noise fluctuations (FWNF). The filter kernel was determined based on the spontaneous walking behavior of the fly (see Results).
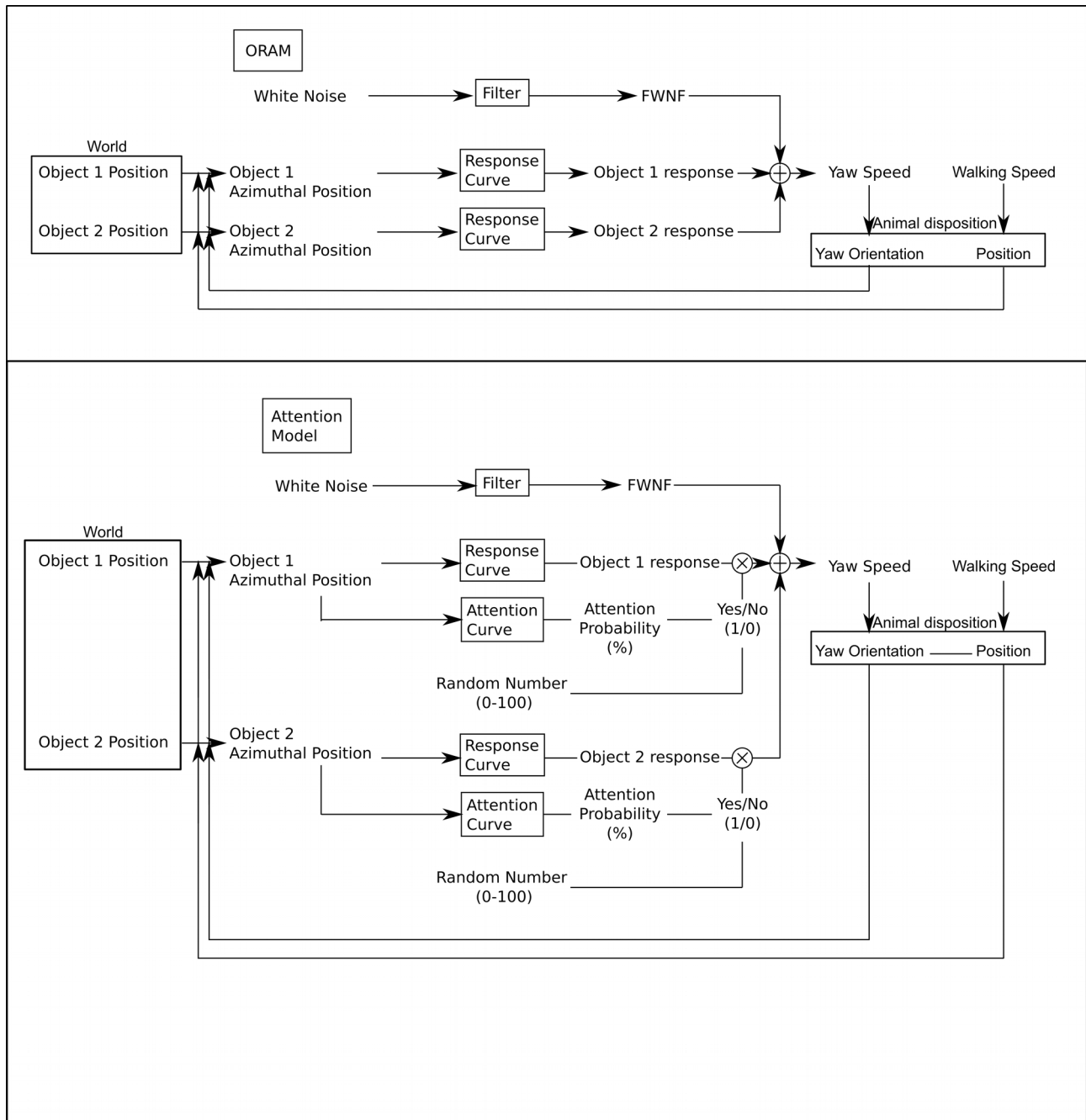
Figure 2. Sketch of models. A) Object response addition model (ORAM). Filter kernel is the inverse of the fast Fourier transform of the yaw speed observed in absence of objects. Filtered white noise fluctuations (FWNF) are added to the response generated by both objects. The resulting change in yaw orientation together with the position updated by a constant speed result in a change in the azimuthal position of the objects. B) Attention model (AM). Same filter kernel as ORAM. Object response of each object is only added to FWNF with a certain probability determined by the attention curve. The resulting yaw speed updates yaw orientation which together with a constant walking speed updates the animals position. The resulting position and yaw orientation update the azimuthal position of the objects.

To allow for a comparison between the model performance and the corresponding experimental data, we modeled the equivalent of 10 flies performing 10 walks each. The modeled animal was initially located at the end of the walkway because we aimed to model the behavior of the animal once it was no longer constrained by the walkway. The exact position of the animal and its orientation at the start of a simulated trajectory was chosen to equal the position and orientation of the experimental animals under the corresponding condition.

This model assumes that at any given time point the animal walks forward in the direction it is oriented. The walking speed was constant throughout the simulations and corresponded to the average walking speed of 6.4 cm/s of our experimental animals along their trajectories (see Results). The simulated trajectory was updated at a 90 Hz frequency, that is, in time steps of roughly 11.11ms duration. The orientation of the animal was updated as determined by its yaw speed, which is in turn controlled by two components: the FWNF and the object response.

The FWNF were derived from the behavior recorded in the absence of objects. We determined the mean fast Fourier transform (FFT) of the yaw speed for all recordings lasting at least 512 frames. This limitation allowed us to use 47 out of a total of 100 recordings. This decision was a compromise between the lowest frequencies that could be determined and the number of traces that could be used for the FFT. We modeled random yaw speed profiles with frequencies corresponding to those characteristic of walking flies in the absence of objects by convolving a white noise signal with the iFFT of the mean FFT of the yaw velocity traces and by normalizing the modeled traces to the mean STD of the yaw velocity of experimentally determined traces. In this way, we generated FWNF of 10.000 time steps length which determined the yaw speed of the animal at each time step in the absence of objects.

The object response was obtained from the behavior of blowflies in the presence of objects, by plotting the yaw speed against the azimuth position of the objects. We fitted the following function to the mean yaw speed at each azimuthal position of the object:

$$f(x) = A \sin(x + B(x))$$

Where f(x) is the yaw speed, x the object azimuth, and A and B are free parameters that control the yaw speed at the moment of peak speed and the position of the peak, respectively.

We assumed a response delay of the fly of 4 frames (~44,44 ms), i.e. the object response was calculated for the object position 4 frames prior. At any given time point the yaw speed of an animal is the sum of the FWNF and the yaw speed determined by the object response. When two objects are present, the response of both objects is added to the FWNF.

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Modeling: Attention Model (AM)

To account for the behavior of walking blowflies (see Results) we concluded that the animals may somehow ignore one of the objects for some time interval. To account for this, we developed the Attention Model (AM) as an elaboration of the ORAM (Figure 2 B): Like in the ORAM the yaw speed was the result of the spontaneous yaw fluctuations, modeled as FWNF, and an object-induced turning response, referred to simply as object response. However, whether the object response was added to the FWNF or not followed a random process that depended on the object's azimuthal position. Thus, at each point in time at which the object response was to be added, the azimuthal position of the object was evaluated. Then the corresponding object response was only added to the FWNF with a certain probability, which depends on the object's azimuthal position. The function describing the probability depending on the position of the object will be called the attention curve. Before the object response was added a random number between 0 and 100 was generated and compared with the corresponding probability (in percent) given by the attention curve. The object response was only added if the number was below the probability, otherwise, the object response was set to 0°/s. The attention curve is bell-shaped and centered around 0° since we observed that animals approached frontal objects with a higher probability than more lateral ones (see Results). The attention curve was generated as a modified von Mises distribution with μ=0 and a scaling factor A. μ=0 makes sure that the maximum probability is at 0° and the scaling factor A allows the maximum probabilities to reach values of up to 100%. The function looks as follows:

$$f(x) = A \frac{e^{k \cos x}}{2 \pi I_0(k)}$$

where f(x) is the attention probability as a percentage, x is the azimuthal position of the object, A is the scaling factor and I_0 is the modified Bessel function of order 0.

Results

To gain insight on how walking blowflies select their goal, we analyzed their behavior in a walking paradigm where they were allowed to move freely and then developed a model capable of reproducing key features of their behavior.

Object preferences depend on azimuth position.

We first determined whether the frequency of object approaches depends on its azimuthal position as was described for walking fruit flies (Mronz 2004). We released a single animal in each

experimental run into an arena. The view of the surroundings was initially occluded by a blinder, except for a narrow walkway, which the fly had to walk along to get, at its end, an unimpeded view into the arena. While walking along the walkway, their orientation was constrained until the objects became visible, allowing us to evaluate whether objects were approached differently often depending on where in the visual field they were initially visible.

As a reference, we first evaluated the walking behavior of blowflies in an environment without any obvious visual object. Flies were allowed to walk freely in the largely featureless walking arena and their trajectories were recorded. In such a scenario, blowflies move out of the walkway and then proceed along either fairly straight trajectories or in some cases on a curved path (Figure 3 A). This is consistent with the behavior observed in *Drosophila* (Soibam et al. 2012). One might have expected spiraled exploration walks instead of the relatively straight trajectories. This kind of
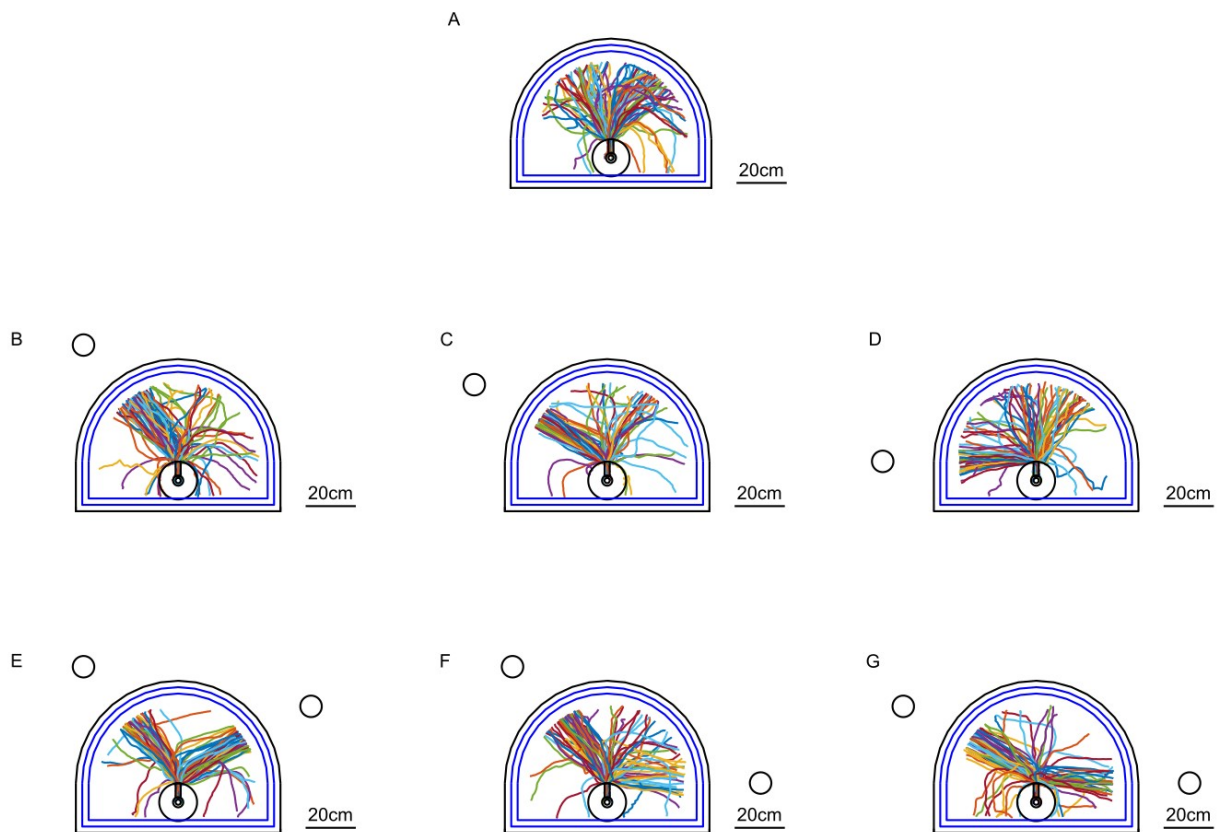

Figure 3. Trajectories of walking blowflies. Objects when placed are located at 60 cm distance and a certain azimuth position, with 0° being in front of the catwalk exit. A) In absence of any objects. B) With one object at 37° C) With one object at 60° D) With one object at 90° E) With two objects, one at 37° and one at 60° F) With two objects, one at 37° and one at 90° G) With two objects, one at 60° and one at 90°

behavior has been observed in flies after finding a food source, such as a drop of sugar water (Takahashi et al 2008).

Next, we evaluated the behavior of walking blowflies in the presence of a single object and assessed if they showed a preference based on the object's azimuthal position. Therefore, the object's position in the arena was varied in different experiments (Figure 3 B-D). Irrespective of object position, most animals approached it in a very obvious manner along paths that are fairly straight right from the moment they leave the walkway. However, other animals walked in different directions on similarly straight trajectories, although some were curved along most of their trajectory. Few flies even turned away from the object after initially heading towards it. The objects were approached with a variable probability depending on their position in the arena (Figure 4 A-C). An object at 37° or 60°, as seen from the perspective of a fly at the end of the walkway when aligned with the walkways axis, is approached more frequently than an object at 90°; objects at 37º and 60º are approached similarly often (compare Figure 4 A and B to C).

When confronted with two objects, most flies approached one of them along paths that appeared to be similar to the ones observed in the presence of only a single object (Figure 3 E-G). Again, a variable proportion of flies walked to none of the objects (Figure 4 D-F) or turned away from what initially seemed to be a path towards an object (Figure 3 E-G). Flies preferred a more frontal object
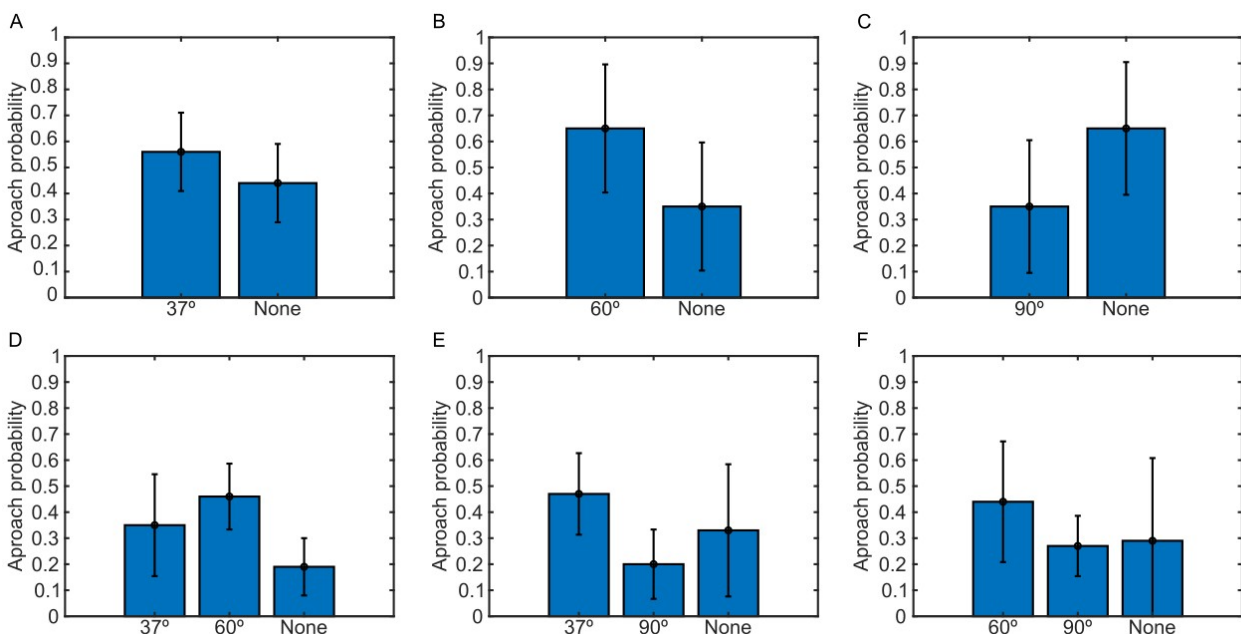
Figure 4: Object preference in walking drosophila. Mean +- S.E.M of the proportion of approaches to an object, or to no object, across flies (N=10). A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°

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(37º or 60º) over a lateral one (90°), in accordance with the object preferences determined in the single-object situation (Fig 4 D-F). In addition, we observed similar approach probabilities for the two frontal objects (37º and 60º).

We conclude that walking blowflies show a preference for objects depending on their initial azimuthal position, with frontal objects being preferred over lateral ones, both when only a single object is presented and when the flies were allowed to choose between two. Despite reacting to objects in an obvious manner, walking blowflies ignored any object in a sizable proportion of walks for all object configurations and walked in other directions.

When approaching objects flies react quickly, regardless of object position

To assess what determines the decision-making of flies with the option to orient themselves to one or the other object, we analyzed what the flies have seen before selecting an object. This makes it necessary to find out when the animals decide. As a proxy for a performed decision, we determined when and where flies start fixating the object.

When blowflies start moving towards their final goal they mostly keep moving in that direction (Figure 3), suggesting that flies fixate an object and maintain it frontally. Thus, we defined the start of object fixation as the onset of the time window where at least one of the object's edges has been kept in the frontal visual field, i.e. within ± 30° relative to the midline of the animal, for at least 100
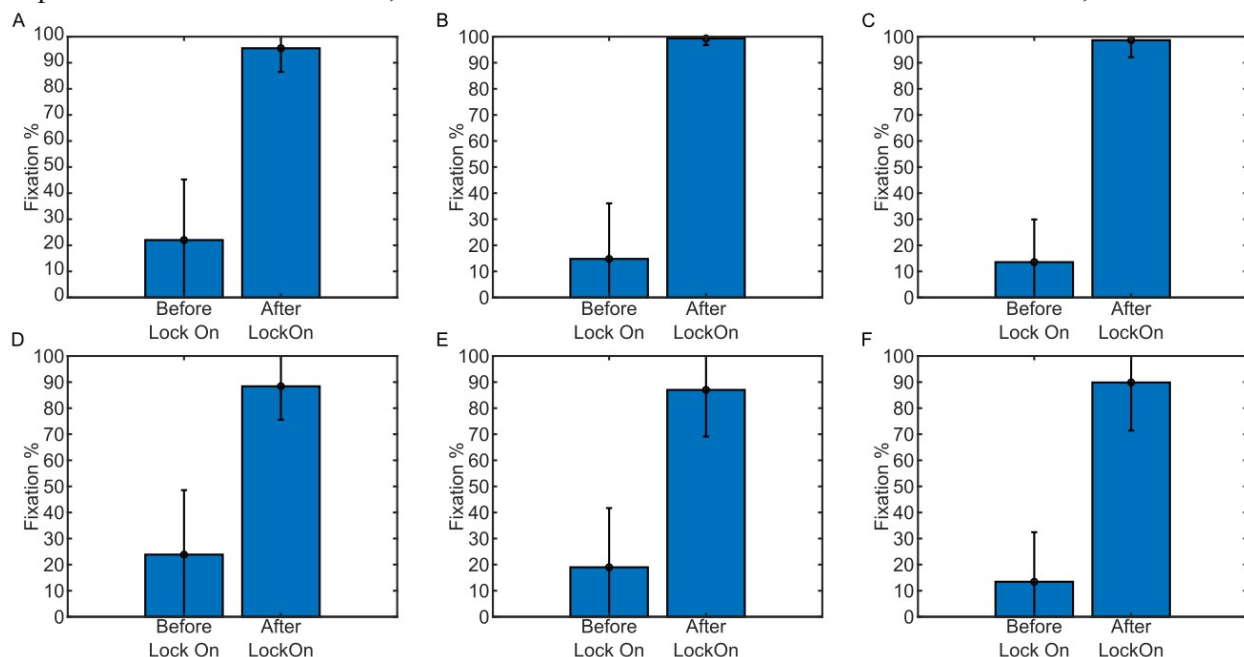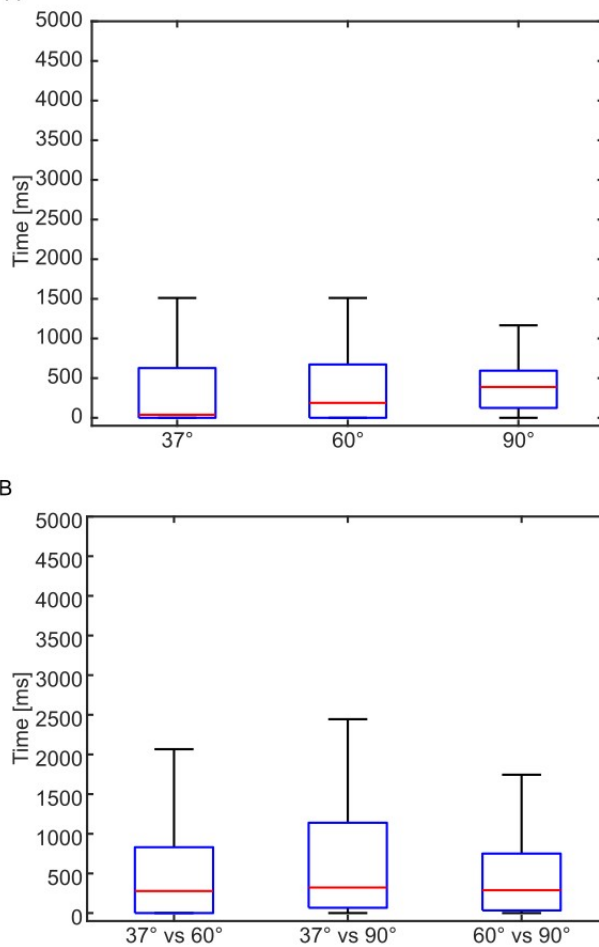

Figure 5: Robustness of fixation in walking blowflies. Mean +- S.E.M. of the mean % of time spend fixating the goal (object or object edges at +- 30°) across flies (N=10) before and after the lock on. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°

frames ($\sim$ 1.11s). To assess whether a fixation
onset as defined in this way is a good proxy,
we calculated the percentage of time the fly
keeps the object in this retinal area before and
after fixation onset. Before that point in time,
there is little object fixation, whereas
afterward fixation is maintained for most of
the time in all our recordings, independent of
object position (Figure 5). This finding
suggests that once flies start fixating their goal
they tend to stick to this decision. Therefore,
we consider the time point determined in this
way as a good indicator of when the animal
has completed its decision to approach an
object.

The decision to approach an object appears, in
most cases, to be made quickly after leaving
the walkway, as the trajectories in the
presence of objects are relatively straight
between close to the end of the walkway and
the object (Figure 3). We verified this
impression by determining the time between
leaving the walkway and the onset of object

**Figure 6: Time passed since leaving the
catwalk till lock on.** Boxplot of time in ms. A)
In presence of a single object, at 37°, 60°, or 90°.
B) In presence of two objects at 37° and 60°, 37°
and 90°, and 60° and 90°.

fixation (Figure 6). When facing a single object, the time to fixation onset is very similar
irrespective of object position, with only a slight tendency to take longer as the object is more
lateral. When two objects are present the time to fixation onset is equally similar irrespective of
object position and is only slightly larger compared to the single object experiments (Figure 6 A).
This finding suggests that flies take a very similar amount of time to respond to the objects
regardless of their position and that, even in a two-alternative choice situation, they only take
slightly longer to decide.

We further quantified the locations on the walking platform of the onset of fixation and whether this
location depends on the object's position (Figure 7). Following the above conclusion that the
decision process is usually very quick, fixation onset takes place in many cases already on the
walkway or immediately upon leaving it irrespective of object position; only in few cases are

fixation onsets located at some distance away from the walkway (Figure 7). Hence, the object to be approached appears to be selected on the walkway or soon after leaving it.
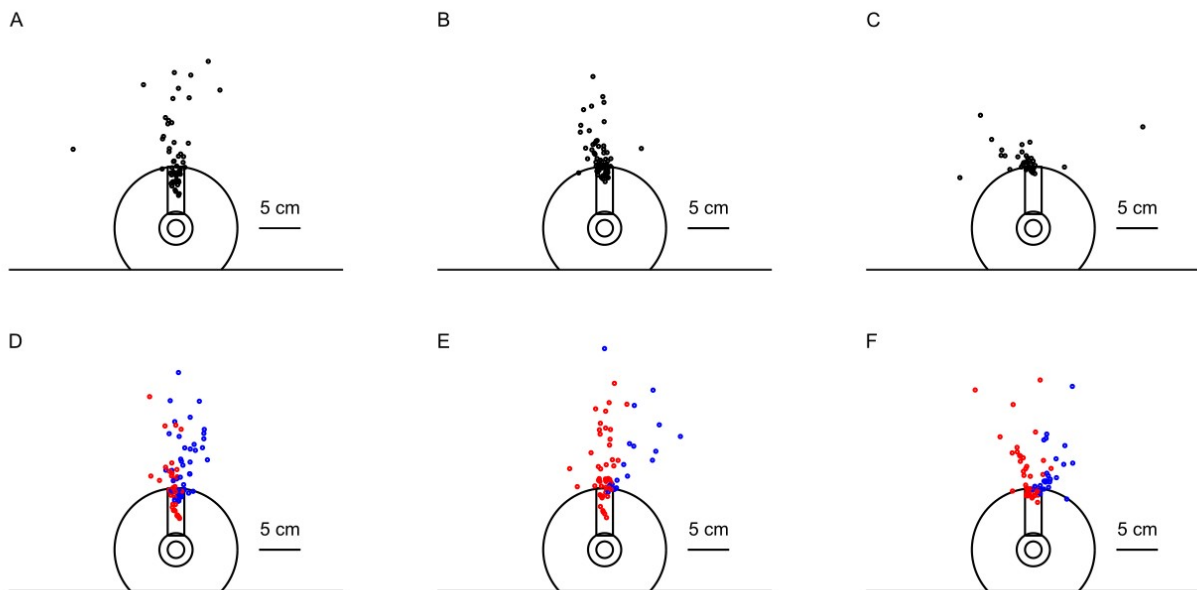
Figure 7: Location of lock on moment. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60°, lock on to 37° in red, to 60° in blue E) With two objects, one at 37° and one at 90°, lock on to 37° in red, to 90° in blue F) With two objects, one at 60° and one at 90°, lock on to 60° in red, to 90° in blue

Possible determinants of object choice

To understand how blowflies select the object they will approach, we determined whether the animals tend to select the object they saw first. We calculated the phi-coefficient as a measure of the correlation between an object seen first and the object eventually approached (Table 1). When the two objects are in a frontal position (37° and 60°) there is no correlation between seeing one of them first and approaching it (Phi-coefficient= 0.14). Furthermore, if one of the objects is placed at 90° it is never seen first but is still approached roughly in a third of the times (Table 1) (compare Figure 3 A to Figure 3 D, F, G). Hence, whether an object is seen first is unlikely to be a major determinant of object choice.

37° vs 60°	Saw 37° first	Saw 90° first	
Approached 37°	47	0	47
Approached 90°	20	0	20
N	67	0	**Phi**
80			0.1412
37° vs 90°	Saw 37° first	Saw 90° first	
Approached 37°	47	0	47
Approached 90°	20	0	20
N	67	0	**Phi**
37			Undefined
60° vs 90°	Saw 37° first	Saw 90° first	
Approached 37°	44	0	44
Approached 90°	27	0	27
N	71	0	**Phi**
71		0	Undefined

Table 1. Correlation between first seen object and approached object

We then analyzed whether walking blowflies tend to approach the more frontal object by calculating the correlation between an object being the more frontal and being approached. Because this correlation is likely to change along the walk, we calculated the correlation at three different time points: (1) when leaving the walkway, which is the first time animals have an unrestricted view into the arena; (2) at the last turn before onset of object fixation, i.e. just before the final turn of the animal leading to object fixating, and (3) at the onset of object fixation, which serves as a control as the approached object is then frontal by definition. For all these points, we calculated the phi coefficient as a measure of correlation (Figure 8). The
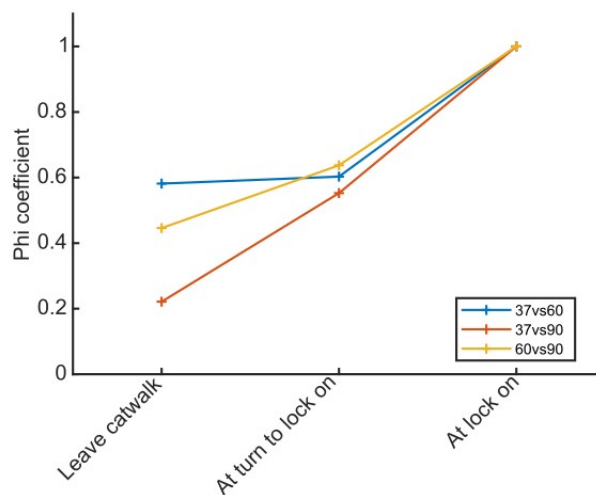
Figure 8: Correlation between being most frontal and being approached. Phi correlation between an object being the most frontal and an object being approached upon leaving the catwalk, at the turn to lock on, and at the lock on start, when choosing between two objects at 37° and 60°, 37° and 90°, or 60° and 90°.

correlation between an object being frontal and being approached increases steadily once the animal leaves the walkway. Upon leaving the walkway the correlation is positive but moderate and varies between object constellations (Phi=0.58 when objects are at 37° and 60°, Phi=0.45 when objects are at 37° and 90°, Phi =0.58 when objects are at 60° and 90°). At the time of onset of the final turn before object fixation, we find fairly similar and moderate Phi-coefficients for all object constellations (Phi=0.64 when objects are at 37° and 60°, Phi=0.60 when objects are at 37° and 90°, Phi =0.55 when objects are at 60° and 90°). After the animal started turning to eventually reach the onset of fixation, the correlation is, by definition, 1. Thus, we conclude that which object is seen more frontally predicts to some extent the choice of the animal.

Object response addition model (ORAM)

Walking blowflies make remarkably quick choices, often starting to approach their goal as soon they leave the walkway. How can this behavior be explained? In tethered flight Poggio and Reichardt (1973) explained object choice as the result of each of the present objects independently leading to a torque component that is added to spontaneous torque fluctuations. The torque caused by an object ('object response'), follows a characteristic curve with the torque induced by an object depending on its azimuthal position in the visual field (Reichardt and Poggio 1976). We analyzed whether such a model can explain the quick decisions we observed for freely walking blowflies. Therefore, we developed a model we call Object Response Addition Model (ORAM) as an adaptation to the walking behavior of Reichardt and Poggio's model (Figure 2 A).

Since our model is intended to describe the behavior of a free-walking animal, rather than exclusively the rotation of a tethered animal, we need to make assumptions regarding its translation velocity. Thus, we determined the mean walking speed of blowflies before and after the onset of fixation, to find out how much the walking speed varies and if it changes once the animal started fixating the object. Across all experiments, the mean walking speed before and after fixation onset does not differ systematically (Figure 9), suggesting that flies do not change their speed once they decided to approach an object. Since we also did not find any systematic differences in speed across object constellations, we set the modeled walking speed at the constant value of v=6.4 cm/s, i.e. at the average speed of blowflies recorded across all object constellations.

In contrast to the studies on tethered flying flies where yaw torque could be measured (Poggio and Reichardt, 1973) we could only determine yaw velocity in our video footage. Thus, we used yaw speed for our model analysis. Moreover, we generated filtered white noise fluctuations (FWNF) to reproduce the spontaneous changes in yaw speed present in the absence of objects. To generate the
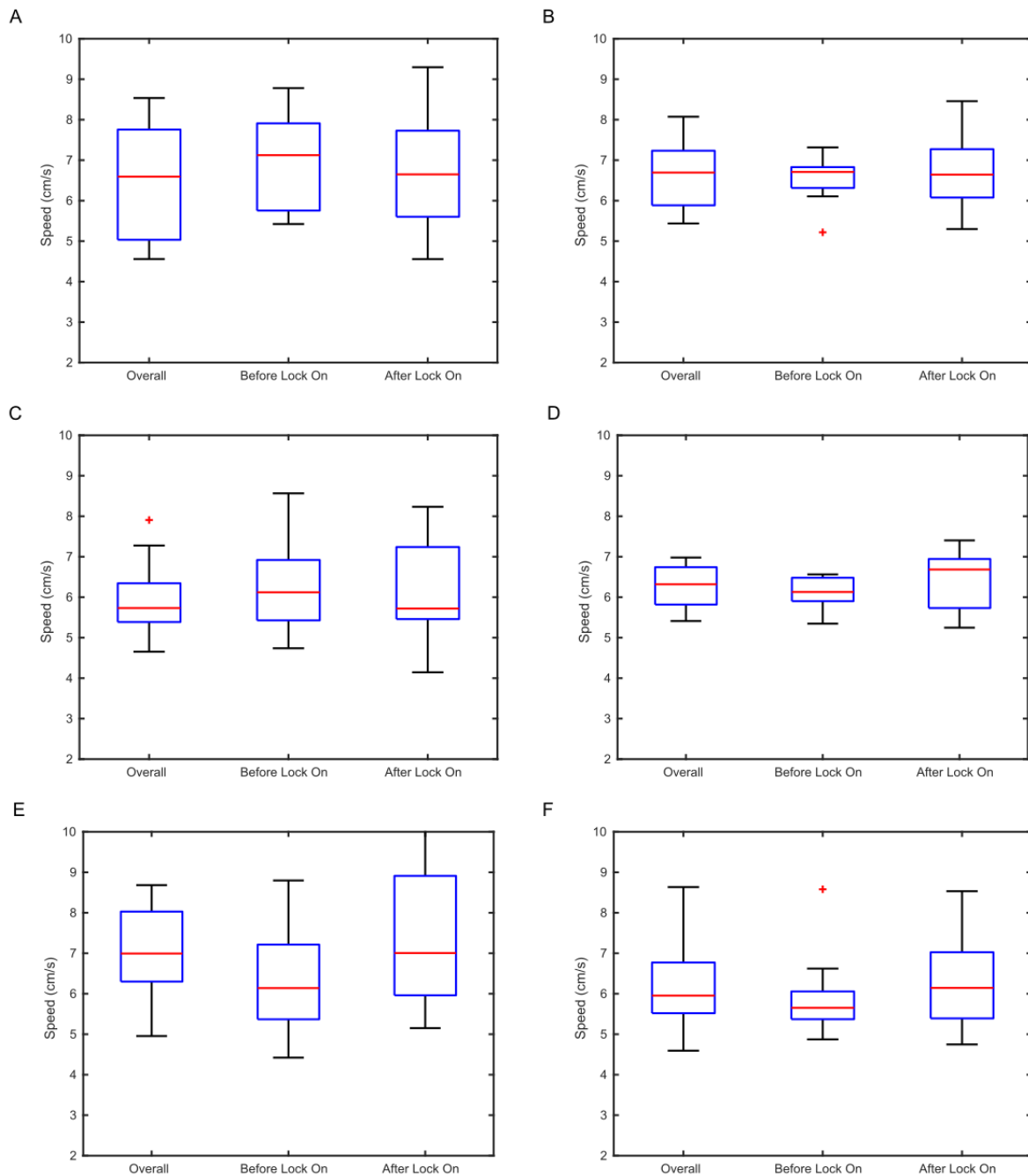
Figure 9: Comparison of walking speed of blowflies before and after lock on. Boxplot of the mean walking speed of blowflies, over the whole trace, before the lock on, and after the lock on. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°

FWNF, we performed a Fourier analysis of the time-dependent yaw speed of walking blowflies recorded when no objects were present. The amplitude spectrum (Figure 10) is characterized by two peaks, one at 1.059 Hz and another at 10.59 Hz. The first peak reflects that some flies walk along slightly curved trajectories. The second peak fits the frequency expected for stride-coupled oscillations of the body (Kress and Egelhaaf, 2012, and Kress and Egelhaaf, 2014). Both have been
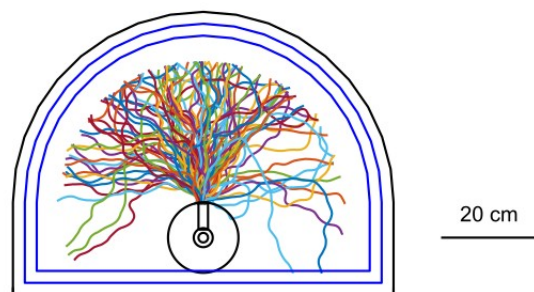
previously described as being present both when approaching a vertical bar and when exploring a homogeneous arena in walking *Calliphora* (Horn and Mittag, 1980). We generated a linear filter by inverting the mean Fourier Transform of yaw velocities across all walking trajectories and generated spontaneous yaw speed profiles by convolving a white noise signal with the filter kernel determined in this way (for details see Methods). Due to the properties of the Fourier transform the resulting simulated yaw velocity fluctuations were
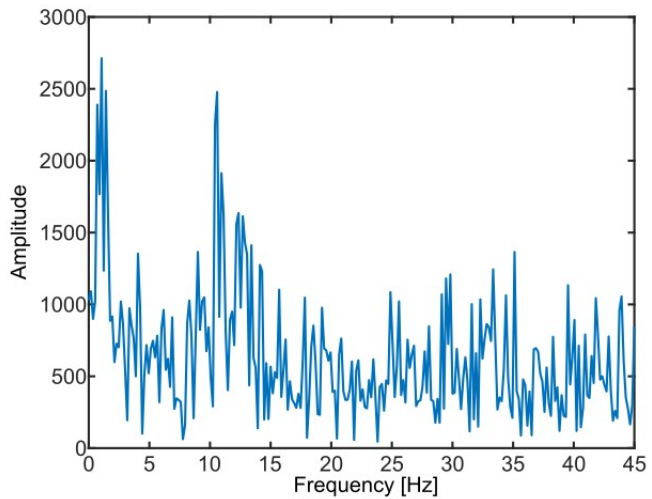
Figure 10: Frequency content of yaw speed in absence of objects. Amplitude spectrum of the mean fast Fourier transform of all recordings with at least 512 frames length after leaving the catwalk.

characterized, on average, by the same frequency spectrum as the corresponding experimentally determined walking traces, but they differed from each other as a consequence of the added noise.
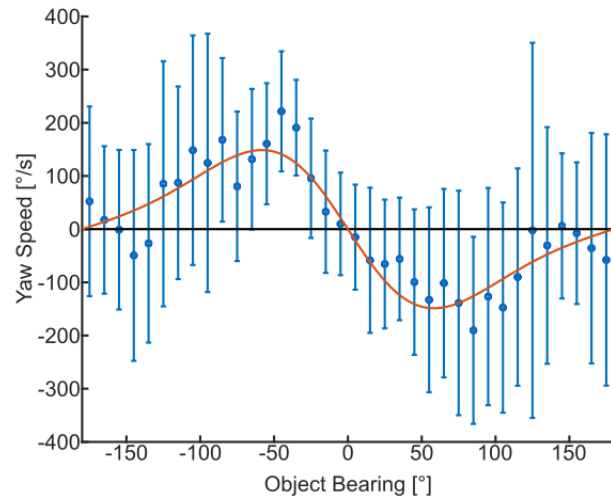
To assess whether the modeled yaw speed traces lead to similar trajectories as the ones observed in walking blowflies, we simulated 100 walks, using the bearing and position of 10 flies at the start at the end of the walkway of 10 walks each, recorded in the absence of objects (Figure 3 A). The modeled trajectories (Figure 11) consist of a mixture of rather straight paths as well as trajectories that turn slowly over their duration, like the ones we observed in walking blowflies (Fig 3 A). We conclude that this procedure leads to an adequate approximation of the spontaneous behavior of blowflies in the absence of objects.

To model the object response, we calculated the mean yaw speed of the animal for the azimuthal positions of the object as tested in the corresponding experiments. Since we aim to capture the yaw speed caused by the presence of the object, we analyzed only recordings where

Figure 11: Modeled trajectories in absence of objects. Displayed is a sample of 100 individual trajectories, each corresponding to the the starting location of one of the 100 trajectories recording on real data. Each trajectory is a different color.

an object was approached. We started the modeled trajectories only after the animal leaves the walkway and, thus, can see the object irrespective of its position in the arena. Furthermore, to capture the yaw speed at all possible azimuthal positions, we used the data obtained when a single object was initially present at 90º, as this was the only condition in which the object was present in some time windows at even the more dorso-lateral positions before being fixated. The plot of yaw speed versus azimuthal position generated in this way follows an anti-symmetrical curve (Figure 12), just as observed in tethered flying *Musca* (Reichardt and Poggio, 1975) and walking *Drosophila* (Horn and Wehner 1975). We conclude that the turning speed of walking blowflies is affected by the retinal position of an object, with the object causing larger yaw speeds when the object is more frontal until it has reached the front, at which point the yaw speed drops drastically to stabilize the object at this position. To model the response generated by an object at a given azimuthal position we used the function
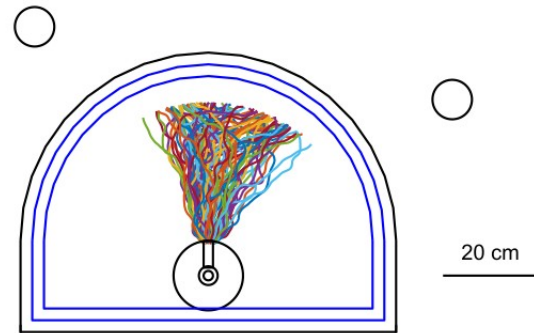
$$v_y(x) = A \sin(x + B \sin(x))$$

where x is object azimuth, v_y the resulting yaw velocity, and A and B are free parameters adjusted by a least-square fit to the experimental data ($R^2 = 0.852$).

Figure 12: Yaw speed depends on object azimuth. Mean +- S.E.M. of the yaw speed when the object is at different azimuth positions, across all recordings were a single object at 90° was approached

Although flies are able to react very quickly to visual stimuli, with delays of less around 30 ms during chases (Land and Collet 1974), a delay remains between the presentation of a visual stimulus and the reaction of an animal. The delay between the presentation of a visual stimulus and the corresponding response in motion-sensitive neurons in the third visual neuropile is roughly 25 ms for a high-contrast stimulus such as ours (Warzecha and Egelhaaf 2000). Under the assumption that it would take the walking apparatus some time to implement the response, we decided to model a delay of 44 ms.

When confronted with two objects, one at 37° and one at 60°, the trajectories modeled using the ORAM consist mostly of rather smooth curves that initially head towards the midpoint between the objects and eventually lead to one of them (Figure 13). This behavior is to be expected based on

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previous literature (Reichardt and Poggio 1976), as the tendency to turn towards one object is canceled initially, i.e. when the retinal position of the objects is relatively close, by the tendency to turn towards the other. However, this is at odds with the behavior observed in walking blowflies in the same situation (Figure 3 E), where in most cases the animals started moving towards one of the objects from the moment they left the walkway. We conclude that the summation of the yaw speed elicited by multiple objects is
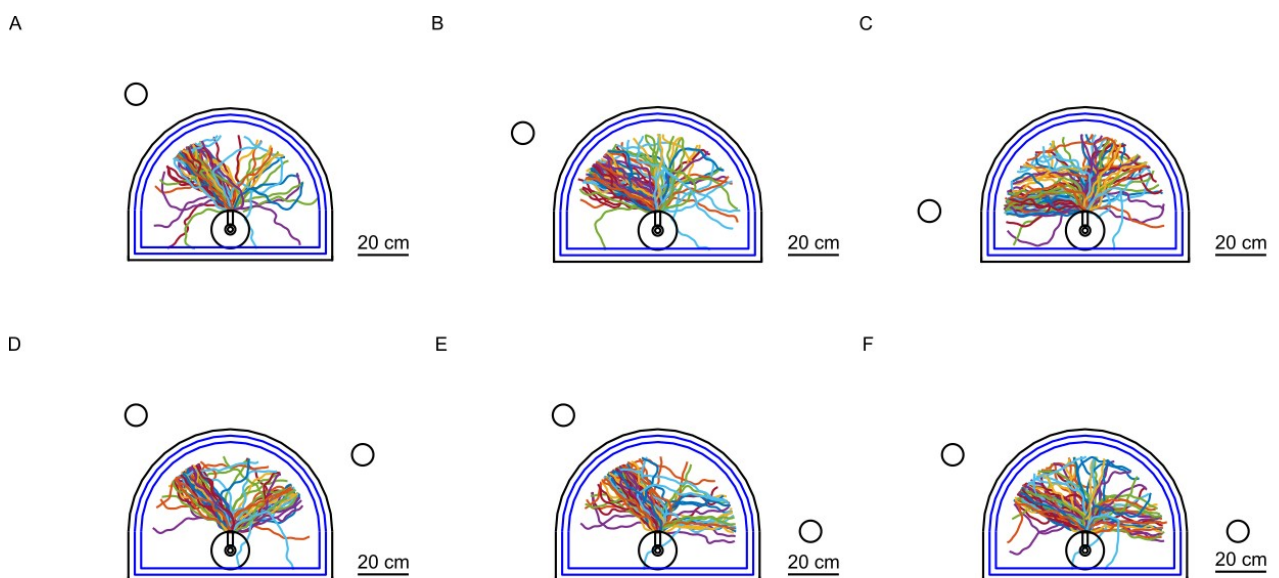

Figure 13: Modeled traces using sum of spontaneous behavior and object response. Displayed is a sample of 100 individual trajectories, each corresponding to the position of yaw of one of the recordings of real flies in presence of two objects, one at 37° and one at 60°.

not sufficient to explain the quick decision to move towards one of the objects observed in walking blowflies, where the trajectories leading to either object split early once the animals leave the walkway.


Attention model

In order to generate the early decision to walk towards one of the objects after the animal leaves the walkway, we hypothesized that the animal may ignore for some time one of the objects, i.e. does not react to it. This ability to ignore objects has been described for fruitflies (Wolf and Heisenberg 1980, Sareen et al 2011). We analyzed whether incorporation of an attention-like mechanism into our model could allow us to reproduce the quick object choice observed in walking blowflies. Therefore, we elaborated ORAM into our Attention Model (AM). The AM (Figure 2 B) modifies ORAM in one important respect: whether the object response is taken into account for generating the overall behavior depends on a random process. Depending on the azimuthal retinal position of the object, the response is taken into account only with a certain probability. Since blowflies are less likely to approach lateral objects, the probability of the object response being used is modeled by an attention curve, i.e. a modified von Mises distribution centered at 0. The attention curve has a k parameter controlling its width and an A parameter controlling the maximum probability. For each

object constellation, we modeled the walks of 10 flies with 10 runs each, using the bearing and position of the walking flies corresponding to the experimentally determined object positions and bearings when of blowflies leaving the walkway and facing the same constellation. We then calculated for each modeled fly the probability of approaching each of the two objects and of not approaching any object. We used 10 different sets of seeds to generate white noise for each starting position. To parameterize the attention curve, we systematically varied the k and A parameters and assessed by visual inspection the similarity of the modeled trajectories to the experimentally determined ones, in the first step (compare Figures 14 and 15 to Figures 3 and 4). Eventually, we decided on k=5.518 and A=96.37. The resulting curve is the circular equivalent of a Gaussian with sigma=25 and a maximum approach probability of 87.5%.

The trajectories generated by this model (Figure 14) describe mostly straight or slightly curved paths that often lead to the object in the one-object constellation, or to one of the objects when two are present. Most important, the AM accounts for the early decision to approach an object in a similar way as observed in the experimental data. Moreover, a substantial number of trajectories do not lead to an object (Figure 15) and even a few modeled trajectories seem to change direction after the simulated animal first moved for some time on a fixation course. Both findings correspond to what has also been observed in experimentally determined trajectories.

Figure 14: Modeled traces combining spontaneous response and attention gated object response. Each plot depicts the trajectories modeled using the yaw and position of one fly when exiting the catwalk. The same list of seeds was used in all conditions to assure the effect of the model was comparable. Displayed are the results of one seed list. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°
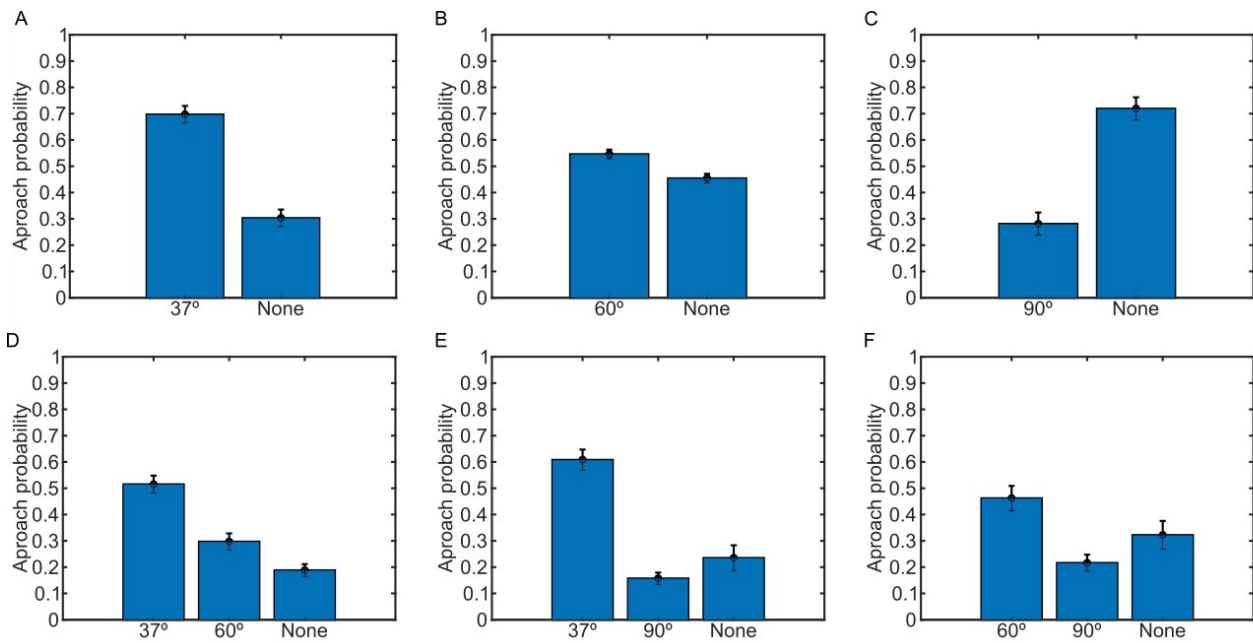
Figure 15: Object preference in modeled data. Approach probability was calculated from the trajectories generated by the position and yaw of real flies in their equivalent scenarios. To account for the effect of the model rather than that of the noise of the spontaneous behavior 10 different list of seeds where used to generate 10 sets of 100 trajectories, corresponding to 10 flies with 10 recordings each. Displayed is the mean +- SEM of the mean approach probabilities generated across the 10 seed lists. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°
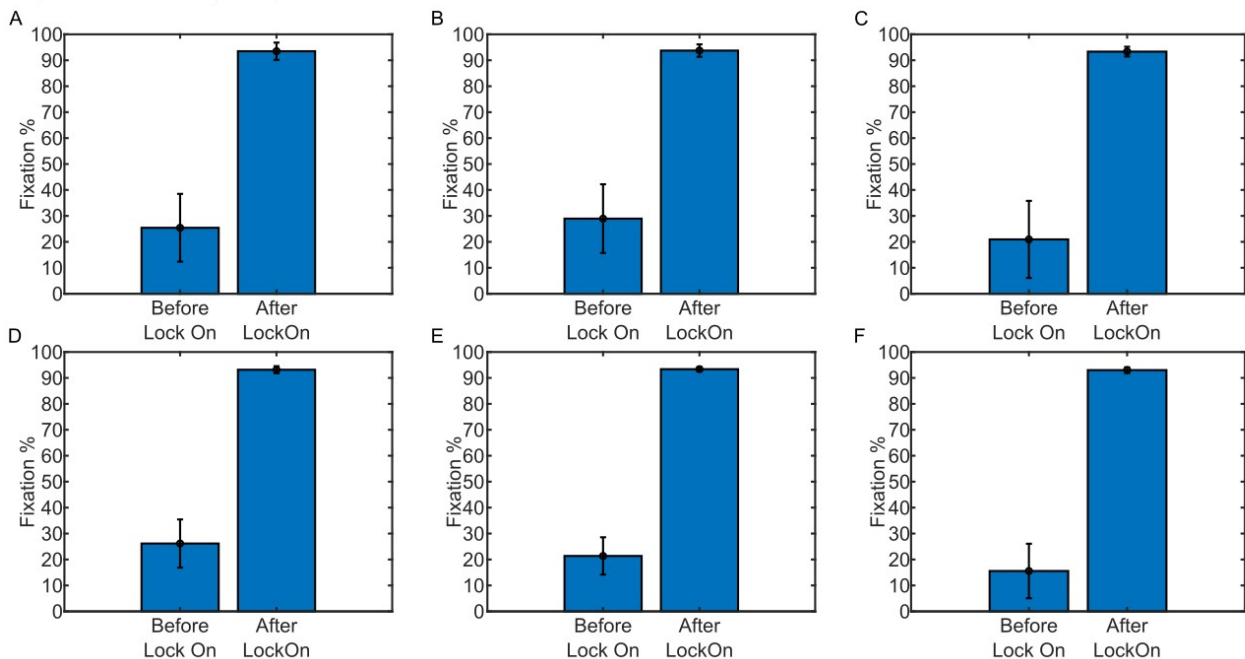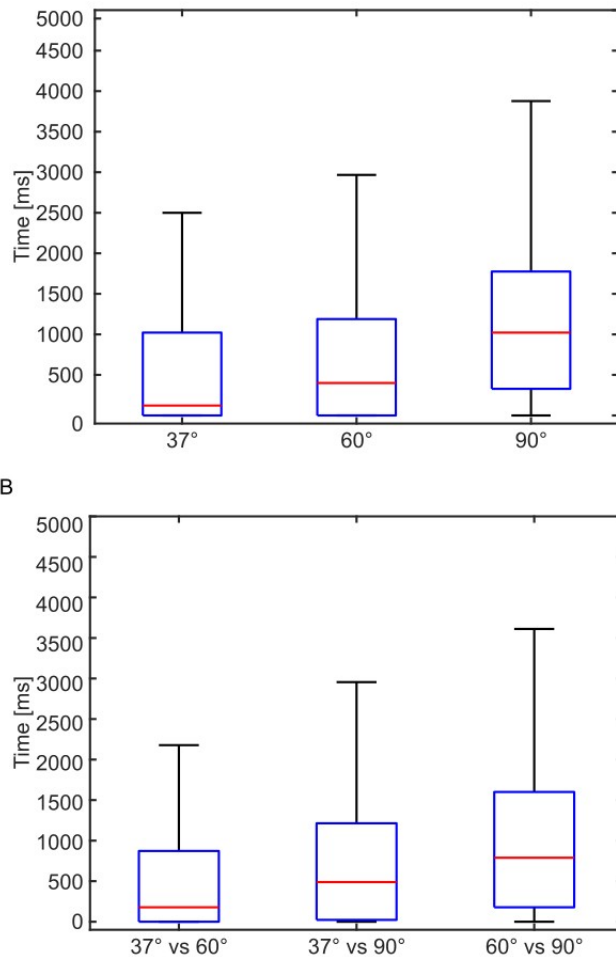

Figure 16: Robustness of lock on modeled data. To account for the effect of the model rather than that of the noise of the spontaneous behavior 10 different list of seeds where used to generate 10 sets of 100 trajectories, corresponding to 10 flies with 10 recordings each. Displayed is the mean +- S.E.M. of the mean % of time spend fixating the goal (object or object edges at +- 30°) across the 10 seed lists. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°

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In addition, the model consistently generates a preference for individual frontal and fronto-lateral objects, i.e. objects at 37° or 60°, over lateral objects, as observed in walking blowflies. This preference is also observed when the modeled animal can choose between two objects at 37° or 60° versus one object at 90°. However, the model also produces a preference for an object at 37° over one at 60° in both the one-object and the two-object constellation. This model result does not reproduce quantitatively this aspect of the corresponding experimental data.

Overall, these trajectories appear to be remarkably similar to the ones observed in walking blowflies and, in particular, seem to display the same quick choice of an object to approach as we observed in walking blowflies. To assess the similarity between experimental and model data, we first determined if we could find a clear onset of fixation in our modeled trajectories by using the same method as we used before

Figure 17: Time passed since leaving the catwalk till lock on in modeled data. Boxplot of all data obtained with 10 different seed list A) In presence of a single object, at 37°, 60°, or 90°. B) In presence of two objects at 37° and 60°, 37° and 90°, and 60° and 90°.

on the experimental data. Thus we defined the fixation onset point as the start of the first segment of recording of at least 100 frames (~ 1.11s) length where the modeled animal fixated its final goal, i.e. keeps at least one of its edges within ± 30°, and then determined the percentage of time spent fixating before and after the onset. To ensure that our conclusions are not biased by the particularities of the noise sequence determining the FWNF, we simulated trajectories based on 10 lists of random seeds and determined fixation percentages, their mean, and their SEM. Like in freely walking blowflies, there is not much fixation before fixation onset and afterward almost perfect fixation independent of the object's position (Figure 16). Having established that our model also generates a clear fixation onset we assessed quantitatively whether it could also replicate the time and location of fixation onset of the corresponding experimental data. Therefore, we

determined the time passed since leaving the walkway till fixation onset for each modeled trajectory that resulted from an approach across all 10 lists of random seeds used. We did not observe any systematic effect of the individual seed lists and, therefore, pooled the results to help highlight the effect of the model over the effect of the individual seeds, i.e. the effect of the white noise used to generate the FWNF. The time to fixation onset (Figure 17) is fairly quick in the one-object constellation if the object is at 37° and increases slightly for more lateral positions of the object. In the two-object constellation the effect is the same, with the time to fixation onset being shorter when the objects are at 37° and 60° and somewhat longer for object positions at 37° and 90° and longest for a combination of 60° and 90°. Although this position dependence is not very strong, it differs to some extent from what was observed in walking blowflies, where the time to fixation onset was almost constant independent of object position. Also, the time to onset tended to be shorter in the experiments even when compared to the shortest times observed in the model simulations.

We also plotted the locations of the fixation onset for the modeled blowflies (Figure 18). For both the one- and two-object constellation, fixation onset is located close to the start of the trajectory at the end of the walkway, but in quite some cases it is located at some distance to the end of the walkway, especially when one object is at 90°. In these cases, the fixation onset locations are fairly widely spread in the half of the arena where the object is placed. This again differs to some extent
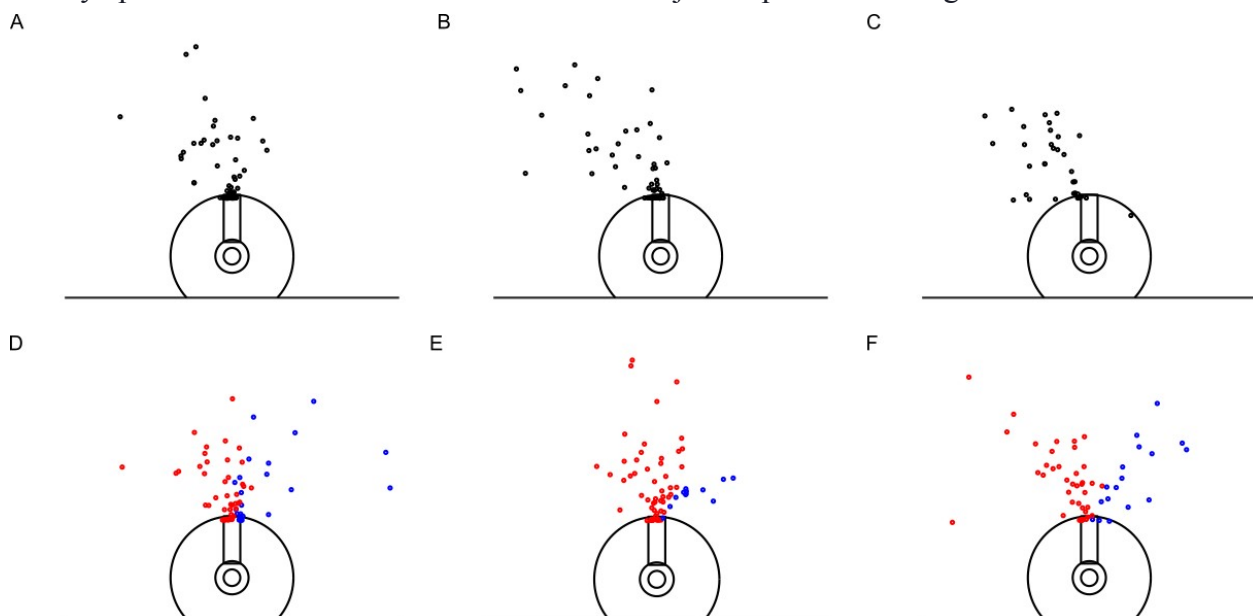

Figure 18: Location of lock on in modeled data Sample of the modeled data obtained using one seed list. Same seed list was use in all object conditions. A) With one object at 37° B) With one object at 60° C) With one object at 90° D) With two objects, one at 37° and one at 60° E) With two objects, one at 37° and one at 90° F) With two objects, one at 60° and one at 90°

from what we observed in walking blowflies,
where the fixation onsets are located mostly
close to the end of the walkway, irrespective of
object position.

We conclude that our Attention Model can
generate a quick response to objects similar to
walking blowflies, but takes, on the whole,
more time and in particular requires slightly
more time to fixate a more lateral object when
compared to walking blowflies. Most
importantly, we show that a simple reactive
fixation mechanism in combination with
random processes can generate trajectories that
a naïve human observer might interpret as
active choice behavior.

The Attention Model reproduces the increasing predictive power of the most frontal object along the walk

In walking blowflies, we observed that the
most frontal object when leaving the walkway
is, to some extent, a predictor of the object that
is eventually approached. The predictive power
increased when the animal was about to
perform the turn that would initiate object
fixation but only reached a truly strong
predictive value once it was about to fixate its
final goal. To assess whether the AM can
reproduce this feature of blowfly choice
behavior, we determined the phi-coefficient,
as a measure of correlation, between an
object being the most frontal in the two-
object constellation and being approached by
the modeled blowflies and compared this
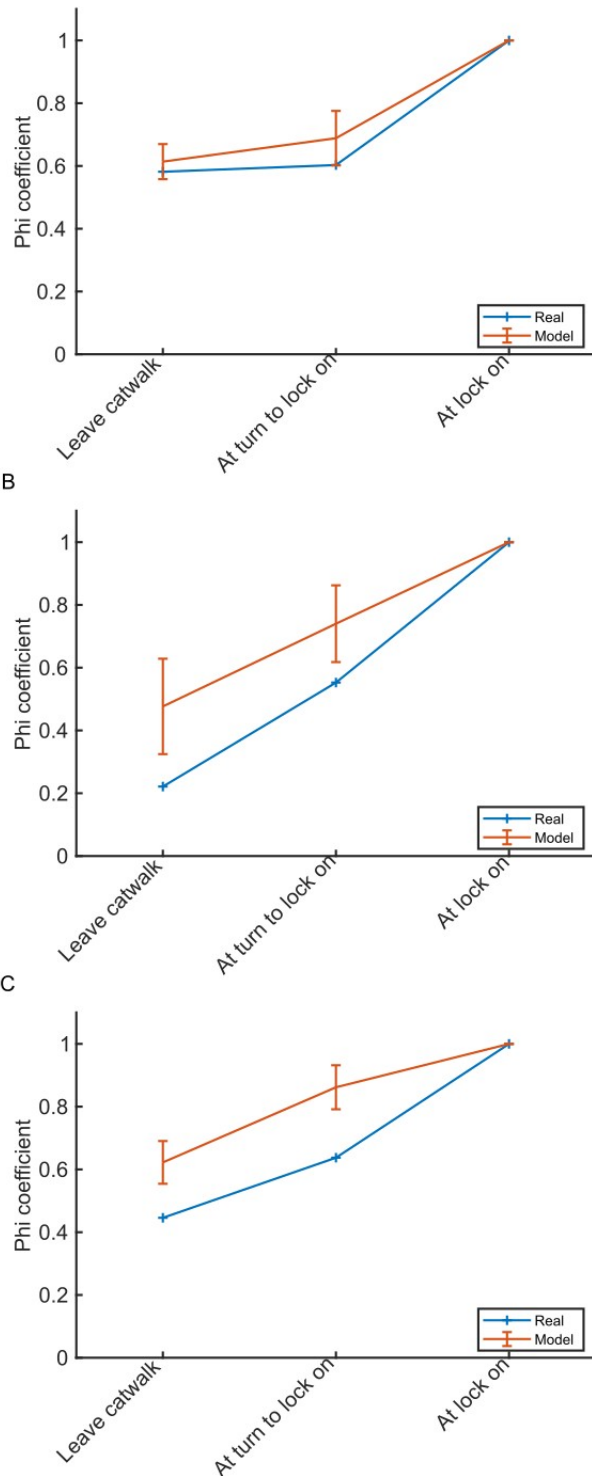
Figure 19: Correlation between most frontal and approached object in model. Mean +- SEM of the phi coefficient across 10 different seed lists, in red, and phi coefficient of the real data, in blue, at different time points. The same seed lists were used in the different experimental setups. A) With two objects, one at 37° and one at 60° B) With two objects, one at 37° and one at 90° C) With two objects, one at 60° and one at 90.

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value to the corresponding correlation obtained for freely walking blowflies (Figure 8). As we did for our experimental data, we did this at 3 different time points: at the beginning of the trace at the end of the walkway, at the last turn before the onset of object fixation, and at the onset of object fixation. Furthermore, to ensure that we captured the genuine performance of the model and not the effect caused by idiosyncratic noise sequences, we calculated for all FWNFs and all object constellations the mean and SEM of the correlation across 10 simulations of the trajectories employing 10 different lists of random seeds.

At the end of the walkway modeled blowflies show a moderate positive correlation between an object being the most frontal and it being approached (Figure 19), though it is variable across object constellations (Phi=0.61 when objects are at 37° and 60°, Phi=0.48 when objects are at 37° and 90°, Phi =0.62 when objects are at 60° and 90°). At the time the animal is about to initiate the final turn leading to the onset of object fixation, the correlation has increased (Phi=0.69 when objects are at 37° and 60°, Phi=0.74 when objects are at 37° and 90°, Phi =0.86 when objects are at 60° and 90°). The correlation is 1 at the onset of fixation. We observed the same increase in correlation in walking blowflies, though the correlations observed were all somewhat smaller, especially when the objects were more separated. We thus can conclude that our model reproduces, at least qualitatively, the initially variable and, along the walking trajectory, progressively increasing the predictive power of the most frontal object for the object that will eventually be approached.

Discussion

We recorded blowflies in a free-walking choice paradigm and developed a model for choice behavior based on the animal's performance. Walking blowflies quickly select their goal, with the initial azimuthal position of the potential goals influencing their attractiveness but having little impact on the time needed to decide which object to fixate and to approach. Which object was seen first also does not play an obvious role in affecting the final decision. Computational modeling revealed that it is necessary to take into account attention-like processes to explain the quick decision process and to account for our behavioral data.

Decision time is minimally affected by object position or the possibility to choose between goals

Walking blowflies start moving towards their final goal almost as soon as they have an unimpeded view of their surroundings. The time till fixating the object that is eventually approached does not

depend much on where the objects were located (Figure 6). By observing behavior we cannot pinpoint exactly when the animal makes its decision, but rather have to rely on behavioral indicators, such as the fixation onset, as a proxy for decision. Also, we started measuring the time when the animal leaves the walkway and not when the object becomes visible, because only then can the animal see the whole test arena unhindered. Thus, an animal should take longer to fixate an initially more lateral object, as his object is seen later than the more frontal objects and its fixation requires a larger turn. However, all objects are fixated within similar time windows irrespective of their initial position. Thus, despite inevitable limitations in our methodology, we can be confident that the retinal position of the objects has little effect on the time the animal takes to decide to approach them. This is in accordance with a previous study (Mronz 2004) where the reaction time of walking *Drosophila* towards objects presented at different azimuthal positions remained largely constant for objects in the fronto-lateral part of the visual field (0°-90°), though the reaction times observed in this study were with around 1s, much larger than the ones we observed (Figure 6 A). This difference could be the result of the different experimental paradigms, as Mronz (2004) presented to the flies a bar which then disappeared while a new one was presented at another location to measure the reaction time to it. In contrast, in our paradigm a single bar became visible and we presented no goal previous to this. Alternatively, the difference in fixation times between *Lucilia* and *Drosophila* could be the result of a difference between the species.

When confronted with two objects the time until fixation increases a bit. A delay in the choice when choosing between multiple objects has been described before for flies in the context of attention (van Swinderen 2011), arguing that the presence of additional objects, referred to as distractors, draws attention away from the target objects. For *Drosophila,* in particular, it has been shown that when confronted with stripe patterns rotating in different directions in each half of the visual field the animal reacts to only one of the patterns, but that the response onset is be delayed when compared with the known response to a single rotating panorama (Tang and Juusola 2010). Our recordings would be analogous to a task where the fly has to select and approach one object, with the other acting as a distractor, with the caveat that either of the objects could be goal or distractor. It is beyond the scope of this study to provide an accurate measure of the choice delay caused by the presence of distractors.

Attention is required to explain quick choices displayed by walking blowflies

Walking blowflies quickly choose a goal between two available objects (Figure 3 E-G). We asked if models derived from the behavior of walking blowflies could reproduce this quick choice.

It has been proposed that choice behavior between different objects is the result of the animal adding the turning response generated by all available objects to spontaneous noise-like turning tendencies (Reichardt and Poggio 1976). We adapted this hypothesis into our object response addition model (ORAM). We found that this model fails to generate a quick fixation decision when the objects were close to each other (Figure 13). This is a consequence of the characteristic dependence of the turning speed of the animal on the object's azimuthal position. This characteristic curve produces an increasing turn speed towards the object the more frontal it is until the object is within a certain frontal window, at which point the turn speed quickly drops to 0°/s stabilizing the object in the front (Figure 12). This curve can generate a choice between two objects if they are separated enough. However, if the two objects are not sufficiently distant, such as when initially presented at an azimuthal position of +37° and -60° (Figure 13), both objects generate similar turning speeds in opposite directions so that the animal walks towards an intermediate position. This phenomenon has been described before (Reichardt and Poggio 1975). We conclude that, while we can see individual cases of traces leading to an object from the start, in most cases the ORAM is unable to generate a quick decision (Figure 13) as we observed in walking blowflies (Figure 3).

Thus, we concluded that to quickly fixate an object in a two-object paradigm, it might be necessary for the animal to ignore the other object for at least some time. This ability had already been concluded for *Drosophila* in a scenario involving two vertical objects in an open-loop paradigm (Wolf and Heisenberg 1980) and thus seems to be a reasonable assumption. The implementation in our attention model (AM) of a probability of reacting to each object that depends on its azimuthal position (Figure 2. B) allowed the model to qualitatively reproduce the quick responses observed in walking blowflies (Compare Fig. 3, 5 & 6 to Fig. 14, 17 & 18). This finding revealed that reducing the gain of parts of the visual input is sufficient to explain the observed behavior. This will immediately evoke the notion of attention, i.e. the ability to focus on parts of the visual input while ignoring the rest. Indeed, our two-object paradigm leads to conclusions reminiscent of the ones of studies used to showcase and study competitive attention (Van Swinderen 2011, Nityananda 2016). In these studies, an animal had to respond to a visual stimulus while suppressing the response to the other. In our scenario, the blowfly needs to suppress the response generated by one of the objects, at least until one of the objects is frontal, to achieve fixation. For the AM we simulated that each object had an independent probability of being taken into account and, conversely, of being ignored at each time step. The probability varied depending on the object's azimuthal position, with a maximal probability at 0°. Alternatively, one might have implemented a probability of selecting each of the available goals, followed by a probability of sticking with the current decision, switching the attention to the other object, or stop reacting to any object. However, such a method

would have required to keep track of states, stating which object is being fixated if any at all, essentially requiring a memory component. Our model does not need to keep track of animal states or memorize the decisions taken, generating object fixation and decision-like processes as emergent properties (compare figures 5 and 16). Thus, our AM provides a simpler implementation while generating similar effects. Furthermore, the AM can reproduce other features of the animal's behavior: despite producing a robust fixation, it allows for significant proportions of modeled flies to not approach any of the objects as well as to occasionally switch to a different goal after apparently starting to walk to an object, as observed in walking blowflies.

However, the AM still shows quantitative differences compared to the behavior of walking blowflies (see Results). It might be possible to fine-tune our AM to better reproduce the quantitative features of the observed behavior. As a precondition, this would require, among other things, the development of a qualitative measure of similarity between modeled behavior and recorded behavior, an issue that is beyond the scope of the current paper. Our goal was to qualitatively reproduce the quick decision which of the two objects to fixate and eventually to approach.

Multiple other factors affect choice and fixation behavior of flies apart from the azimuthal position of objects in the visual field. For example, *Drosophila* shows different preferences for bars depending on how broad and wide they are (Wehner 1972), and *Lucilia* has different preferences for different colors (Fukushi 1989). The AM could be extended to address other preferences by tuning the object response curve to objects of variable characteristics or adapting the attention curve to reflect preferential attention based on other stimulus parameters than the azimuthal position.

References

Geurten, B. R., Jähde, P., Corthals, K., & Göpfert, M. C. (2014). Saccadic body turns in walking Drosophila. *Frontiers in behavioral neuroscience*, *8*, 365.

Fukushi, T. (1989). Learning and discrimination of coloured papers in the walking blowfly, Lucilia cuprina. *Journal of Comparative Physiology A*, *166*(1), 57-64.

Horn, E., & Wehner, R. (1975). The mechanism of visual pattern fixation in the walking fly, Drosophila melanogaster. *Journal of comparative physiology*, *101*(1), 39-56.

Horn, E. (1978). The mechanism of object fixation and its relation to spontaneous pattern preferences in Drosophila melanogaster. *Biological cybernetics*, *31*(3), 145-158.

Horn, E., & Mittag, J. (1980). Body movements and retinal pattern displacements while approaching a stationary object in the walking fly, Calliphora erythrocephala. *Biological cybernetics*, *39*(1), 67-77.

Kress, D., & Egelhaaf, M. (2012). Head and body stabilization in blowflies walking on differently structured substrates. *Journal of Experimental Biology*, *215*(9), 1523-1532.

Kress, D., & Egelhaaf, M. (2014). Gaze characteristics of freely walking blowflies Calliphora vicina in a goal-directed task. *Journal of Experimental Biology*, *217*(18), 3209-3220.

Land, M. F., & Collett, T. S. (1974). Chasing behaviour of houseflies (Fannia canicularis). *Journal of comparative physiology*, *89*(4), 331-357

Mronz, M. (2004). Die visuell motivierte Objektwahl laufender Taufliegen (Drosophila melanogaster)-Verhaltensphysiologie, Modellbildung und Implementierung in einem Roboter.

Nityananda, V. (2016). Attention-like processes in insects. *Proceedings of the Royal Society B: Biological Sciences*, 283(1842), 20161986.

Poggio, T., & Reichardt, W. (1973). A theory of the pattern induced flight orientation of the fly Musca domestica. *Kybernetik*, *12*(4), 185-203

Reichardt, W. (1973). Musterinduzierte flugorientierung. *Naturwissenschaften*, *60*(3), 122-138.

Reichardt, W., & Poggio, T. (1975). A theory of the pattern induced flight orientation of the fly Musca domestica II. *Biological cybernetics*, *18*(2), 69-80.

Reichardt, W., & Poggio, T. (1976). Visual control of orientation behaviour in the fly: Part I. A quantitative analysis. *Quarterly reviews of biophysics*, *9*(3), 311-375.

Sareen, P., Wolf, R., & Heisenberg, M. (2011). Attracting the attention of a fly. *Proceedings of the National Academy of Sciences*, 108(17), 7230-7235.

Schuster, S., Strauss, R., & Götz, K. G. (2002). Virtual-reality techniques resolve the visual cues used by fruit flies to evaluate object distances. *Current Biology*, *12*(18), 1591-1594.

Soibam, B., Mann, M., Liu, L., Tran, J., Lobaina, M., Kang, Y. Y., ... & Roman, G. (2012). Open-field arena boundary is a primary object of exploration for Drosophila. *Brain and behavior*, *2*(2), 97-108.

Takahashi, H., Horibe, N., Shimada, M., & Ikegami, T. (2008). Analyzing the house fly's exploratory behavior with autoregression methods. *Journal of the Physical Society of Japan*, *77*(8), 084802-084802.

Tang, S., & Juusola, M. (2010). Intrinsic activity in the fly brain gates visual information during behavioral choices. *PLoS One*, *5*(12), e14455.

van Swinderen, B. (2011). Attention in drosophila. In *International review of neurobiology* (Vol. 99, pp. 51-85). Academic Press.

Warzecha, A. K., & Egelhaaf, M. (2000). Response latency of a motion-sensitive neuron in the fly visual system: dependence on stimulus parameters and physiological conditions. *Vision research*, *40*(21), 2973-2983.

Wehner, R. (1972). Spontaneous pattern preferences of Drosophila melanogaster to black areas in various parts of the visual field. *Journal of insect physiology*, *18*(8), 1531-1543.

Wolf, R., & Heisenberg, M. (1980). On the fine structure of yaw torque in visual flight orientation of Drosophila melanogaster. *Journal of comparative physiology*, *140*(1), 69-80.