Chapter 6

RADAR-DOPPLER AS A TOOL TO MEASURE BEHAVIOURAL RESPONSES OF FEMALE HOUSEFLIES

Abstract

A radar-Doppler actometer is described with which movements of individual body parts of *Musca domestica* flies can be recorded. Movements of the insect's head were used to monitor the behavioural responses of houseflies to (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. A comparison was made with The results were compared with EAG recordings obtained previously on stimulation with the same substances. It was found that, although (Z)-9-heneicosene evoked high EAG responses in males as well as females, both sexes did not show behavioural responses to this substance. Our results compared with those of field experiments described in the literature suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating behaviour in males.

Introduction

Buchnan and Sattelle (1979) and Buchnan and Moreton (1981) were the first to use actometers equipped with a radar-Doppler detector for quantitative analysis of insect activity. They were able to monitor the locomotor activity of several insect species (*Periplaneta americana* L., *Musca domestica* L., *Calliphora erythrocephala* Mg. and *Drosophila melanogaster* Mg). Since then the locomotor activity of several other insect species have been studied with various types of actometers fitted with radar-Doppler systems. These actometers have been shown to be sensitive enough to record flight and locomotor activity of individual insects, such as tsetse flies (Van der Goes van Naters and Den Otter (1992), moths (Den Otter *et al.*, 1996; Renou *et al.*, 1998), *Anopheles* mosquitoes (Van de Broek *et al.*, 1999) and fruit flies (Knoppien *et al.*, 2000).

In this study we describe a radar-Doppler actometer with which movements of individual body parts of (fixed) *Musca domestica* flies can be recorded. We have used this actometer to measure behavioural responses of the flies to semiochemicals. For that purpose we recorded the head movements occurring after application of (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture (by weight) of these substances. (Z)-9-tricosene is supposed to function both as a sex attractant and a sex stimulant in males (Carlson *et al.*, 1971, 1974). According to Mansingh *et al.* (1972) (Z)-9-heneicosene may induce and maintain sexual behaviour in male *Musca domestica* when combined with (Z)-9-tricosene.

Materials and methods

Insects

Behavioural experiments were done with *Musca domestica* L. flies from 2 different strains: A laboratory strain (WHO Ij2), which was obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark) and is cultured in the laboratory since 1961, and a wild-type strain obtained from a cow-house with pig-sty (Pesse) in The Netherlands. The latter strain had been cultured in the laboratory for 6 (Pesse-6) months. The flies were reared in cages (30 x 30 x 40 cm) in a L12:D12 regime at 25

°C and r.h. 60%. They were fed (ad libitum) a mixture of sugar, powdered milk and yeast (5 : 5 : 1 by weight). In addition, a fountain filled with tap water was present.

Registration of the insects' movements.

The movements of the flies were detected by an actometer (Syntech, Hilversum, The Netherlands) consisting of a radar-Doppler sensor (Alpha Industry, Type GOS 2780), the output of which (24 GHz, 3 µW) was directed to the insect through a 4-cm-long wave-guide (Fig. 1). Opposite of the outlet of the wave-guide a microwave absorber was placed. Both the sensor and absorber were mounted on magnetic clamps fixed to a metal base plate.

The dorsal side of the thorax of the fly was fixed to the head of a pin using super glue, and the fly positioned horizontally in front of the wave-guide's outlet with its head at about 1 cm from the latter (Fig. 1). In this way the combined movements of the legs, wings and head could be recorded.

To study the movements of the legs, wings or head separately, body parts which were not subject to examination, were immobilized with glue. For recording head movements during olfactory stimulation the fly was positioned in a plastic pipette tip with the head protruding from the tip's narrow end. The fly was placed vertically with the head up and the lateral side of the head directed to the wave-guide outlet.

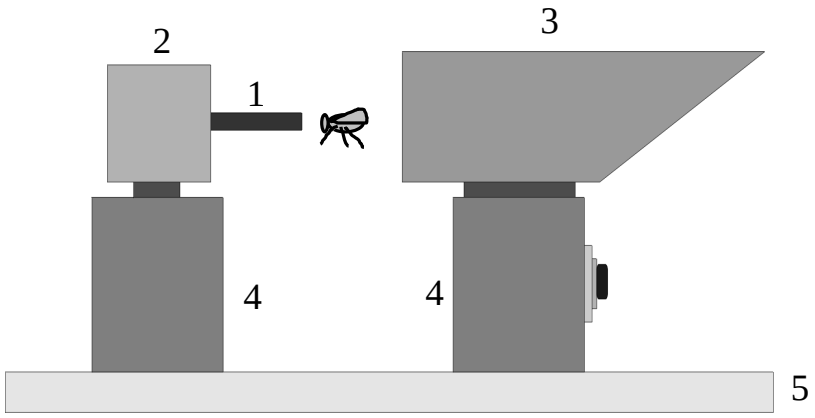

Figure 1. radar-Doppler actometer (Syntech, Hilversum, The Netherlands).
1: wave-guide, 2: radar sensor, 3: microwave absorber, 4: magnetic clamps,
5: base plate.

The microwave beam passing over and along the insect was partially reflected by its body. The frequency and phase shifts of the reflected waves induced by the insect's movements were detected by mixing with the emitted wave. The signal from the detector was electronically filtered (bandwidth 0.1-10 Hz), amplified, rectified and integrated (time constant 0.5 s). The signal was digitized (IDAC, Syntech), stored and evaluated with the software programme EAD (Syntech).

Stimuli

Stimuli were 0.01 and 10 µg (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture (by weight) of these two chemicals dissolved in 25 µl silicon oil. The solutions were pipetted onto pieces of filter paper (1.5 cm^2). In addition, papers loaded with 25 µl silicon oil (control stimuli) were prepared. Each individual paper was put into a Pasteur pipette. The pipette served as an odour cartridge.

Stimulation was achieved by injecting, during 1.5 s, the vapour content of an odour cartridge into a continuous, charcoal-filtered, humidified airstream (0.8 m/s) passing over the insect's head. Each fly was stimulated with 10 pulses of one and the same substance with intervals of 15 s, after which 10 pulses of another substance were applied. First all lower doses of each substance were tested. Before the series of lower and before that of the higher doses 10 pulses from the cartridge with silicon oil and from a cartridge containing clear air were given.

Results

'Spontaneous' movements

Figure 2A shows an actogram of 'spontaneous' head movements of a female WHO fly of which the legs and wings were fixed. Every time the head moved, a steep, high peak was seen followed by peaks which gradually declined in height. The fly spontaneously moved its head about 16 times per minute with intervals of about 2.25 s. The average duration of a head movement was 1.5 s. Figure 2B shows the movements of the six legs of a female fly with wings and head fixed. It appears that the number, duration and interval of the leg movements were about the same as those of the head. The shape of the leg actogram peaks, however, was different from that of the head movements. A high peak occurs at the start of the movements which stays at

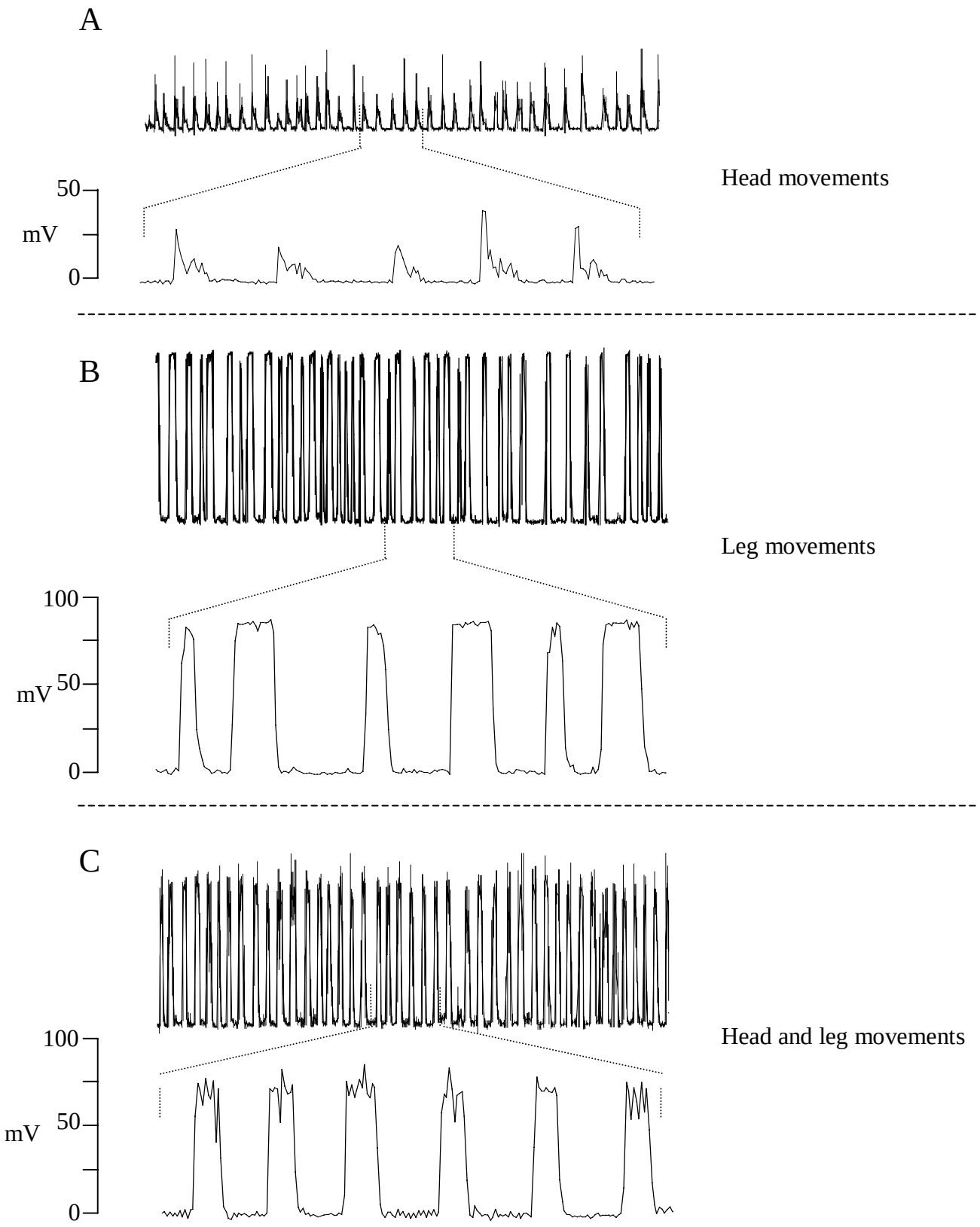

Figure 2. Actographs of spontaneous movements of head (A), legs (B), and head and legs (C) of individual *Musca domestica* females during periods of 150 s (upper traces). Lower traces show some individual movements in more detail. In A legs and wings, in B head and wings and in C the wings of the fly were fixed.

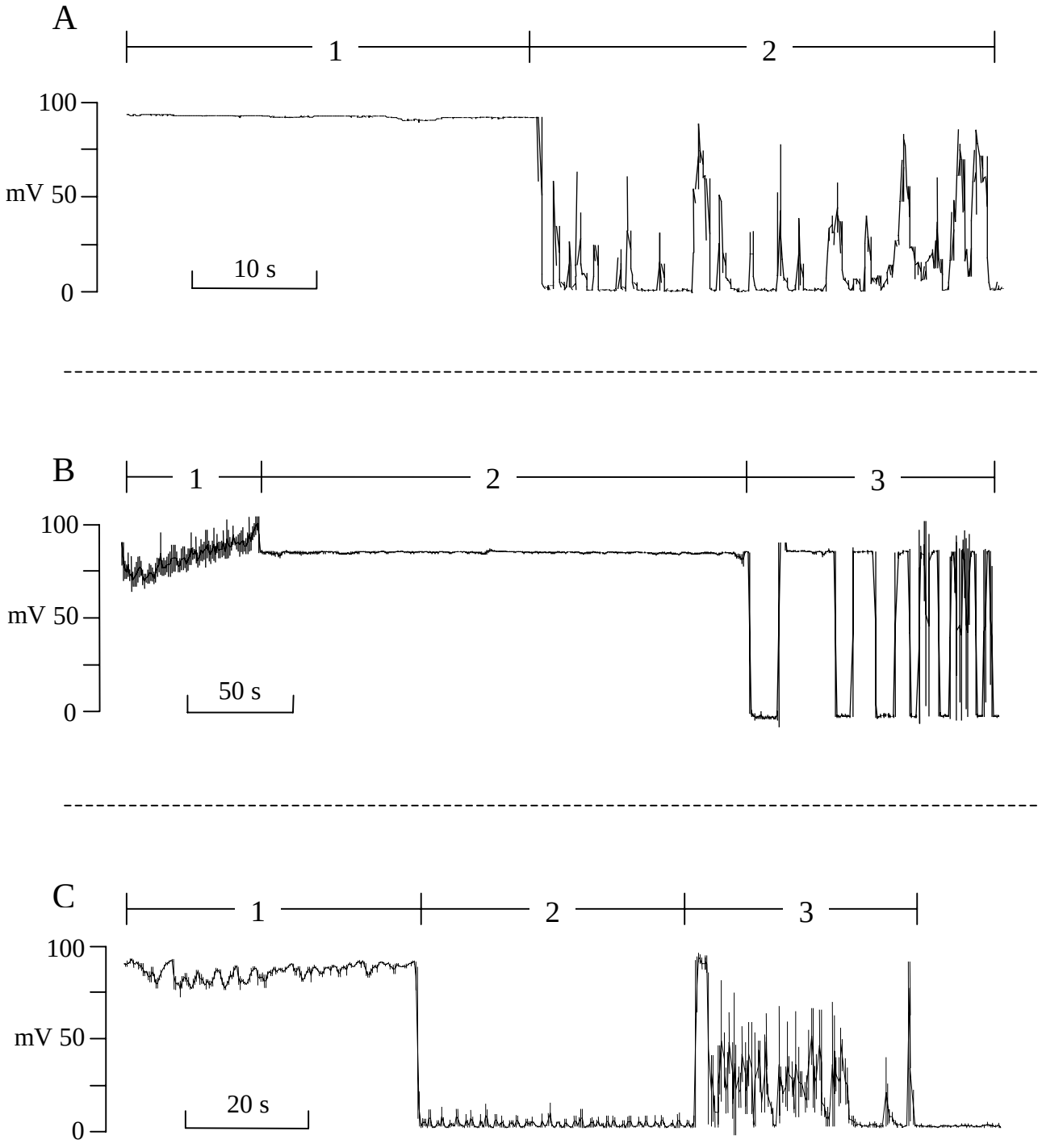

Figure 3. Spontaneous movements of wings (A), wings and legs (B), and wings, head and legs (C) of individual *Musca domestica*.
A: Continuous flight (1) and short movements of wings (2) of a female. 70 seconds recording.
B: Continuous flight of male with dangling legs (1), continuous flight (2) and short periods of flight followed with short flights combined with leg movements (3). 400 seconds recording.
C: Continuous flight of male with dangling legs (1), head movements (2) and combined wing and leg movements (3). 120 seconds recording.

a relatively constant high level after which its falls sharply to the original level. In Fig. 2C simultaneous movements of legs and head are shown of a female of which the wings were fixed. Visual observation of the movements learned that the legs and head moved synchronously. This is also reflected in the actogram, in which now clear peaks are seen.

Figure 3A shows the actogram of wing movements of a female fly with the legs and head immobilized during constant flight and when the wings were moving during short periods. In Fig. 3B recordings of leg and wing movements of a male fly with immobilized head are shown. Figure 3C presents actograms of a male fly able to move head, wings and legs.

Short movements of the wings (Fig. 3A:2) can be clearly distinguished from the wing movements during flight. In the latter case the actogram shows a nearly straight line at a relatively high level (Figs. 3A:1, 3B:2). When flight was combined with 'uncontrolled' dangling movements of the legs, small deviations of the almost straight line occur (Figs. 3B:1, 3C:1), whereas flight and 'controlled' leg movements resulted in a combination of the high-peak flight and leg movement recordings (cf. Figs. 2B and 3B:3). Short movements of wings plus 'controlled' leg movements resulted in a series of sharp, relatively high peaks (Fig. 3C:3). The head movement's actograms in Fig. 3C:2 show the same characteristics as in Fig 2A.

The results show that movements of the various body parts of a fly can be separately detected by the actometer. We have used this technique to investigate whether behavioural reactions (head movements) to semiochemicals occur and we have compared these results with those of EAG recordings (Chapter 3).

Head movements on stimulation with semiochemicals

Figure 4 shows that head movements occur on application of pulses of (Z)-9-tricosene. Responses to the control (silicon oil) and pure air were small and varied from 0 to 2 per 10 pulses. In Fig. 5 the average numbers of head movements of Pesse males and females of different age to 10 stimulus pulses are shown. For both males and females of all ages tested, the responses to the low doses of (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals did not significantly differ from each other and also not from the silicon oil control (not shown) (paired t-test) (Fig. 5A). At the higher doses, however, the responses to (Z)-9-tricosene and to the mixture of (Z)-9-tricosene and (Z)-9-heneicosene were

significantly higher than the responses to (Z)-9-heneicosene alone; the latter did not differ significantly from the control (paired t-test, $p<0.01$)(Fig. 5B). No significant differences existed between the responses to (Z)-9-tricosene and the mixture. In addition, the responses of males and females of the same age did not differ (t-test). It is obvious that the responses decreased when the flies grew older than about 6 days. In 12 days and older flies the responses to (Z)-9-tricosene and the mixture were no longer different from the control (paired t-test).

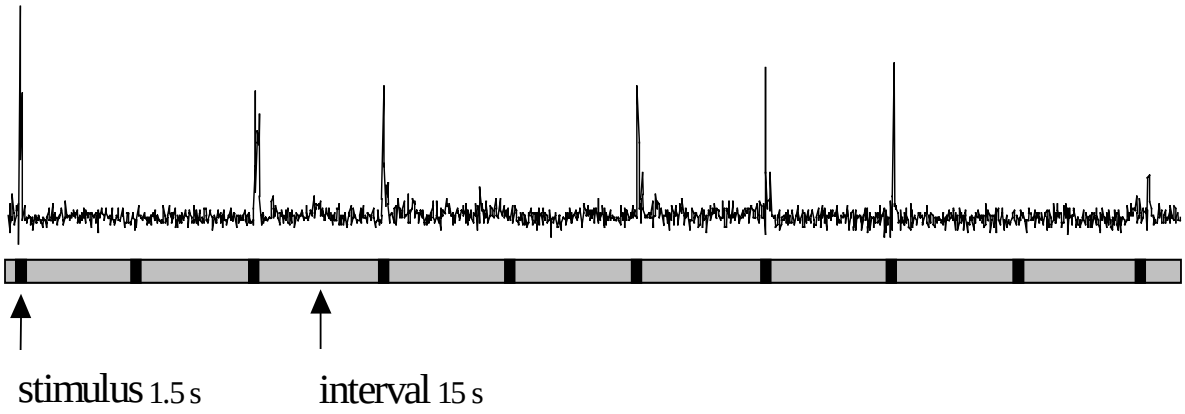

Figure 4. Head movement recording of a 6-day-old female Pesse fly in response to 10 µg (Z)-9-tricosene.

Figure 6 presents results of similar experiments with WHO flies. It can be seen that the responses of WHO flies tended to be somewhat higher, although not significantly (t-test), than those of the Pesse flies of the same age. Again the responses to the lower doses did not differ from those to the control, as was also the case with the higher doses of all three substances for 1-day- and 9-day-old flies. As in the Pesse flies (Z)-9-heneicosene never evoked significant responses in WHO flies. For flies of all ages the responses to (Z)-9-heneicosene did not differ from the control. The responses of flies 2-5 days of age were equally high.

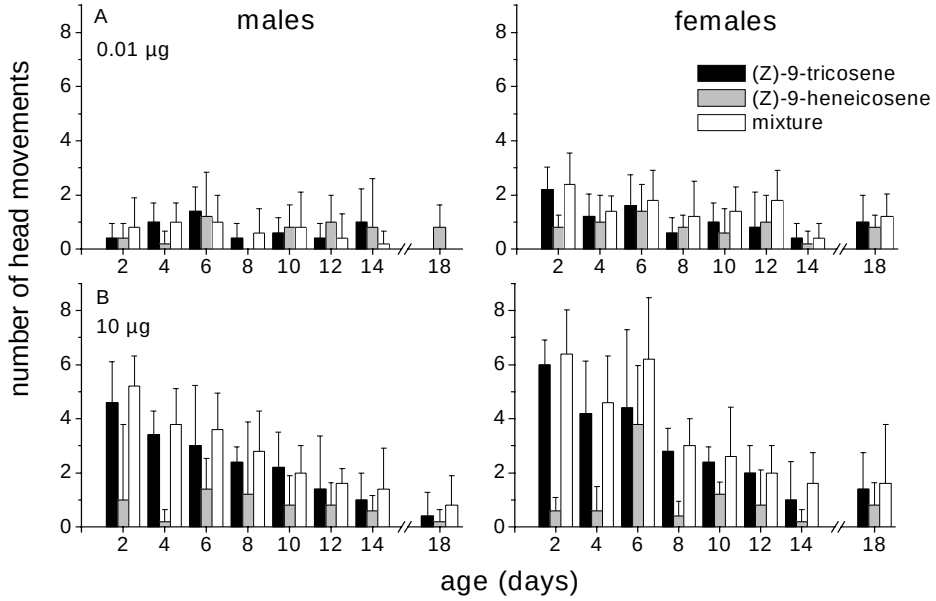

Figure 5. Average numbers of head movements of 2- to 18-day-old Pesse males and females to 10 pulses of the vapours of 0.01 µg and 10 µg (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. n=8 for both sexes of all ages tested. Error bars denote standard deviations.

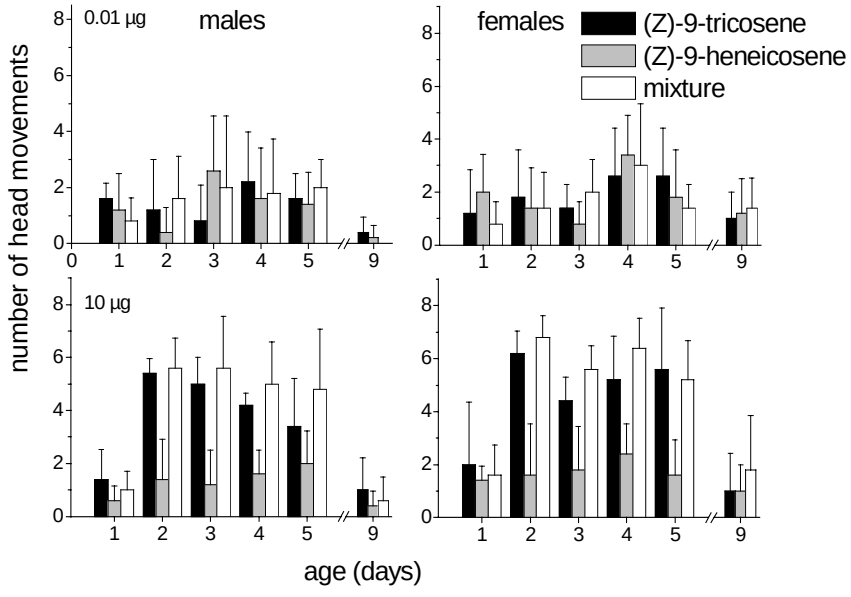

Figure 6. Average numbers of head movements of 1- to 9-day-old WHO males and females to 10 pulses of the vapours of 0.01 µg and 10 µg (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. n=6 for both sexes of all ages tested. Error bars denote standard deviations.

Discussion

Although EAG's indicate which chemicals an insect can smell no conclusions about the insect's behaviour towards those chemicals can be drawn from these recordings. In the present study we used the movements of the insect's head in order to obtain an indication of the behavioural responses of *M. domestica* to certain semiochemicals by means of an actometer equipped with a radar-Doppler detector. This technique has several advantages: it works at low light intensity or in complete darkness, the radar beam can pass through glass and plastic walls of containers without interference, it has a low sensitivity to noise, it is cheap and easy to handle and, moreover, it provides very reliable recordings of the timing and sequence of responses (Renou *et al.*, 1999).

In the present study we tested the same chemicals ((Z)-9-tricosene and (Z)-9-heneicosene) as we used before in EAG recordings (Chapter 3) in order to be able to compare between the two techniques. Comparison of the EAG recordings and the actograms shows that, although (Z)-9-heneicosene evoked high EAG responses, no behavioural responses to this substance occurred. (Z)-9-tricosene, however, evoked high responses in both the EAG and actometer studies, as did the 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene. As to the males, these results agree with the common opinion that (Z)-9-tricosene attracts and sexually excites male houseflies (see e.g. Carlson *et al.*, 1971, 1974). It also may explain the results of Mansingh *et al.* (1972) who found that the mixture of (Z)-9-tricosene and (Z)-9-heneicosene induced high excitement and mating responses in male flies, whereas only low responses were found to (Z)-9-heneicosene alone. Carlson *et al.* (1974), in olfactometer tests, also found that (Z)-9-heneicosene was not very attractive to males. These authors, however, could not confirm the findings of Mansingh *et al.* (1972) that this substance, when mixed with (Z)-9-tricosene enhanced sexual activity in males. Finally, La-France *et al.* (1989) showed that mixtures of 5 :g of (Z)-9-tricosene and 5 :g of other (Z)-9-alkenes when applied to washed dead females induced the same striking activity in males as compared to females that were treated with 10 :g of (Z)-9-tricosene alone.

The sensitivity of female houseflies to (Z)-9-tricosene may explain that in the field, traps loaded with this substance not only may attract males but also about equal numbers of females (Carlson and Beroza, 1973; Mitchell *et al.*, 1975; Chapman *et al.*, 1998). We suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating

behaviour in males. So far, the effects of (Z)-9-heneicosene and of the mixture of (Z)-9-tricosene and (Z)-9-heneicosene on the behaviour of females is unknown.

The finding that the head responses to (Z)-9-tricosene and a mixture of (Z)-9-tricosene and (Z)-9-heneicosene declined with age, may be contributed to a general decline in olfactory sensivity with age as has also been found in several other insect species (Den Otter *et al.*, 1991; Roelofs and Comeau, 1971). However, Kelling (2000) found that the response of single olfactory cells of 1-day-old male and female *M. domestica* did not differ significantly from those of flies up to 28 days of age. A reason for the age-related decrease in head responses may be due to an increasing number of inoperative number of cells as has also been found for labellar taste hairs in the fly *Phormia regina* (Rees, 1970; Stoffolano, 1973). The fact that the number of head responses to (Z)-9-tricosene and a mixture of (Z)-9-tricosene and (Z)-9-heneicosene of 1-day-old WHO males and females did not differ from the control may be due to the fact that 1-day-old flies are still sexually immature.

We have demonstrated that movements of different body parts of a fly can be distinguished by actometry, even when they occur simultaneously. The new head movement measuring technique is a relatively easy way to investigate behavioural reactions towards odours. More detailed studies are necessary, however, in order to relate these responses to the behaviour of free moving insects and such studies may lead to discrimination of actograph patterns evoked by repellent and attractive chemicals.

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