

A visual horizon affects steering responses during flight in fruit flies

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ABSTRACT

To navigate well through three dimensional environments, animals must in some way gauge the distances to objects and features around them. Humans use a variety of visual cues to do this, but insects, with their small size and rigid eyes, are constrained to a more limited range of possible depth cues. For example, insects attend to relative image motion when they move, but cannot change the optical power of their eyes to estimate distance. On clear days, the horizon is one of the most salient visual features in nature, offering clues about orientation, altitude, and for humans, distance to objects. We set out to determine if flying fruit flies treat moving features as farther off when they are near the horizon. Tethered flies respond strongly to moving images they perceive as close. We measured the strength of steering responses while independently varying the elevation of moving stimuli and the elevation of a virtual horizon. We found responses to vertical bars are increased by negative elevations of their bases relative to the horizon, closely correlated with inverse of apparent distance. In other words, a bar that dips far below the horizon elicits a strong response, consistent with using the horizon as a depth cue. Wide-field motion also had an enhanced effect below the horizon, but this was only prevalent when flies were additionally motivated with hunger. These responses may help flies tune behaviors to nearby objects and features when they are too far off for motion parallax.

INTRODUCTION

To navigate with precision and speed, flying insects must approximate the distances of the visible features of their environment. But retinal images don't contain inherent distance information (Land and Nilsson, 2012), so animals infer distance with secondary cues. Every distance cue has limitations, and only provides good estimates when certain conditions are met (Howard, 2002). Humans use several depth cues, some of which are reliable in circumstances where others are not (Landy et al., 1995). The horizon is one of the most conspicuous features of the outdoor world, and both humans and insects use its image for a variety of purposes. Our goal was to determine if fruit flies, like humans, are able to use angular distance from the horizon to judge features as closer.

Images are captured when light from the three dimensional world focuses onto a two dimensional surface. This encodes the relative angles between features, but not the distances to them, and images that appear close together on the retina may represent objects that are far

apart in the world (Land and Nilsson, 2012). Depth cues allow animals some ability to reconstruct the distance information necessary to model the three dimensional environment (Howard, 2002).

When scanning a landscape, or catching a ball, or watching a movie, we rely on an array of depth cues that give scenes an intrinsic quality of distance (Landy et al., 1995). Insects, with rigid exoskeletons, cannot use ocular convergence or accommodation of focal length to judge distance (Srinivasan, 1992). Further, although several insects have binocular overlap (Beersma et al., 1977), only a few groups have been shown to use stereopsis to determine depth (Rossel, 1983; Eriksson, 1985), and the tiny separations between their eyes limits its use to short distances (Collett, 1987).

For familiar objects, some insects use larger retinal image sizes to indicate proximity, such as bees searching for flowers (Cartwright and Collett, 1983). For insects in motion the best depth cue is motion parallax (Lehrer et al., 1988), in which changing the viewing position displaces the images of nearby objects. This is related to stereopsis, but rather than a fixed offset between two eyes, the viewer's own motion provides the offset. Several insects generate motions specifically to produce parallax, such as locusts peering before a jump (Wallace, 1959; Collett, 1978), and hymenopterans performing intricate orientation flights before foraging (Wehner, 1981; Zeil, 1993). In other cases the parallax generated from natural flight allows insects to gauge distances (Kirchner and Srinivasan, 1989; Srinivasan et al., 1991) and modulate flight speed (Preiss, 1987). Fruit flies use motion parallax while walking on the ground (Schuster et al., 2002) and during flight (Cabrera and Theobald, 2013).

However, when animals are not translating, or visual elements are far off, motion parallax provides no distance information. Humans retain depth perception by a range of minor depth cues (Landy et al., 1995), for example when viewing photographs (Benson and Yonas, 1973). One such indirect cue is an image's elevation, or elevation away from a visible horizon. Frogs use retinal elevation as a distance indicator when striking at prey (Collett and Udin, 1988), Fiddler crabs use visual elevation above the horizon as the crucial test to distinguish conspecifics from predators (Layne et al., 1997; Zeil and Hemmi, 2006). Tiger beetles prefer prey farther from the horizon, presumably judging it as closer (Layne et al., 2006). Insects additionally use the horizon to correct attitude deviations (Berry et al., 2007) and use nearby horizontal edges to regulate altitude (Straw et al., 2010). For humans, an image's angular

offset from the horizon is an indication of distance, with objects near the horizon appearing farther off (Ooi et al., 2001). This is one common, though probably incomplete, explanation for the moon illusion, in which the full moon near the horizon seems substantially larger (presumably because it seems farther off) than it does high in the sky a couple hours later (when its actual angular extent is slightly larger) (Rock and Kaufman, 1962; Kaufman and Kaufman, 2000).

We set out to determine if flying fruit flies, like humans, perceive visual features as farther off when they appear near the horizon. *Drosophila melanogaster* steer in response to image motion in their frontal visual region, and are particularly adept at tracking vertical bars (Reichardt and Wenking, 1969a; Maimon et al., 2008). Flies respond more strongly to images they perceive as close objects (Wehner and Horn, 1975; Cabrera and Theobald, 2013), probably because they represent the features most relevant to making good steering decisions. We hypothesized that, in the absence of other depth cues, flies would steer more strongly to moving images placed farther from the horizon. To test this we measured steering effort of tethered fruit flies while independently varying the elevations of a virtual horizon and a moving visual features.

RESULTS

We tethered fruit flies to the center of a projection based visual flight arena (Fig. 1A) (Cabrera and Theobald, 2013) where they viewed perspective-corrected images of vertical bars and an artificial horizon (Fig. 1B). The figures show approximate intensities only, as actual intensities in the arena differ from those viewed on a monitor or printed on a page. Tethered flies respond to lateral bar motion by varying left and right wing beat amplitudes, producing a difference (Δ WBA), which generates yaw torque (Fig. 1C) (Götz, 1987; Tammero et al., 2004). Our bars were 11 degrees wide, spanned 60 vertical degrees, and were shaded such that the contrast above and below the horizon was the same, but with opposite sign. The bar moved left and right 45 degrees from the center line at 1 Hz. We took the correlation between the bar motion and Δ WBA as a measure of steering effort. In closed-loop, flies can track light and dark bars with different dynamics (Reiser and Dickinson, 2008), but open-loop tracking of vertically centered bars in our arena against the light or dark backgrounds produces statistically indistinguishable correlations (dark background mean

correlation = 0.268 ± 0.066 , $n = 42$, bright background mean correlation = 0.267 ± 0.028 , $n = 49$, $t = 0.199$, $P = 0.842$).

Bar tracking effort varies with the angle between visual horizon and bar bottom

We varied the center elevation of a bar between -30 and 30 degrees, both with and without a horizon at 0 degrees (Fig. 2A), then varied the elevation of the horizon between -30 and 30 degrees, with a bar whose center was at 0 degrees (Fig. 2B). This created two analogous sets of stimuli, in which the bar and horizon elevation didn't match, but their relative positions did. Responses to horizontal bar motion are robust, and flies track multiple cycles with little delay (Fig. 2C, D). But both the actual and apparent elevation of the bar determines how strongly wing beat amplitudes correlate to bar motion (Fig. 2E and F). A bar elevated by 30 degrees, such that the bottom meets the horizon, produces relatively weak tracking, but when the bottom is 60 degrees below the horizon it generates much stronger tracking (Fig. 2E, white markers). Because both relative and actual elevation might affect responses, we removed the horizon to examine the effect of intrinsic elevation (Fig. 2E, gray markers). Elevated bars produce a response that is indistinguishable with or without a horizon, and lower bars still produce stronger tracking, but tracking at the lowest elevation is significantly stronger in the presence of a horizon, ($t = 3.11$, $P = 0.0015$, $n = 49$). This relatively minor effect of the visual horizon may imply that when a horizon is not visible, flies behave as if it were central. But the horizon effect is strongly apparent when it is shifted up or down. When we hold the bar elevation to the middle and move the horizon, a higher horizon elicits greatly increasing tracking responses (Fig. 2F).

Multiple wing beat changes accompany tracking correlation

Several factors could affect the correlation values we observed. To investigate the aspects of raw traces (Figs. 2C and D) that produce the correlations (Figs 2E and F), we extracted potentially relevant factors. We estimated phase and amplitude by fitting our data to the triangular waveform of the bar motion, optimizing with a Levenberg-Marquardt gradient method to minimize the least squares error. The amplitude has no direct effect on the Pearson's' correlation, which is scale free, but higher positions relative to the horizon correspond to decreased tracking amplitude (Fig. 3A), and phase lag generally increases (Fig 3B). Both correspond to weaker tracking at greater apparent distances. The effects are more pronounced when the bar is elevated (white markers), and the phase lag effect does not hold for negatively elevated horizons (black markers). The residual variance in the Δ WBA, or the

turns that don't correspond to bar motion, show no clear correspondence to relative bar elevation (Fig 3C), although the highest horizon elevation produced reduced tracking variation (leftmost black marker). In free flight, high horizontal edges induce flies to increase altitude (Straw et al., 2010). To determine if this affected tethered flies, we examined the sum of wing beat amplitudes (Σ WBA), which increases when flies compensate for upwards optical flow (Theobald et al., 2010). Mean Σ WBA slightly increases as the horizon appears higher (Fig. 3D, $n = 49$). To determine if the increased Σ WBA might create stronger tracking correlations itself, we examined the Σ WBA and tracking responses of individual flies viewing a central horizon, the middle bar of figure 3D (Fig. 2E, $n = 49$). Σ WBA appears uncorrelated with tracking, implying these are separate effects. However, variation partly results from differences in tethering and fly placement, which affects the strength of the signal over the sensor. To account for this effect, we subtracted out the initial Σ WBA value to plot only the change in sum of wing beat amplitude that followed a stimulus. This similarly showed that the change in Σ WBA, (or $\Delta \Sigma$ WBA) from the onset of the stimulus had no clear link to tracking (Fig. 2F, $n = 49$).

Angle below the horizon can indicate distances

If a few assumptions are met, a viewer can estimate the distance to an object that touches the ground simply by measuring the angle it subtends below the horizon. If the ground is flat, the viewer is near the ground, and the horizon is far off, then the horizontal distance to the object (d) is simply: $d = a/\tan \theta$, where a is the viewer's altitude and θ is the angular distance from the horizon to the object bottom (Fig. 4A).

To better evaluate the effect of horizon and elevation on tracking, we examined the fits of a series of simple linear regressions. We first considered the angle of the bar bottom below the horizon to fit tracking responses (R) from Fig. 2E, F: $R = m\theta + b$. These produce good fits (Fig. 4B) for both the variable bar elevation (white markers, dotted line, $r^2 = 0.81$, intercept = 0.140, slope = -0.004, slope different from 0: $t = 5.56$, $P < 0.001$, $n = 49$), and variable horizon elevation (black markers, solid line, $r^2 = 0.83$, intercept = 0.172, slope = -0.003, slope different from 0: $t = 3.39$, $P < 0.001$, $n = 49$). The intercepts here represent the tracking response expected when bar bottoms are on the horizon, and the slopes are the rate at which they increase as the angle becomes more negative.

However the variable horizon data in Fig. 4B (black markers) noticeably curves, dipping below our straight line fit. To address this we considered the apparent distance implied by a bar meeting the assumptions above: $R = ma / \tan \theta + b$. We used a constant value of 1 for a , and removed the condition of bar bottoms touching the horizon, since this implies an infinite distance (which produces an outlier effect). Even so, the fits are considerably worse (Fig. 4C), for both variable bar elevation (white markers, dotted line, $r^2 = 0.44$, intercept = 0.324, slope = -0.020, slope different from 0: $t = 2.33$, $P = 0.010$, $n = 49$), and variable horizon elevation (black markers, solid line, $r^2 = 0.45$, intercept = 0.270, slope = -0.015, slope *not* statistically different from 0: $t = 1.62$, $P = 0.053$, $n = 49$). Here the intercepts represent the responses expected if the bar were at zero distance, and the slopes describe the decrease to bars farther off.

Some behaviors increase disproportionately with nearness, so we finally considered the fit to the inverse of apparent distance: $R = ma \tan \theta + b$. This produced better fits than apparent distance (Fig. 4D) with the variable bar elevation slightly lower than bar angle alone, (white markers, dotted line, $r^2 = 0.76$, intercept = 0.163, slope = 0.129, slope different from 0: $t = 5.35$, $P < 0.001$, $n = 49$) but an excellent fit for the variable horizon elevation, much better than bar angle alone, (black markers, solid line, $r^2 = 0.95$, intercept = 0.182, slope = 0.112, slope different from 0: $t = 4.29$, $P < 0.001$, $n = 49$). In this case the intercepts represent the response to a bar at infinity, and the slopes describe the increasing response, inversely proportional to decreasing distance. Because this produced the best fit for the variable horizon experiment, and the best overall, we illustrated these fits in Fig. 2E and F. The slopes of variable bar and variable horizon fits are not statistically distinguishable ($t = 0.47$, $P = 0.682$)

Reversing contrast decreases the horizon's effect on lateral tracking

On land, natural light generally arrives from the sky, then illuminates the ground, which absorbs some of it. Because of this the sky above the horizon is frequently, but not always, brighter than the ground, even at night. To assess the importance this arrangement to the horizon's effect on tracking responses, we reversed contrast, displaying darker regions above and brighter regions below. Although lower bars still produce greater responses, a reversed horizon reduces tracking at each bar elevation (Fig. 5A, intercept = 0.233, slope = 0.121, slope different from 0: $t = 3.349$, $P < 0.001$, $n = 42$). The response to the highest bar can not be distinguished from zero ($t = 1.13$, $P = 0.263$, $n = 42$). When the elevation of a reversed

horizon, its effects on tracking are minimal, and possibly absent entirely, as the slope of the regression can not be distinguished from zero (Fig. 5B, intercept = 0.137, slope = 0.031, slope not statistically different from 0: $t = 0.898$, $P = 0.815$, $n=42$).

Fixing the head in place reduces the horizon's effect on lateral tracking

Changing head position is a straightforward behavior by which the horizon could modulate tracking. If head pitch angle were fixed by the horizon then objects far from the horizon would produce retinal images far from the midline. This is how fiddler crabs identify predators (Layne, 1998). To determine the contribution of head motion, we fixed fly heads to the thorax during tethering. Importantly, this blocks lateral as well as vertical head motion, and will inhibit behaviors that depend on lateral gaze shifts. But recent studies have shown that if background motion is absent, head fixation doesn't impair fruit flies from tracking vertical moving features (Fox and Frye, 2014). Consistent with this, bars elevated against the horizon produce tracking comparable in flies with free and glued heads, but for bars lower against the horizon, flies with glued heads showed a muted increase in tracking (Fig. 6A, B compared to responses from Fig. 2E, F shown in gray). The linear fit for the bar elevation conditions produced an intercept and slope of 0.255 and 0.083, (Fig. 6A, slope different from 0: $t = 2.81$, $P = 0.002$, $n=62$). The fit for the horizon elevation conditions had an intercept and slope of 0.207 and 0.044, and no significant support for a slope different from 0 (Fig. 6B, $t = 1.48$, $P = 0.06$, $n=62$). To determine if retinal location explains the reduced response, we took videos of fly heads from the side during bar tracking with a variable height horizon. We used custom software to estimate the angle of head tilt in each frame, for 100 frames per second (images shown in Fig. 6C). The results showed highly variable head angles that do not strongly correlate with horizon angle (Fig. 6D, $n = 15$). Although a horizon below 0 degrees is associated with a lower head tilt, so is a horizon above 0 degrees, implying a complex relationship between horizon and head tilt.

Wide-field tracking responses can also vary with the visible horizon

Both small visual features, such as isolated spots or vertical bars, and wide visual fields, such as the optic-flow produced by self-motion, elicit robust responses in flies (Krapp et al., 2012; Maimon et al., 2008). But natural scenes contain intermediate visual stimuli, and responses with blocked motion-sensitive T4-T5 cells imply the neural processing is more nuanced than simple small-field and wide-field motion detection (Bahl et al., 2013; Fenk et al., 2014). We set out to determine whether the visible horizon modulates wide-field optic flow responses

differently than bar tracking responses. Wide fields of dots that simulate translational or rotational motion produce changes in Δ WBA which work to correct the apparent deviation from a forward heading (Theobald et al., 2010). These responses are robust and repeatable, although the correlation between motion and tracking is often lower than for bars (Mazo and Theobald, 2014). Further, translational motions produce stronger responses than rotational motions, but provide the independent depth cue of motion parallax, and additionally generate responses that inherently vary with elevation (Mazo and Theobald, 2014). To avoid these effects, we presented rotational flow fields. We produced flow fields as described in previous work (Cabrera and Theobald, 2013), but limited to a spherical segment of varying elevation, that, if wrapped all around, would represent a 2 steradian solid angle. Fitting non-overlapping, 2 steradian segments required us to modify the elevations tested compared to the bars. The dots simulated oscillation of 22 degrees around a vertical axis. We measured the effect of displaying the horizontally moving segment in the upper, middle, or lower regions of the arena with a visible horizon (Fig. 7A, B). The dots were shaded like the bars to have the same contrast against the light background above the horizon and the dark background below.

Dots generated weaker responses that initially varied little with horizon elevation. To enhance the responses we removed flies from bottles two hours before tethering and provided them with water but no food. The hungry flies tracked dot fields with amplitudes greater than tracking bars (Fig. 7C, D, $n=39$), and correlations that were comparable (Fig. 7E, F, black markers). This contrasts with the unmotivated responses of flies that were on food immediately before tethering, which showed low correlations with the bar motion under all experimental conditions (Fig. 7E, F, gray markers, $n=156$). Responses grow as the stimulus lowers relative to the horizon, similar to bar tracking, but with differences, as they are poorly fit, in both cases, with an inverse of apparent distance equation. Our bars always extended down to the horizon, but dot segments were sometimes completely above it. We originally predicted that this positive angular distance from the horizon would induce stronger responses, just as distance below the horizon does, but this is not the case. Responses are stronger to dot fields below the horizon, and weaker to dot fields above, regardless of whether the dot field or horizon has shifted. Altering the equation for fitting the line produces little improvement because the responses seem to saturate both above and below the horizon.

DISCUSSION

These results indicate that fruit flies track visual features with greater fidelity when they dip below a visual horizon (Fig. 2). Since flies track apparently nearer features more strongly (Schuster et al., 2002; Cabrera and Theobald, 2013), this result is consistent with their using angular distance to the horizon as a minor depth cue, as humans do (Ooi et al., 2001). The tracking correlations are partly by variations in the phase and amplitude of bar tracking (Fig. 3), and are well modeled by an inverse response to apparent distance (Fig. 4). This modulation of tracking strength is reduced if the contrast between the sky and ground is reversed, or if flies' heads are glued in place (Figs 5 and 6). Initial data showed that gluing heads does not affect the base response to bars, but impairs the enhanced tracking to bars below the horizon, suggesting that flies might modulate responses by tilting their heads to alter retinal images (Fig. 6, A, B). But examining head tilts directly showed a more complex relationship (Fig. 6D), in which flies lift their heads most for horizons at middle elevations. Another possibility is that flies execute brief head motions up to the horizon, not easily seen in our analysis, but important for steering modulation. Finally, when vertical bars are replaced with wide-field patterns, the responses were more nuanced. Our initial flies showed reduced tracking that was not modulated by elevation from the horizon, but motivating them with mild starvation strongly enhanced responses to dots below the horizon. (Fig. 7). This response is qualitatively similar to bar tracking, but with subtly different aspects, and could imply a more complex role for the horizon when the stimulus is not a target. It additionally raises questions about the conditions under which tracking responses vary with internal physiological states. Bar tracking in the arena is already strong, but it may also be modified by hunger. Other states, such as thirst, virginal status, or age, may further alter responses. This capacity to alter behavior, sometimes greatly, with internal state, may be one key to the surprising success of insect brains, and elevation may offer a promising direction for further experiments.

In experimental settings the horizon may produce unintended effects.

In laboratory settings, visual stimuli for flies are often delivered with cylinders, such as patterned drums or LED arrays. These arenas can generate, from the perspective of the fly, a line of contrast with the rest of the room at the upper and lower boundaries. Our results imply that this arrangement could alter steering responses. For example, if a moving bar were presented in a dark cylinder in a bright room, we might expect enhanced tracking, since the

bar sinks below a bright horizon. If the same stimulus were delivered in a fully enclosed arena, or with room lights off, the tracking response might be muted. Most behavioral experiments will properly hold elements like room lighting constant between experiments, so the visual horizon is unlikely to affect the qualitative results. But in some designs this may be an artifact, and introduce unwanted variation. In certain situations it could provide a way to enhance otherwise subtle tracking responses.

In nature horizon effects will depend on the characteristics of the visual scene.

Our experimental flies viewed a highly simplified visual world. In natural environments the coupling between distance and the horizon is more nuanced than a simple line of contrast. Except at sea, the visual horizon is rarely the Earth's horizon. Rather we see mountain ranges, tree lines, and haze, that elevate, blur, and obscure the line between ground and sky. In many cluttered visual scenes, such as in a forest, the horizon may be completely invisible. But busy scenes such as those may frequently be rich with parallax information, in which slight body motions will reveal distances with much more certainty than horizon cues could anyway. Fly brains may, similar to our own brains, somehow weigh the reliability of different depth cues depending on the characteristics of a visual scene.

Natural images have other characteristics that signal distance and may be discernible to a fly eye. Objects appear to have lower contrast, smaller size, and are more likely to be occluded when they are far off. However, these properties are likely to reduce fundamental motion detection, and therefore inherently reduce tracking strength. A fly can't robustly track an image that makes no contrast against the background, for example. But the effect measured here may function in situations where a visual feature may be difficult to range based on size or contrast. Trees are the obvious vertical object that will motivate a fruit fly to approach, but they can vary in size by orders of magnitude, and often produce good visual contrast even when far off. In other words, unless a tree is near enough to determine its distance by motion parallax, it can be difficult, even for a human, to decide how far off it is. In addition to tracking, horizon cues could potentially affect tuning. The amplitude of fly steering responses is tuned to different oscillation frequencies (Duistermars et al., 2007a; Duistermars et al., 2007b), which means visual features moving at different speeds. Object speed is a minor depth cue for humans, as in some circumstances slower objects seem more distant (especially objects in free fall). If the optimal tuning is adaptive, flies might benefit by shifting it to slower speeds when the horizon indicates objects are farther off. This hypothetical effect

might be nullified, though, if object motion appears to be a result of self rotation, in which case distance would not affect image speed.

Depth cues allow both animals and computer vision systems to model the three dimensional world with two dimensional images. They have potential relevance to nearly any sighted organism, but flying insects are a special case. They continue to pose a paradox by using small brains and poor vision to seemingly control sophisticated aerial navigation. They can navigate around obstacles with quick precision, or steadfastly head to far off destinations. Even tiny fruit flies can travel over great distances (Coyne et al., 1987), but to travel efficiently they must keep a constant heading as wind gusts alter their position and heading. Fruit flies are now known to be able to use the polarized sky to maintain a constant direction over long distances (Weir and Dickinson, 2012). A depth cue that functioned beyond the range of motion parallax could help to both choose distant targets and enhance flight stability in wide open spaces. Here we have shown that visual elevation below the horizon could serve as a sensory depth cue for fruit flies when other cues are absent.

MATERIALS AND METHODS

Subjects

We chose flies from a colony derived from wild-caught *Drosophila melanogaster* (Meigen), fed on standard media and kept on a 12:12 light cycle. We cold-anesthetized females, 3-6 days after eclosion, and attached a rigid tungsten tether, 0.02 mm in diameter, to the dorsal prothorax. We then placed them upside down with a small piece of paper at their feet, which keeps them from beating their wings before an experiment begins. After rewarming for approximately an hour, we suspended them in the center of the flight arena, a cube of projection surfaces, and removed the paper, causing them to initiate tethered flight.

Visual Stimulus

The flight arena is a perspex cube, 200mm on a side, inlaid with back-projection screen material. Four first-surface mirrors, angled at 45 degrees to each side, allow a single projector (Lightspeed Designs DepthQ 360) to illuminate 5 sides of the cube (Fig. 1A). We use custom OpenGL code to drive a high-end graphics card (GeForce GTX 480) and animate the cube with perspective-corrected, three dimensional scenes at 360 frames per second. From the

center of the cube the scene appears continuous, even as shapes move from one surface to another (Fig. 1A). The four rendered scenes that reflect off mirrors are mathematically reversed to account for the reflection. The front face displays 229x229 pixels, (2.5 pixels/degree) and the side faces display 200x200 (2.2 pixels/degree). The small difference is due to the shorter path between the projector and the front surface, which displays an image of the same size but at a higher pixel density. These experiments were performed with room lights on, producing a maximal contrast between dark and light areas in the arena of 85%.

Each experiment consisted of open loop presentations of bar motion, interspersed by three second bouts of closed loop fixation of a striped bar. During fixation the fly's wing beats control the position of a rotating vertical bar, which improves their responsiveness to experimental presentations (Heisenberg and Wolf, 1979; Reichardt and Wenking, 1969b). During experimental presentation, the striped bar was replaced by a gray bar, usually 11 degrees wide and 60 degrees long, and an artificial horizon appeared at some elevation, generally brighter above and dimmer below, to match natural conditions. The intensity of the bar was scaled to produce the same contrast against the bright background above the horizon and the dark background below. The bar moved left and right following a triangle waveform, at 1 Hz, for two full cycles.

Steering Responses

The center of the visual arena situates a tethered fly between an infrared light emitting diode above, and a pair of photodiodes below. Each wing casts an infrared shadow onto a photodiode through each beat cycle, 200 times per second, with larger wing beats occluding more light (Tammero et al., 2004). These signals feed into a dedicated wing beat analyzer which outputs a voltage proportional to the wing beat amplitude for each stroke. Although this does not capture the three dimensional dynamics of a wing stroke, the difference between the left and right amplitudes (Δ WBA) is proportional to yaw torque when flies are connected to a torque meter (Götz, 1987; Tammero et al., 2004). The amplitude difference is visible to the naked eye when flies are exposed to involuntary optic flow, and the resulting Δ WBA voltage signal is accurate enough that they can precisely fixate the rotating stripe in closed loop feedback between tests. In addition to wing stroke size, the brightness of the light emitting diode also affects the measured amplitude, and we turned the brightness up for the experiments in Fig. 7, as initial trials yielded weaker responses. As the absolute amplitude is only comparable within experiments, we used the Pearson's correlation, which is scale

independent, between bar motion and Δ WBA as a measure of tracking response.

List of symbols and abbreviations

Δ WBA = Difference between left and right Wing Beat Amplitudes

Σ WBA = Sum of the left and right Wing Beat Amplitudes

θ = angular distance from the horizon

Competing interests

The authors declare no competing financial interests.

Author contributions

JCT, CM, and JC planned the experiments and analysis. JC and CM performed the experiments. IRP performed the image analysis. JCT, JC, CM, and IRP wrote the manuscript.

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Figures

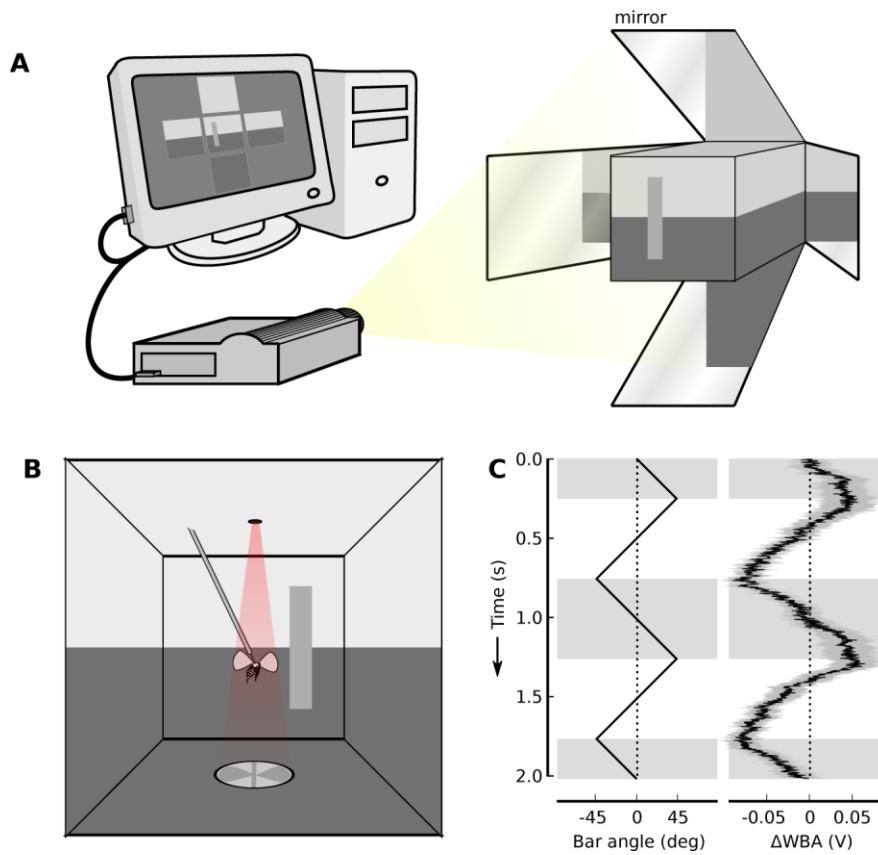


Figure 1. Flies track moving bars in a projection based arena. A. A dedicated computer graphics card connects to a high-speed projector, casting images onto an array of mirrors which then cover 5 sides of a visual arena. B. A fly rigidly tethered inside the arena views images that are perspective corrected to simulate immersion in a virtual environment, here with a vertical bar against a horizon. An infrared light-emitting diode casts shadows of the wings onto a pair of photodiodes below, to measure left and right wing beat amplitudes. C. Flies modulate their relative wing beat amplitudes to create a difference, Δ WBA, that follows the bar displacement. The trace shows the mean and standard error of 49 flies tracking a moving bar.

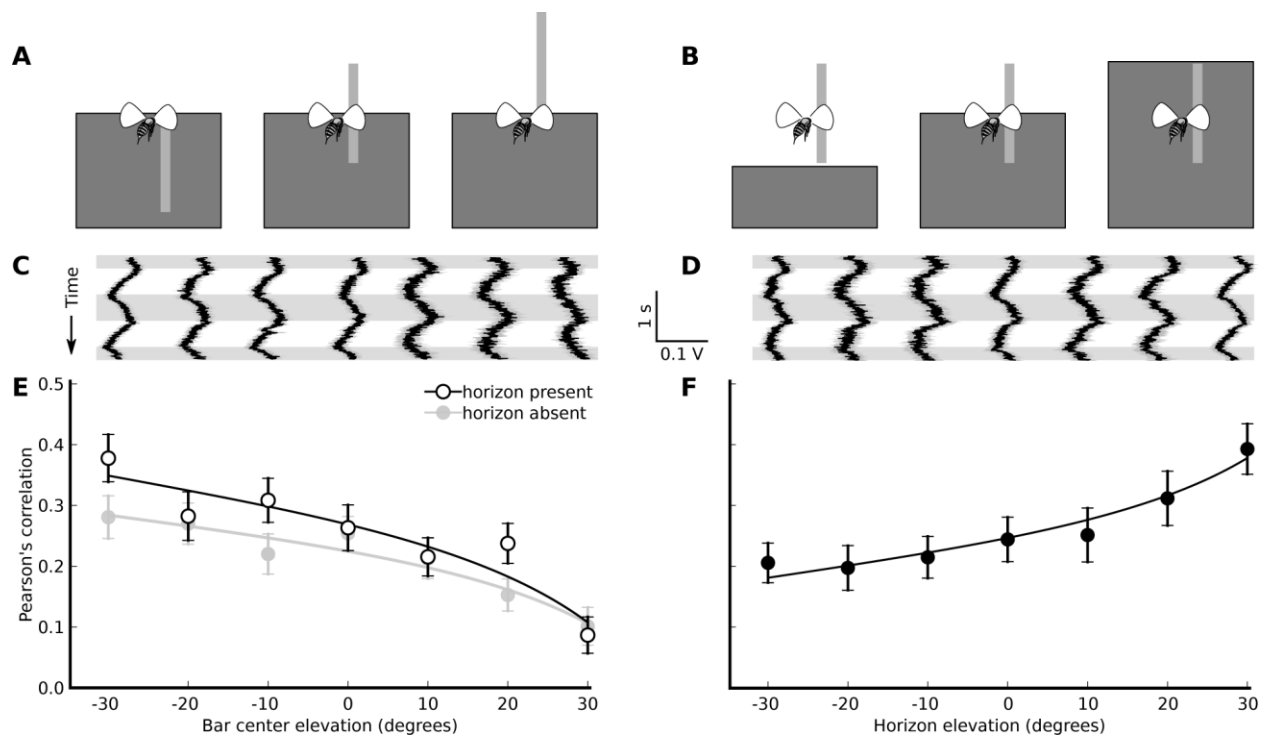


Figure 2. Tracking varies with bar and horizon elevation. A. We varied the elevation of a vertical, horizontally moving bar, against a horizon of constant elevation, or B. held the bar's elevation constant and manipulated the horizon's elevation. C. Flies modulated Δ WBA in response to two cycles of bar motion against variable bar elevation, or D. variable horizon elevation. Horizontal gray bars indicate rightward motion, white indicates leftward motion, black traces are the mean response of 49 flies, flanked with light gray to indicate standard error. E. White markers show correlation between bar displacement and Δ WBA, bars indicate standard error. Gray markers show the response to bar motion when no horizon is present. F. Black markers show the change in response correlation as the horizon elevation changes. The lines in E and F are linear regressions in which the elevation was transformed to the inverse of apparent distance, detailed later.

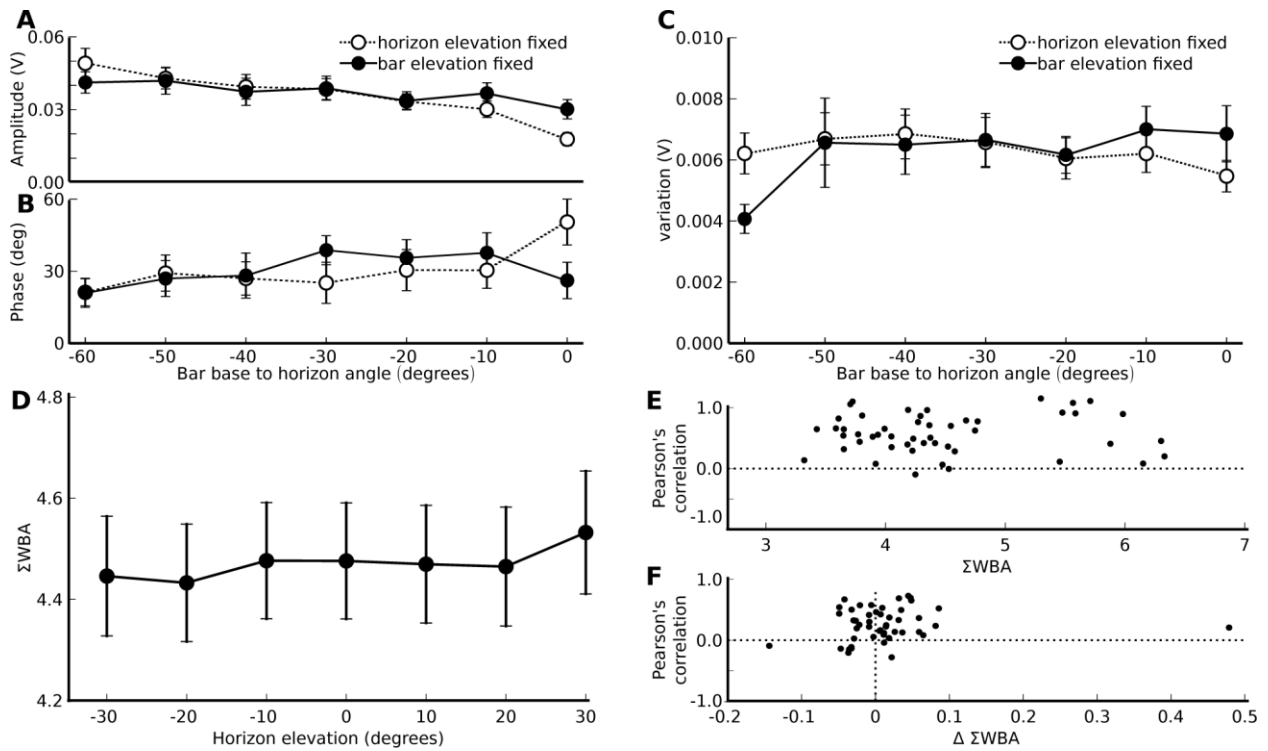


Figure 3. Multiple factors affect tracking. A. The amplitude of Δ WBA drops as the bottom of the bar approaches the horizon, and B. the phase lag increases. C. The residual variance shows no clear trend, but is suppressed when the horizon was at its highest mark. In A-C white markers represent the varying bar elevations, black markers represent varying horizon elevations. D. Σ WBA increases with horizon elevation, bars show standard error. E. When the horizon is central, increased Σ WBA does not correspond to higher tracking correlations for individual flies. F. Shows the relative change in Σ WBA values from E, with the initial Σ WBA value subtracted out to account for variations in tethering.

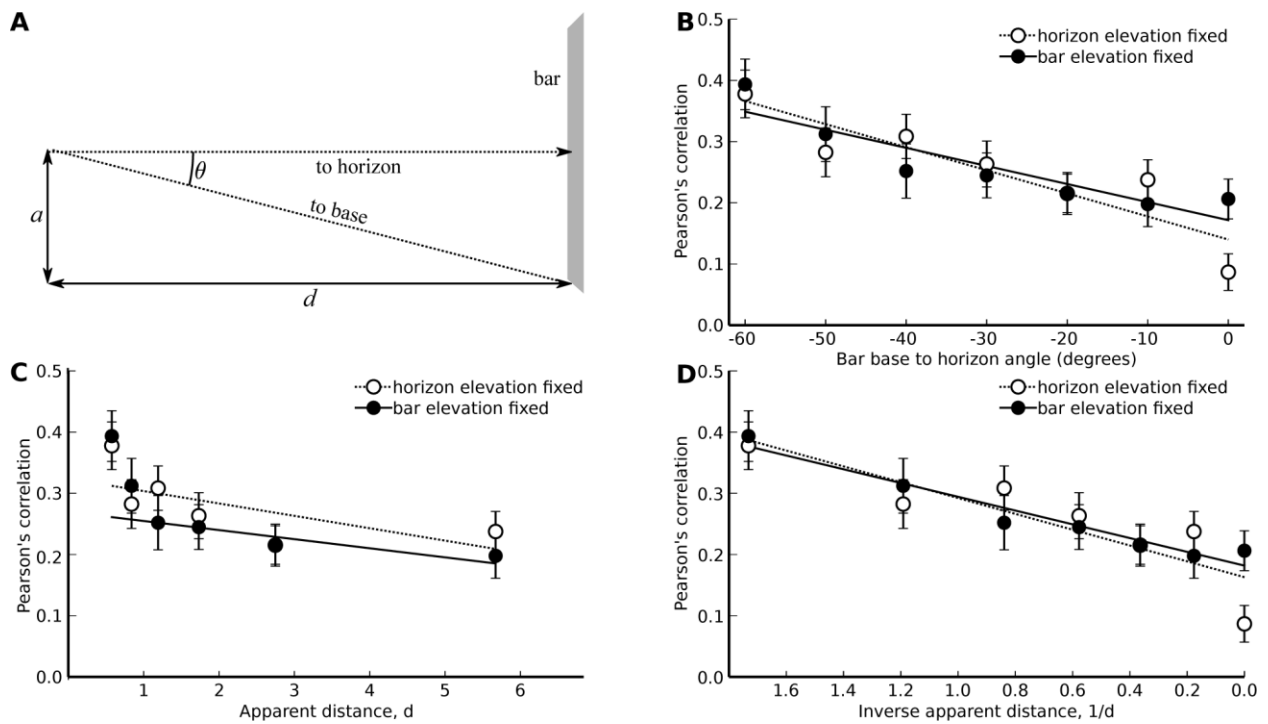


Figure 4. Regression fits for horizon mediated responses. A illustrates the geometry of a vertical object for which the base dips below the horizon, when the horizon is far off. B. shows the regressions of tracking responses against the elevation of the base of the bar, θ . C. shows the regressions when the angle of the base of the bar is transformed by $1/\tan \theta$, giving the apparent distance to the bar, in arbitrary units, assuming a height of 1. D. shows the regressions when the angle of the base of the bar is transformed by $\tan \theta$, giving the inverse of apparent distance. In B-D, white markers and the dotted line are the data and regression for variable bar height against a central horizon, Figure 2E, black markers and solid lines show the data and regression for variable horizon height against a central bar, Figure 2F.

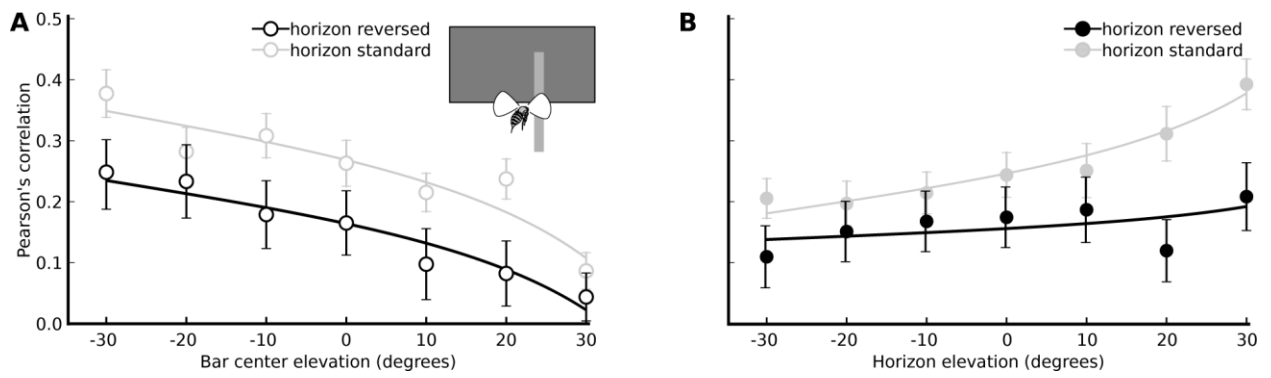


Figure 5. Reversed contrast horizons reduce horizon-mediated tracking. A. The strength of lateral tracking a bar of varying elevation while the horizon is held constant at 0 degrees. The gray markers are for comparison from Fig. 2E, the same experiment with the brightness above and below the horizon reversed. B. The effect of varying the horizon elevation, while the bar elevation was held constant, spanning 30 degrees above and below the horizon. The gray markers are for comparison from Fig. 2F.

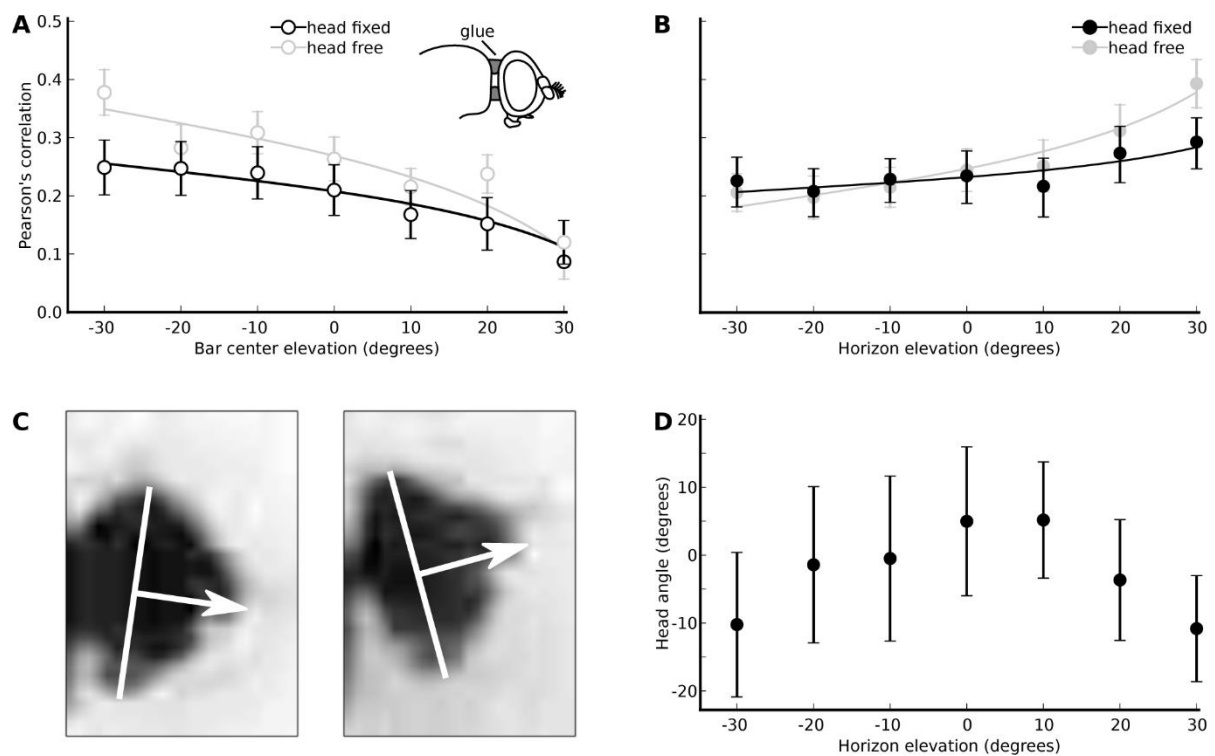


Figure 6. Head tilt affects lateral tracking responses. A. Tracking a horizontally moving bar of varying elevation against a horizon at 0 degrees. The black markers show the response with the head glued in place, and the gray markers show the response from Fig. 2E, with the head free. B. Tracking a horizontally moving bar vertically centered at 0 degrees against a horizon at varying elevations. The black markers again show responses from individuals with glued heads, while the gray markers show the free head response reproduced from Fig. 2F. C. Images of a *Drosophila* head, shot from the side at 100 frames per second, showing tilts as she watches a bar move against a horizon. D. The average head tilt as horizon elevation changed, $n = 15$.

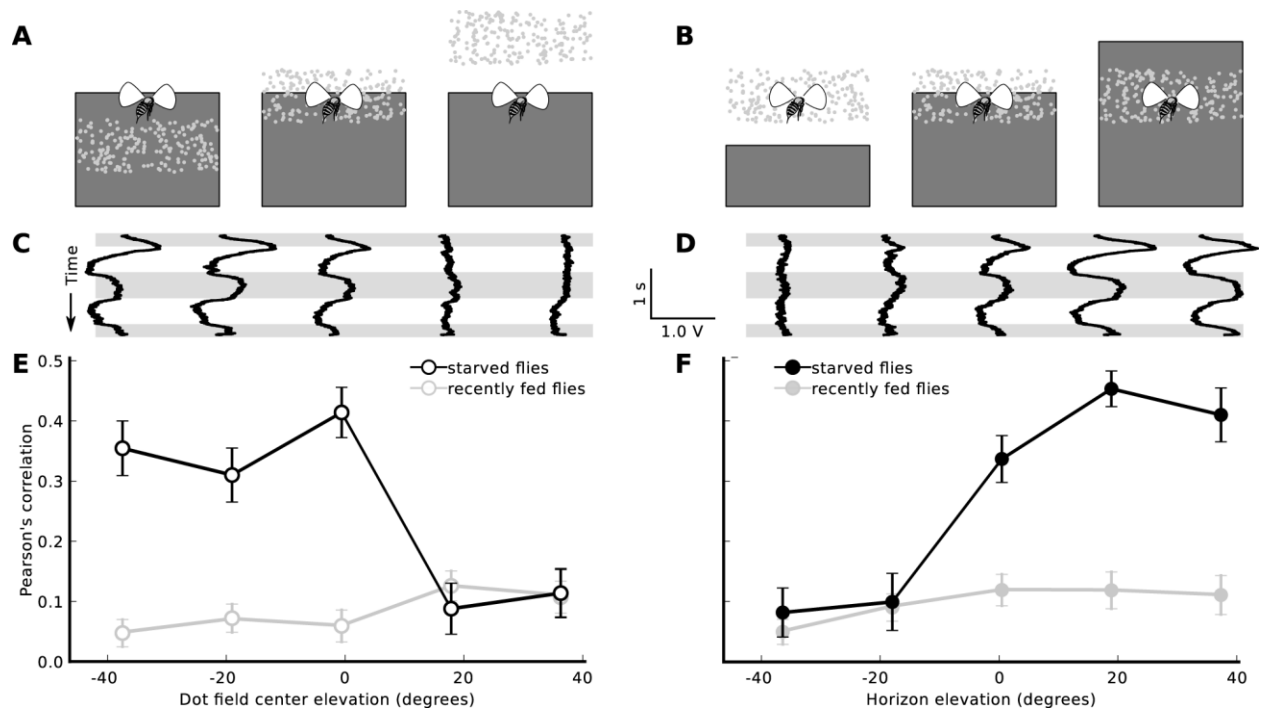


Figure 7. A horizon can have a similar effect on tracking a spherical segment of dots.

A. Flies track the random dot array as it moves laterally, and the elevation of the segment containing the dots varies. The dots were rendered to fill a solid angle of 2 steradians at each elevation, but weren't displayed in the back where there is no projection, as shown in Figure 1B. B. Analogous to Figure 2B, the dot field moves in a restricted segment and the elevation of the virtual horizon varied. C. Mean tracking response of 39 individual fruit flies, starved for about 3 hours to enhance tracking responses, viewing dots at five mean elevations. D. The mean responses of the same flies to central moving dot fields at five horizon elevations. E. Black markers show mean correlations of tracking responses to dot motion at variable dot elevations, with vertical bars to show standard error. Gray markers show the mean correlations of 156 flies that were not starved before testing. F. The response to motion of a segment of dots with a mean 0 degree elevation, while the elevation of the horizon varied.