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Free-running Bimodal (Circadian Rhythm) in the Tsetse Fly (*Glossina longipennis*)

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In nature, the tsetse fly, *Glossina longipennis* Corti, restricts its activity to ~1 h around sunset and a lesser peak at dawn. This markedly crepuscular rhythm was investigated in actographs under constant conditions in the laboratory. In LD 12:12 with 30-min dawns and dusks, the evening activity started as the lights dimmed, peaked in the first hour of darkness, and then declined rapidly to near zero, to peak again for ~1 h at dawn. Virtually no activity occurred through the central 8 light hours of the photophase. In constant darkness, this pattern of a major 'dusk' and minor 'dawn' peak was repeated in the males for at least three cycles, with a free-running period of ~23 h. This is the first demonstration of a fully endogenous bi-modal rhythm in tsetse flies.

Glossina longipennis Tsetse fly Circadian rhythm Bimodal rhythm Endogenous control Activity

INTRODUCTION

Several species of tsetse fly are bimodally active in nature, with sub-equal morning and evening peaks in their availability to sampling systems such as bait traps, fly-round catches and traps (see Crump and Brady, 1979; Gouteux and Monteny, 1986; Brady, 1988a). Only a few have had the endogenous control of this rhythm examined under constant laboratory conditions. These are *Glossina m. morsitans* West., studied extensively by Brady (1970 *et seq.*, see 1988b), *G. fuscipes* Newst., and *G. p. palpalis* (R.-D.) by Crump and Brady (1979), and *G. pallidipes* Aust. by van Etten (1982).

Whereas the bimodal pattern typical of the field is usually still evident under 24-h light cycles in the laboratory, only one of the two peaks apparently ever survives in fully constant conditions. No-one has yet managed to demonstrate that both arms of the U-shaped activity rhythm persist in constant darkness or constant light, the crucial test of full endogenous control by an internal circadian 'clock' (Brady, 1988b). Indeed, in *G. morsitans*, although it is the evening peak that survives in DD in mature flies, it is the morning peak that survives in young flies (Brady, 1988a), which confuses the situation still further.

So far, it has been only flies from the epidemiologically more significant 'morsitans' and 'palpalis' subgroups of the genus that have been studied. However, none of the supposedly less important 'fusca' group

tend to have even more markedly bimodal activity in nature than do 'morsitans' or 'palpalis' flies. *G. brevipalpis* Newst., for example, performs most of its activity around dusk and dawn (Harley, 1965), and *G. longipennis* Corti is even more extremely crepuscular, typically restricting 90% of its activity to less than an hour after sunset and a similarly brief peak at dawn (Power, 1964; Kyorku, 1989).

Quite apart from the interest of studying a 'fusca' fly in the laboratory for the first time, therefore, it seemed that the apparently tight constraint of *G. longipennis* activity into two completely discrete peaks might be a suitable model in which to examine the bimodality of typical tsetse fly activity rhythms—to see whether both dawn and dusk peaks are under endogenous, circadian control. We report here on this study.

MATERIALS AND METHODS

Insects and conditions

The *G. longipennis* used were kindly supplied weekly as pupae by Dr S. K. Moloo of the International Laboratory for Research in Animal Diseases, Nairobi [the strain originated from the area studied by Kyorku (1989)]. The pupae were kept, adults maintained, and all experiments conducted in a controlled environment room, at $26.5 \pm 0.5^\circ\text{C}$, 70 ± 5 r.h., and a 12 h light:12 h dark cycle (with 30-min dawns and dusks at ~08.00 and ~20.00 h local time), unless otherwise specified. Flies received at least one blood meal from a rabbit before testing. The sexes were kept separate from emergence, so the females tested were unmated.

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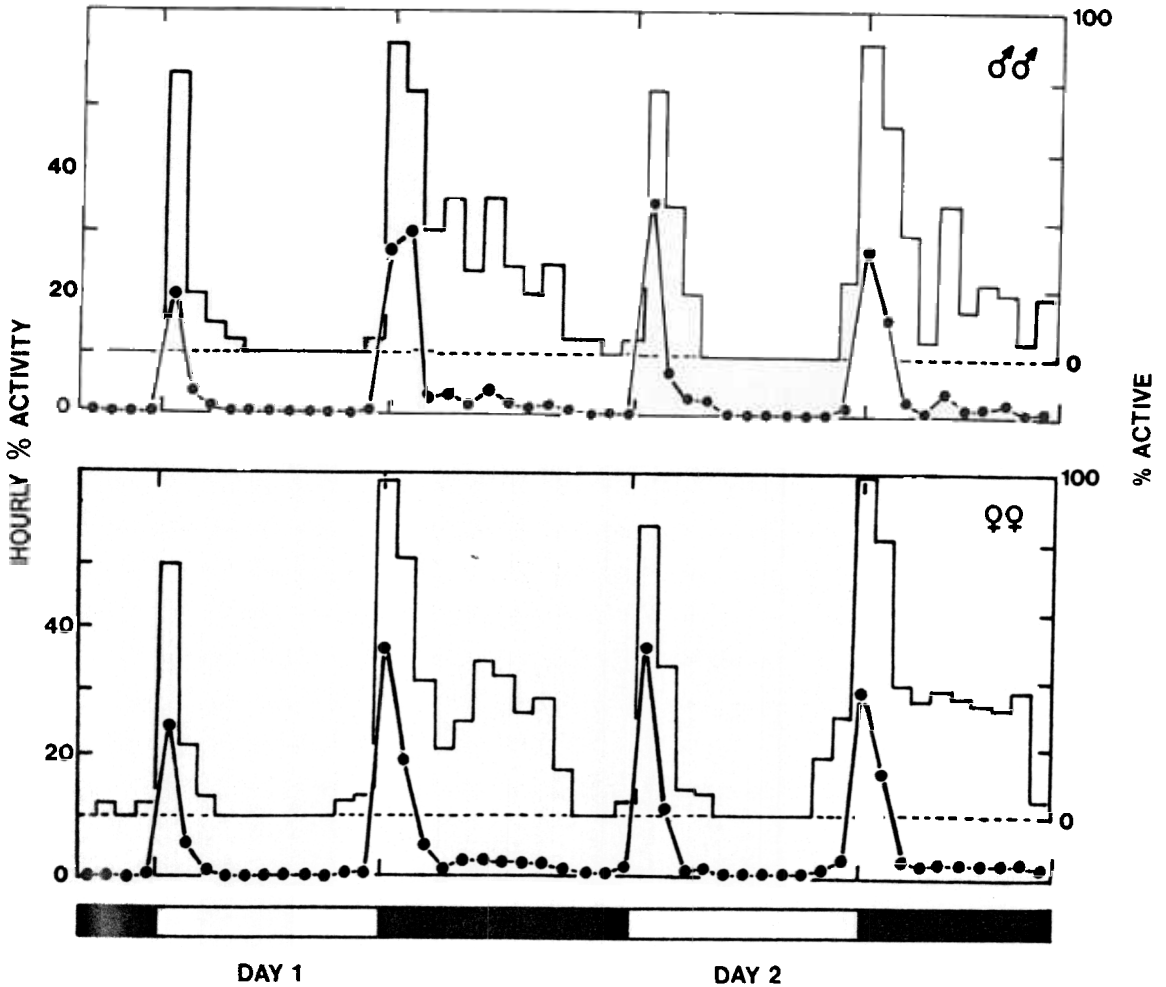


FIGURE 1. Diel activity rhythm in LD 12:12 of male (upper) and female (lower) *G. longipennis*; first two full days in actographs since last fed (= Day 0); based on three replicates of 8 flies for each sex. Dotted curves (and left-hand ordinates) show the mean number of minutes' activity/h expressed as the per cent of each fly's total activity/24 h (see Table 1); histograms (and right-hand ordinates) show the mean per cent of flies active each hour. Abscissa shows the light cycle used in both the culture room and the actographs.

Actographs

Locomotor activity (almost exclusively flight) was monitored in individual 18-cm-long, rocking-cage actographs (Brady and Gibson, 1983). Each cage contained one fly and was isolated within its own wooden compartment in a bank of 8 compartments. As a fly flew from end to end of its cage, the cage rocked and activated an i.r. light switch, and thence a pen on an event recorder. Charcoal-filtered air from the general room circulation was pumped separately through each compartment. The compartments were illuminated from above by a bank of 60-W tungsten strip lamps diffused through two sheets of opal Perspex.

Under the full light of the LD 12:12 cycle, the intensity at the actographs was between 400 and 650 mW/m², depending on actograph position. When dim light was required, the tungsten strips were removed, and the fluorescent lights of the CE room allowed to shine

through the Perspex diffusers, to provide 30–50 mW/m² at the actographs.

Analysis

As in other tsetse species, flight activity tended to occur in short bursts (see Brady, 1970, 1988b; Crump and Brady, 1979), so each fly's record consisted of discrete clumps of pen marks. The traces were therefore analysed as the number of minutes per hour which contained any activity (Brady and Gibson, 1983). These data were then averaged by expressing each fly's number of minutes of activity per hour as a percentage of its total number of minutes of activity per 24 h, a procedure that normalized the wide range of activity levels between different individuals (Brady, 1972).

To avoid any effects of disturbance from handling, all analysed records start at midnight (00.00 h) on day 1;

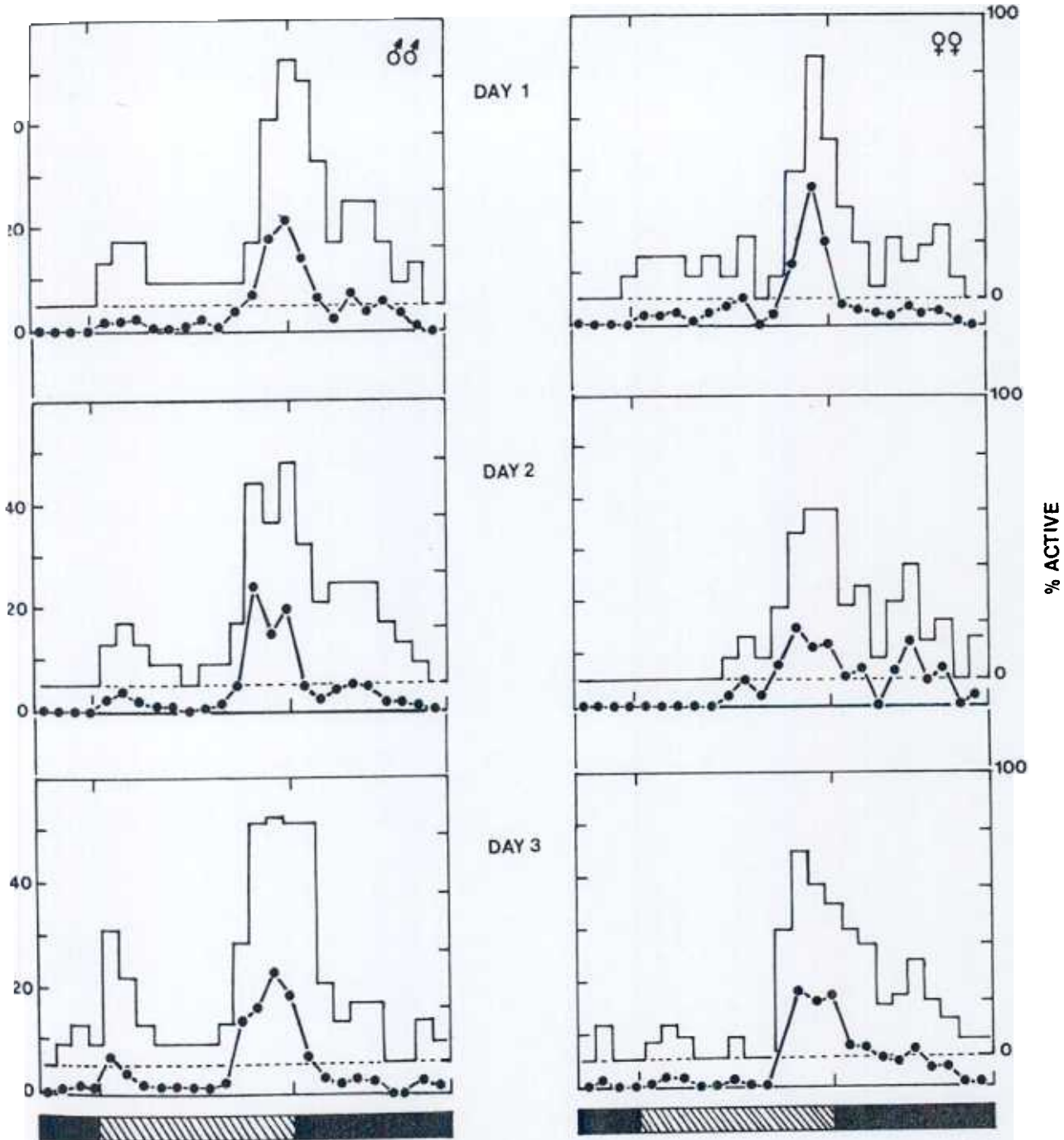


FIGURE 2. Circadian activity rhythm of male (left) and female (right) *G. longipennis*; first three full days in DD (day fed and last LD day = Day 0); based on two replicates of 8 flies for each sex. Abscissa bars show timing of previous LD cycle; other details as in Fig. 1.

flies were inserted into their actographs soon after feeding on the afternoon of day 0.

Experiments

The experimental measurements were done:

- (i) in the standard 12 h light:12 h darkness cycle (LD) with 30-min dusks and dawns [see Brady (1987) for details];
- (ii) in constant darkness (DD) with all the lights switched off so that the flies were effectively in

- total darkness (leaked light was at $< 1 \text{ mW/m}^2$);
- (iii) in constant light (LL) at two light levels (in the full LD intensity and in the dim light described above);
- (iv) with the LD light cycle advanced 6 h to examine entrainment of the rhythm.

RESULTS

The mean activity levels in the different experiments are shown in Table 1. Flies began to die of starvation by

TABLE 1. Mean (\pm SE) minutes of spontaneous flight activity per day by *G. longipennis* under different light régimes

Light cycle	Males			Females		
	Day	Day 2	Day 3	Day 1	Day 2	Day 3
	26 \pm 4	51 \pm 11	—	32 \pm 4	56 \pm 6	—
	13 \pm 3	15 \pm 3	26 \pm 5	7 \pm 1	9 \pm 2	13 \pm 5
	6 \pm 1	10 \pm 1	27 \pm 8	—	—	—

n: LD males = 22, females = 34, DD and LL = 13–15 each.

the end of day 3 in the light cycle, but they survived longer in DD or LL, because of their lower activity levels. Accordingly, data for only two days in the light cycle but three in DD and LL are shown. In all light régimes, these *G. longipennis* (of both sexes) were about twice as active as *G. morsitans* (cf Crump and Brady, 1979; Brady and Gibson, 1983), and they generally died within three days rather than the four or five typical of *G. morsitans*.

Activity in LD 12:12

The rhythm in the light cycle is shown in Fig. 1. The daily pattern of activity of males and females in the actographs was closely similar, but rather more of the females than of the males were active in any 1 h, typically about 40% of them in the quiet phase of the night, compared with some 25% in the males. Neither sex showed any significant activity during the central 8 h of the light phase.

Both generally started their activity during the last half of the 30-min dusk [i.e. at below \sim 25% of full light intensity—see Brady (1987)]. They then showed a marked peak of activity during the first 2 h of full darkness, with most activity in the first hour, followed by a steep decline to near zero activity for the remaining 8 or 9 dark hours. This 'inactivity' in the night was different from that in the light phase, however, because, except just before dawn, there were usually several flies active every hour, each performing a few minutes' activity.

During the first third of the 30-min dawn (i.e. at less than \sim 10% full light) the flies began to be active again, and for the first hour of full light they were as active as in the first hour of darkness. However, unlike the progression into the dark phase, activity after this first hour fell sharply towards zero, by the third hour of the light phase. Such a dawn peak also occurs in nature (Power, 1964; Kyorku, 1989), but is normally much smaller than the dusk peak, presumably because of the low temperatures at dawn in the field. Overall, about 50% of all the activity occurred during the first 2-h of full darkness, and 35% during the first two light hours.

Activity in DD

In constant darkness, the most obvious element in both sexes was the persistence of the dusk activity peak (Fig. 2), although this broadened out considerably from its sharp 2 h in LD. Moreover, whereas virtually all flies

were active in the LD dusk peak, only about 80% were in the DD 'dusk' peak.

This persistence of the activity rhythm in constant conditions implies control by a circadian clock, although a free-running drift is not immediately obvious in these 'form estimate', averaged curves. However, as the males' activity rose most steeply at hours 17, 16 and 15 on the successive DD days, compared with hour 18 in LD, a free-running period for the rhythm of about 23 h was implicit. This was confirmed by analysis of the individual records for the time of the males' first rise in activity, which showed the mean interval between the 'dusk' peaks in DD to be 23 h 6 min (SE \pm 7 min), virtually the same as reported for *G. morsitans* in DD [23 h 0 min \pm 12 min; Brady (1988a)].

This 'dusk' peak is closely reminiscent of the evening peak in DD in *G. morsitans* (Brady, 1988a, b). However, quite unlike the situation in *G. morsitans*, or in *G. palpalis* or *G. austeni* (Crump and Brady, 1979), the *G. longipennis* males (though perhaps not the females) also showed a small, clearly persistent 'dawn' peak in DD. Moreover, this 'dawn' peak apparently free ran at about 23 h, like the 'dusk' peak.

Activity in LL

In constant light at the full intensity of 400–650 mW/m², virtually no activity occurred, but when the light was reduced to 30–50 mW/m², the males performed a low level of activity for at least three days (Table 1). This activity was more or less continuous, however, generally involving 10–30% of the flies each hour, and showed no detectable sign of being rhythmic (other than in first starting at about 'dusk' on day 1).

Entrainment

When the light cycle was advanced so that dusk and dawn occurred 6 h earlier on day 2 than on day 1, the dusk activity peak did not advance by $>$ 1 h within the first new zeitgeber cycle. This transient phase shift further implies the stability of the rhythm, and hence the endogenous basis of the oscillator(s) underlying it. This stability in the face of an earlier zeitgeber setting seems to be greater than that of *G. morsitans*, which advanced about 3 h within the first new cycle under similar circumstances (Brady and Crump, 1978). More rigorous measurement of entrainment in tsetse flies is impractical because of the sporadic nature of their activity (see Brady, 1988a, b).

DISCUSSION

G. longipennis has been reported, along with one or two other 'fusca' group tsetse flies, as sometimes being active at night (Power, 1964; Kyorku, 1989), behaviour that is unusual for what is otherwise a markedly diurnal genus. Figure 1 shows that it readily flies in total darkness, and that it is in fact strongly inhibited from activity during the light phase of a light:dark cycle, even at the unnaturally low intensity of around 500 mW/m².

is inactivity during the day is presumably a combined response to light *per se* and to the fly's circadian time-keeping.

Since the main activity peak of *G. longipennis* starts at dusk, it occurs just as other tsetse species indulge their 'sunset' activity of flying out to the ends of the wings and leaves for the night (Brady, 1987). It is tempting to speculate as to whether the activity pattern of *G. longipennis* has therefore evolved as an extended version of this brief, but highly characteristic bout of sunset activity. What advantages accrue to *G. longipennis* flying in very low light intensities, and at a time when the air is scarcely moving so that odour plume clues to wind direction are at their worst (Brady *et al.*, 1989), is not clear.

That *G. longipennis* has a free-running circadian rhythm of activity is scarcely surprising, most animals do. What is interesting is that the males in Fig. 2 showed free-running 'dusk' and 'dawn' activity peaks in constant darkness. It is thus the first tsetse species in which both arms of the typically bimodal rhythm have been revealed to be under true circadian control (*cf* Brady, 1988a). And this is true for *G. longipennis*, it is probably true also for the other species, but in their case the lesser peak disappears within the 'noise' of the low level of their free-running activity in constant darkness.

G. longipennis is a rare example of an insect in which full circadian control of a bimodal rhythm has been demonstrated (see Brady, 1988a). Many insects show such bimodal activity rhythms in normal light cycles, but what usually happens is that one or other of the peaks disappears in the absence of either the lights-on or the lights-off signal when the rhythm is tested in constant conditions.

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