Olfactory Sensitivity in Tsetse Flies: a Daily Rhythm

Wynand M. Van der Goes van Naters, Cornelis J. Den Otter and Frans W. Maes

Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA Haren (Gn), The Netherlands

Correspondence to be sent to: W.M. van der Goes van Naters, Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

Abstract

The diurnal tsetse Glossina morsitans morsitans bites especially in early morning and late afternoon; around midday feeding is at a low. In laboratory apparatus that measures the amount of locomotion under constant conditions over the photophase, the flies display a similar patterning of activity levels. The profile of daily rhythms for G. morsitans reported in the literature includes a number of motor and sensory motor systems that fluctuate cophasically. Lacking is a study on the patterning of the senses' response levels. In this paper we present the first instance of a daily modulation in the sense of smell. We stimulated the antennae with concentration series of host-derived odours and measured the spiking rate of cells at different times during the photophase. The concentration-response curves suggest that the sensitivity of antennal olfactory cells flows in parallel with the other daily rhythms. This was also reflected in electroantennograms (EAGs). The electroantennography was extended to G. fuscipes fuscipes, whose level of spontaneous locomotor activity—instead of following a U-shaped pattern—rises gradually over the photophase. Again, the EAGs appeared to parallel the species' locomotor activity. What we believe happens is that the organism tones down the sensitivity of its odour receptors during periods of anticipated inactivity for reasons of economy.

Introduction

Sense organs are inconstant. Besides the changes due to adaptation which take place within seconds to minutes after onset of a stimulus, there may be slower, endogenously induced shifts in sensitivity. The time course of such shifts, for taste and olfaction in insects, varies from several hours to many days. In the locust Locusta migratoria, for example, the apical pore of gustatory sensilla on the maxillary palps is occluded upon feeding, thereby impeding access to taste stimuli, and reopens within 2 h thereafter (Bernays et al., 1972); in the stable fly Stomoxys calcitrans olfactory sensitivity as measured through electroantennograms (EAGs) increases twofold in a 3 day period of starvation (Warnes and Finlayson, 1986); and the blowflies Phormia regina and Protophormia terraenovae gradually lose taste sensitivity (for salt and water) in nearly half of their labellar sensilla over a period of 3 weeks of aging (Rees, 1970; Stoffolano et al., 1978).

In some cases a close correlation exists between sensory and behavioural change. Female Aedes aegypti mosquitoes, for example, start to exhibit host-seeking behaviour as their lactic acid odour receptors become functional 30–100 h after emergence (Davis, 1984a), and the sensitivity of the lactic acid receptors in Culex pipiens is depressed during diapause (Bowen et al., 1988). Thus far, changes in the sensitivity of insect chemoreceptors have been shown to accompany ageing, diapause, starvation, feeding (including dietary change) and reproduction (for review see Blaney et al., 1986; see also Warnes and Finlayson, 1986; Abisgold and Simpson, 1988; Bowen et al., 1988; Den Otter et al., 1991; Simmonds et al., 1992). Though some of the changes in an animal's physiology and behaviour are predictably patterned over the day, literature on diel rhythms in chemoreceptors is uniformly negative. Electroantennograms of the noctuid moth Trichoplusia ni (Payne et al., 1970) and the male spruce budworm Choristoneura fumiferana (Worster and Seabrook, 1989) to their sex pheromones are not affected by time of day; the responses to sucrose of taste cells on the legs of Phormia regina and Culex pipiens mosquitoes does not vary over the day (Bowen, 1992).

The circadian organization of behaviour in the tsetse fly Glossina morsitans has been particularly well studied (Brady, 1988). Tsetse flies are diurnal, haematophagous insects native to Africa; they feed off mammals (including man), birds and reptiles. Those of the species G. morsitans bite especially in early morning and late afternoon (Brady and Crump, 1978); around midday, feeding is at a low. Though ambient temperature can modulate the biting pattern (Brady and Crump, 1978), records of the spontaneous locomotor activity (Brady, 1972) suggest that its shape may be
generated endogenously. The flight responsiveness to odours and to visual stimuli, landing behaviour, defecating and 'singing' parallel the pattern of biting activity over the photophase (Brady, 1988). These behaviours involve sensorimotor systems. The activity of the sense organs alone cannot be gauged from behaviour but must be measured electrophysiologically.

In this paper we examine whether the odour reception of *G. m. morsitans* is patterned over the photophase as is its behavioural repertoire. The olfactory response of *G. m. morsitans* is compared with that of *G. f. fuscipes*, a species for which a different daily activity pattern has been reported (Harley, 1965; Mwangelwa et al., 1990).

**Materials and methods**

**Insects**

Pupae of *G. m. morsitans* and *G. f. fuscipes* were obtained from the Département d’Elevage et de Médecine Vétérinaire (CIRAD-EMVT) at Montpellier, France. The pupae were kept individually in glass vials until the emergence of the flies, whereupon the flies were grouped in cages by sex and age. Pupae and flies were kept at 25°C and 80% relative humidity in a 12:12 h light:dark cycle with lights-on at 6:00 a.m. Flies were fed off rabbits' ears on alternate days beginning the day after emergence.

**Registration of locomotor activity**

The locomotor activity of the flies was recorded by placing them individually into actograph units fitted with a milliwave-Doppler system (Syntech, Hilversum, The Netherlands). The sensitivity of the units was adjusted to trigger pulses when the flies walked or flew but to remain silent when they groomed or were occupied otherwise. Pulses were fed on-line into a computer and counted over 15 min bins. The computer's clock and the timer switch for the light regime were not connected so that dawn and dusk fell within a bin rather than at bin boundaries. The flies were placed in the units on day 5 and were not fed thereafter.

**Electrophysiology: single cell recording and EAG**

After the third blood meal on day 5, flies were deprived of food for 3 days before testing. Preliminary actograph registrations showed that after 3 days of starvation the pattern of locomotor activity is most pronounced and none of the flies will yet have died. The flies were mounted with their heads protruding from a hollow plastic cone and placed in a current of humidified, charcoal-filtered air of the airstream.

Stimuli were 1.5 ml of the vapour content of a disposable syringe injected within 0.1 s through the tube's aperture using a spring-powered device (type MS-02, Syntech). Each syringe held a 1 cm² piece of filter paper charged with one of three known tsetse attractants: 1-octen-3-ol (syringes with 0.5 mg and with decade steps down to 5 × 10⁻⁷ mg), 3-methylphenol (with 0.5 and 0.005 mg) or acetone (with 20 and 2 mg). Control cartridges were also prepared. See Den Otter and Van der Goes van Naters (1992) for further details on the preparation of stimuli and their controls.

Spikes from antennal receptors were picked up using the surface-contact technique (Den Otter and Van der Goes van Naters, 1992) whereby the recording electrode, an Ag–AgCl wire sheathed by a Beadle–Ehrussi ringer-filled pipette, is brought with its tip (<3 μm diameter, >5 MΩ impedance at 1000 Hz) into contact with the cuticular surface near the base of a sensillum. The indifferent electrode (tip diameter 50 μm) of similar make was inserted in the head just proximate to the antennae. Spike trains were stored on tape and analysed by computer with software developed by Vervoort (1993). A response was defined as the maximum number of spikes in a sliding 0.1 s interval of the registration.

EAGs were recorded from the distal end of the funiculus relative to an electrode implanted near the base of the antennae (for details see e.g. Den Otter et al., 1988). The signal was digitized, stored on diskette and analysed with the Syntech PC-EAG program. EAG amplitude was defined as the maximum negative deviation of the signal from the baseline potential.

**Olfactory response over time**

We measured olfactory responses to tsetse kairomones at different times of the photophase in three experiments.

(i) To determine how the response level of a random sample of cells is distributed over the day, we recorded spike responses from antennal receptors of *G. m. morsitans* to a single dose each of 1-octen-3-ol (0.005 mg), 3-methylphenol (0.005 mg) and acetone (2 mg). A response was noted if the stimulus elicited at least two spikes more than the control. When cells did not respond to a stimulus, they were tested for sensitivity to the odour at the higher dose (0.5, 5 and 20 mg respectively); the recordings of both doses were excluded from the data if the higher dose failed to excite.

After presentation of the three odours a new fly was taken so that all points in the data set were independent. The data set comprised responses from 141 cells on as many flies (75 females and 66 males).

(ii) To determine whether dose–response curves of olfactory receptors shift over the photophase, we selected a distinct class of cells. The antennae of *G. m. morsitans* harbour cells which are highly sensitive to 1-octen-3-ol. These are collocated with cells remarkably responsive to 3-methylphenol and have a comparatively slow return of spike frequency to pre-stimulus levels after stimulation. Responses to an ascending concentration series of 1-octen-3-ol (syringes charged with 5 × 10⁻⁷ to 5 × 10⁻³ mg in
decades) were measured from 33 cells in 16 females and 17 male flies at different times between dawn and dusk.

(iii) To determine whether EAGs reflect the patterns found in (i) and (ii), and to compare G. m. morsitans with a second species, EAGs were taken from both G. m. morsitans (18 females and 17 males) and G. f. fuscipes (25 females and 24 males) on stimulation with an ascending concentration series of 1-octen-3-ol (syringes charged with $5 \times 10^{-7}$ to $5 \times 10^{-1}$ mg in decades).

Results

Locomotor activity

Registrations of locomotor activity for the two tsetse species 3 days after the third blood meal are given in Figure 1. As the duration of food deprivation increases, the activity intensifies though the gross daily pattern remains unchanged (not shown). Patterns of activity for the two species differ: G. m. morsitans is active especially in the early morning and late afternoon, with a midday trough in between, while G. f. fuscipes shows a slight but steady rise in activity throughout the photophase. Both species show a salient jump at dusk (shaded in Figure 1).

Electrophysiology

(i) Of the 141 antennal olfactory cells, 54 responded to 1-octen-3-ol only, 50 to 3-methylphenol only, 14 to acetone only, 13 to both 1-octen-3-ol and 3-methylphenol, 9 to both 1-octen-3-ol and acetone, and 1 to all three. Grouping responses by odour and analysing against time of day shows that the response levels are lowest around midday (graph not given), though the spread of data is considerable. Parabolas (U-shaped), fit by a least-squares algorithm, describe the data significantly better than do linear regressions. 1-octen-3-ol: $F(1,76) = 18.7$, $P < 0.0001$; 3-methylphenol: $F(1,65) = 23.1$, $P < 0.0001$; acetone: $F(1,23) = 7.8$, $P = 0.0102$; but successively higher order polynomials do not give significantly closer fits ($P > 0.05$). The parabolas' vertices (minima) are centred within half an hour of noon. We defined three periods in the photophase (cf. Figure 1a) to determine how the dose–response curves of olfactory receptors change over the day. Periods 1 and 3 cover the activity peaks while period 2 holds the trough.

(ii) Dose–response curves for the distinct class of 1-octen-3-ol sensitive cells, averaged by period, are given in Figure 2a. They rise slowly at first with increasing stimulus intensity, then climb more steeply before levelling off to a maximum. Due to range fractionation, however, the steepness of the dose–response incline for averaged data may be biased: a Hill coefficient of a sigmoid fitted to averaged dose–response data is generally lower than the average Hill coefficient of sigmoids fitted to the dose–response data of cells individually. By fitting sigmoids to the dose–response data of cells individually, and then averaging the four sigmoidal parameters in each period, we therefore obtain more representative curves (Figure 2b). The function fitted was $R = R_{\text{min}} + (R_{\text{max}} - R_{\text{min}})(1 + K/C)^{-h}$, where $C$ = dose of the stimulus, $R_{\text{min}}$ = asymptote when $C \to 0$, i.e. the spontaneous firing rate, $R_{\text{max}}$ = asymptote when $C \to \infty$, i.e. the maximum firing rate, $K$ = the concentration at the inflection point and $H$ = the Hill coefficient which is proportional to the slope at $C = K$ in the logC plot. The spontaneous activity of the cells, their maximum response and the Hill coefficient appear to stay the same (ANOVA, $P > 0.5$); rather, it is the $K$ which is modulated over the day (Table 1). LogK-values differ significantly for periods 3 and 2 [ANOVA with Bonferroni post-tests, $P = 0.0128$ (see Sokal and Rohlf, 1997)] though not for periods 1 and 2, nor for periods 1 and 3 ($P > 0.1$). As the Hill coefficients are approximately equal, the $K$-values reflect threshold values and the differing $K$-values translate to changing thresholds of up to 1 log unit for the receptors over the photophase.

(iii) As in Figure 2, the EAG dose–response curves for G. m. morsitans are constructed by grouping fly responses into the three periods defined in Figure 1a and averaging (Figure 3a). To facilitate comparison with G. m. morsitans, the data is similarly formatted for G. f. fuscipes (Figure 3b),

![Figure 1](http://chemse.oxfordjournals.org/) (a) Locomotor activity 8 days after emergence and 3 days after the last blood meal for (a) G. m. morsitans and (b) G. f. fuscipes. The data are the average of 10 and seven individuals respectively. The maximum activity of each individual was set to 100 before averaging. For analysing the electrophysiological results, three periods were defined in the photophase (6:00–18:00) to cover the peaks and trough of activity in (a): 1 (6:00–10:00), 2 (10:00–14:00) and 3 (14:00–18:00). The error bar shows size and location of largest SEM. The shaded area is the activity in the bin which contains lights-off.

![Figure 3b](http://chemse.oxfordjournals.org/) (b) As in Figure 2, the EAG dose–response curves for G. m. morsitans are constructed by grouping fly responses into the three periods defined in Figure 1a and averaging (Figure 3a). To facilitate comparison with G. m. morsitans, the data is similarly formatted for G. f. fuscipes (Figure 3b),
Table 1  Averages (± SEM) of parameters of sigmoidal functions fitted to the dose–response curves in three periods (cf. Figure 1a)

<table>
<thead>
<tr>
<th>Period</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{min}}$</td>
<td>46 ± 3</td>
<td>48 ± 6</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>275 ± 11</td>
<td>264 ± 10</td>
</tr>
<tr>
<td>log$K$</td>
<td>-3.76 ± 0.27</td>
<td>-3.38 ± 0.26</td>
</tr>
<tr>
<td>$H$</td>
<td>1.02 ± 0.13</td>
<td>1.18 ± 0.12</td>
</tr>
</tbody>
</table>

Discussion

Change in an animal’s physiology and behaviour is in some cases paralleled by its sense organs. For *G. m. morsitans*, the sensitivity of olfactory receptors appears to fall and rise over the photophase in synchrony with much of its behavioural repertoire. A comparison of Figures 1b and 3b suggests that the olfactory responses and the spontaneous locomotor activity are correlated for *G. f. fuscipes* also. The spontaneous locomotor activity of *G. m. morsitans* as measured in our actographs (Figure 1a) is similar to that reported by Brady (1972) for mature, food-deprived flies. The final jump in activity for the bin which contains lights-off, shaded in Figure 1, is reminiscent of a phenomenon that has been noted in several Glossina species [the ‘sunset’ activity in *G. m. morsitans*, *G. m. submorsitans*, *G. swynnertoni*, *G. pallidipes*, *G. palpalis* and *G. tachinoides* (for references see Brady, 1987)]. According to Brady, the peak is exogenously induced at sundown and appears to be functionally unrelated to the morning and afternoon peaks of locomotion, though it extends smoothly from the latter in *G. m. morsitans*. A surprising feature of the spontaneous locomotor activity in *G. m. morsitans* is the near-total loss of the morning peak from the bimodal pattern in constant darkness (Brady, 1988, figure 4); only a vestige remains. The ‘afternoon’ peak of mature flies persists in constant darkness, which suggests its coupling to the circadian
oscillator is the more rigid of the two. This could explain why, though the morning peak of spontaneous locomotor activity in Figure 1a is at least as large as the afternoon one, it is in the afternoon that the sensitivity of the olfactory receptors is highest (lowest K and threshold).

The afternoon sensitivity is nearly a factor of 10 higher than around midday. This is a large change in a short time when compared to the lactic acid sensitivity of host-seeking mosquitoes, for example, which drops by only a factor of 2–5 over 48 h after a blood meal (Davis, 1984b). As Blaney et al. (1986) note in their review on chemoreceptor sensitivity variations, most of the literature until then described even slower processes, which led them to suggest that 'periods of 1–2 days are needed to change chemo-sensory responses'.

The prime examples of fast endogenous change in sense organs come from studies of vision. In Limulus at least 13 properties of the retina wax and wane in an endogenous circadian rhythm to prepare the visual system for the 8-log-unit transition of light intensity between day and night (Barlow et al., 1989). Retinal sensitivity, as measured from electroretinogram amplitudes, fluctuates daily in many other arthropods as well, including scorpions (Fleissner and Fleissner, 1985: 4 log units), spiders (Yamashita and Tateda, 1981: 1 log unit), beetles (Koehler and Fleissner, 1978: 3 log units; Fleissner, 1982), and cockroaches (Wills et al., 1985: 0.6 log units). In Manduca sexta the diel rhythm of the adults develops prior to eclosion from an even faster cycling one in which the visual sensitivity goes up and down once every 6 h (Bennett, 1983). The common transduction mechanisms underlying vision and olfaction, which are only now becoming apparent from studies of mutants in Drosophila (e.g. Carlson, 1991; Riesgo-Escovar et al., 1994), suggest that sensitivity in chemoreception is as amenable to regulation as sensitivity in vision (Weckström and Laughlin, 1995).

It is in accessory structures that we may find a mechanism for the chemosensory changes observed in Glossina. Reversible binding of stimulus molecules to acceptor sites (Hollenberg, 1978), enzymatic reactions (Dixons and Webb, 1964) and the 'self-shunting membrane' model (Lipetz, 1971) are bases for theories of chemoreceptive transduction (Maes, 1985). In their simplest forms, these theories all predict a sigmoidal relationship between dose and response. The relationship has been characterized here by four parameters (Table 1). The $R_{\text{min}}$ (spontaneous firing rate) is a function of resting membrane potential of the cell and the cell's spike generator. The steepness of the dose–response incline, as reflected in the Hill coefficient $H$, is thought to be influenced by the multiformity of acceptor sites and their interaction. Maximum response $R_{\text{max}}$ is due to saturation of the acceptor population, though other stages in the transduction process may be rate-limiting as well (Hollenberg, 1978). The spontaneous firing frequency of the cells studied here shows no change (Figure 2b), nor does the Hill coefficient or the maximum response. This suggests the intrinsic neuronal properties are invariant and perireceptor events (Carr et al., 1990) change sensitivity. A plausible regulation site would be the transport facility of odour molecules across the sensillum lymph. Odorant-binding proteins (general OBP) are now thought to act as the ferry, taking the often hydrophobic ligands through the hydrophilic lymph to the receptor dendrites (Pelosi and Maida, 1995). The thresholds are probably under endocrine influence as efferent neural control of arthropod chemo-receptors is yet to be demonstrated. Endocrine influence has been demonstrated in the closing of apical pores of Locusta after feeding (Bernays and Chapman, 1972), in the blowfly (Angioy et al., 1983), in the spruce budworm Choristoneura fumiferana, where a juvenile hormone analogue reduces EAG responses to the female sex pheromone (Palaniswamy et al., 1979), and in mosquitoes, where a haemolymph-borne factor modulates the lactic acid and oviposition site odour receptors in synchrony with a 3–5 day behavioural cycle of blood-feeding and egg-laying (Davis and Takahashi, 1980; Davis, 1986). The consequence of a shift in threshold is that—in an odour-laden environment—the cells will fire fewer spikes around midday than during early morning or late afternoon.

In one view of the function of sensitivity changes, this modification of output from the cells is the path by which behaviour is modulated. The change in sensitivity of peripheral chemoreceptors is seen as the action of a neuro-endocrine feedback loop which controls the expression of behaviour (more or less explicitly suggested in Omand, 1971; Omand and Zabara, 1981; Davis, 1986; Bowen et al., 1988; Den Otter et al., 1991; Simmonds et al., 1991). This hypothesis could have been tested for mosquitoes by determining (i) the lactic acid cells' firing rate which is required for host-seeking to occur during the periods of high sensitivity; (ii) the stimulus intensity needed to effect this same spike rate when the cells are in their insensitive mode; and (iii) whether this stimulus intensity evokes host-seeking behaviour during the lactic acid-insensitive mode. Much behaviour is, however, regulated without a corresponding change in sensory properties. Pertinent to this paper are e.g. Hall (1980), who reports a lack of correlation between taste responses to sucrose and the circadian rhythm of proboscis extension in the blowfly, and Bowen (1992), who studied the lactic acid sensitivity of mosquito olfactory receptors over the day with the same result (see Introduction).

A different view on the functional significance of chemoreceptor inconstancy is one of economy. It may be cheaper in terms of energy and cell material to have less responsive cells (as in this study), or even inactive cells, during periods of low foraging activity. Many of the examples cited in the literature to bolster the view that the sensitivity changes serve to modulate behaviour may also be interpreted in this light. To what extent expenses are cut by
fluctuating the olfactory thresholds is difficult to estimate, especially for anabolic processes. That it pays to delay the build-up of sensory machinery until it is needed is plausible. Mosquitoes, for example, will delay development of their lactic acid receptors until they are ready to take blood (data from Davis, 1984a). Once the senser organs are up and working, anabolism is still a cost because they need to be maintained. The reduced lactic acid sensitivities of mosquitoes after a blood meal and during diapause (data from Bowen et al., 1988) may reflect a temporary slacking of maintenance, though no data exist to support this. Besides resources, energy may be conserved. The energy required to generate spikes is at least the cost of building the electrochemical gradients they annihilate. A third of an animal’s energy budget is taken by the Na-K ATPase that pumps sodium and potassium across the membranes of all body cells (Alberts et al., 1983). For neurons ~70% of the cells’ energy budget is used by the pump. As Na+ leakage across a non-excited membrane is low, a large part may go to compensate sodium influx due to spike generation. The low proportion of odour-inhibited cells, which generally have a high spontaneous rate of spike discharge, relative to odour-excited cells in the antennae of Glossina is striking (<2% for both G. m. morsitans and G. f.fuscipes (Van der Goes van Naters, 1997)). Interestingly, in the mosquitoes’ lactic acid system (where the proportion of odour-inhibited neurons is larger) the fluctuation of sensitivity is limited to the lactic acid-excited neurons and does not extend to the lactic acid-inhibited ones (Davis, 1984b); this is a strategy that appears to minimize spike discharge in an odour-laden environment. Thus energy may be saved, and possibly resources too, by heightening thresholds of odour-excited neurons when the senses’ information is less needed. Sensory inconstancy may therefore not only be a route to control the expression of behaviour, as suggested by other authors, but may also be part of a more general programme of toning physiological celerity according to a prediction of need. Fluctuations in chemoreceptors may contribute especially to an organism’s zenith (i) when the cycles of the behaviour they mediate are of relatively long duration, allowing more of the sensory transduction machinery of a cell to be involved, and (ii) when a large number of neurons participate.

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