



Visual attention in *Drosophila melanogaster*

(Visuelle Aufmerksamkeit bei *Drosophila melanogaster*)

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1. Introduction

Our visual environment is cluttered with information. In spite of this, we manage to perform common visual tasks without any difficulties by focussing our attention to process only the useful information. The rest of the information is discarded. This “focusing on one source of sensory inputs to the exclusion of others is termed selective attention” (Luck and Mangun 2009). Attention has been extensively studied in humans and non-human primates (NHPs) (Wurtz and Goldberg 1972; Moran and Desimone 1985) where it has often been compared to a “spotlight” (LaBerge 1983) and a “zoom lens” (Eriksen and St James 1986). Attentiveness is generally reflected by an increase in the efficiency of the response detection and hence the enhanced visual performance in various forms like shorter response times, higher accuracy, and lower thresholds of neurons in the corresponding projection area of the brain.

It is well known that attention can be guided by external cues and also voluntarily in humans and NHPs. Studies using cues to guide spatial attention in humans were pioneered by Posner. A cue preceded a detection stimulus (test) and the reaction times were measured. The reaction times for the test in a valid cue trial, where the cue informed about the correct position of the test, were shorter than in an invalid cue trial showing that the reaction times are shorter if

attention is directed to the test (Posner 1980).

The voluntary or covert shifts of attention were already observed in the 19th century by Helmholtz. He projected a printed page for a brief moment while fixating in the center of the page and found that he could perceive several letters from any pre-chosen region of the page without shifting his gaze to that region (Helmholtz 1909–1911).

Similar restriction of the behavior is also shown by male hoverflies which choose and track single flies while ignoring other possible target flies in the vicinity (Collett and Land 1975). In flying *Drosophila*, attention has been studied at the torque meter (Wolf and Heisenberg 1980; Heisenberg and Wolf 1984). In this experimental set-up flies cannot move their eyes because their head and thorax are fixed in space, which makes this preparation well suited for the study of visual attention. If a single stripe is oscillated at a fronto-lateral position, flies show a characteristic torque pattern. They generate a fast sustained torque response of the same polarity as the front-to-back motion of the stripe. But for the back-to-front motion of the same stripe, the flies produce torque spikes which are indicative of body saccades. These saccades are directed towards the side of the stripe but against its direction of motion. This asymmetric torque pattern helps to show where the focus of attention (FoA) of the fly lies. When two such stripes

are coherently oscillated in the left and the right visual fields, the flies sometimes show the torque pattern as if only one of the stripes was presented and sometimes as if only the other stripe was presented by producing a mirror-symmetrical pattern. Similar behavior is seen when instead of two stripes a random dot pattern is shown. Flies produce the characteristic torque pattern as if sometimes they follow the motion of a constellation of dots on only one side of the panorama. Flies seem to shift their attention endogenously in these cases. But the FoA of the flies can also be shifted through the use of external cues. For instance, an air puff with the scent of fermenting banana from the side of one of the oscillating stripes or a fast wiggling stripe in front of the random dot panorama would prompt the flies to attend to the visual information on the side of these cues (Wolf and Heisenberg 1980; Heisenberg and Wolf 1984).

Drosophila can also restrict some of its responses to parts of the visual field under closed loop conditions. If a stripe is displaced from front-to-back rapidly (loop is opened during this displacement), the flies respond with a short burst of torque which brings the stripe back to about its initial position. The flies respond to the displacement of a single stripe about twice as often as compared to when there is a second stripe also present but not displaced. Although the angular separation and the positions of the stripes are variable in this

experiment there is one stripe in each frontal quadrant at the onset of the displacement. In another experiment, the angular separation between the two stripes is kept constant while displacing both the stripes together (one front-to-back and one back-to-front). This keeps the displaced stripe in the frontal part of the visual field but still the response frequency is significantly lower in the presence of a second stripe. During stationary flight, patterns that are not apparently salient are not ignored and can draw attention away from the more salient stimuli (Heisenberg and Wolf 1984).

There are several recent studies on *Drosophila* that claim the involvement of attention and attention-like processes in various behaviors. Changes in local field potentials produced in relation to novel visual stimuli and motion processing in a multiple choice point maze have been studied. These behaviors are shown to be modulated by short-term memory genes *dunce* and *rutabaga* and an involvement of attention-like processes has been hypothesized here (van Swinderen 2007). Same studies with the memory consolidation mutant *radish*¹ attributed the variations in the behavior of these flies when compared to the wild-type flies to attention-like defects. Some of these defects were rescued by the administration of methylphenidate, a drug that is commonly given to attention deficit hyperactivity disorder patients (van Swinderen and Brembs 2010).

Some other studies relate the orientation ability of the flies towards a visual object during tethered flight to attention-like behavior (Ye et al. 2004). These studies also talk about the role of long-term and short-term blockade of dopamine release in this orientation behavior (Ye et al. 2004). In another study the effect of salience of an object, depending on the contrast between the object and the background and the sharpness of the visual object, on fixation behavior for this single target object is related to attention (Xi et al. 2008).

Although in these more recent studies attention has been hypothesized to underlie various behavioral and electrophysiological phenomena, no attempts have been made to show that these phenomena correspond to a behavior conforming to an operational definition of attention. The present study tries to investigate different aspects of attention in *Drosophila* while revolving around a working definition that is used in human and NHP studies. Some of the basic characteristics of the cued and the covert attention and the neuronal underpinnings of the cued attention have been studied here.

2. Materials and Methods

2.1. Flies

Flies were reared on standard corn meal-molasses medium at 25° C and 60% relative humidity under 14 h/10 h light/dark cycle. For tethering, individual female flies (2-3 days old) were briefly immobilized by cold anesthesia. A triangular shaped holder made of copper wire (0.05 mm diameter) was positioned using a micromanipulator between the fly's head and thorax. Dental composite (3M ESPE Sinfony®, DO3) was used to glue the hook to the fly. The composite was polymerized with a 10 s pulse of blue light from an LED dental curing light source (distance < 0.5 cm). Flies were then kept in separate chambers supplied with sucrose and water for a day before the experiment.

Canton S (CS) flies were used for all the experiments related to wild-type behavior, unless otherwise specified. For some experiments wild-type Berlin (WTB) were also used. TH-GAL4 and dumb² flies were kindly provided by Andreas Thum. UAS-shi^{ts1} flies were taken from the Department of Neurobiology and Genetics stock originally procured from Troy Zars. TH-GAL4 flies had a p-element insertion on Chromosome 2, in white background. UAS-shi^{ts1} flies had a p-element inserted in Chromosome X (alpha).

dumb² had a piggyBac insertion on Chromosome 3.

2.2. Hydroxyurea (HU) treatment

Approximately 1 hour after larval hatching (ALH), 100 WTB larvae were fed HU in a heat-killed yeast suspension (50-60 mg HU/ml yeast) for 4 hours. 1 hour ALH, control larvae were fed only the yeast suspension without any HU. Both HU-treated and control larvae were then washed in water and transferred to culture bottles with standard corn meal-molasses medium for further development at 25 °C and 60% relative humidity under 14 h/10 h light/dark cycle. 2-3 old female flies were then used for the experiments.

2.3. Heat shock treatment

2-3 days old male TH-GAL4 flies were crossed with 2-3 days old virgin female UAS-shi^{ts1} flies. 2-3 days old female TH-GAL4/UAS-shi^{ts1} flies were incubated at the permissive (25 °C) or the restrictive (30 °C) temperature for 10 minutes in a humid incubator and then immediately measured at the torque meter in the LED arena maintained at the permissive or the restrictive temperature, respectively. For the 18 °C experiments, 2-3 days old female TH-GAL4/UAS-shi^{ts1} flies were incubated at 18

°C for a day before the experiment and then measured at 18 °C at the torque meter.

2.4. Setup

A cylindrical arena made of 180 x 48 green LEDs was used to present the visual stimuli. If not otherwise stated, a circular disk of black cardboard was positioned at the lower edge of the LED panels, forming the floor of the arena. For a 'Low Bright Floor', the fly could see a ring of grey plastic underneath the arena (on which the arena rested) and, about 6 cm further down, a grey metal plate diffusely reflecting some of the light of the LEDs (Fig. 1.16).

Control of the sequence of pattern presentation and timing of the experiments was accomplished by custom made software written in C++ on a Linux environment (by Andreas Eckart). Flies were clamped with their triangular copper wire holders to a small metal clip which was attached to a torque meter. While doing so, the flies were positioned in the center of the LED arena (Fig 1A) in an otherwise dark room. Yaw torque of the stationary flying flies was digitalized (resolution 12 bit) and recorded on the controlling computer's hard disk at a sampling frequency of 100 Hz. These torque data were later evaluated using Microsoft Excel. All experiments were carried out under open loop conditions, i.e., the flies' yaw

torque responses were only recorded and had no retroactive effect on the stimulus itself.

2.5. Stimulus conditions

Stripes used in all the experiments were 6° wide (3 vertical columns of LEDs) unless otherwise specified. For the test of visual attention, two stripes were presented in the fly's fronto-lateral visual field at symmetrical positions with respect to the fly's longitudinal body axis. Stripes were displaced by $\Delta\psi = 30^\circ$ with fast front-to-back motion of 150°/s and then moved back slowly to the initial positions at 20°/s. For cuing, one of the stripes was oscillated prior to the displacement with a frequency of 10 Hz and a peak-to-peak amplitude (A_{p-p}) of 4°. Unless otherwise mentioned, the stationary starting positions of the stripes were at $\psi_0 = \pm 42^\circ$. A typical experimental frame without a cue had the following sequence: presentation of stationary stripes at the initial position ψ_0 for 5 s, displacement over $\Delta\psi = 30^\circ$ for 0.2 s, stripes returning back to ψ_0 in 1.5 s. In a typical experiment involving cue presentation, the following sequence was used: stationary stripes presented at ψ_0 for 5 s, cue presentation for 5 s, displacement over $\Delta\psi = 30^\circ$ for 0.2 s, stripes returning back to the initial position ψ_0 in 1.5 s. This sequence was repeated 8 times for the same fly, if the fly did not stop flying. Responses to the fast front-to-back displacement were readily recognizable (steep rising phase in one or the

other direction and somewhat slower decline). A response was scored when the yaw torque modulation in response to the front-to-back displacement was more than 10×10^{-10} Nm and started within 40 to 200 ms after the onset of the displacement (Fig 1.1B). The response was called syn-directional when the steep rising phase was of the same polarity as the front-to-back displacement. When it was of the opposite polarity, it was called anti-directional. Syn- and anti - directional responses as well as the responses to bilateral displacements had all about the same dynamics and remained similar in all the cases whether or not there was a cue present. For the experiments of Fig 1.9A, the stationary positions of the stripes were varied from $\psi_0 = 0^\circ$ to $\psi_0 = \pm 90^\circ$. In the experiments with temporal delays between the cue and the displacements (Fig 1.10), the stripes were visible already during the delay before their displacement. Here, a *Response Index* was defined as $RI = [p(s) - p(o)] / [p(s) + p(o)]$, with $p(s)$ being the probability of the response toward the cued side and $p(o)$ the probability of the response away from the cued side. Both WT strains, CS and WTB, were tested in these experiments. Data from both WT strains were pooled as they did not show any significant differences. For the spatial separation of the cue from the displacement (Fig 1.11), during cue presentation two extra stripes appeared at variable positions. These stripes had the same size as the displaced stripes. The stripes to be displaced were always presented at $\psi_0 = \pm 42^\circ$.

For the flickering stripe as the cue, just like the oscillating stripe, one of the stripes that was later displaced, was flickered at 5 Hz. For flickering, the corresponding LEDs of the arena were switched off and on. The white LED column used for the experiments of Fig. 1.16 was not completely opaque and was narrower (column width $\approx 3^\circ$) than the width of the dark stripe ($\delta = 10^\circ$ in this experiment) on the display such that the latter was easily visible on both sides of the LED column. Stripes were shown in the entire visual field for these experiments and the flicker was presented for 5 s at 2Hz. In the experiments of Fig 1.12, four stripes were presented always while only one was ever displaced at any given time. The cuing duration was 3 s in these experiments.

In the experiments with fixed windows in which the stripes were oscillating (Fig. 3.1), the width of the stripes was 10° and the distance between each stripe was also 10° . The stripes were moved in one direction for 5 s at $8^\circ/s$. For the motion processing experiments (Fig. 3.2, 3.3), the optomotor response was calculated with respect to the motion of the upper field where the stripes first moved counterclockwise and then clockwise.

2.6. Statistical analysis

Since some data were not normally distributed (Kolmogorov-Smirnov test),

Wilcoxon Matched Pairs test for dependent pairwise comparisons was used to test the frequency of response to one side (for e.g., the 'cued' side) against the frequency of response to the other side (for e.g., the 'not-cued' side). The one-sample Wilcoxon Signed Rank test was used to compare the response frequencies with a random value (for landing response). $p\text{-value} < 0.05$ was taken as significant.

3. Results

3.1. Cued shifts of attention

A fly can focus its attention on a part of the visual field. This focus of attention (FoA) can be steered through external visual or non-visual cues (Heisenberg and Wolf 1984). A systematic study of visual attention and in particular of external cuing was carried out here.

3.1.1. Focusing on part of the visual field

A test was developed where the response of a fly could be measured, with or without the external guidance of attention to check which stimulus, one presented in each of the two visual halves, the fly was responding to. Initially, a single stripe was presented to the fly at a fronto-lateral position (Fig. 1.1A; height, $H = 96^\circ$; azimuth, $\psi_0 = \pm 42^\circ$). This stripe was then displaced in a fast front-to-back motion ($\Delta\psi = 30^\circ$ at $150^\circ/\text{s}$). Flies showed a typical phasic yaw torque modulation to this fast displacement (Fig. 1.1B, gray area is the time period of front-to-back displacement). There was no detectable response to the slow back-to-front motion of the stripe. The yaw torque responses mostly had the same polarity as the motion of the stripe ('syndirectional' responses; Fig. 1.2A, Single, One-of-two). In the real world, the

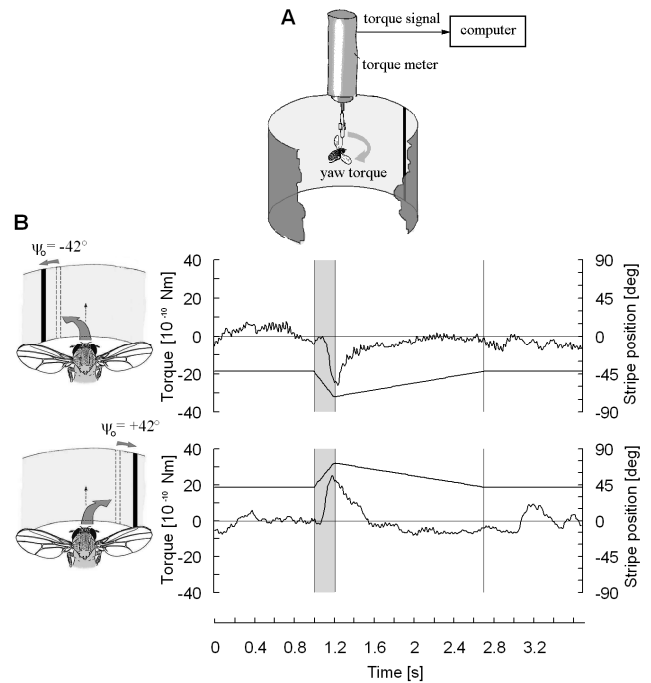


Fig. 1.1. Phasic yaw torque response to fast displacement of a stripe. (A) Experimental setup. A single fly is attached to a torque meter and centered in a cylindrical arena made of LEDs on which the visual stimuli are presented. (B) Representative clockwise and counterclockwise torque responses of single flies. When a stripe is displaced front to-back ($\Delta\psi = 30^\circ$; $150^\circ/\text{s}$; gray area), the fly responds with a phasic modulation of torque most often with the same polarity as the front-to-back motion. The jagged line represents the yaw torque and the smooth line the stripe position over time. The vertical line (at 2.7 s) indicates the time when the stripe returns to the initial position after displacement (return speed = $20^\circ/\text{s}$). The small average torque response to the slow back-to-front motion cannot be seen in the single traces. ψ , azimuth.

front-to-back motion of the stripe could have been caused by passive self-rotation and so, possibly, the flies tried to correct for it by producing syndirectional torque responses. Occasionally, the response had the opposite

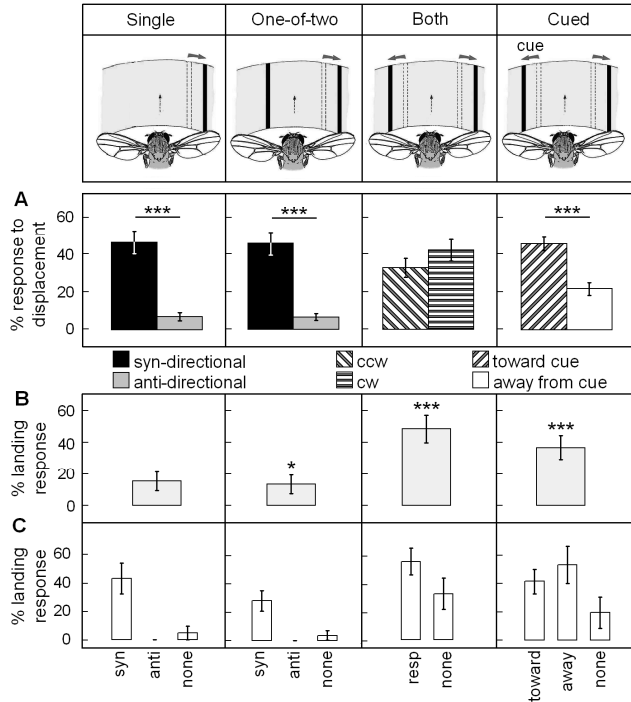


Fig. 1.2. Attention can be guided to one or the other visual half-field by a cue. (A) When a single stripe is displaced (Single, One-of-two), flies mostly respond with syndirectional yaw torque to the front-to-back stripe motion. When both the stripes are displaced simultaneously (Both), flies respond equally often to either stripe. If a 10-Hz oscillation of one stripe (cue) precedes the displacement of both stripes (Cued), flies respond more often to the displacement on the cued side ($n = 29$). ccw, counterclockwise; cw, clockwise. (B) Frequency of landing responses. Flies often show landing responses which may occur for the same displacement event that leads to the phasic yaw torque response. ($n = 17$; flies are a subset of those in A; see frequency of yaw torque responses for this subset in Fig. 1.3). (C) Frequencies of landing responses associated with the different yaw torque responses ($n = 17$). syn, syndirectional yaw torque (torque response with the same polarity as the fast front-to-back motion of the stripe); anti, antidirectional yaw torque (torque response with the opposite polarity as the fast front-to-back motion of the stripe); resp, all yaw torque responses; toward/away, toward or away from the

cued side. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars are SEM.

polarity as that of the motion of the stripe ('antidirectional' responses; Fig. 1.2A, Single, One-of-two). These antidirectional responses had similar dynamics as the syndirectional responses suggesting that the yaw torque responses had a fixed action pattern like all-or-none responses.

When two such stripes were displayed at symmetrical fronto-lateral positions in the two visual half-fields ($\psi_0 = \pm 42^\circ$) but only one of them was displaced (Fig. 1.2A, One-of-two), the response probabilities were similar to those of the single-stripe case. Then, if both the stripes were displaced front-to-back simultaneously (right one clockwise and left one counterclockwise), the flies produced equally frequent responses of both the polarities (Fig. 1.2A, Both). If the flies were to respond only to the vector sum of the two equal but opposing motion stimuli no phasic torque responses would have been seen. However, the responses were observed for $\sim 78\%$ of the displacements. The flies responded as if out of the two stripes they were selectively following only one at times and the other one at other times. There were also times when the flies did not respond at all. Among these no-response cases, it is not possible to distinguish when the flies might have responded to the vector sum of the two motions from when the flies just did not respond.

3.1.2. Guiding the focus of attention

To guide the FoA of the flies, one of the stripes was oscillated before both were displaced simultaneously (frequency, $f = 10$ Hz; peak-to-peak amplitude, $A_{p-p} = 4^\circ$; Fig. 1.2A, Cued). The oscillation stopped right before the displacements started. This resulted in more syndirectional (toward cue) responses to the motion of the stripe on the cued side than the antidirectional (away from cue) responses. The total response frequency increased by about 20% when two instead of one stripe was displaced (Fig. 1.2, compare Single and One-of-two with Both and Cued). It is only natural that there would be more responses if there would be more stimuli to which the flies could respond to. The total response frequencies were almost equal for the Both and the Cued conditions showing that the cuing stimulus did not have a significant impact on the total response frequency for the two-stripe displacements but it only redistributed the frequencies.

3.1.3. Same stimulus leads to both yaw torque and landing responses

Apart from the phasic yaw torque responses and the no-responses, landing responses were also observed for the same stimulus. These landing responses were seen more frequently when both the stripes were displaced as compared to when only one was displaced (Fig. 1.2B). For the two-stripe displacements,

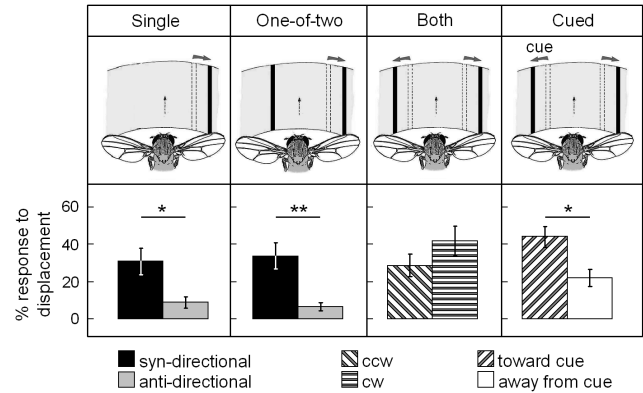


Fig. 1.3. Steering of the focus of attention, as in Fig. 1.2A, for the $n = 17$ flies evaluated for the landing response in Fig. 1.2 B and C. * $P < 0.05$, ** $P < 0.01$. Error bars are SEM.

when a yaw torque response was produced a landing response was also produced in more than half of these cases (Fig. 1.2C Both). So, during the same event when both the stripes were displaced, the flies evaluated motion of both the stripes for the production of the landing response but only that of a single stripe for the torque response. Since the flies showed both the behaviors for the same stimulus, the local stimulus suppression i.e., the FoA must be behaviorally selective.

Interestingly, if the flies produced the landing responses for the single-stripe displacements, they also produced the syndirectional torque responses in most of these cases (Fig. 1.2C, Single, One-of-two). They never produced an antidirectional response with a landing response. This means that if the flies tried to land on a stripe they also tried to turn towards it. If the antidirectional responses would be startle or

escape responses, concomitant landing responses would be intuitively inappropriate.

3.1.4. Cuing affects the outcome of the single-stripe displacement

The cuing effect was further investigated by checking if the FoA could be steered by shorter stripes and stripes at different heights in the visual field. First, a stripe of half the height of the arena ($H = 48^\circ$; $\psi_o = \pm 42^\circ$) was presented such that half of it lied above the equator and half below (Fig. 1.4A, Middle Stripe). When this stripe was displaced, as expected, the flies responded more frequently with the syndirectional torque modulations than with the antidirectional ones. Similar response frequencies were obtained when the same stripe was displayed only in the upper visual field (UVF) (Fig. 1.4A, Upper Stripe). But if the same stripe was presented only in the lower visual field (LVF) (Fig. 1.4A, Lower Stripe), equally frequent syn- and antidirectional responses were observed. This effect was specific for the normal contrast of the scene. When the contrast was inverted, the responses were mainly syndirectional (Fig. 1.4A, Inverted Lower Stripe). This observation is in line with the differences in the yaw torque responses observed in other studies for inverted contrast conditions (Heisenberg and Wolf 1984).

Since for the displacement of a single stripe presented only in the LVF the antidirectional

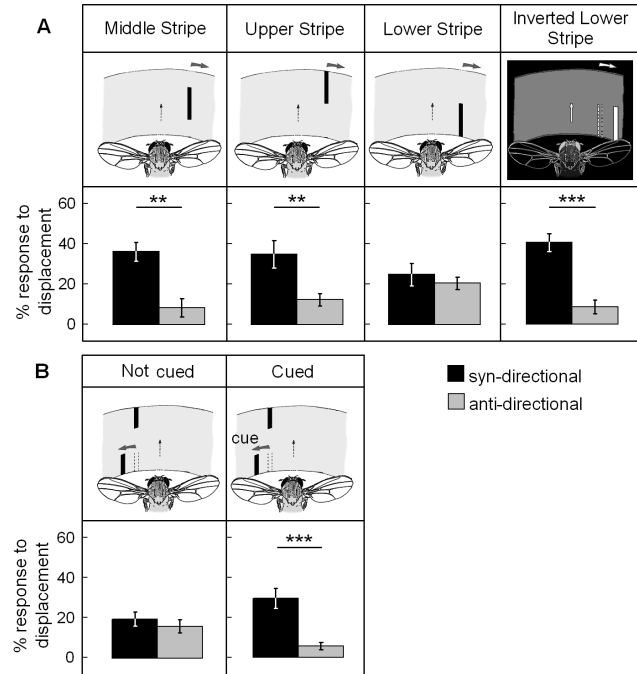


Fig. 1.4. Different response frequencies are observed for the displacement of a stripe at different vertical positions. (A) Flies respond more often syndirectionally when a single stripe which lies half in the upper (UVF) and half in the lower visual field (LVF) or only in the UVF is displaced (Middle Stripe and Upper Stripe, resp.; $n = 20$). Surprisingly, equally often syndirectional and anti-directional responses are seen if the stripe is confined to the LVF (Lower Stripe, $n = 20$). The responses become more syndirectional in the LVF when the contrast is inverted (Inverted Stripe, $n = 22$). (B) Cuing increases frequency of the syndirectional responses. When the stripe in the LVF is oscillated before the displacement, the frequency of the syndirectional responses is increased, that of the antidirectional responses decreased. ($n = 24$; part of data obtained with Low Bright Floor arena.) $**P < 0.01$, $***P < 0.001$. Error bars are SEM.

responses were as frequent as the syndirectional ones, the effect of cuing on these frequencies was investigated. This

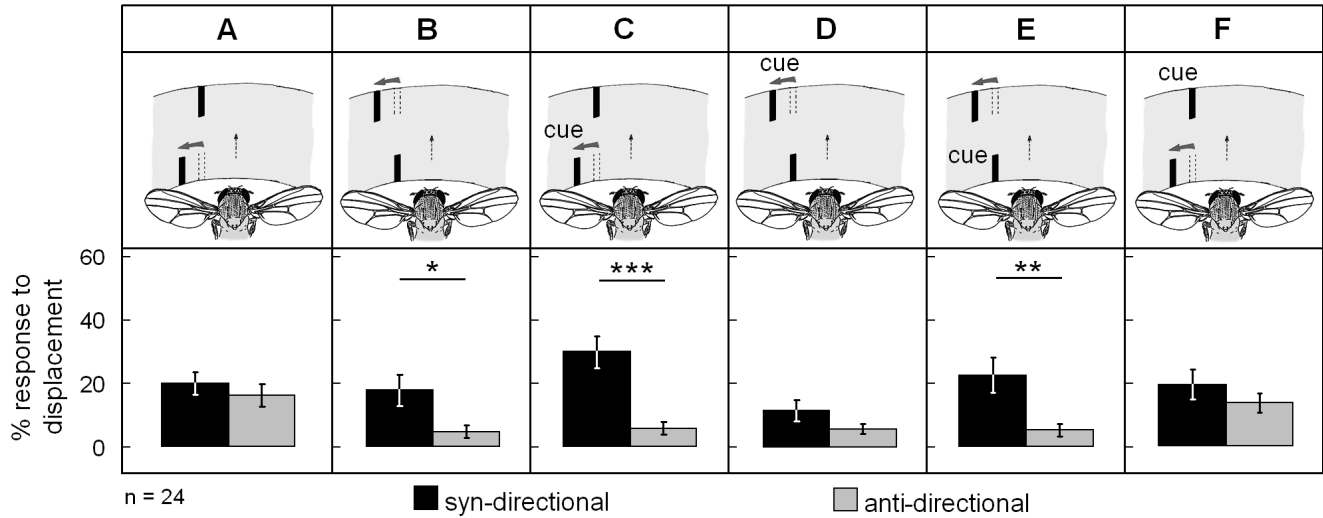


Fig. 1.5. Cuing affects the test only in the LVF. (A) When a stripe is presented in the LVF and one in the UVF at the same azimuth, and only the lower stripe is displaced, equally frequent syn- and antidirectional responses are observed. (B) When only the upper stripe is displaced, more syndirectional responses are observed. (C) If the lower stripe is cued before its displacement then more syndirectional responses are seen. (D) But cuing the upper stripe before its displacement does not have any major effects. Cuing the lower stripe and displacing the upper one (E) or vice versa (F) does not have any effects either. $n = 24$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars are SEM.

experiment was initially a part of another study (Fig. 1.5) where two stripes of equal heights ($H = 28^\circ$) were displayed at the same azimuth, one in the LVF and the other in the UVF. Like earlier, the displacement of a single stripe in the LVF led to equally frequent syn- and antidirectional responses (Fig. 1.4B, Not cued). But when the same stripe was oscillated before the displacement, the syndirectional responses became more frequent (Fig. 1.4B, Cued). This could be because the cuing somehow changed the meaning of the test stimulus or the FoA was guided to the cued stripe and whenever the attention was focused on a stripe, more syndirectional responses were produced (see section 3.1 for more discussion).

3.1.5. Cuing operates exclusively in the LVF

In the next set of experiments, two stripes were shown like in the experiments of Fig. 1.4B. First, the single-stripe displacements without cuing were done either in the LVF (Fig. 1.5A) or in the UVF (Fig. 1.5B). The response frequencies were as found previously for these conditions. Then, the lower stripe was cued and displaced like for Fig 1.4B and the frequency of the syndirectional responses increased and became more than that of the antidirectional responses (Fig. 1.5C). Whereas, there was no apparent increase in the frequency of the syndirectional responses when the upper stripe was cued and

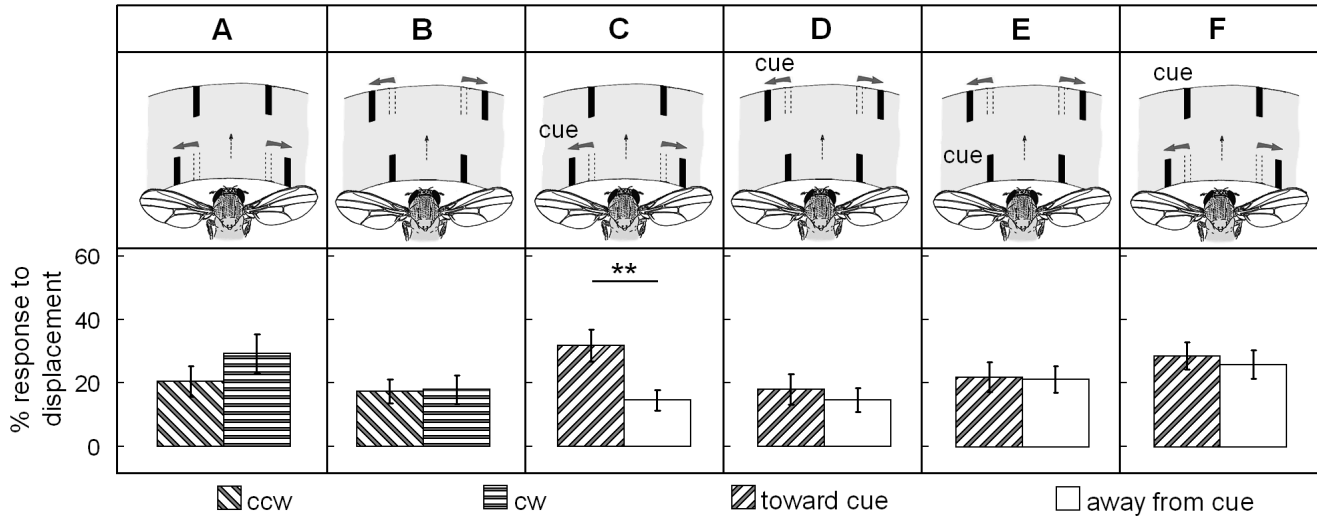


Fig. 1.6. External guidance of attention works only in the LVF. (A) When two stripes are presented in the UVF and two in the LVF and only the lower two are displaced, equally frequent torque responses of either polarity are observed. (B) If only the upper two stripes are displaced, the fly again generates equally frequent clockwise and counter-clockwise responses. (C) Now, if one of the lower stripes is oscillated (cued) and then both lower stripes are displaced, flies respond more to the cued side. (D) Interestingly, when one of the upper stripes is cued and then both the upper stripes are displaced, no guidance effects are seen. (E and F) If one of the lower stripes is oscillated and both the upper ones are displaced (E) or one of the upper stripes is oscillated and both the lower ones are displaced (F), again no cuing effect is found. The cue can guide the attention only when both the cue and the displacements are presented in the LVF. $n = 19$. ccw, counterclockwise; cw, clockwise; $**P < 0.01$. Error bars are SEM.

displaced (Fig. 1.5D) as compared to when there was no cuing (Fig. 1.5B). In fact, cuing the upper stripe seemed to decrease the syndirectional response frequency (Fig. 1.5D). This observation might not hold true on increasing the sample size but if it did then it would be a very interesting observation and would call for more investigation. It would again point to how different stimuli are processed differently depending on whether they lie in the UVF or in the LVF. Next, the lower stripe was cued and the upper stripe was displaced (Fig. 1.5E) or the upper stripe was cued and the lower one was displaced (Fig. 1.5E). Both

these conditions produced results like if there was no cue at all (compare with Fig. 1.5B and A, respectively).

This set was later extended to both the left and the right visual half-fields instead of showing the stimuli in just one visual half-field at a time (Fig. 1.6). When both the lower stripes were displaced but the upper ones remained stationary (Fig. 1.6A) or when both the upper stripes were displaced but the lower ones remained stationary (Fig. 1.6B), the results were like those seen earlier i.e., equally frequent clockwise and counter-clockwise responses. Then, one of the lower

stripes was oscillated before both the lower ones were displaced (Fig. 1.6C). Again, as seen earlier, more responses towards the cued side were observed. But when one of the upper stripes was oscillated before the displacement of both the upper stripes (Fig. 1.6D), no cuing effects were seen i.e., the frequency of the responses to the cued side did not increase. These results showed that the cuing was effective only in the LVF. These experiments also excluded the possibility that the response frequency for the two-stripe displacement condition was just a sum of the frequencies for the cued single-stripe displacements (compare Fig. 1.5C with 1.6C).

To check if any cross-talk occurred between the LVF and the UVF, one of the lower stripes was oscillated and then both the upper ones were displaced (Fig. 1.6E) or one of the upper stripes was oscillated and both the lower ones were displaced (Fig. 1.6F). The response frequencies remained as if no cuing was presented in these cases (compare with Fig. 1.6B and A, respectively).

Even if the vertical gaps between the upper and the lower stripes were varied from no gap (Fig. 1.7, No gap) to up to a gap of 20° (Fig. 1.7, 20° gap), while keeping the height of the lower stripe constant, no cuing effects were transferred from the lower to the upper field when one of the lower stripes was oscillated and the upper ones were displaced (Fig. 1.7).

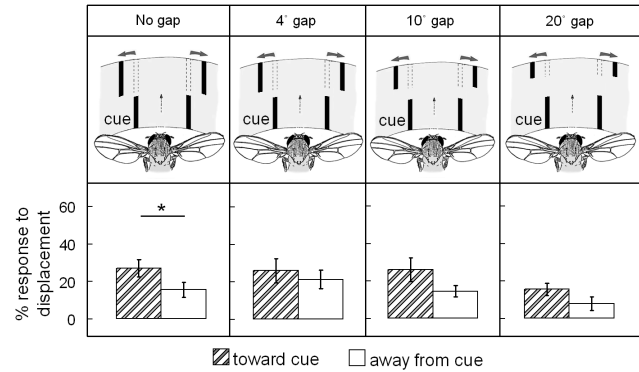


Fig. 1.7. Cuing effects are not transferrable between the LVF and the UVF even if there is no gap between the stripes. When the vertical gaps between the upper and the lower stripes are varied from no gap to up to a gap of up to 20°, while keeping the height of the lower stripe constant, no cuing effects are transferred from the lower to the upper field if one of the lower stripes is oscillated and both the upper ones are displaced. $n = 13$. $*P < 0.05$. Error bars are SEM.

Though in the No gap condition the difference between the frequency of the responses towards the cue and those away from the cue was statistically significant the p-value was ~ 0.04 with a sample size of 13 (Fig. 1.7, No gap). This difference might not remain significant if the sample size is increased. Taken together with the results of Fig. 1.5E, F and Fig. 1.6E, F, these results indicate that for the cue to be effective, both the test and the cue have to be in the LVF and that irrespective of the vertical distance between the upper and the lower stripe, the cuing effect is never transferred from the LVF to the UVF. Alternatively, if the difference between the response frequencies in the No gap condition stays significant then it would mean that the continuity of the

stripes in the UVF and the LVF before cuing and test leads to a transference of the cuing effect from the LVF to the UVF. In such a situation, the upper and the lower stripes might be treated as one object by the flies and therefore even if only the lower part of this object is cued the upper part also carries the cuing effect with it.

3.1.6. Cuing is equally effective at various lateral positions

Since the cuing operated only in the LVF, experiments in the LVF were made standard to test further characteristics of the cued attention (Fig. 1.8; $H = 48^\circ$; $\psi_o = \pm 42^\circ$). (Note: all further experiments were performed only in the LVF, unless otherwise specified). As seen previously, there were equally frequent syn- and antidirectional responses for the single stripe displacements (Fig. 1.8A, Single, One-of-two). When both the stripes were displaced without cuing, equally frequent clockwise and counter clockwise responses were seen (Fig. 1.8A, Both). On cuing one of the stripes, the guidance of attention was very prominent (Fig. 1.8A, Cued). The landing response frequencies for this condition (Fig. 1.8B) matched the frequency trends seen for the conditions where the stripes were presented in the whole visual field (Fig. 1.2). There were significantly more landing responses when two stripes were displaced (Fig. 1.8B,

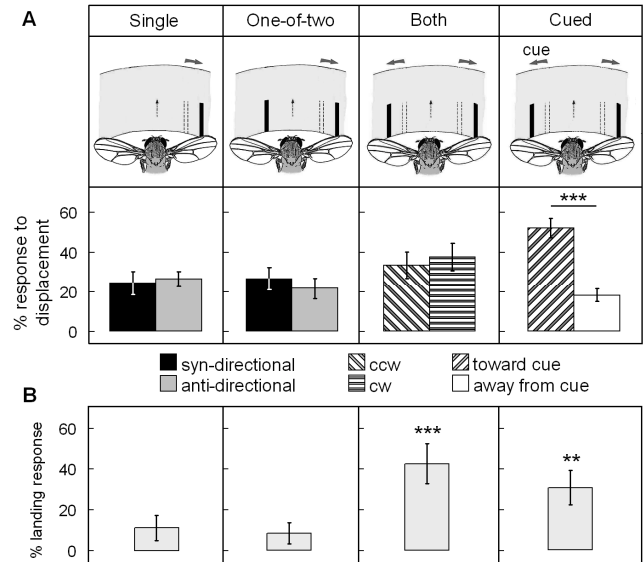


Fig. 1.8. Standard attention test conditions. The cuing operates only in the LVF and therefore the experiments restricted to the LVF are treated as standard. For the single stripe displacements, equally frequent syn- and antidirectional responses are seen (A, Single, One-of-two). Equally frequent clockwise and counterclockwise responses are seen when both the stripes are displaced simultaneously (A, Both) and significantly more responses are seen to the cued side when one of the stripes is wiggled before the displacement of both (A, Cued). Significant number of landing response are also observed when both the stripes are displaced (B, Both, Cued). $n = 18$. ccw, counterclockwise; cw, clockwise. ** $P < 0.01$, *** $P < 0.001$. Error bars are SEM.

Both, Cued) instead of a single stripe (Fig. 1.8B, Single, One-of-two).

To see if and how the cuing effects depended on the lateral position, two stripes ($H = 48^\circ$) were presented at various positions ranging from $\psi_o = 0^\circ$ to $\psi_o = \pm 90^\circ$ (Fig. 1.9A). When both the stripes were displaced, the expected equally frequent clockwise and

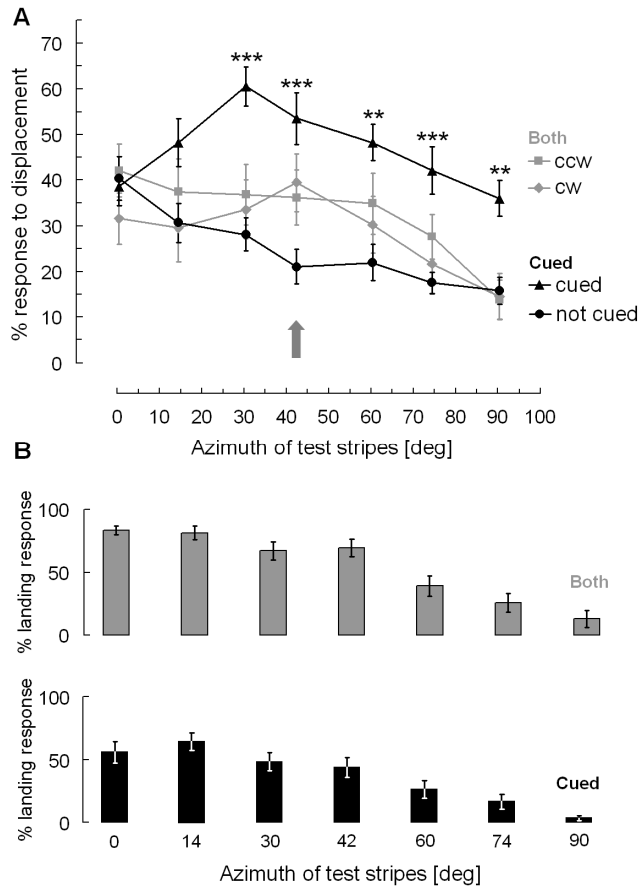


Fig. 1.9. Guidance of attention in the LVF. (A) The guiding cue is effective at various azimuths from $\pm 30^\circ$ to $\pm 90^\circ$. Arrow indicates the azimuth used in most of the experimental conditions like in Figs. 1.1–1.8. (B) Landing response is also observed at all of these azimuths. The total landing response frequencies decrease as the stripes are placed more laterally whether or not there is any cuing just like the torque responses. $n = 19$. ccw, counterclockwise; cw, clockwise. $**P < 0.01$, $***P < 0.001$. Error bars are SEM.

counterclockwise responses were observed at all the lateral positions even though the total response frequency decreased when the stripes were placed more laterally (Fig. 1.9A, Both). The cuing effects were observed from $\psi_o = \pm 30^\circ$ to $\pm 90^\circ$ (Fig. 1.9A, Cued) even if

the total response frequency was sometimes different from the corresponding Both condition (Fig. 1.9A, Both). At $\psi_o = \pm 14^\circ$, the cuing effect was not significant may be because this position lied in the region of binocular overlap or within the spotlight of attention created by the cue on the other side. Landing responses were also measured at all of these positions (Fig. 1.9B). Like for the torque responses, the total response frequencies for landing also decreased as the stripes were placed more laterally whether or not there was any cuing (Fig. 1.9B, Both, Cued). Lower total response frequencies for both the torque and the landing responses indicate that the flies miss a stimulus more frequently if this stimulus is placed more laterally in their visual field. Also, if the stripes are already so far apart, their further separation during the displacement might not look like much of an expansion stimulus to evoke a landing response.

3.1.7. Cuing effect lasts longer than 2 seconds

How long does the cuing effect persist after the removal of the cue? Delays of different lengths were inserted between the presentation of the cue and the test thus effectively separating the two in time. Like in the standard situation, two stripes were presented and one of them was oscillated to act as the cue. Then, both the stripes

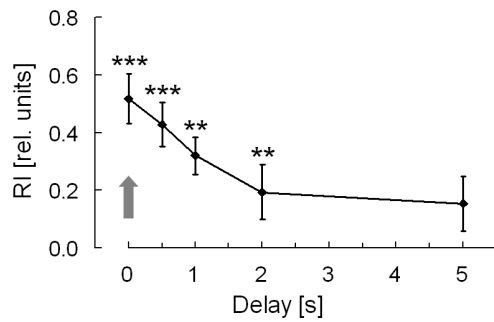


Fig. 1.10. Temporal separation of the cue from the displacement in the LVF. When the cue precedes the displacement temporally by several seconds, it can still guide the attention. The effect of the cue diminishes as the delay increases and disappears around a delay of 5 s ($n = 36$). RI is the response index (Materials and Methods). Arrow indicates the condition where the displacement follows the cue immediately. $**P < 0.01$, $***P < 0.001$. Error bars are SEM.

remained stationary for variable delays (from 0 – 5 s) after which both were displaced. The cuing effect diminished with time but was effective at least till a delay of 2 s (Fig. 1.10). At a delay of 5 s, the effect could not be seen anymore (Fig. 1.10). Apart from informing about how long the cuing effect persists this temporal separation of the cue and the test also eliminates the possibility of any kind of interaction between the two stimuli.

3.1.8. Cuing and test stimuli can be spatially separated; spatial extent of FoA is smaller than one lower visual half-field

The horizontal spatial extent of the cuing

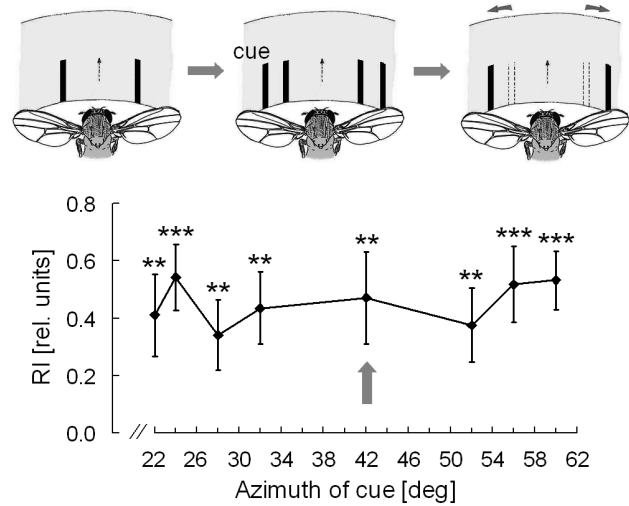


Fig. 1.11. Spatial separation of the cue from the displacement in the LVF. When the cue is presented at variable azimuths whereas the position of the displacements remains constant, irrespective of the distance of the cue from the displaced stripes (in the same visual half-field), the guidance of the attention is equally effective ($n = 18$). RI is the response index (Materials and Methods). Arrow indicates the condition where the cue and the displaced stripes are at the same azimuth. $**P < 0.01$, $***P < 0.001$. Error bars are SEM.

effect was measured. Two test stripes were always presented at $\psi_o = \pm 42^\circ$ whereas two additional cuing stripes were presented at various positions from $\psi_o = \pm 22^\circ$ to $\pm 60^\circ$ (Fig. 1.11). During these experiments, the test stripes were always visible while the cuing stripes appeared only at the time of the cue presentation. One of the cuing stripes oscillated and then both disappeared followed by the normal test. The attention guiding effects of the cue were not affected by the sudden appearance and disappearance of the extra pair of stripes and the guidance was equally effective at all of the

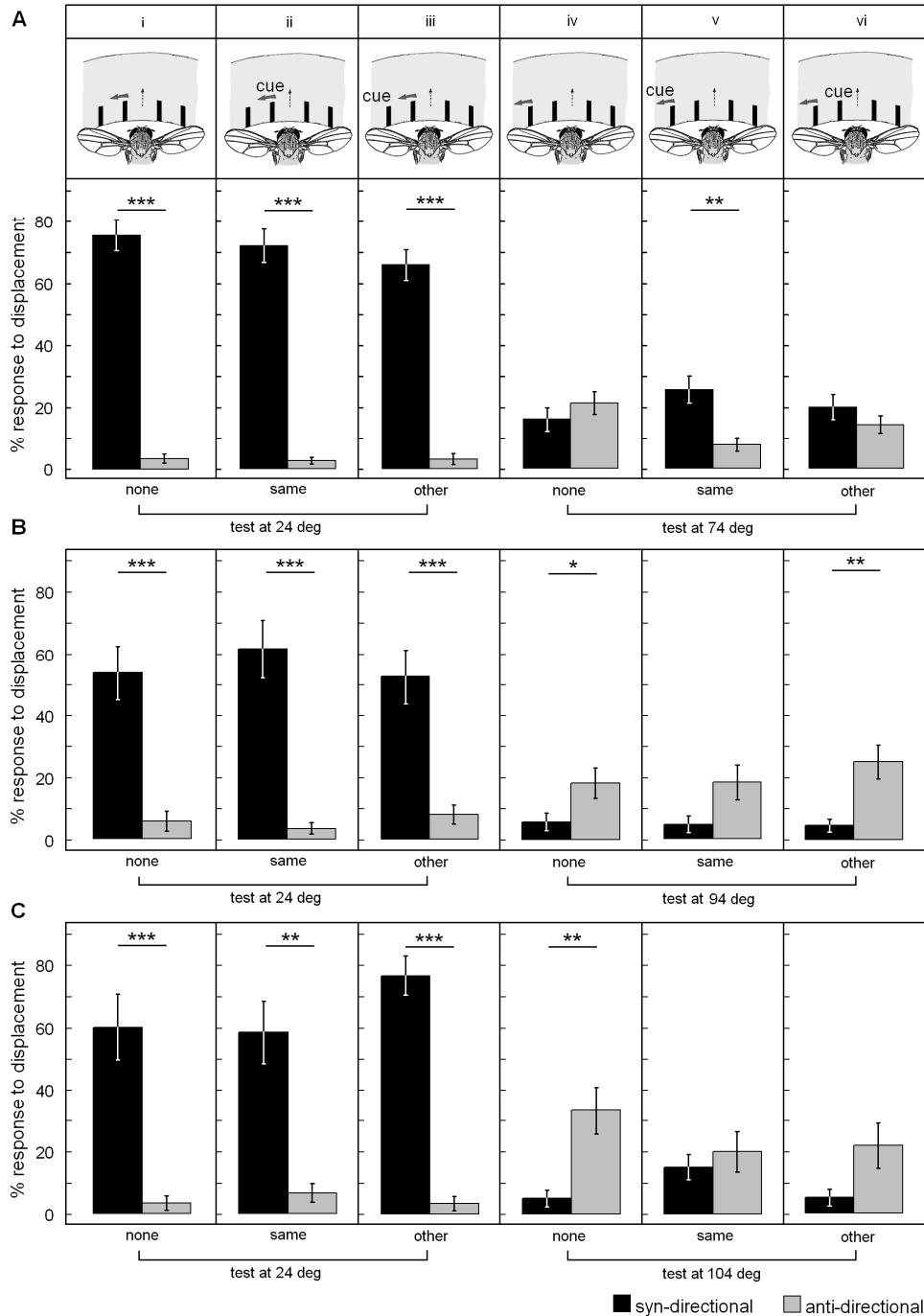


Fig. 1.12. Spatial extent of the focus of attention (FoA) is smaller than one lower visual half-field. (A) When a stripe is displaced at a more frontal azimuth ($\pm 24^\circ$), flies always respond syndirectionally [A(i)]. When the same stripe is cued and then displaced [A(ii)] or another more laterally placed stripe (at $\pm 74^\circ$ in the same half-field) is cued before the more frontal stripe's displacement [A(iii)], flies mostly respond syndirectionally. However, when the more lateral stripe is displaced flies generate equally frequent syndirectional and antidirectional responses [A(iv)]. Cuing this same stripe before its displacement results in significantly more frequent syndirectional responses [A(v)], whereas cuing the other stripe in the same half-field does not [A(vi)]. $n = 49$.

(B, C) When the stripes at $\psi_o = \pm 74^\circ$ are placed farther back at $\psi_o = \pm 94^\circ$ (B, $n = 22$) or $\psi_o = \pm 104^\circ$ (C, $n = 15$), the antidirectional responses become more frequent but the cuing of the same stripe does not seem to increase the proportion of the syndirectional responses (B(v), C(v)) more than when no cue or cue on the other stripe is presented (B(iv, v, vi), 12C(iv, v, vi)). $**P < 0.01$, $***P < 0.001$. Error bars are SEM.

tested positions ($\psi_o = \pm 22^\circ$ to $\pm 60^\circ$; Fig. 1.11). This indicates that the cue and the test need not be the same object for the cuing to be effective.

As after the spatial separation of the cue from the test the guidance was equally effective at all the tested positions, experiments were done to find out if the FoA was as wide as one lower visual half-field. Four stripes ($H = 40^\circ$) were presented, two each at $\psi_o = \pm 24^\circ$ and $\psi_o = \pm 74^\circ$ (Fig. 1.12A). Only a single stripe was displaced in each condition. First, a stripe (say at $\psi_o = -24^\circ$) was displaced while other stripes remained stationary. In this situation, the flies almost always responded syndirectionally (Fig. 1.12A(i)). After this, either the same stripe (Fig. 1.12A(ii)) or the stripe at $\psi_o = -74^\circ$ (Fig. 1.12A(iii)) was oscillated and then the stripe at $\psi_o = -24^\circ$ was displaced. The syndirectional responses remained more frequent in both these cases. Then, the stripe at $\psi_o = -74^\circ$ was displaced and equally frequent syn- and antidirectional responses were seen (Fig. 1.12A(iv)). When the same stripe was oscillated before it was displaced, the syndirectional responses became more frequent (Fig. 1.12A(v)). Whereas, when the stripe at $\psi_o = -24^\circ$ was oscillated before the displacement at $\psi_o = -74^\circ$, the cuing did not result in more

frequent syn- than anti-directional responses (Fig. 1.12A(vi)).

When the stripes at $\psi_o = \pm 74^\circ$ were placed farther back at $\psi_o = \pm 94^\circ$ (Fig. 1.12B) or $\psi_o = \pm 104^\circ$ (Fig. 1.12C), the antidirectional responses became more frequent (Fig. 1.12B(iv, v, vi), 12C(iv, v, vi)) for the displacements at these more lateral positions. The cuing of the same stripe did not seem to increase the proportion of the syndirectional responses (Fig. 1.12B(v), 1.12C(v)) more than when no cue or cue on the other stripe was presented (Fig. 1.12B(iv, vi), 1.12C(iv, vi)). The experiments above (Fig. 1.4B) indicate that the cuing of a single stripe in the LVF results in more syndirectional than antidirectional responses and this may happen due to the FoA on the cued stripe (see section 3.1 for discussion). If in the present experiment the window of attention was as wide as the distance between the two stripes in the same half-field, the cuing should have increased the syndirectional responses irrespective of which stripe was oscillated. Although the results are not fully conclusive (see Fig. 1.12Bv), they suggest that the FoA is smaller than the full lower visual half-field.

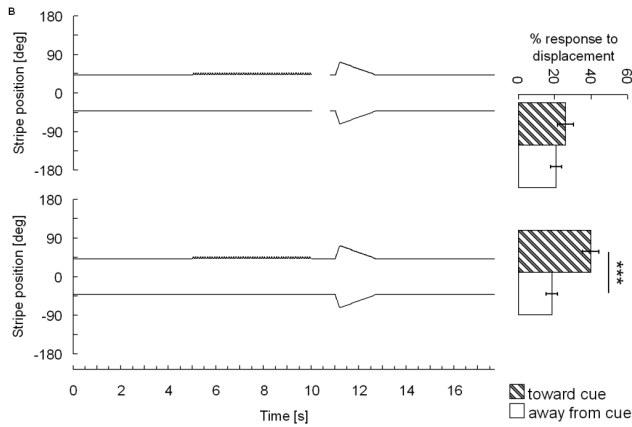


Fig. 1.13. Discontinuity in the display of the cue and the test stripes abolishes the cuing effect. When the test stripes are presented after 0.8 s of the disappearance of the cue, the cuing effect is abolished (Upper) but when the test stripes are continuously visible after the cue presentation, the cuing effect persists (Lower). $n = 27$. *** $P < 0.001$. Error bars are SEM.

3.1.9. Continuous visibility of test stripes is necessary for effective cuing

The experiments above showed that the test and the cue can be separated in time (Fig. 1.10) and space (Fig. 1.11) and the cuing could still be effective. Also, the cue can be invisible for most part of the experiment and appear only at the time of the cue presentation and still guide the attention (Fig. 1.11). Interestingly, if the test stripes were not visible for 0.8 s between the presentation of the test and the cue, the cuing effect was abolished (Fig. 1.13, upper panel). This condition was the same as the 1 s temporal separation of the test and the cue (Fig. 1.10) except that the test stripes

were invisible for 0.8 s and reappeared 0.2 s before the displacement. Continuous visibility of the test stripes was necessary for the cuing to be effective. Since the cuing effect was not altered by the disappearance and reappearance of the cuing stripes during the spatial separation experiments (Fig. 1.11) this indicates that the cue can be temporarily invisible but it is the test that needs to be visible for the entire duration of the experiment for the cuing to be effective. Alternatively, the sudden absence of any landmarks in the visual field might have abolished the cuing effect. In this case, if the cuing stripes were to always remain visible and the test stripes were to disappear and appear only at the time of the test, the cuing would still be effective.

3.1.10. Differences in the frequencies of responses due to inverted contrast of the scene

As already reported in the experiment of Fig. 1.4A (Inverted Lower Stripe), changes in the frequency of torque responses were seen for the single-stripe displacements in the LVF when the contrast of the scene was inverted. To learn more about this effect, the entire standard experiment was repeated in the LVF with inverted contrast (Fig. 1.14). The flies almost always produced syndirectional responses for the single-stripe displacements with (Fig. 1.14, Single) or without (Fig. 1.14, One-of-two) a second stationary stripe.

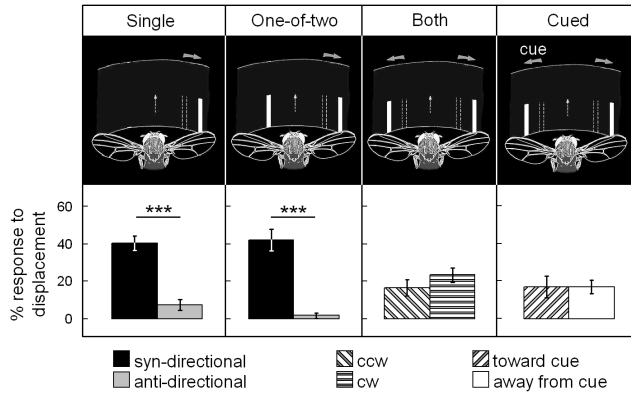


Fig. 1.14. Contrast of the scene affects the response frequencies in the LVF. When the contrast of the scene is inverted, the flies almost always produce syndirectional responses for the single-stripe displacements (Single, One-of-two). For the simultaneous displacement of both the stripes, equally frequent clockwise and counterclockwise responses are observed (Both). But no cuing effect is seen when one of the stripes is oscillated before the displacement of both (Cued). $n = 22$. $***P < 0.001$. Error bars are SEM.

This is in contrast with what was observed for the normal contrast of the scene where the syn- and the antidirectional responses were equally frequent for the single-stripe displacements (Fig. 1.8A, Single, One-of-two). As expected for the simultaneous displacement of both the stripes, equally frequent clockwise and counterclockwise responses were observed with the inverted contrast (Fig. 1.14, Both). Surprisingly, no cuing effect could be seen when one of the stripes was oscillated before the displacement of both (Fig. 1.14, Cued).

Since in the LVF with the inverted contrast the cuing was ineffective but was effective

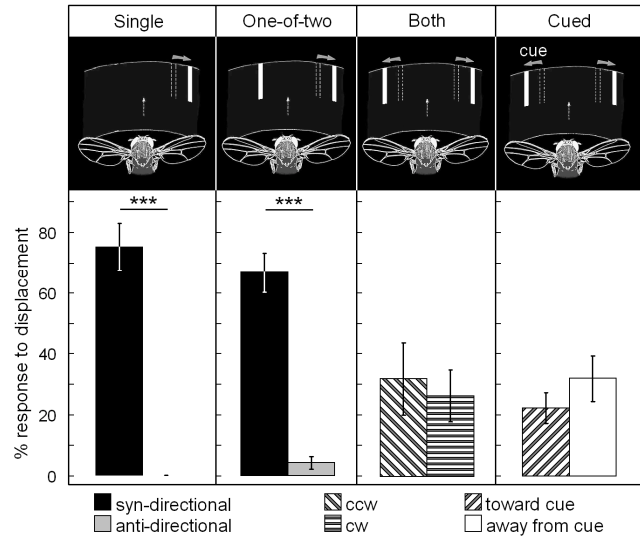


Fig. 1.15. Response frequencies are unaffected by the contrast of the scene in the UVF. With the inverted contrast, the responses in the UVF under all the conditions are the same as with the normal contrast. More frequent syndirectional responses are seen for the single-stripe displacements (Single, One-of-two) and equally frequent syn- and antidirectional responses are seen for the two-stripe displacements, with (Both) or without (Cued) cuing. $n = 9$. $***P < 0.001$. Error bars are SEM.

with the normal contrast, similar experiments were done in the UVF (Fig. 1.15) to see if by inverting the contrast one would now obtain cuing in the UVF. With the inverted contrast, the responses in the UVF under all the conditions (Fig. 1.15) mirrored those for the corresponding conditions in the inverted contrast LVF (Fig. 1.14) and the normal contrast UVF (Fig. 1.4A, Upper Stripe; Fig. 1.6B, D) including the ineffectiveness of the cue. Even though in the LVF the response frequency for the single-stripe displacements changed and the cuing effect was abolished, no major effect on the response frequencies

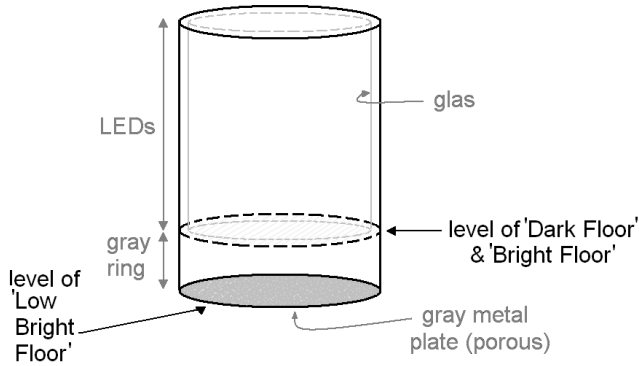


Fig. 1.16. Details of the experimental setup. The figure schematically shows the 'Dark Floor', 'Bright Floor' and 'Low Bright Floor' conditions in the LED-arena.

for any condition was seen by inverting the contrast in the UVF.

3.1.11. Differences in the frequencies of responses due to different experimental setup conditions

Experiments with different experimental setup details were performed and were found to have some effect on the torque response frequencies. In most of the experiments, the floor of the arena was a dark cardboard disk just below the LED panels (standard setup, 'Dark Floor', see Fig. 1.16) but if this dark disk was removed and some of the light of the arena was diffusely reflected by a gray ring (which made the ground level lower) and a metal plate underneath ('Low Bright Floor', see Fig. 1.16), the response frequencies in most cases were different. For instance, with the Dark Floor and stripes spanning the whole height of the arena, the

overall response rate for the two-stripe displacements was higher ($f_{\text{both}} = 73\%$) than for the single-stripe displacements ($f_{\text{single}} = 51\%$; in Fig. 1.2A compare Both with Single and One-of-two), whereas with the same stripes and the Low Bright Floor, the response rates were $f_{\text{both}} = 60\%$ (Fig. 1.17, Both) and $f_{\text{single}} = 79\%$ (Fig. 1.17, Single, One-of-two). Even more surprisingly, cuing was ineffective with the Low Bright Floor but effective with the Dark Floor condition.

Also, with the Dark Floor, the syn- and the antidirectional responses were equally frequent for the single-stripe displacements in the LVF (Fig. 1.8A, Single, One-of-two) but with the Low Bright Floor these frequencies were altered to make the syndirectional responses more frequent (Fig. 1.18, Single, One-of-two). These findings indicate that the yaw torque modulations in response to the front-to-back displacement of one or two stripes might serve other purposes besides visual course control, such as escape or collision avoidance.

To distinguish between height and brightness among the factors responsible for these alterations in the response frequencies, experiments were done with variations in the brightness and the level of the floor as these were the two main changes in the new setup. When a dark cardboard was placed back into the setup just below the LEDs at the level of the Dark Floor (see Fig. 1.16, level of 'Dark Floor'), the response frequencies returned to

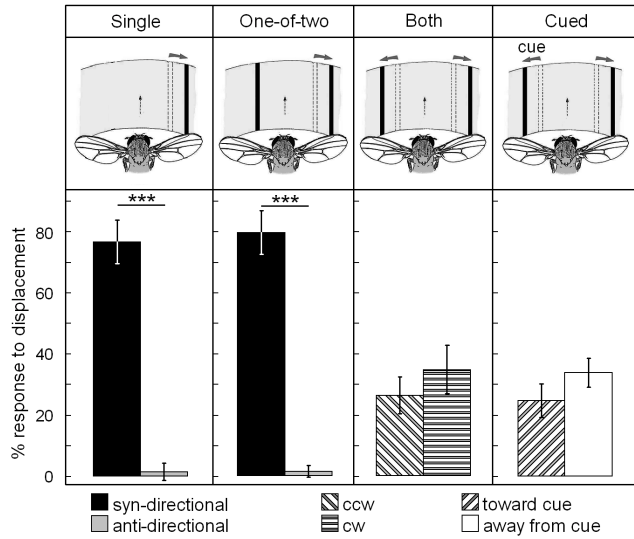


Fig. 1.17. Low Bright Floor and the full visual field. In most of the experiments, the floor of the arena is a dark cardboard disk just below the LED panels (Dark Floor). But if this dark disk is removed and some of the light of the arena is diffusely reflected by a gray ring (which made the ground level lower) and a metal plate underneath (Low Bright Floor) the total response frequencies are higher for the single-stripe displacements (Single, One-of-two) than for the two-stripe displacements (Both, Cued) which is in contrast to the standard Dark Floor condition. Also, the cuing is ineffective (Cued). $n = 30$. *** $P < 0.001$. Error bars are SEM.

the previous trend for both the full visual field (Fig. 1.19A) and the LVF (Fig. 1.19B) conditions. The cuing was also effective again for the full visual field (Fig. 1.19A, Cued). The same flies were used in both the full- and the LVF experiments.

To check if the brightness of the floor played any role, experiments were done using the same flies with both a dark cardboard and a bright cardboard placed just beneath the level of the LEDs (see Fig. 1.16, level of ‘Dark

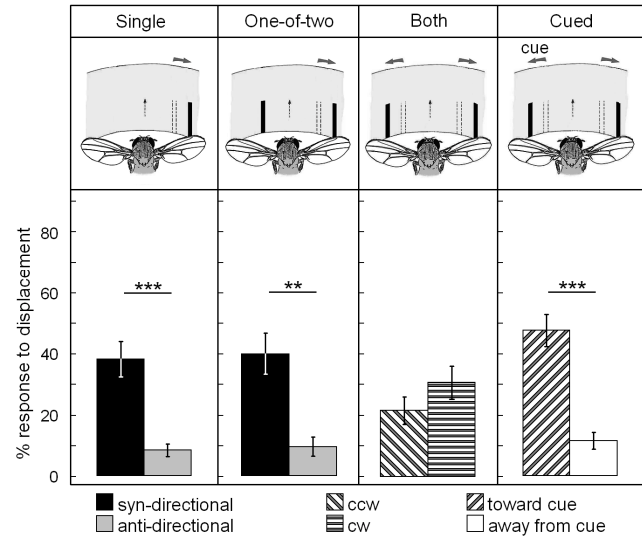


Fig. 1.18. Low Bright Floor and the LVF. In contrast to the standard Dark Floor condition, the syndirectional responses are more frequent for the single-stripe displacements (Single, One-of-two). The two-stripe displacements with (Both) or without cuing (Cued) give similar results as with the Dark Floor condition. $n = 33$. ** $P < 0.01$, *** $P < 0.001$. Error bars are SEM.

Floor’ & ‘Bright Floor’). The level of the floor was the same in both these conditions but the brightness of the floor was different. For both the Dark Floor (Fig. 1.20A) and the Bright Floor (Fig. 1.20B) the response frequencies were similar to each other and to the standard setup condition. These results indicated that the brightness of the floor was not an important factor but its level from the end of the LEDs was. It is possible that when the floor was placed lower (Low Bright Floor), the level of the equator was shifted lower pushing the LVF even lower and resulting in the response frequency changes. This is also supported by the results from Fig. 1.21A and B.

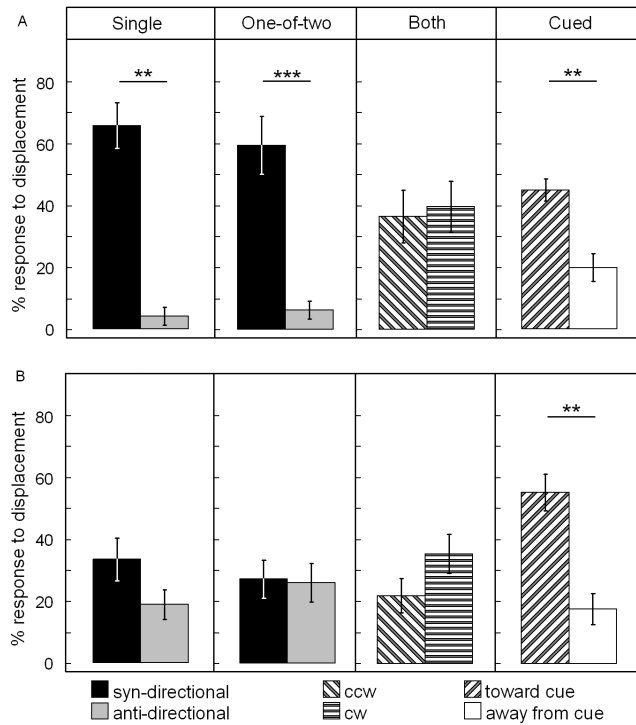


Fig. 1.19. Dark Floor condition returns the usual response frequency trends. When a dark cardboard is placed back into the setup just below the LEDs at the level of the Dark Floor, the response frequencies return to the previous trend for both the full visual field (A) and the LVF (B) conditions. The cuing is also effective again for the full visual field (A, Cued). The same flies are used in both the full- and the LVF experiments. $n = 12$. ** $P < 0.01$, *** $P < 0.001$. Error bars are SEM.

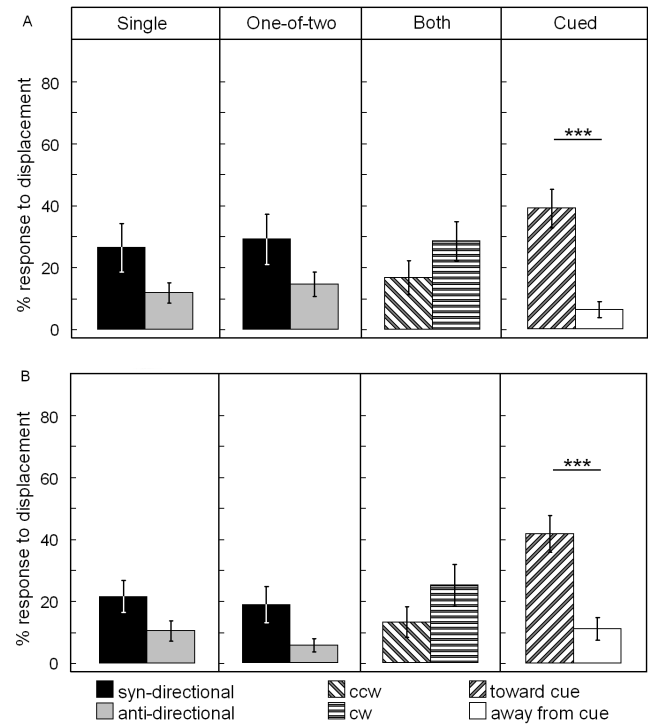


Fig. 1.20. Brightness of the floor does not affect the response frequencies. When experiments are done in the LVF using the same flies with a dark cardboard (A, Dark Floor) and a bright cardboard (B, Bright Floor) placed just beneath the level of the LEDs, the response frequencies are similar to each other and to the standard setup condition in the LVF. The level of the floor is the same in both of these conditions but the brightness of the floor is different. $n = 18$. *** $P < 0.001$. Error bars are SEM.

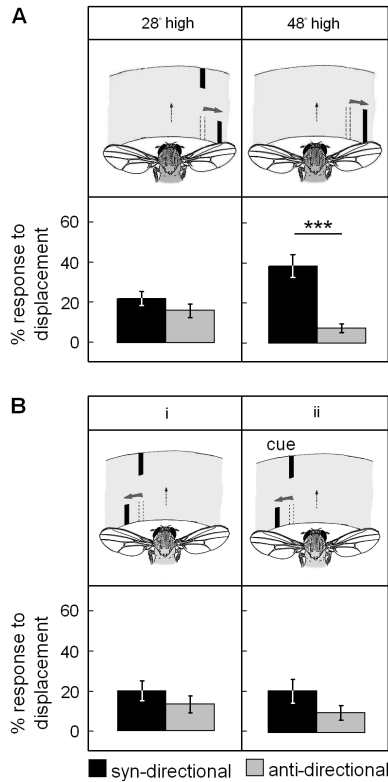


Fig. 1.21. (A) When with the Low Bright Floor, the stripe heights are varied, the displacement of a single shorter stripe (28° high) gives equally frequent syn- and antidirectional responses like the standard setup but the longer stripe does not (A, 48° high). $n = 35$. (B) The equally frequent syn- and antidirectional responses for the single-stripe displacements are again obtained with the Low Bright Floor and shorter stripes (28° high) as part of another experiment. $n = 15$. *** $P < 0.001$. Error bars are SEM.

When with the Low Bright Floor, the stripe heights were varied, the displacement of a single shorter stripe (Fig. 1.21A, 28° high) gave equally frequent syn- and antidirectional responses like the standard setup but the longer stripe did not (Fig. 1.21A, 48° high). The longer stripe probably was high enough to reach partly into the UVF as the equator

was now probably lower than in the standard setup. The equally frequent syn- and antidirectional responses for the single-stripe displacements were again obtained with the Low Bright Floor and shorter stripes (28° high) as part of another experiment (Fig. 1.21B).

Since the Low Bright Floor was developed to control the temperature inside the arena with airflow through a porous floor, a dark mesh was placed at the same level as the standard setup floor (Dark Floor). Now, the floor level was restored while the temperature could still be controlled. The standard experiment in the LVF was repeated with this condition and the response frequencies were as with the standard setup. The frequency of the syndirectional responses was slightly higher but not significantly different from that of the antidirectional responses for the One-of-two condition. For the Single condition the syndirectional response frequency was slightly higher than for the antidirectional with a p-value of 0.032 and $n = 14$. This difference was statistically significant but would probably disappear by increasing the sample size since a reliable result for the standard experiment is generally obtained with a sample size of about 30 flies. Overall, these observations indicate that even slight changes in the experimental conditions have major effects on the responses of the flies.

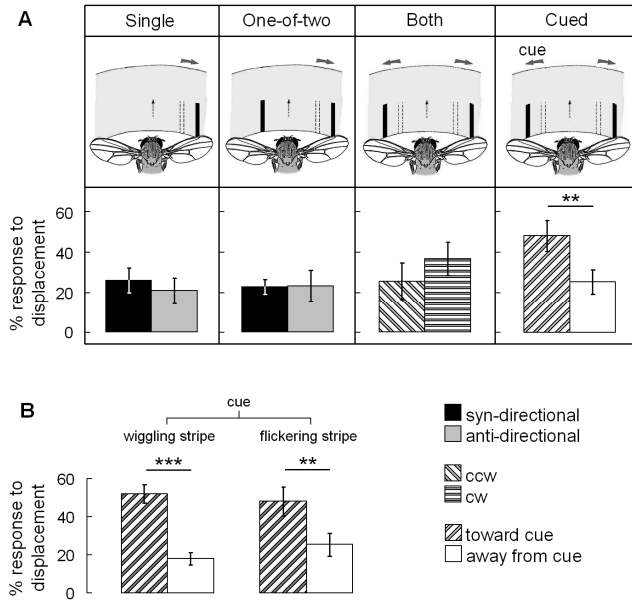


Fig. 1.22. Motion stimuli are not essential for cuing. (A) A flickering stripe (flicker frequency, $f = 5$ Hz) is also guiding the attention. $n = 27$. (B) When one of the stripes is flickered before the test (flickering stripe), the attention is guided as effectively as with the 10 Hz oscillation (wiggling stripe) used in all other conditions. $n = 18$ for the wiggling stripe and $n = 27$ for the flickering stripe. $**P < 0.01$, $***P < 0.001$. Error bars are SEM.

3.1.12. Different cuing stimuli

A few experiments were done to find out what kind of visual stimuli would be suitable to guide the attention in the paradigm used in this study. In the experiments so far, the cue was the 10 Hz, $A_{p-p} = 4^\circ$ oscillation of one of the stripes. This is a motion stimulus like the fast displacement of the test stripe. To check if motionless stimuli could also guide the attention, one of the stripes was flickered (flicker frequency $f = 5$ Hz; $H = 48^\circ$; $\psi_0 = \pm 42^\circ$) before the test (Fig. 1.22A,

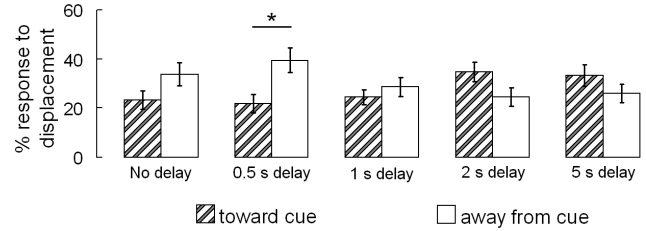


Fig. 1.23. Highly salient cues can escape attention. When a vertical strip of LEDs is placed, one in front of each stripe ($\psi_0 = \pm 42^\circ$, $H = 96^\circ$; width, $\delta = 8^\circ$), and one of these LED strips is flickered at 2 Hz, no guidance effects are observed. $n = 24$. $*P < 0.05$. Error bars are SEM.

Cued). This flicker was as effective as the 10 Hz oscillation in guiding the attention (Fig. 1.22B).

Surprisingly, when a vertical strip of LEDs was placed, one in front of each stripe ($\psi_0 = \pm 42^\circ$, $H = 96^\circ$; width, $\delta = 8^\circ$), and one of these LED strips was flickered at 2 Hz, no guidance effects were observed (Fig. 1.23). To check for aftereffects of this flicker, delays of different durations were inserted between the presentation of the cue and the test. These LED strips emitted white light which was of much higher intensity than that from the green LEDs of the arena. The flies responded with the characteristic torque modulation to the stripe displacement. The overall response frequency and the response dynamics were not altered which shows that the visibility of the stripe was not hampered. It is remarkable that such highly salient visual stimuli can escape the attention system.

3.2. Neuronal underpinnings of the cued shifts of attention

To further understand the phenomenon of cued attention its neuronal underpinnings would need to be elucidated. Such an investigation might give indications about possible common phylogenetic origins or co-evolution of these pathways in invertebrates and vertebrates. Also, developing disease models for attention related disorders would be more meaningful if evolutionarily conserved genes and molecules would underlie attention in flies and humans.

3.2.1. Mushroom bodies are dispensable for the cued shifts of attention

Mushroom bodies (MBs) are thought to be implicated in decision making (Xi et al. 2008) and context generalization (Liu et al. 1999) in flies. They seemed a good candidate for involvement in visual attention as well. So, newly hatched WTB larvae were fed hydroxyurea (HU) to ablate the MB-neuroblasts. This procedure specifically prevents larval development of the MBs (Prokop and Technau 1994). As a result, adult flies with only embryonic MBs were obtained. HU control flies were treated the same way as the HU treated flies except that no HU was fed to the control flies. When these HU treated flies were tested under the standard test conditions with different

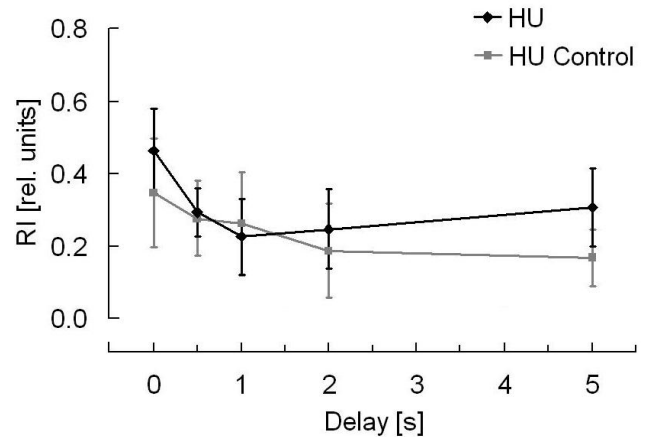


Fig. 2.1. Mushroom bodies are dispensable for the cued shifts of attention. When HU treated flies are tested under the standard test conditions with different delays between the cue and the test presentation, their behavior is not different from that of the HU control flies indicating that the presence of MBs is not essential for guiding the FoA in the LVF. $n = 12$ for HU and $n = 11$ for HU control. Error bars are SEM.

delays between the cue and the test presentation, their behavior was not different from that of the HU control flies (Fig. 2.1). These results indicated that the presence of MBs was not essential for guiding the FoA in the LVF using external visual cues.

3.2.2. Dopamine mutants do not produce torque or landing response

Dopamine is hypothesized to be involved in attention and attention deficit-hyperactivity disorder in humans (Swanson et al. 2007). To test the role of dopamine in the cued shifts

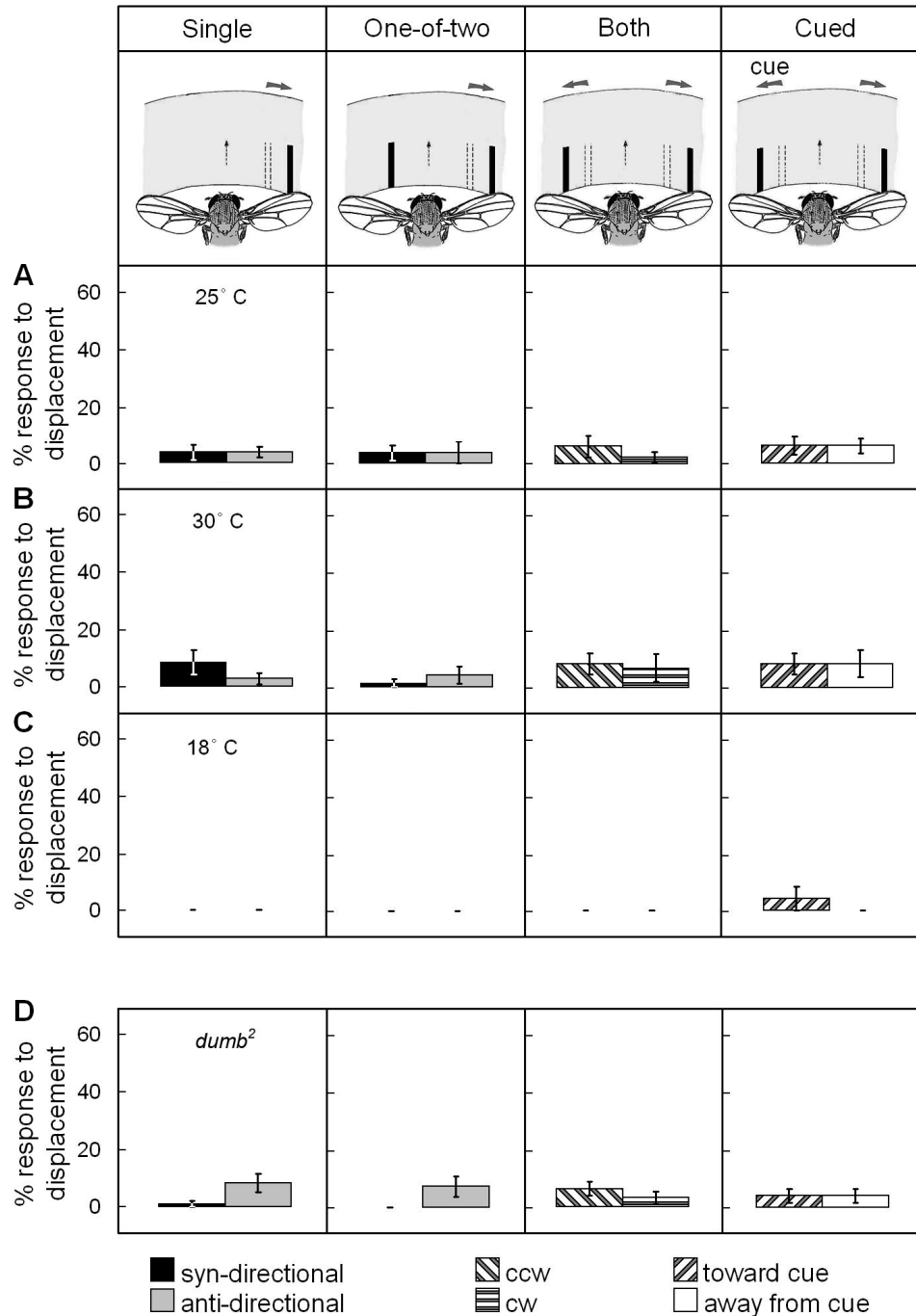


Fig. 2.2. Dopamine mutants do not produce torque or landing responses. When TH-GAL4/UAS-shi^{ts1} flies are incubated at the permissive (25 °C, A, n = 10) and the restrictive (30 °C, B, n = 9) temperatures for 10 min and then tested at the 25 °C and 30 °C, respectively, the flies rarely produce any phasic torque response or landing response at either temperature. (C) To check if even 25 °C is restrictive, if these flies are kept at 18 °C for a day and then tested at 18 °C they again do not show any significant torque or landing responses. n = 3. (D) *dumb*² flies also do not produce significant number of torque and landing responses. n = 12. Error bars are SEM.

of attention in flies, synaptic output of dopaminergic neurons was blocked at the adult stage. 2-3 days old TH-GAL4/UAS-shi^{ts1} flies were incubated at the permissive (25 °C) and restrictive (30 °C) temperatures for 10 min and then tested at 25 °C and 30 °C, respectively. Surprisingly, these flies rarely produced any phasic torque response or landing response at either temperature (Fig. 2.2A, B). To check if even 25 °C was restrictive, flies were kept at 18 °C for a day and then tested at 18 °C. But these flies did not show any significant torque or landing responses either (Fig. 2.2C). Since these flies did not produce the basic response to the test, the guidance of attention could not be tested with the present paradigm.

dumb² flies were also tested. dumb² mutants have abnormal D1 dopamine receptor (dDA1) expression with negligible immunoreactivities in the MB and the central complex (CX) (Kim et al. 2007). These flies did not produce a significant number of torque and landing responses either (Fig. 2.2D) and therefore could not be tested for the guidance of attention. A different paradigm would be needed to test TH-GAL4/UAS-shi^{ts1} and dumb² flies for cued attention. The possibility that the genetic background might be responsible for this lack of the basic responses is unlikely because both CS and WTB flies performed alike in these tests. Despite the lack of further controls, the finding that interference with dopaminergic neurons as well as with the

dDA1 receptor suppresses torque and landing responses argues that dopamine is involved in regulating the frequency of these responses.

3.3. Covert shifts of attention

It is easy to observe shifts of attention guided by external stimuli. But flies are able to shift their attention endogenously as well, in the absence of any guiding stimuli (covert attention). The covert shifts of attention were already demonstrated in *Drosophila* in 1980 (Wolf and Heisenberg 1980) but a systematic analysis was missing in those studies.

3.3.1. Windows with oscillating stripes and the typical torque pattern

Attempts were made to reproduce the results of the experiments performed by Wolf and Heisenberg in 1980 (Wolf and Heisenberg 1980). In these experiments two stripes were oscillated in phase and flies produced a typical torque pattern corresponding to the motion of one stripe at times and the other stripe at other times as if following only one stripe occasionally while ignoring the other. The setup conditions were mimicked as closely as possible but the experimental results could not be reproduced. Several other conditions were then tried and instead of single stripes, fixed windows with several moving stripes gave the desired torque

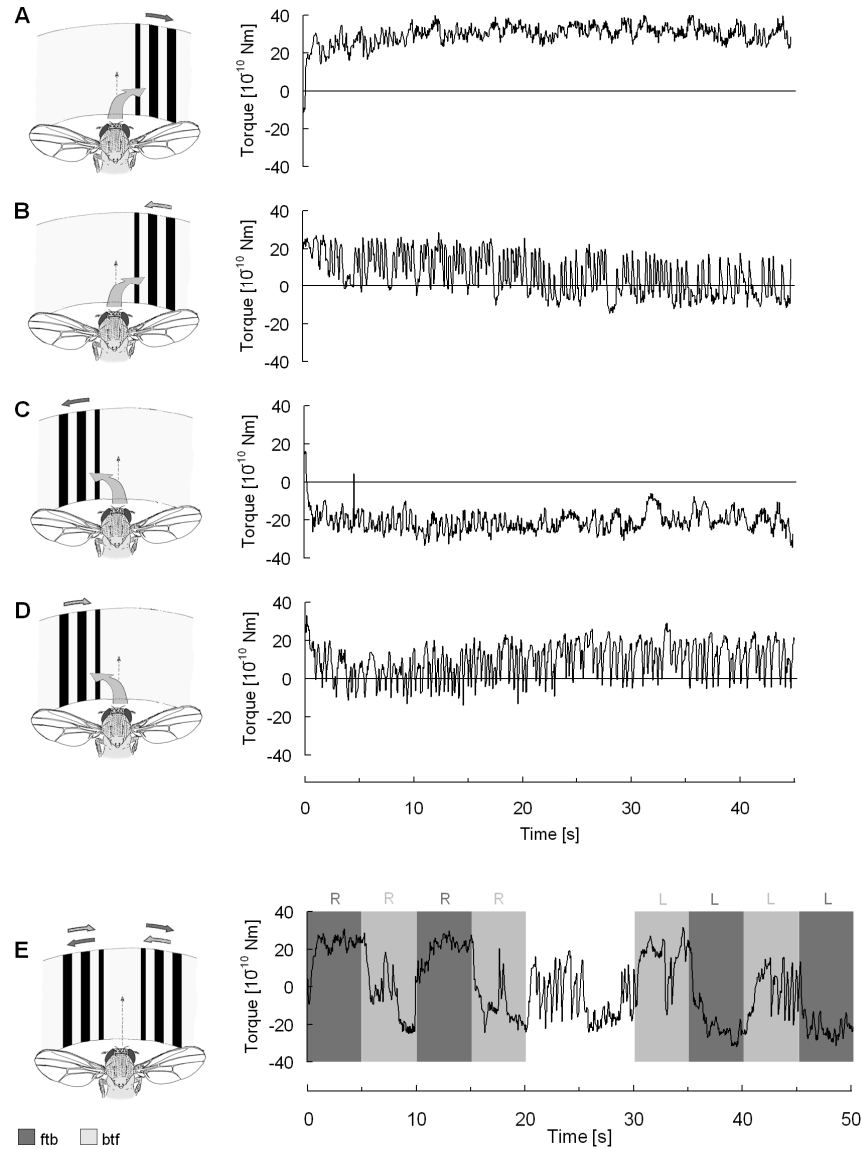


Fig. 3.1. Characteristic torque pattern reveals the covert FoA. (A, C) When the stripes move front-to-back inside the right window, the flies produce sustained torque with the same polarity as the motion of the stripes and the side of the window. (B, D) For the back-to-front motion of the same stripes, the flies produce torque with an average of zero magnitude superimposed by torque spikes. These torque spikes are opposite in polarity to the motion of the stripes but have the same polarity as the side of the window. (E) When both the windows are presented together, due to the in phase nature of the oscillations of the stripes in the two windows, when there is front-to-back motion in one window there is back-to-front motion in the other window. This makes it easy to distinguish when the flies produce the torque pattern corresponding to a particular window. For instance, there is the typical torque pattern for the right window for the first two cycles and the typical pattern for the left window for the last two cycles in E. ftb = front-to-back, btf = back-to-front. R = torque corresponding to the stripe motion in the right window; L = torque corresponding to the stripe motion in the left window.

patterns. Two 60° wide windows were shown, one in the left and one in the right visual field. These windows had a constant boundary (from $\pm 30^\circ$ to $\pm 90^\circ$) while the stripes within each window moved front-to-back and back-to-front for 5 s each (at a spatial frequency of 20°). This cycle was repeated many times for each fly. Several flies showed the characteristic torque pattern similar to the 1980 study. For the front-to-back motion of the stripes, the flies produced sustained torque with the same polarity as the motion of the stripes and the side of the window (Fig. 3.1A, C) whereas, for the back-to-front motion, the flies produced torque with an average of zero magnitude superimposed by torque spikes (Fig. 3.1B, D). These torque spikes were opposite in polarity to the motion of the stripes but had the same polarity as the side of the window. Due to the in phase nature of the oscillations of the stripes in the two windows, when there was front-to-back motion in one window there was back-to-front motion in the other window. This made it easy to distinguish when the flies produced the torque pattern corresponding to a particular window due to the temporal correlation of the torque pattern with the motion of the stripes. During the entire duration of the experiment all the flies that produced this typical torque pattern also produced torque without this pattern in the presence of the same stimulus. The typical torque pattern appeared irregularly and intermittently with the ‘unpatterned’ torque.

Although these experiments provided a clear cut demonstration of the flies’ ability to focus on part of the visual field and shift this focus endogenously without the help of any external cues the typical torque pattern could not be observed after the few initial days of the experiments. Since the production of these torque patterns was under the control of the flies it was difficult to command when such patterns appeared.

3.3.2. Motion processing in the UVF and the LVF

In order to find a condition where the covert shifts of attention could be more frequently observed, stripes were shown in the entire upper and lower visual fields while there was a vertical gap between the two striped patterns to visually demarcate them from each other (Fig. 3.2). The hypothesis was that when these patterns would be oscillated in antiphase and the motion processing for the two patterns would be counterbalanced flies might follow the motion of one pattern at times and then shift to the other pattern to follow its motion. If this would happen then two peaks would appear in the average torque histograms. But the flies should be following one or the other field for most part of the experiment for these peaks to be clearly seen. To test this, the upper and the lower patterns were oscillated in antiphase ($f = 0.1$ Hz). So, when there was clockwise rotation of one pattern there was

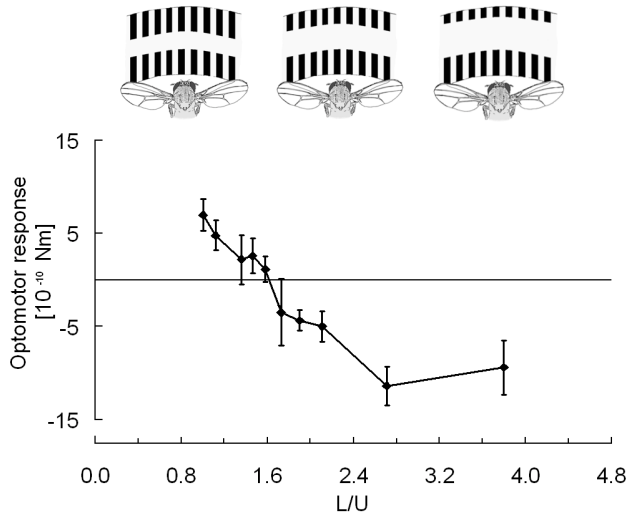


Fig. 3.2. Motion processing of the same stimulus is stronger in the UVF than in the LVF. When the heights of the upper and the lower patterns are equal ($H = 38^\circ$) with a gap of 20° in between, the OR is positive and shows that the flies almost always follow the motion of the upper pattern (at $L/U = 1$ where $L/U = \text{height of the lower pattern} / \text{height of the upper pattern}$). The height of the upper field gradually decreases while the height of the lower pattern remains constant, to find the point of equilibrium (PoE) where the OR would be zero. The height of the lower pattern has to be 1.58 times that of the upper pattern to get to a point closest to the PoE. The motion of the lower pattern is processed more strongly after this point. Error bars are SEM.

counterclockwise rotation of the other pattern. The optomotor response (OR) was calculated with respect to the motion of the upper pattern as $OR = (\text{average torque for the first half cycle}) - (\text{average torque for the second half cycle})$.

Since the upper pattern rotated counterclockwise in the first half of the cycle an OR with a positive polarity would mean that the

flies were following the motion of the upper pattern. If the OR would be negative then the flies would be following the lower pattern. At zero magnitude of the OR, the two fields would be completely counterbalanced. At the beginning of the experiment, the heights of the upper and the lower patterns were equal ($H = 38^\circ$) with a gap of 20° in between (both the patterns had a gap of 10° from the equator). The OR for this situation was positive and showed that the flies were almost always following the motion of the upper pattern (Fig. 3.2, at $L/U = 1$ where $L/U = \text{height of the lower pattern} / \text{height of the upper pattern}$). The height of the upper field was gradually decreased while keeping the height of the lower pattern constant, to find the point of equilibrium (PoE) where the OR would be zero and to find when the motion of the lower pattern would be processed strongly than that of the upper pattern. The height of the lower pattern had to be 1.58 times that of the upper pattern to get to a point closest to the PoE even though the spatial frequency of the two patterns was the same (Fig. 3.2). Incidentally, at this point the height of the upper pattern (24°) was also half the total height of the upper field in the LED arena (48°). The motion of the lower pattern was processed more strongly when the height of the lower pattern was more than 1.58 times that of the upper pattern (Fig. 3.2). Due to the size restriction of the single LEDs (one LED = 2°) an exact PoE could not be found.

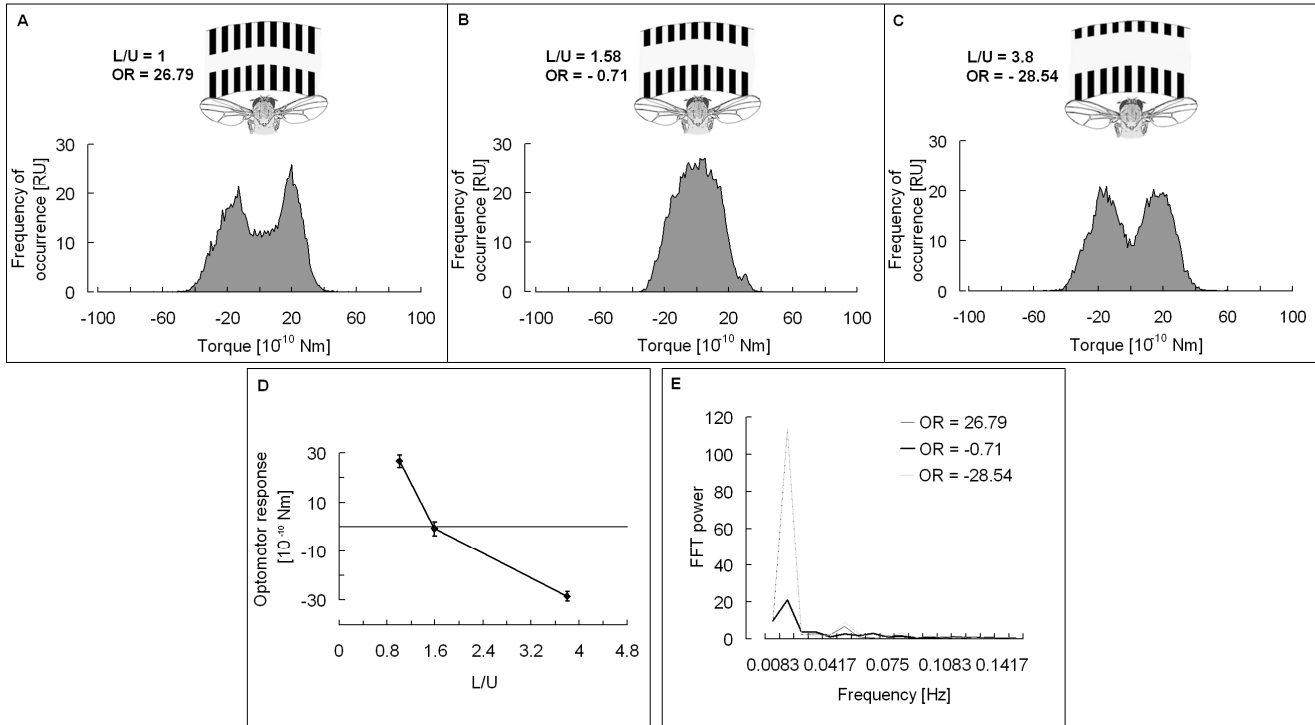


Fig. 3.3. Flies respond to the vector sum of the motions of the patterns in the UVF and the LVF. The average torque histograms show two distinct peaks for the extreme conditions where the flies follow the motion of either the upper (A) or the lower pattern (C). At the point nearest to the PoE (B) only a single peak appears and is centred at zero. (D) ORs for the corresponding conditions in A, B, and C. (E) Fourier analysis shows a frequency peak corresponding to the frequency of the oscillation of the two patterns even for B meaning that the flies do follow the motion of one or the other pattern (both the patterns oscillated at the same frequency). The OR is slightly negative for this condition indicating that the flies follow the lower pattern. But the amplitude of the oscillations is so small that two distinct peaks can not be seen. R.U. = relative units. L/U = height of the lower pattern/ height of the upper pattern. OR = optomotor response. Error bars are SEM.

To get more informative average torque histograms the frequency of the pattern oscillation was decreased ($f = 0.01667$ Hz) so that the magnitude of the torque produced for one half cycle would be as large as possible. The average torque histograms showed two distinct peaks for the extreme conditions where the flies followed the motion of either the upper or the lower pattern (Fig. 3.3A and C, respectively). At the point nearest to the PoE (Fig. 3.3B, D)

only a single peak appeared and was centered at zero. Fourier analysis showed a frequency peak corresponding to the frequency of the oscillation of the two patterns even at this point (Fig. 3.3E) meaning that the flies did follow the motion of one or the other pattern (both the patterns oscillated at the same frequency). The OR was slightly negative at this point indicating that the flies were following the lower pattern. But the amplitude of the oscillations was so small

that two distinct peaks could not be seen. The maximum magnitude of torque produced was lesser than in the extreme cases and the single peak was centered at zero which was calibrated before the start of the experiment. It seems that even if the actual PoE would be found two distinct peaks would still not be visible because the flies would almost always produce a response for the vector sum of the motions of the two patterns.

Although these experiments were not telling of attention they did point out a property or two of motion processing in the flies. In general, motion processing of the same pattern was stronger in the UVF than in the LVF. The magnitude of the OR depended on the ratio of the heights of the two patterns. The OR decreased as the height of the upper pattern decreased showing that the final output of the flies was a vector sum of the motion of the patterns in both the fields.

3.3.3. Simultaneous opposing motion of two stripes

In the stripe displacement experiments for studying cued attention, apart from the cued condition two identical stripes were also displaced without any cue. These stripes were displaced simultaneously in opposing front-to-back motions (one clockwise and the other counterclockwise) (e.g., Fig. 1.2, Both). These opposing motions did not cancel out

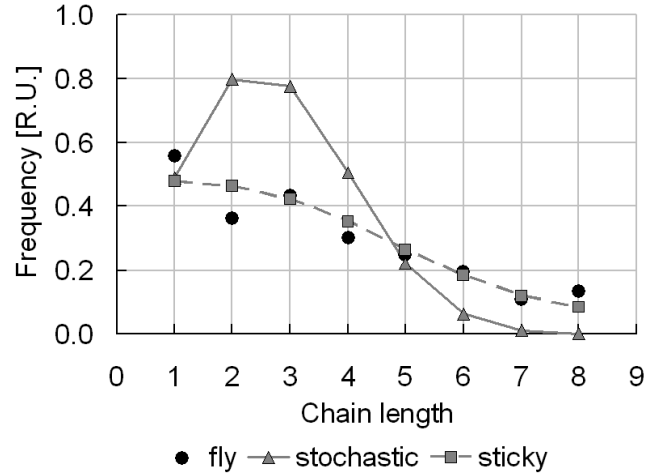


Fig. 3.4. Attention increases the chances of producing the same kind of response for two-stripe displacements. The distribution of the number of the same kind of responses generated by one fly i.e., the chain length (fly) is different from the one generated by a stochastic event (stochastic). The fly chain length distribution is closely emulated by the computer generated data with 35% probability that the next response would be the same as the previous one (sticky). fly = real data produced by the flies; stochastic = simulated random data generated by the computer; sticky = computer generated data with a stickiness factor of 35. R.U. = relative units.

each other's stimulatory effects but equally frequent phasic yaw torque responses of either polarity were observed. If the flies were responding only to the sum of the two opposing motions then only the no response situation would have occurred. That does not happen and the flies respond as if they sometimes follow the motion of only one stripe and the motion of the other stripe at other times. It is as if attention was allocated to one stripe at a time restricting the behavior of the flies to the motion of that stripe. The attention then would switch to the other

stripe endogenously, without the aid of any cues, restricting the behavior to the motion of the other stripe. Such a trend of equally frequent responses of both the polarities could also be obtained by a hypothetical mutually inhibiting network of central pattern generators (CPGs) for the right and the left turns. This kind of a network would always produce a response frequency pattern that would be stochastic in nature. Even with attention playing a role in this condition the fly data could be stochastic. If attention were to stay on one side for periods longer than one test event then there would be a tendency to have more responses to that side and the data would no longer be stochastic.

For the three response types, clockwise, counterclockwise and no response, the frequencies of the occurrence of chains of the same response type were counted. If a single response was followed by a different response type this was scored as a 1-chain event. Similarly, the frequencies of 2 consecutive responses of the same kind (2-chain) and so on were scored till the maximum possible chain length of 8 (8-chain). A stochastic dataset was then generated using a random number generator and the same mean frequencies of the three response types to match the real fly data. This random number generator worked like a three sided die that was rolled eight times in a row. The frequencies of the response chain lengths were calculated in the same way as for the real fly data. The frequencies of the different

chain lengths for the real data differed clearly from those of the stochastic data (Fig. 3.4, compare fly with stochastic) showing that the flies did not produce these responses in a stochastic manner. In fact, the probability of the next response being the same as the previous one was 35 – 40% larger than the mean probability (Fig. 3.4, sticky). This was calculated by introducing a ‘stickiness’ factor during the production of the stochastic data. The stickiness factor defined the probability of the next response being the same as the previous one. For example, if a response was to the left, a stickiness factor of 35 made the probability of occurrence of the next response to be $p = 0.35$ plus the mean frequency of that response type. Different stickiness factors were used and the response chain lengths were calculated and compared to the real data. A stickiness factor of 35 produced data that very closely emulated the real data scored in the fly experiment (Fig. 3.4, sticky) suggesting that it was attention that substantially increased the chance of a response type being the same as the previous one. (The analysis shown in Fig. 3.4 was done by Wolf R.).

4. Discussion

4.1. Cued shifts of attention

A systematic analysis for the cued visual attention was started and a solid approach was developed for its study. An earlier account of the basic phenomenon (Wolf and Heisenberg 1980) had certain ambiguities since the test and the cue stimuli overlapped temporally. Due to this overlap the interactions between the two stimuli could not be ruled out. The present study avoids such alternative interpretation as the test and the cue were never presented simultaneously and the cuing was effective even with a delay of 2 s between the test and the cue presentation (Fig. 1.10). In fact, the two stimuli were even spatially separated and the cuing effect could still be observed (Fig. 1.11) thereby, excluding any kind of interference between the two. Moreover, the present paradigm is simple yet robust and the results are easily quantifiable. This paradigm should easily lend itself to the genetic dissection of the neuronal and the functional architecture of visual attention in flies.

The simultaneous but opposing front-to-back displacements of two stripes, one in the left and one in the right visual field, should intuitively annihilate each other's stimulatory effects on the yaw torque response production. That does not happen and the flies respond as if only one stripe were

displaced. This phenomenon should by itself meet the operational definition of attention i.e., restriction of a behavioral response to a certain part in the visual field (FoA). But such a restriction of this behavior might also be produced through a mutual inhibition network of CPGs for right and left turns. The one that would be randomly triggered first would suppress the other (see section 2.3.3 and 3.3 for more discussion). However, visual attention serves more than just preventing simultaneous activation of antagonistic motor patterns. Attention enables the organism to process the putatively salient and behaviorally relevant information from its surroundings.

Such parceling of visual information is what is reflected in the cuing experiments. The FoA and its externally driven steering reveal one aspect of this internal choice process. At first, the cue attracts the FoA. Then, the cue presentation ceases and the test appears. Though the cue is no longer present, the FoA persists and influences the test outcome. This excludes a direct interaction of the cue and the test. It means that the FoA is established before a CPG for turning would be activated by the test. Most strikingly, the cue and the test need not be the same visual object. The cue can be spatially separated from the test by at least 20° horizontally without losing its effectiveness. The horizontal extent of the FoA appears to be $d_{(\text{hor})} > 40^\circ$. The cuing effect is however lost if the two test stripes disappear after cue presentation and reappear

at the onset of the test. Disappearance of all the objects or landmarks in the visual field and/or sudden appearance of a visual object that had not been there before seem/s to override the attentional restrictions.

Interestingly, apart from eliciting the phasic yaw torque response the test was also a stimulus for producing a landing response. The probability of the landing response was quite low when only a single stripe was displaced whereas the probability more than tripled when both the stripes were simultaneously displaced (Fig. 1.2B, 1.8B). This indicated that the flies were more likely to take the two-stripes displacement as an approach as compared to the single-stripe displacement. Also, when the two stripes were displaced simultaneously and a yaw torque response was produced, a landing response was also produced in more than half of these cases (Fig. 1.2C Both). Since during the same event of a two-stripe displacement the displacement of a single stripe was effective for generating the torque response but two stripes were more potent for generating the landing response, this coincidence of the two kinds of responses excluded the possibility that for production of the torque response visual input was blocked already at the level of retina or lamina. The optomotor pathways for turning and landing are not separated at this level of the visual system (Fischbach 1983; Rister et al. 2007). So, the frequencies for the intended turning responses might be

modulated somewhere after the processing pathways of landing and turning have separated. Alternatively, there might be a common processing step for turning and landing with a different delay on the cued side which might suppress the yaw torque response of opposite polarity but not the landing response.

The relevance of these phasic yaw torque responses for the flies is not clear, especially due to the occurrence of the antidirectional responses for the single-stripe displacements. Flies coordinated the syndirectional torque responses with the landing responses for the single-stripe displacements (Fig. 1.2C Single, One-of-two). In such cases the front-to-back motion of the stripe was perhaps taken as self rotation and so possibly the flies tried to correct for it. There were no landing responses concomitant with the antidirectional responses (Fig. 1.2C Single, One-of-two). Also, cuing changed the response frequency for the single-stripe displacements in the LVF to more syndirectional (Fig. 1.4B, Cued) from equally frequent syn- and antidirectional. This change in the frequencies of the syn- and the antidirectional responses could be explained in two ways. First, the cue somehow changed the meaning of the test stimulus leading to more syndirectional responses. Second, the cue shifted the FoA to the cued stripe and whenever the attention was focused on the stripe, flies produced a syndirectional response. The latter explanation would fit

with the idea that the antidirectional responses are more like startle or escape responses. When the flies are not attentive to the stripe they are startled by the sudden stripe displacement and try to turn away from the stripe (antidirectional response). This hypothesis is also supported by the absence of concomitant landing and antidirectional responses (Fig. 1.2C Single, One-of-two conditions). A landing response accompanying an antidirectional torque response would be inappropriate if the antidirectional response was due to startle or for escape.

The data from this study can be interpreted to indicate that visual attention in *Drosophila* is like a spotlight that can be directed to regions in the visual space. Another compatible and mechanistically simpler model would be where the visual space would be divided into fixed windows that could be opened and closed. The width of the spotlight at the test azimuth position, $\psi_o = \pm 42^\circ$ is $d_{(\text{hor})} > 40^\circ$ but it does not fill out one whole lower half-field (Fig. 1.12).

A major finding of this study is about the marked differences in the processing of visual information and the deployment of attention through certain cues in the LVF and the UVF. The response frequencies for the single-stripe displacements were different for the LVF and the UVF. The syn- and the antidirectional responses were equally frequent in the LVF (Fig. 1.4A, Lower

Stripe) whereas the syndirectional responses were significantly more frequent in the UVF (Fig. 1.4A, Upper Stripe) or when the stripe was displayed symmetrically above and below the equator (Fig. 1.4A, Middle Stripe, Fig. 1.2, Single, On-of-two). On inverting the contrast of the scene, these frequencies changed only in the LVF (Fig. 1.4A, Inverted Lower Stripe) but not in the UVF (Fig. 1.14, Single). Also, with the cues that were tested, attention could only be guided in the LVF. The ecological significance of these differences might be related to the fact that during flight most of the objects of interest for the fly are in the LVF like food and landmarks. Moreover, predators like robber flies and dragonflies generally attack from below. So, the guidance of attention would, intuitively, be more important in the LVF during flight. These findings are somewhat consistent with those found in humans and other primates where some visual attributes are differentially processed in the LVF and the UVF (Previc 1990; Hagenbeek and Van Strien 2002) and the attentional resolution is finer in the LVF (He et al. 1996).

Whether effective cuing in the UVF would be found with other stimuli is an open question. Antidirectional responses for the unilateral and directional responses for the bilateral test are also found in the UVF like in the LVF so, this part of the visual system may not be any more 'hardwired' than the LVF. It is possible that in the UVF cuing just follows different rules and requires different

kind of stimuli. It will also be interesting to see if the differences between the UVF and the LVF also exist during walking as during walking most of the visual stimuli should be at the level of the horizon or in the UVF. The cuing of the upper stripe seemed to decrease the syndirectional response frequency (Fig. 1.5D). If this observation would hold even after increasing the sample size then this would mean that the cue is not completely ignored in the UVF and that the cuing rules are just different in the UVF.

It was shown here that the size of the spotlight is smaller than one lower visual half field (Fig. 1.12) but it has still to be determined if attention can be guided within one visual half field. Continued visibility of the test stripes is required for cuing to be effective. Testing whether this requirement is absolute or if there is a minimum duration for which the stripes can be invisible while keeping the cuing still effective would also be interesting to know.

4.2. Neuronal underpinnings of the cued shifts of attention

The neuronal correlates of the spatially cued attention would provide important information about the attention pathways in flies. Pilot experiments with HU treated flies showed that mushroom bodies (MBs) are dispensable for the kind of guidance of attention that was tested in this study (Fig.

2.1). To substantiate this finding, other MB mutants like *mbm*¹ (Raabe et al. 2004) could be tested. Visual attention has been postulated to play roles in visual pattern recognition at the torque meter (Tang et al. 2004). Also, fan-shaped body is thought to be involved in pattern recognition (Liu et al. 2006) and represent visual space (Heinze and Homberg 2007). It is possible that a neuronal substrate for attention could be found in these regions of the fly brain.

Dopamine and serotonin have been hypothesized to be involved in attention deficit-hyperactivity disorder in humans (Robert 2008). It is pertinent to test the role of these biogenic amines in attention in flies. Octopamine is the presumed arthropod homolog of norepinephrine but the available octopamine mutants do not fly and therefore could not be tested in the present paradigm. However, dopamine mutants were tested in the standard experiment in the LVF. TH-Gal4/UAS-*shi*^{ts1} flies were tested at the permissive (25 °C) and the restrictive (30 °C) temperatures. Surprisingly, these flies flew as well as the wild-type flies and also showed normal optomotor response during the initial calibration phase of each experiment but did not show any phasic torque or landing response at either of these temperatures (Fig. 2.2A, B). To rule out the attribution of the absence of these responses to the permissive temperature (25 °C) also acting as the restrictive temperature, TH-Gal4/UAS-*shi*^{ts1} flies were kept at 18 °C for a day before the

experiment and then tested at 18 °C. Still no phasic torque or landing responses were observed (Fig. 2.2C). *dumb*² flies were also tested and like TH-Gal4/UAS-*shi*^{ts1} flies did not show any phasic torque or landing responses (Fig. 2.2D). Both CS and WTB flies were tested previously and gave similar ‘normal’ results in the standard experiment which excludes the possibility that the genetic background might be the cause of this anomaly. Also, TH-Gal4/UAS-*shi*^{ts1} and *dumb*² flies might have different genetic backgrounds. So, the genetic background might not be playing a role here. Although, testing the parental controls for TH-Gal4/UAS-*shi*^{ts1} flies might help to confirm this notion.

It is possible that some developmental defects associated with the dopamine pathway might be occurring in these flies that lead to the loss of the phasic yaw torque and the landing response. *dumb*² flies have almost no dDA1 receptor expression in the MBs and the CX (Kim et al. 2007) but the absence of MBs did not alter these responses in the HU-treated flies (Fig. 2.1). So, a malfunction of the dopamine pathway in the CX should be responsible for the absence of these responses. One might investigate this aspect further but it would not inform about attention in flies.

Whether dopamine is involved in attention can not be deduced from these observations. Another paradigm where the dopamine mutants would produce the basic response

would have to be devised to test the role of dopamine in cued attention. The experiments here only represent pilot studies and a comprehensive investigation to find the exact neuronal correlates of cued attention would require investment of more time.

4.3. Covert shifts of attention

The experiments involving windows with oscillating stripes nicely demonstrate the phenomenon of covert attention in flies. However, the fact that the phenomenon is not easily quantifiable and reproducible in these experiments makes it inadequate for the analysis of a population of flies, the study of the frequency and the duration of the attentive phases and the comparison of the wild-type flies with different mutants for unravelling the underlying neuronal machinery.

A more systematic approach is required here. The simultaneous opposing displacement of two stripes is a promising avenue for this problem. It shows that the covert attention is involved in the production of the phasic yaw torque responses. Flies restrict their behavior to one stripe or the other, which results in equally frequent responses of both the polarities. A no-response situation is also seen sometimes. Flies either ignore both the stripes or respond to the vector sum of the two motions in these situations. Flies have a higher tendency to deliver one type of

response than what the tendency would be if the responses were produced stochastically suggesting that attention substantially increased the chance of a response type being the same as the previous one.

Once a more quantifiable approach is standardized it would be easier to compare and contrast the two kinds of attention, the cued and the covert. There might be completely different neuronal pathways for these two types of attention or there could be certain common and other uncommon parts. It would be interesting to know how these central (covert) and peripheral (cued) systems of attention work and coordinate, if they do.

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6. Summary

There is such vast amount of visual information in our surroundings at any time that filtering out the important information for further processing is a basic requirement for any visual system. This is accomplished by deploying attention to focus on one source of sensory inputs to the exclusion of others (Luck and Mangun 2009). Attention has been studied extensively in humans and non human primates (NHPs). In *Drosophila*, visual attention was first demonstrated in 1980 (Wolf and Heisenberg 1980) but this field remained largely unexplored until recently. Lately, however, studies have emerged that hypothesize the role of attention in several behaviors but do not specify the characteristic properties of attention. So, the aim of this research was to characterize the phenomenon of visual attention in wild-type *Drosophila*, including both externally cued and covert attention using tethered flight at a torque meter. Development of systematic quantifiable behavioral tests was a key aspect for this which was not only important for analyzing the behavior of a population of wild-type flies but also for comparing the wild-type flies with mutant flies. The latter would help understand the molecular, genetic, and neuronal bases of attention. Since *Drosophila* provides handy genetic tools, a model of attention in *Drosophila* will serve to the greater questions about the neuronal circuitry and mechanisms involved which might be analogous to those in primates. Such a model might later be used in research involving disorders of attention.

Attention can be guided to a certain location in the visual field by the use of external cues. Here, using visual cues the attention of the fly was directed to one or the other of the two visual half-fields. A simple yet robust paradigm was designed with which the results were easily quantifiable. This paradigm helped discover several interesting properties of the cued attention, the most substantial one being that this kind of external guidance of attention is restricted to the lower part of the fly's visual field. The guiding cue had an after-effect, i.e. it could occur at least up to 2 seconds before the test and still bias it. The cue could also be spatially separated from the test by at least 20° and yet attract the attention although the extent of the focus of attention (FoA) was smaller than one lower visual half-field. These observations excluded the possibility of any kind of interference between the test and the cue stimuli. Another interesting observation was the essentiality of continuous visibility of the test stimulus but not the cue for effective cuing. When the contrast of the visual scene was inverted, differences in response frequencies and cuing effects were observed. Syndirectional yaw torque responses became more frequent than the antidirectional responses and cuing was no longer effective in the lower visual field with inverted contrast. Interestingly, the test stimulus with simultaneous displacement of two stripes not only effectuated a phasic yaw torque response but also a landing response. A

landing response was produced in more than half of the cases whenever a yaw torque response was produced.

Elucidation of the neuronal correlates of the cued attention was commenced. Pilot experiments with hydroxyurea (HU) treated flies showed that mushroom bodies were not required for the kind of guidance of attention tested in this study. Dopamine mutants were also tested for the guidance of attention in the lower visual field. Surprisingly, TH-Gal4/UAS-shi^{ts1} flies flew like wild-type flies and also showed normal optomotor response during the initial calibration phase of the experiment but did not show any phasic yaw torque or landing response at 18 °C, 25 °C or 30 °C. dumb² flies that have almost no D1 dopamine receptor dDA1 expression in the mushroom bodies and the central complex (Kim et al. 2007) were also tested and like TH-Gal4/UAS-shi^{ts1} flies did not show any phasic yaw torque or landing response. Since the dopamine mutants did not show the basic yaw torque response for the test the role of dopamine in attention could not be deduced. A different paradigm would be needed to test these mutants.

Not only can attention be guided through external cues, it can also be shifted endogenously (covert attention). Experiments with the windows having oscillating stripes nicely demonstrated the phenomenon of covert attention due to the production of a characteristic yaw torque pattern by the flies. However, the results were not easily quantifiable and reproducible thereby calling for a more systematic approach. Experiments with simultaneous opposing displacements of two stripes provide a promising avenue as the results from these experiments showed that the flies had a higher tendency to deliver one type of response than when the responses would be produced stochastically suggesting that attention increased this tendency. Further experiments and analysis of such experiments could shed more light on the mechanisms of covert attention in flies.

7. Zusammenfassung

Zu jedem Zeitpunkt stellt unsere Umgebung eine so große Menge an visueller Information zur Verfügung, dass das Herausfiltern der wichtigen Informationen für eine weitere Verarbeitung eine grundlegende Herausforderung für jedes komplexe visuelle System darstellt. Bewerkstelligt wird dies u.a. mittels der selektiven Aufmerksamkeit, die die sensorischen Inputs einer Quelle, unter Ausschluss aller anderen, hervorhebt (Luck und Mangun 2009). Aufmerksamkeit wurde an Menschen und nichtmenschlichen Primaten bereits ausgiebig untersucht. Visuelle Aufmerksamkeit bei *Drosophila* konnte 1980 zum ersten Mal nachgewiesen werden (Wolf und Heisenberg 1980), jedoch blieb dieses Feld bis heute großen Teils unerforscht. In jüngster Zeit tauchten Studien auf, die der Aufmerksamkeit eine Rolle bei verschiedenen Verhaltensleistungen zuweisen, ohne jedoch die charakteristischen Eigenschaften von Aufmerksamkeit zu spezifizieren. Es ist das Ziel dieser Arbeit, das Phänomen der sowohl durch externe Reize ausgelöst, als auch endogen erzeugten (covert attention) visuellen Aufmerksamkeit bei wildtypischen *Drosophila* im stationären Flug am Drehmoment-Messgerät zu charakterisieren. Hierbei ist ein wesentlicher Aspekt durch die Entwicklung von quantitativen Tests das Verhalten von wildtypischen Fliegen so zu analysieren, dass es mit dem Verhalten genetischer Varianten verglichen werden kann. Ein solcher Vergleich würde helfen, die molekularen, genetischen und neuronalen Grundlagen der Aufmerksamkeit zu verstehen, da bei *Drosophila* für solche Untersuchungen einfach anwendbare genetische Werkzeuge zur Verfügung stehen. Ein Modell der Aufmerksamkeit bei *Drosophila* könnte auch für die visuelle Aufmerksamkeit bei Primaten relevant sein, falls diese Systeme homolog sind, d.h. in der Stammesgeschichte einen gemeinsamen Ursprung haben.

Mittels äußerer Reize lässt sich die Aufmerksamkeit auf einen bestimmten Ort im visuellen Feld führen. In dieser Arbeit wird die Aufmerksamkeit einer Fliege durch visuelle Reize auf jeweils eines der beiden visuellen Halbfelder gelenkt. Es wird ein einfaches und robustes Paradigma entwickelt, dessen Ergebnisse ohne viel Aufwand quantifizierbar sind.

Eine wesentliche Eigenschaft der exogen gelenkten visuellen Aufmerksamkeit, zu deren Entdeckung dieses Paradigma unter anderen beigetragen hat, ist, dass diese Art der Lenkung der Aufmerksamkeit auf den unteren Teil des visuellen Feldes der Fliege beschränkt ist. Der lenkende Reiz hat einen Nacheffekt, das heißt, er kann bis zu zwei Sekunden vor dem Test auftreten und dessen Ergebnis trotzdem beeinflussen. Auch bei einer räumlichen Trennung des Reizes vom Test um mindestens 20° kann er noch die Aufmerksamkeit auf diesen ziehen, wobei hier dann die Ausdehnung des Aufmerksamkeitsfeldes kleiner als ein unteres visuelles Halbfeld

ist. Durch diese Beobachtungen wird eine mögliche Interferenz zwischen Reiz und Test ausgeschlossen. Eine weitere interessante Beobachtung ist, dass für ein effektives Lenken der Aufmerksamkeit der Teststimulus aber nicht der lenkende Reiz durchgehend sichtbar sein muss. Eine Invertierung des Kontrastes der visuellen Reizgebung führt zu veränderten Antwortfrequenzen und Effekten der Aufmerksamkeitslenkung. So treten syndirektionale Drehmoment-Antworten häufiger auf als antidirektionale und die Lenkung der Aufmerksamkeit im unteren visuellen Feld durch einen vorhergehenden Reiz tritt nicht auf.

Interessanterweise kann der Teststimulus, die simultane Verschiebung zweier Streifen nicht nur eine phasische Drehmoment-Antwort, sondern auch einen Landeversuch auslösen. Dieser wird in mehr als der Hälfte aller Fälle, in denen eine Drehmomentantwort gezeigt wird, beobachtet.

Eine Untersuchung der neuronalen Korrelate der reizgelenkten Aufmerksamkeit wurde begonnen. In Pilotexperimenten mit Fliegen, die mit Hydroxyharnstoff (HU) behandelt worden waren, zeigte sich, dass die adulten Pilzkörper nicht für diese Art der Lenkung der Aufmerksamkeit, wie sie in der vorliegenden Arbeit untersucht wird, benötigt werden. Des Weiteren wurden auch Fliegenmutanten mit Defekten im Dopamin-System getestet. Überraschenderweise flogen TH-Gal4/UAS-shi^{ts1} Fliegen wie wildtypische Fliegen und zeigten auch ein normales optomotorisches Verhalten während der anfänglichen Kalibrierungsphase des Experimentes. Sie zeigten jedoch weder phasische Drehmoment-Antworten noch Landeversuche bei 18°C, 25°C oder 30°C. Auch dumb² Fliegen, die so gut wie keine D1 Dopaminrezeptoren in den Pilzkörpern und im Zentralkomplex exprimieren (Kim et al. 2007), zeigten die gleichen Verhaltensdefekte wie TH-Gal4/UAS-shi^{ts1} -Fliegen. Da bei den Dopaminmutanten die phasische Drehmomentantwort fehlte, konnte die Bedeutung von Dopamin für Aufmerksamkeit aus diesem Test nicht abgeleitet werden. Um diese Mutanten zu testen, bedarf es eines anderen Paradigmas.

Die Richtung der Aufmerksamkeit kann nicht nur durch äußere Reize gelenkt, sondern auch endogen verändert werden (covert attention). Experimente mit zwei oszillierenden Streifenmustern in der rechten und linken Sehfeld-Hälfte verdeutlichen das Phänomen der endogen gesteuerten Aufmerksamkeit anschaulich, da die Fliegen hierbei charakteristische Drehmomentmuster für das eine oder andere Muster erzeugen. Weil diese Einzelbeobachtungen aber nicht leicht quantifizierbar sind, ist hier ein neuer Ansatz notwendig. Die obigen Experimente mit zwei einzelnen Streifen, die gleichzeitig nach rechts und links versetzt werden, versprechen systematischere Ergebnisse. Die Fliegen neigen stärker dazu einen bestimmten Antwort-Typ (Drehmoment nach links bzw. nach rechts)

beizubehalten, als eine statistische Verteilung annehmen ließe. Es ist zu vermuten, dass dieser Effekt durch die Aufmerksamkeit hervorgerufen wird. Die Analyse solcher Experimente könnte also die endogene Steuerung der Aufmerksamkeit beleuchten.

8. Annex

8.1. Affidavit

I hereby confirm that my thesis entitled “Visual attention in *Drosophila melanogaster*” is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Wuerzburg, 22.11.2011

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation „Visuelle Aufmerksamkeit bei *Drosophila melanogaster*“ eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Würzburg, 22.11.2011

8.3. Publications and important conference contributions

Publications

Sareen P., Wolf R., and Heisenberg M. (2011). Attracting the attention of a fly. *Proc Natl Acad Sci U S A* 2011 Apr 26; 108(17):7230-5

Conference contributions

Sareen P., Wolf R., and Heisenberg M. (2010). Visual selective attention in *Drosophila*. Poster presented at the 9th International Congress of Neuroethology, Salamanca, Spain

Sareen P., Wolf R., and Heisenberg M. (2009). Visual selective attention in *Drosophila*. Talk presented at the Neurobiology of *Drosophila* Conference, Cold Spring Harbor Laboratory, NY, USA

Sareen P., Wolf R., and Heisenberg M. (2008). Guidance of visual selective attention in *Drosophila*. Talk presented at the *Drosophila* Regional Meeting, Max Planck Institutes of Biochemistry and Neurobiology, Martinsried-Munich, Germany

Sareen P., Wolf R., and Heisenberg M. (2008). Guidance of visual selective attention in *Drosophila*. *J Neurogenet* 2009 Feb; Vol. 23 Suppl 1: 78. Neurofly 2008. Abstracts of the 12th European *Drosophila* Neurobiology Conference. September 6-10, 2008. Wuerzburg, Germany

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