Osmotropotaxis in larvae of *Drosophila melanogaster*

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The larvae of *Drosophila* exhibit osmotropotaxis. At high concentrations of the odorant, chemotactic response is strongly oriented. At low concentrations oriented crawl is replaced by quasi-random turning. If the olfactory sensillum is ablated unilaterally, the larvae turn so as to present the ablated side to the odour source.

In order to orient towards sources of odours, bilaterally-symmetrical animals usually employ a pair of odour detectors. The underlying mechanism, called *osmotropotaxis*, involves a comparison of sensory output from the two sense organs. The animal turns clockwise or counter-clockwise until the detectors are symmetrically placed in relation to the stimulus and their outputs are equalized. If one of the detectors is ablated, the animal keeps turning in circles. Flugge[1] first employed unilateral ablation of antenna to demonstrate osmotropotaxis in the imago of *Drosophila melanogaster*. Borst and Heisenberg[2] observed that the fly can orient towards attractants but does not turn away from benzaldehyde, a repellent. This observation implies that the imago employs two different mechanisms in its olfactory response. The larva of *Drosophila* is attracted by a variety of odours including some that repel adults[3,5], but the mechanism of orientation in the larva has not been analysed. In this paper we show that the larva is capable of osmotropotaxis at high concentrations of an attractant ethyl acetate (EA). At lower stimulus concentrations, orientation response is lost and the larval track tends to become wayward.

The response of larva to odorants can easily be measured on petri dishes. About a hundred larvae are placed at the centre of the dish and a measured amount of odorant is spotted near the edge. As the petri dish is covered, a gradient of odorant concentration forms. The larvae which at first crawl in all directions rapidly reorient and move towards the odour source. After a fixed time, the number of larvae on the two sides of the plate is counted. The larval plate test is a population test and yields an average measure of the response. The test can be used to determine the threshold of response to different odorants and to construct dose-response curves. Larval plate test can also be used to follow tracks of individual larvae[4–7].

We examined tracks of larvae responding to varying concentrations of EA. Selected examples of these tracks are shown in Figure 1. At high concentrations of the odorant, the track is relatively straight and maximally-oriented towards the odour source. Larvae reached the source in less than 3 minutes. As the concentration of EA is reduced, the larvae become markedly disoriented. A fraction of larvae do not reach the odour source and the track ends with these larvae burrowing in agar. At 10^{-3} dilution of EA, for instance only 60% had reached the source. At a yet lower concentration, 10^{-7} EA, the larval walk becomes entirely wayward and the majority fail to arrive at the source.

The orientation response was analysed in the following manner. Each track was divided into 2.5 mm segments, approximated by a straight line, joining the ends of the segment. The angle made by each segment with the line joining the larva to the odour source was measured. The orientation angles can vary between zero (directed

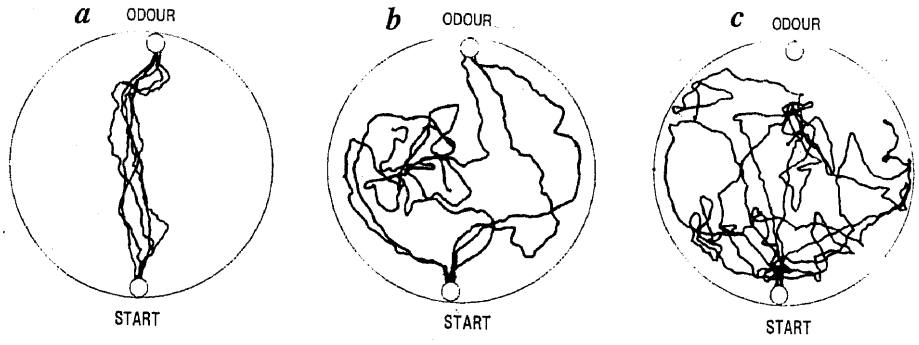

Figure 1. Tracks of early third-instar larvae responding to ethyl acetate (EA): The larva was placed at the start on a 10 cm petri dish filled with 1% agar. The tracks were marked with a pen on a transparent sheet. Specified dilutions of odorant were dispensed in 25 µl of paraffin on a filter paper disc. 20 larvae were tracked at each concentration, for clarity only 5 tracks are shown. *a*, 10^{-1} EA; *b*, 10^{-3} EA; *c*, 10^{-7} EA.

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towards the source) and 180 degrees (diametrically opposite). Clockwise is positive and anticlockwise, negative. The measured orientations were grouped in bins of 20 degrees and are presented in Figure 2. As concentration of EA is reduced, the tracks become unoriented, the track length increases and the proportion of larvae reaching the source falls (Figure 3).

Although the orientation declined greatly at 10^{-7} EA, the threshold of chemotactic response is much lower. It lies between 10^{-8} and 10^{-9}. The question arises whether at low-odorant concentrations the larvae employ osmokinesis rather than tropotaxis as a means of reaching the odour source. Kinetic response can arise from a stimulus-dependent change in turning frequency or a change in the speed of locomotion[8]. We have measured both these parameters. Turning frequency increases greatly with dilution, from 0.18 ± 0.05 turns/cm at 10^{-1} to 0.511 ± 0.04 turns/cm at 10^{-7} of EA (Figure 4). The speed of locomotion on the other hand seems to change in the opposite direction. The change in speed, is not so marked.

The dorsal organs (DO) of *Drosophila* are a part of the antenno-maxillary complex. They consist of dome-shaped sensory structures, 5 μm in diameter, located on two sides of the anterior segment. Anatomical studies by Singh and Singh[9] on *Drosophila* and Chu and Axtell[10] on other dipterans, suggest that the DO mediates olfactory response.

The dorsal organ on one side was crushed in cold-anesthetized third-instar larvae and the larvae were allowed to recover for 15 min in Ringer's solution. The ablated and control larvae were tested for chemotactic response. In the absence of an odour cue, both ablated and control larvae crawl in an unoriented manner; there is no marked difference in the two. In the presence of 10^{-1} EA, the normal larva shows an oriented crawl headed towards the odour source. The larva with one side ablated does not move towards the source. It turns repeatedly in the direction of the intact side, presenting the ablated side to the odour source. In a small proportion of larvae (about 10%), clockwise or counter-clockwise circular movements, depending upon the side that was ablated, are observed. The effect of unilateral ablation upon track direction is shown in Figure 5.

The effect of ablation on turning behaviour was analysed. The track was divided into 5 mm segments and the angle each segment made with the preceding segment was measured. The turns were thus classified into right turn (T_r) and left turn (T_l), if the turning angle exceeded 20 degrees. A differential turning index can be defined as $(T_r - T_l)/(T_r + T_l)$. In the tracks of unablated larvae the turning index is close to zero

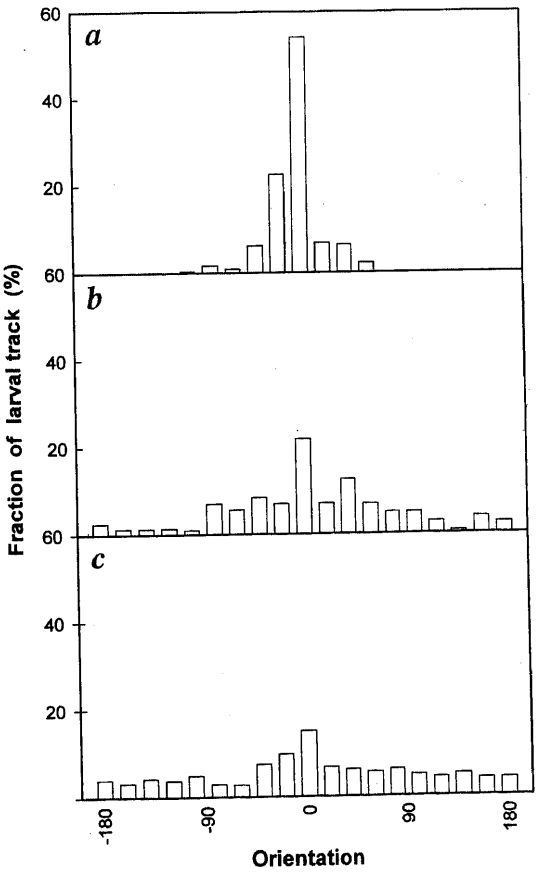

Figure 2. Orientation to odour: Larval tracks were divided into 2.5 mm segments. The angle made by each segment with the line directed towards the odour source was measured. Histograms show the angular distribution of segment orientation. *a*, 10^{-1} EA; *b*, 10^{-3} EA; *c*, 10^{-7} EA.

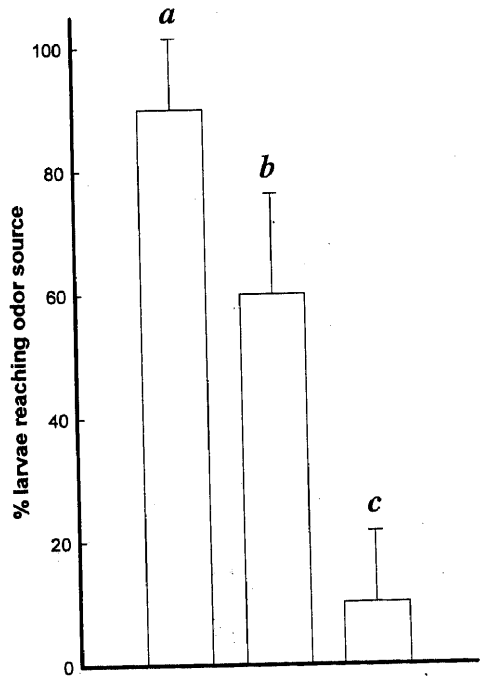

Figure 3. Proportion of larvae reaching the odour source: Odorant ethyl acetate *a*, 10^{-1} EA; *b*, 10^{-3} EA; *c*, 10^{-7} EA.

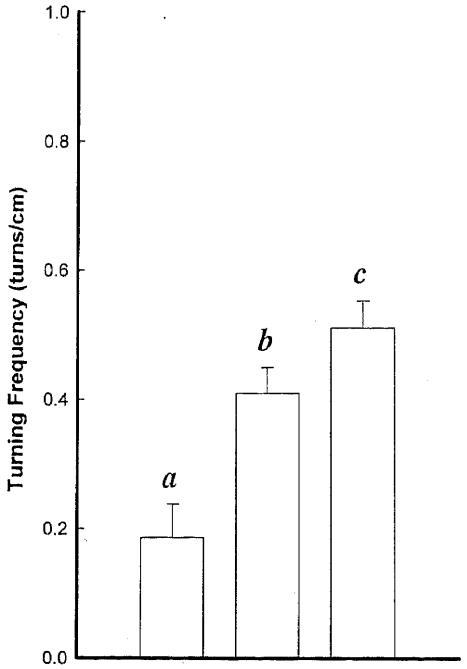

Figure 4. Turning frequency: Each 2.5 mm segment of the track was classified as right turn (T_r) or, left turn (T_l) if the angle of the turn exceeded 45 degrees. Frequency represents number of turn per cm of the track. Bars indicate standard errors.

Figure 5. Tracks of unilaterally ablated larva. Odorant 10^{-1} ethyl acetate. Right ablated (red); left ablated (black); normal control (green).

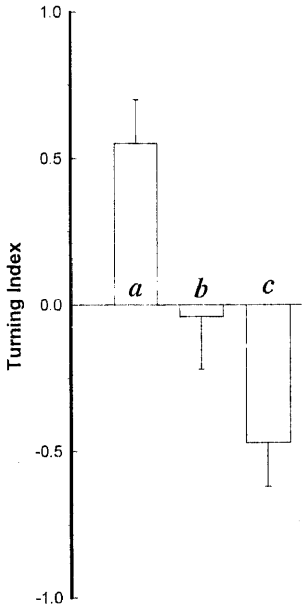

Figure 6. The turning response of larvae with unilateral ablation: Turning index is defined as $(T_r - T_l)/(T_r + T_l)$. *a*, left organ crushed; *b*, normal and *c*, right organ crushed. Bars indicate standard errors.

showing no right vs left bias. In the ablated larvae, turning is strongly biased towards the unablated side (Figure 6). The frequency of turning was somewhat greater in ablated larva (82%), compared to the normal (54%).

Odour, when spreading by diffusion, is not a directional stimulus. It consists of a moving front of concentration gradient. The animal may respond by orienting towards the source or by a biased random walk modulated by the odour. The neural mechanisms underlying either of these strategies are not fully understood. The experiments described here show that the larva of *Drosophila* is capable of osmotropotaxis at high concentrations of an attractant. At lower concentrations, tropotaxis is replaced by a kinesis-based response.

The unilateral ablation experiment provides evidence that the dorsal organ is involved in osmotropotaxis. However, ablation by crushing is a relatively crude operation and does not exclude the role of the other components of antenno-maxillary complex in orientation to odours.

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Periodically varying sensitivity to melatonin in a mammalian circadian system

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In this communication we examine the effect of the pineal hormone melatonin on the circadian locomotor activity rhythm of the field mouse *Mus booduga*. Phase shifts in the circadian rhythm of locomotor activity were measured and a phase response curve was constructed following a single dose of 2 µl melatonin (1 mg/kg) at various circadian times. Melatonin administered from circadian time 4 (CT4) to CT16 induced delay phase shifts whereas at phases CT18 to CT2 advance phase shifts were evoked. The magnitude and direction of the phase shifts evoked were a function of the time of the melatonin injections. The results suggest that the circadian timing system controlling the locomotor activity rhythm in the *M. booduga* is responsive to melatonin and also that the daily endogenous rhythm of melatonin may be involved in phasing or entraining the circadian system of mice.

THE vertebrate circadian system has the melatonin secreting pineal gland as one of its most important components[1]. Periodic administration of exogenous melatonin was found to entrain the activity rhythm in some vertebrates[2–5]. Timed administration of melatonin to some rodents also facilitated re-entrainment[6–9] and caused phase advances in the circadian activity rhythms[10,11]. The reported effects of melatonin on circadian rhythms and the presence of melatonin receptors in the suprachiasmatic nucleus (SCN) suggest a direct action of the hormone on the pacemaker[12,13].

Although in the last two decades the literature on PRCs has become abundant, that for melatonin has been very limited[3,14–16]. Recent work in our laboratory (and other laboratories) emphasizes the need to examine a variety of species to phase-shifting effects of exogenous melatonin administration. Furthermore, to confirm the hypothesis that the pineal plays an important role in phasing the circadian oscillator in a mammalian system, the present study was designed to describe the phase response curve (PRC) for melatonin for the locomotor rhythms in the field mouse *Mus booduga*.

Adult male mice *M. booduga* were captured from the fields near the University campus (9°58′N 78°10′E) and were raised under light/dark (LD) 12 : 12 for two weeks. The body weight of these animals ranged from 8 g to 12 g. Melatonin was procured from Sigma (USA), which was dissolved in 50% dimethyl sulphoxide (DMSO)[17].

The locomotor activity rhythms of individual mice ($n = 65$) was monitored by running wheel, connected to an Esterline Angus event recorder (Model A 620 X). The activity wheels (16 cm diameter) were attached to a plexiglass cage ($h \times l \times b = 6 \times 11 \times 8.5$ cm). The running wheels with mice were kept in light-tight experimental cubicles. Temperature (30 ± 1°C) and humidity (70 ± 5%) of the experimental cubicle were constant throughout the experiment. Food (grains) and water were available *ad libitum*.

The mice were maintained in continuous darkness (DD) throughout the duration of the experiment. After attainment of a steady state free-run, the animals' free-running period (τ) was calculated using linear regression. Activity onset was taken as phase reference point and is referred as CT 12. The phase and the magnitude of phase shifts are expressed in circadian hours. Mice were administered with 2 µl of single injections consisting of vehicle (50% DMSO) or melatonin (1 mg/kg)[14,21] at various circadian times (CTs: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22). Injections were given intraperitoneally in constant darkness under a dim red light of wavelength 640 nm. Mice received a maximum of 3 treatments with an interval of three weeks between each treatment. Each mouse was treated at different CTs.

The phase shifts induced by melatonin (experimental) and vehicle injections (control) were calculated as the differences in time between the linear regression carried out for two steady states prior to and after the administration of melatonin or 50% DMSO. The phase shift values were considered negative (delays) when the onset occurred later than the expected time and positive (advances) when they occurred before. The various phases

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