

Visual Pattern Recognition in *Drosophila* Is Invariant for Retinal Position

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Vision relies on constancy mechanisms. Yet, these are little understood, because they are difficult to investigate in freely moving organisms. One such mechanism, translation invariance, enables organisms to recognize visual patterns independent of the region of their visual field where they had originally seen them. Tethered flies (*Drosophila melanogaster*) in a flight simulator can recognize visual patterns. Because their eyes are fixed in space and patterns can be displayed in defined parts of their visual field, they can be tested for translation invariance. Here, we show that flies recognize patterns at retinal positions where the patterns had not been presented before.

In the flight simulator (Fig. 1A), the fly's (*Drosophila melanogaster*) head and thorax and, hence, its eyes are fixed in space while its yaw torque can still control the angular velocity of a panorama surrounding it (1). If the panorama displays different patterns the fly can be trained to discriminate them (Fig. 1B) (2). The flight simulator lends itself to an investigation of translation invariance as patterns rotate around the fly at a fixed height and can be vertically displaced between training and test. To our surprise, flies failed in such tests to recognize patterns shifted up or down by 9° or more after training. Pattern recognition seemed to require the same retinal coordinates for acquisition and retrieval (3–5). This finding was in line with earlier experiments in ants, which had failed to show interocular transfer for landmark recognition (6).

Subsequent studies (7–9) identified some of the pattern parameters (features) the flies used for discrimination. These were size, color, vertical compactness, and vertical position of the centers of gravity (COGs) of the patterns in the panorama. For many pattern pairs carrying none of these features, no conditioned discrimination could be detected, although flies often discriminated them spontaneously (7). In the earlier quest for translation invariance (3–5), flies had been conditioned to discriminate patterns solely by the vertical position of their COGs. For instance, if in Fig. 1A the vertical positions of the COGs of upright and inverted Ts were aligned, flies were unable to discriminate

them after conditioning [shown for triangles in ref. (7) and discussed in Supplement]. This raised the possibility that perhaps vertical displacement specifically interfered with the feature “vertical position” (10, 11).

Only two of the four parameters, size and color, are independent of the vertical position of the pattern elements in the arena. These, therefore, were chosen in the present study. To test for conditioned discrimination of (horizontal) size (Fig. 2A, left dotted bars), we presented two black rectangles of the same height but differing by about a factor of two in width in neighboring quadrants. They were all shown at the same vertical position in the arena slightly above the fly's horizon. Flies readily learned to avoid the larger or smaller figure after the training ($P < 0.001$). Next, these patterns were vertically displaced between training and test (12). In contrast to the earlier experiments with patterns differing in the vertical position of their COGs, no decrement of the memory score was observed after a vertical displacement of $\Delta H = 20^\circ$ (Fig. 2A, right cross-hatched bars). In the same way, we tested color. Flies remembered blue and green rectangles of the same size and presented at the same vertical position (8, 9). They had no difficulty recognizing them after vertical displacement at the new position (Fig. 2B).

Edge orientation is a feature that has been extensively documented in the honeybee (13–17) to serve in conditioned pattern discrimination. We found a robust conditioned preference for bars tilted +45° and –45° to the vertical [Fig. 2C, left dotted bars; (18); but see (7)]. Vertical displacement of the bars after training had no significant effect on the memory score [Fig. 2C, right cross-hatched bars; (19)].

Next, more complex patterns were tested. The rectangles in the four quadrants in

Fig. 2D were each composed of a blue and a green horizontal bar. They differed only in whether green was above blue or blue above green. In principle, flies had two options to discriminate the two figures. They could combine the two features vertical position and color to give a new feature with relational cues (e.g., “green above blue”). Alternatively, they could evaluate the two colors separately and remember for each whether the high or low rectangles were safe or dangerous. This would have different consequences in the transfer experiment. If the colors were processed separately, flies would have to rely on vertical positions and could recognize neither the green nor the blue patterns at the new retinal positions. For composite figures, however, the vertical positions would be transformed into relational cues, which might still be recognized after vertical displacement. The latter was observed (Fig. 2D, right cross-hatched bars). Apparently, within each rectangle, flies evaluated the positions of the colored pattern elements

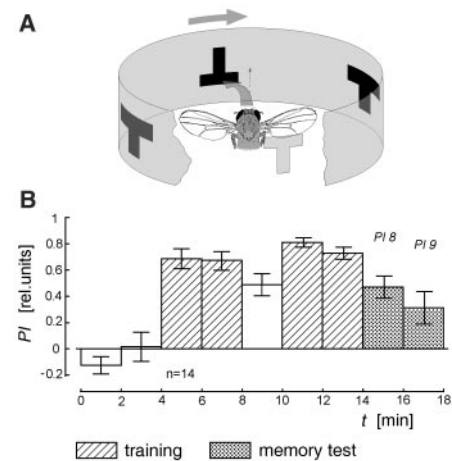


Fig. 1. Visual pattern discrimination learning in the flight simulator. Angular velocity of an artificial visual panorama is made negatively proportional to the fly's yaw torque [gray arrows in (A)], allowing the fly to change its flight direction (yaw torque $\neq 0$), or to maintain stable orientation (yaw torque = 0) with respect to visual landmarks in the panorama (upright and upside-down T-shaped black patterns). During training, a heat beam (not shown in figure), directed to the fly's thorax and head from behind, is switched on or off at the boundaries between quadrants containing the one or the other pattern type in their center. (B) Standard learning experiment. Performance index (PI) is calculated as $PI = (t_c - t_h)/(t_c + t_h)$, where t_c is the fraction of time with heat off and t_h the remaining time with heat on in a 2-min interval. The arena is rotated to a random angular position at the beginning of each 2-min interval. Empty bars indicate test intervals without any heat; hatch bars denote training intervals. PI 8 and PI 9 (dotted bars) quantify the flies' conclusive pattern memory. Error bars are SEMs.

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relative to each other and directed their flight with respect to these cues.

Similar relational cues were effective in Fig. 2E. Each of the alternative figures consisted of two orthogonally oriented oblique bars, one above the other. In the two figures the two orientations were exchanged. In one, the top bar was tilted by +45°, in the other by -45°. If the flies relied on the integral of the orientations of all edges in each composite figure they would not be able to discriminate the two. Flies were able to evaluate the spatial relations in the composite figures. Even for these complex patterns, translation invariance for vertical displacement was found. When the two patterns were rotated by 90°, which placed the two bars side-by-side at the same vertical position, no conditioned discrimination was obtained (Fig. 2F).

The only patterns *Drosophila* failed to recognize after vertical displacement were those that can discriminate only by their vertical position. This suggests a special interference between the feature vertical position and the vertical displacement. Horizontal displacement of these patterns cannot be tested in the flight simulator, because horizontal motion is controlled by the fly, which has to choose a certain azimuth relative to the landmarks for its direction of flight. We therefore developed an alternative paradigm to investigate visual pattern recognition and, in particular, horizontal translation invariance. Flies were conditioned at the torque meter by heat to restrict their yaw torque range to only left

or only right turns (2). Two patterns were displayed at stable retinal positions during training (Fig. 3), for instance at +45° and -45° from the frontal direction. Between training and memory test the patterns were exchanged. Flies shifted their restricted yaw torque range to the other side (i.e. from left turns to right turns or vice versa) (Fig. 3A).

When patterns were shifted to a new position on the same side (from ±30° to ±80° or vice versa), the yaw torque bias stayed on the side of the yaw torque range to which it had been confined during training (Fig. 3B). Obviously, flies recognized the patterns at the new retinal positions after horizontal displacement. The T-shaped patterns used in this experiment could be discriminated only by the vertical positions of their COGs, and they were the very patterns for which no translation invariance had been found in the flight simulator after vertical displacement.

All five pattern parameters tested (size, color, edge orientation, relational cues, vertical position) showed visual pattern recognition in *Drosophila* to be translation invariant. Vertical position was the only parameter that the flies could not recognize after vertical displacement, but they did recognize this parameter after a horizontal shift.

Little is known so far about translation invariance in flies. In the present study, it has been demonstrated for horizontal displacements between +45° and -45° from the frontal direction. These positions are well outside the region of binocular overlap (20). Hence,

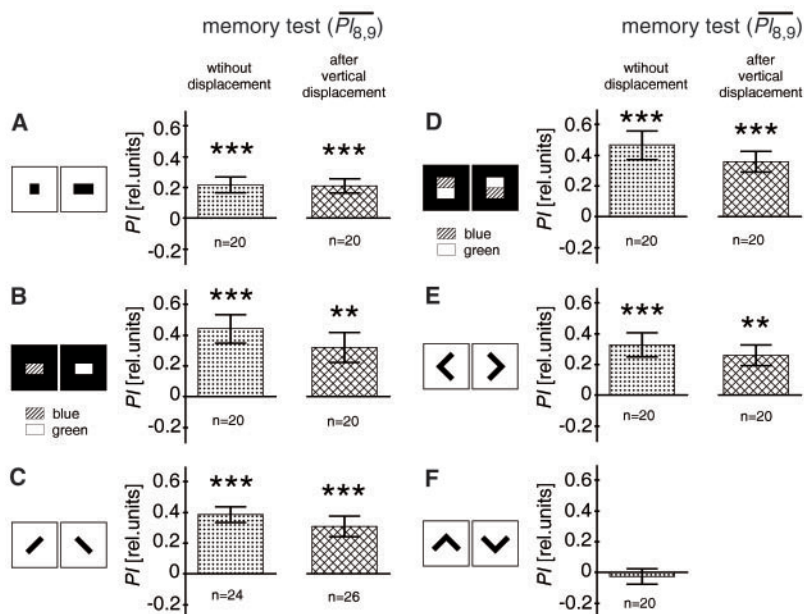


Fig. 2. Pattern discrimination learning and retinal transfer with vertical displacements for various parameters. Flies are trained with different pairs of patterns following the protocol of the standard learning experiment (see Fig. 1). Bar graphs: (dotted) pattern memory tested without vertical displacement; (cross-hatched) the complete panorama was shifted upward by 20° after the last training block (at $t = 14$ min). Bars are averaged means of PI_8 and PI_9 . Error bars are SEMs. *** $P < 0.001$; ** $P < 0.01$ (From a one-sample t test, 2-tailed P value).

the pattern information generalized for position must be made available to both brain hemispheres (interocular transfer).

Our yaw torque learning paradigm reveals intriguing properties of visual processing. First, it shows that visual motion is not a prerequisite for pattern recognition. Flies with their eyes fixed in space can recognize stationary visual objects (21). No motion is required for the perceptual process. Although the fly can still move the optical axes of its photoreceptors by a few degrees (22), this is too little to generate directional motion. Moreover, flies can recognize visual patterns in the flight simulator if during acquisition these are kept stationary (23). In the present experiment, the patterns were stationary even

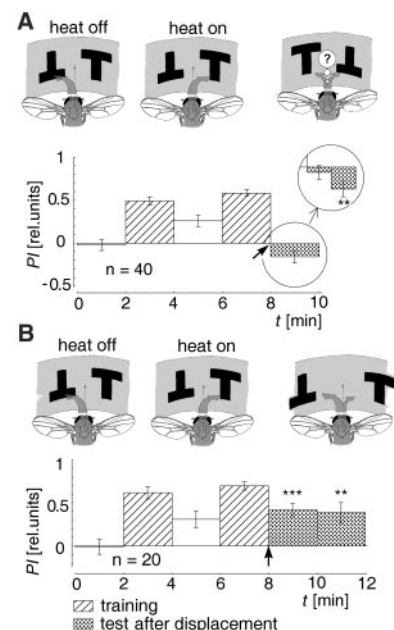


Fig. 3. Yaw torque conditioning with fixed visual patterns. Two stationary patterns are presented to the fly at +45° and -45° from the frontal direction. The fly's yaw torque is recorded and its range is divided in two domains roughly corresponding to intended left and right turns. If torque is to the right, heat is switched on, if torque is to the left, heat is switched off. Flies can learn to keep their torque persistently in the safe domain (for this experiment without visual patterns see ref. (2)). The first test between the two training blocks is carried out with patterns in the training positions. The positive PI indicates that the flies continue to direct their yaw torque to the "safe" side. (A) The two patterns are exchanged after a second training period with the arena light switched off during the shift. Negative PI after the second training indicates that flies recognize the patterns in their new positions. Higher time resolution of PI s shows gradual transition from positive to negative PI s (inset in circle), which indicates different dynamics of visual and motor memory. (B) As a control, patterns were shifted within the same visual half-field (from $\psi = \pm 30^\circ$ to $\psi = \pm 80^\circ$ or vice versa). *** $P < 0.001$; ** $P < 0.01$ (From a one-sample t test, two-tailed P value).

during retrieval. The fly's turning tendency indicated that it recognized the patterns.

Second, during intended turns to one side flies selectively followed the directional motion cues of landmarks on that side and neglected the symmetrical motion cues of a corresponding landmark on the other side (24). From the present experiment, we can deduce that the fly associated the heat with the pattern to which it tried to turn while being heated, and the no-heat condition with the pattern to which it tried to turn while flying in the cold. Because the flies were exposed to the two patterns in equivalent retinal positions, they must be able to activate a gating process for a part of the visual array in the optic lobes corresponding to one or the other of the visual half-fields. Studies of walking flies have provided similar phenomena (25). The ability to confine visual processing to a visual field region of choice is called selective visual attention (26).

Selective attention may be relevant also for the flight simulator experiment. For translation invariance in the flight simulator, the fly has to store not only a feature of a pattern for recognition ("what") but also an azimuth value for orientation ("where"). We propose that, while being heated, the fly associates the pattern that happens to be in the window of attention with the heat. In the flight simulator, the fly most of the time keeps the window of attention in a frontal position (27). In this way, the pattern would be labeled "dangerous if approached."

Third, the flies in the yaw torque learning paradigm not only associated heat with the turning tendency to one side but also with the pattern on the side to which they tried to turn. In the memory test after the patterns had been exchanged, the fly inverted its turning tendency. The preference for the previously "safe" torque domain quickly fades, whereas the pattern preference, expressed by the fly's yaw torque to the side of the attractive pattern, persists. If, for instance, the fly expects yaw torque to the left to entail heat but suddenly finds on that side the previously safe pattern, it overrides its negative predisposition for left turns and tries to turn into that direction. The fading of the behavioral memory component has also been reported for 3-way associations testing colors instead of patterns (23).

Feature detectors for edge orientation are a hallmark of mammalian visual sys-

tems (28) and have also extensively been studied in the honeybee (13–17). Like translation invariance, edge orientation is a further basic property that is shared between the visual systems of *Drosophila* and larger animals. Finally, flies also evaluate relational cues such as {A above B} versus {B above A}. So far this fascinating ability has been demonstrated only for two colors (blue and green) and two edge orientations (+45° and -45°). The negative outcome of the experiment with two horizontally arranged oblique bars in the flight simulator cannot be generalized. As mentioned above, the exclusively horizontal motion in the flight simulator may specifically interfere with this arrangement. Also horizontal compactness is not a discriminating parameter (7), although a grouping effect for vertical bars can be observed in fixation (27). In any case, the discovery of relational cues seems to vastly increase the potential number of pattern parameters the fly might be able to discriminate.

Our data suggest a basic scheme (a minimal circuit) for translation invariance. As mentioned above (10, 11), the experimental paradigms conceptually demand a distinction between orientation and recognition, i.e., a where and what network (28, 29). Both networks must have a centripetal (afferent) and a centrifugal (efferent) branch. The model is outlined in the supplement (fig. S1).

References and Notes

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9. Note that flies discriminated the rectangles by hue rather than brightness, because varying the relative intensities of the two colors by a factor of 10 between training and test has no significant effect on memory performance.
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11. In the flight simulator, an orientation task is used to measure pattern recognition and translation invariance. In order to retrieve a particular flight direction relative to the panorama in the memory test, the fly has to store the respective pattern (feature) during the training not only for recognition but also by an azimuth value for orientation (e.g., direction of flight).
12. In previous experiments (3, 6), the transparency in the arena carrying the figures had been exchanged

between training and test. This procedure required 30 to 60 s. Also, for half of the flies, the pattern changed from a lower to a higher position; for the other half, the sequence was the opposite. In the present experiments, the whole arena was shifted after the final training and the shift was always 20° upward. The new procedure implied that, not only the figures, but also the upper and lower margins of the arena were displaced, but the shift took only an instant and did not entail any visual disturbances from handling. Control experiments showed the same basic results with the old and new procedures. For instance, flies trained with horizontal bars at different heights ($\Delta H = 20^\circ$) to avoid certain flight directions were unable to retrieve this information if, after the training, the whole arena was shifted upward by 20° (30), this inability confirmed that the flies' pattern recognition system does not tolerate the vertical displacement if vertical position is the discriminating pattern parameter.

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Supporting Online Material

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Material and Methods

SOM Text

Fig. S1

References

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