The flicker fusion frequencies of six laboratory insects, and the response of the compound eye to mains fluorescent 'ripple'

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ABSTRACT. The ERG response of the compound eye to single, brief, light pulses, to sustained stimulation for 2 s, and the dark adapted flicker-fusion frequency (FFF) under stroboscopic light was measured in six species: *Locusta migratoria* (FFF range: 40—90 Hz), *Periplaneta americana* (25—60 Hz), *Saturnia pavonia* (65—85 Hz), *Antheraea pernyi* (25—70 Hz), *Glossina morsitans* (85—205 Hz) and *Drosophila hydei* (60—100 Hz). The first four species have typical 'slow-eyed', monophasic ERG responses; the two flies typical 'fast-eyed', biphasic responses. The FFF proved to be dependent on the state of light adaptation, being 40—70% higher than the above figures after only 2 min exposure to as little as 300 lx. Adult male *Glossina*, but not *Locusta* nymphs, showed a clear 100 Hz ERG ripple in response to single-phase, mains fluorescent lighting. To three-phase fluorescent lighting no 300 Hz ERG ripple was detected, but the 100 Hz component was still evident.

Introduction

Because of the nature of both fluorescent tubes and incandescent tungsten-wire lamps, these lights emit a flicker at a frequency of twice that of the alternating current (AC) power source driving them. Although the light may not actually be extinguished between peaks, a substantial sinusoidal ripple in the intensity is produced. This flicker is therefore normally present in rooms used for rearing insects or for observing their behaviour, and may affect that behaviour.

Renner (1957) described a reduction in the gathering behaviour of the honeybee (*Apis mellifera*) in a bee flight room illuminated by fluorescent tubes. Van Praagh (1972) described a bee flight room that can be illuminated with either a 100 or 300 Hz ripple, and has shown (Van Praagh, 1975) that honeybees reared entirely under 300 Hz

conditions display higher wing-stroke frequencies, slower flying speeds and reduced obstacle-avoidance behaviour, when tested under a 100 Hz light-ripple.

Van Praagh (1975) also demonstrated that the honeybee has a flicker-fusion frequency (FFF) of above 100 Hz but below 300 Hz. The FFF is the maximum frequency of flickering light that the eye can resolve as discontinuous, and can be determined by a study of either the optomotor responses or the electroretinogram (ERG) responses of the insect (Fig. 1).

Autrum (Autrum, 1950; Autrum & Stoecker, 1950; Autrum & Gallwitz, 1951) cites FFFs ranging from 180 Hz to over 300 Hz for the honeybee, dragonfly and blow-fly, but of below 60 Hz for the cricket and stick-insect. He designated these as fast and slow eyes, respectively, and both he and Ruck (1958a, b) have described various parameters of the ERG typical of each type.

In Britain, Europe and America the mains electrical supply has either a 50 or 60 Hz AC

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cycle, producing a light ripple of 100 or 120 Hz. These are both considerably lower than the FFFs typical of the fast eyes, and will be present in all experimental situations illuminated by standard, mains-driven lights.

With these problems in mind, this paper describes an ERG-based study of the FFF of several common laboratory insects.

Materials and Methods

Insects

The work was undertaken in two stages: first, a preliminary study involving adults of *Locusta migratoria migratoroides* (R & F), *Periplaneta americana* (L), *Glossina morsitans morsitans* (Westwood), *Drosophila hydei* Sturtevant, *Saturnia pavonia* L. and *Antheraea pernyi* Guérin Ménéville; and second: a more detailed examination of fifth instar *Locusta migratoria* nymphs and adult *Glossina morsitans*. The locusts were obtained from the Centre for Overseas Pest Research, London; the tsetse flies were raised at the Imperial College Field Station from pupae reared by the Tsetse Research Laboratory, Langford, Bristol. Although some changes in the detail of the techniques and equipment were made between the two stages, the methods were basically similar.

Stimulation

Each insect was anaesthetized for *c.* 2 min with CO_2, and then fixed securely by the head and legs to a small metal platform, with black dissecting wax. For recording, this platform was held within a light-proof Faraday cage. Once the electrodes were in place the insect was left in total darkness until stimulation started. The flickering light stimulus was provided by a Flash–Tac transistorized stroboscope (Electronic Applications Ltd) for frequencies up to 250 Hz. For FFF measurements of over 250 Hz a Dawes Strobo-torch 1202D was used. The light was focused on to a 5 mm fibre optic (Barr & Stroud) and further focused within the Faraday cage to produce a well-defined spot of *c.* 2 mm diameter on the insect's compound eye. Accurate measurements of the intensity of this spot were not possible, as the photometers available were not designed to record flickering light. However, using a Lunasix Mark III, values of between 5 and 22 lx were recorded over the range of frequencies used.

Recording

The recording electrode was inserted tangentially in the dorsal hemisphere of the eye, with the tip lying just beneath the cornea. The indifferent electrode was positioned ipsilaterally in the rear of the head or in the thorax. The electrodes were drawn from glass capillary tubing to a tip diameter of *c.* 20 μm. The recording electrode was filled with 2 M potassium citrate, while the indifferent electrode was filled with insect Ringer's solution. The signal was recorded on a Tektronix 564 B twin-beam storage oscilloscope, after impedance matching with a voltage follower (W.P. Instruments, type V.F.1). A trace of the stimulus was provided on the second oscilloscope beam from a photodiode placed near the stroboscope. Permanent records were made on film but in most trials the trace was temporarily stored on the oscilloscope screen. This was particularly important when determining FFFs, as it was often desirable to overlay a number of successive responses, so that any ripple-response present could be identified in the background noise.

Experiments

Each experiment consisted of three sections. First the FFF was determined by studying the ERG while increasing the stimulus frequency in steps of 5 or 10 Hz, and recording the highest frequency at which a ripple was seen (Fig. 1). The duration of stimulation was governed by the levels of background noise, but was generally 2–10 s for any one stroboscope frequency. The ERG waveform was also studied, in response to: (a) a single, brief pulse (40 μs) of light, and (b) a 2-s period of effectively sustained stimulation (see Fig. 3), provided by the stroboscope run at its maximum frequency (250 Hz).

While most references to FFFs in the literature concern the dark-adapted values, it is the light-adapted values that are of practical importance in behavioural experiments, as light tends to raise the FFF above
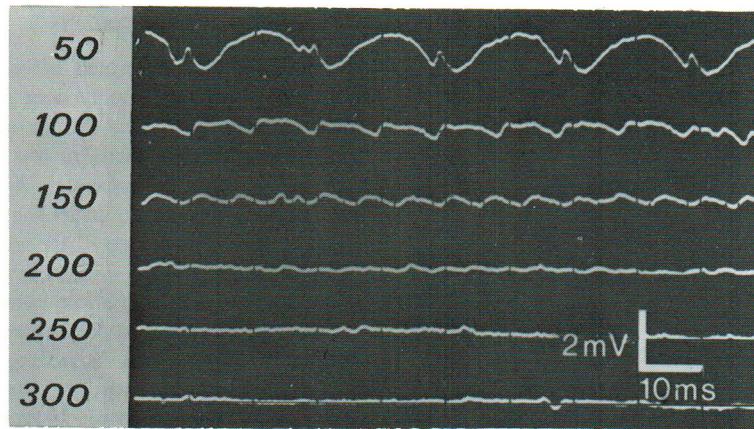
FIG. 1. Reduction and extinction of ripple-responses in male *Glossina* with increasing stimulus frequency. (The flicker-fusion frequency of this individual was 240 Hz). Stimulus frequencies are given on the left.

the dark-adapted levels (Bullock & Horridge, 1965). For this reason the FFF and ERG waveform were studied both after 30 min dark-adaptation and during a subsequent 20 min of light-adaptation. During this latter period the insect's head was illuminated by a ripple-free DC light (from a battery-powered torch), with the beam focused on to a 7-mm light-guide (Barr & Stroud). This beam had an intensity of *c.* 300 lx at the insect, though, because its light was continuous, this measurement is not directly comparable with that of the strobed stimulus light (above). The FFF was measured at intervals during the light-adaptation period by simultaneously stimulating the insect via the flickering (stroboscope) and DC (torch) light-guides. By this method it was possible to determine the increase in FFF as light-adaptation occurred.

Finally the equipment was transferred to an experimental room that provided both 100 and 300 Hz fluorescent lighting, and the ERGs of a number of insects were measured under these conditions, to determine if a ripple-response occurred to mains flicker.

Because of the limited number of trials per species, most results are quoted as ranges with the mean and sample size given only where appropriate.

Results and Discussion

ERG waveform

The responses of the six species were determined, after 90 min dark-adaptation, to a single brief pulse of light. The responses of *Locusta*, *Periplaneta* and *Saturnia* were of the 'slow' type. They were all similar, with little variation in form or speed. All displayed a sharply falling cornea-negative wave, slowly returning to the resting potential, often after a short plateau on the return slope. The response had a latent period of 10–18 ms, and a duration of 250–320 ms (Fig. 2a). The response of *Antheraea* was of a similar form and duration, but no plateau was seen and an initial, brief, cornea-positive component of low amplitude occurred occasionally. All showed a sustained, ripple-free, cornea-negative potential in response to sustained stimulation (Fig. 3a). When stimulation ceased the potential did not return immediately to the resting level, but dropped sharply towards it initially and then gradually travelled the remaining distance.

The responses of *Glossina* and *Drosophila* were of the 'fast' type and were similar to each other, with a fast diphasic response, comprised of a 4–6 ms latent period followed by a positive and a negative component. The potential then slowly returned to the resting level, within 200–300 ms. Although the diphasic components were usually of equal magnitudes, some responses were more complex, with more than one cornea-positive component (Fig. 2b), while others had reduced or absent positive components. The flies' responses to sustained stimulation were also similar (Fig. 3b), with a positive on-response, a sustained negative component (containing
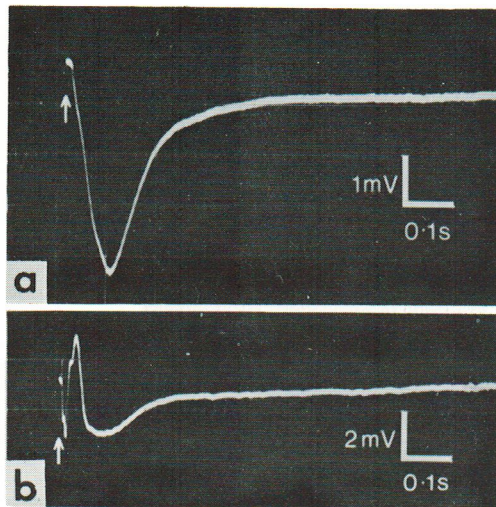
FIG. 2. ERG responses of (a) *Locusta* and (b) *Glossina* to brief (40 µs) light pulses (indicated by white arrows).
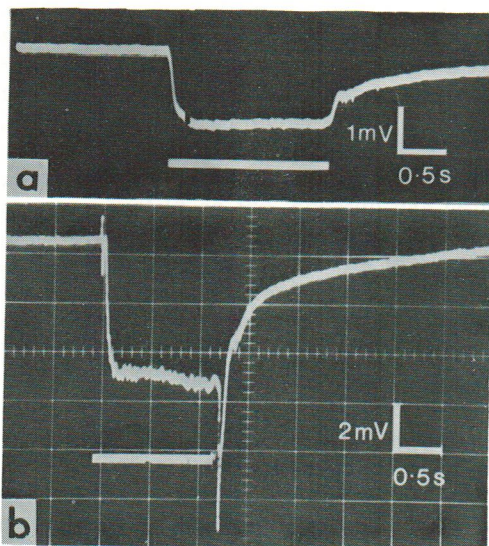

FIG. 3. ERG responses of (a) *Locusta* and (b) *Glossina* to short (2 s) periods of sustained stimulation (indicated by white bars; see text).

the ripple-response in some individuals, but not visible in Fig. 3b) and a fast negative off-response.

These responses are all similar to those found by others (Autrum, 1950; Ruck, 1958a, b, 1961; Yinon, 1970), and tend to fall within Autrum's classification, so that the locust, cockroach and moths could be described as slow-eyed, and the flies as fast-eyed (Table 1).

FFFs of dark-adapted insects

The FFFs (determined after 90 min dark adaptation, and to the nearest 5 Hz) showed large overlaps between the six species (Table 2), although the nocturnal or less active species of each order (*Periplaneta*, *Anthereae* and *Drosophila*) tended to have the lower fusion frequencies. This also fits with Autrum's (1950) classification.

In general the FFFs are comparable with those already reported for the same or related species (see Bullock & Horridge, 1965; Ruck, 1958a). However, those of the Diptera are lower than have previously been reported (e.g. Ruck, 1961). One reason for this may be the relatively low intensity of the stimulating light, since in contrast to the 5–22 lx used here, Ruck (1961) used up to 12,000 lx, which would not only provide a much stronger stimulus, but would also tend to raise the FFF through light-adaptation (see below).

FFF changes during light adaptation

Working only with the fifth instar *Locusta* and adult *Glossina*, it was found that exposure of the dark-adapted insects to even short periods of light resulted in an increase of the FFF. This increase was studied by determining the FFF of individuals as rapidly as possible (to minimize the change caused by the stimulus itself) after 30 min dark-adaptation, and then at frequent intervals during 20 min light exposure, at approximately 300 lx.

Locusta migratoria. The dark-adapted FFFs agree closely with those determined above, with a mean of 49.5 Hz (range 40–80 Hz, $n = 10$). After light adaptation this had increased to a mean of 85.0 Hz (range 55–100 Hz, $n = 10$). The rate of FFF-increase was initially high, and the process nearly complete within 2 min (Fig. 4).

Glossina morsitans. Because the FFFs of the males were over 250 Hz, the higher range stroboscope had to be used (Methods). The light this provided appeared to be comparable in both colour and intensity to that of the original machine, both having Xenon gas discharge tubes as their light source, but no precise measurements could be made.

It was suspected that the lower FFFs found in the unsexed tsetse flies in Table 2 were those of the females, as later trials with

TABLE 1. ERG response characteristics of the adults of six insect species to single light pulses, after a total of 90 min dark adaptation

Species	n	Mono/ biphasic	Latency (ms)		Duration (ms)		Amplitude (mV)	
			Range	Mean	Range	Mean	Range	Mean
Locusta	17	Mono	6–18	10.5	200–450	277.7	6.8–1.2	3.8
Periplaneta	14	Mono	11–25	17.5	180–600	290.0	4.7–0.7	2.4
Saturnia	3	Mono	11–18	14.3	170–320	245.0	7.4–0.2	4.2
Antheraea	4	Mono/bi	13–25	18.0	210–420	295.0	8.4–0.2	4.0
Drosophila	11	Biphasic	3–8	4.8	90–250	173.3	1.5–0.2	0.6
Glossina	14	Biphasic	4–10	6.7	150–350	268.2	3.0–0.9	1.9

n = no. of individuals tested (sex and age unknown). Amplitude refers to major, cornea-negative component only.

TABLE 2. Flicker-fusion frequencies of the adults of six insect species after a total of 90 min dark adaptation

Species	n	Flicker-fusion frequency	
		Range	Mean
Locusta	16	40–90	66.3
Periplaneta	14	25–60	41.8
Saturnua	3	65–85	76.7
Antheraea	4	25–70	42.5
Drosophila	8	60–100	81.9
Glossina	14	85–205	116.4

n = no. of individuals tested (sex and age unknown).

sexed flies showed a marked sex difference. Only five individuals of each sex were tested, but they showed only a slight overlap of their FFF ranges at each determination, and the mean frequencies were clearly separated (Fig. 4). The males had dark-adapted FFFs ranging from 183 to 233 Hz (mean 212.5 Hz, $n = 5$); the females from 150 to 190 Hz (mean 176.0 Hz, $n = 5$). Both ranges are considerably higher than those determined above (Table 2), but agree more closely with FFFs found in other Diptera (Autrum, 1950; Ruck, 1961). The reasons for this increase are unknown, but are not connected with the change in stimulus-
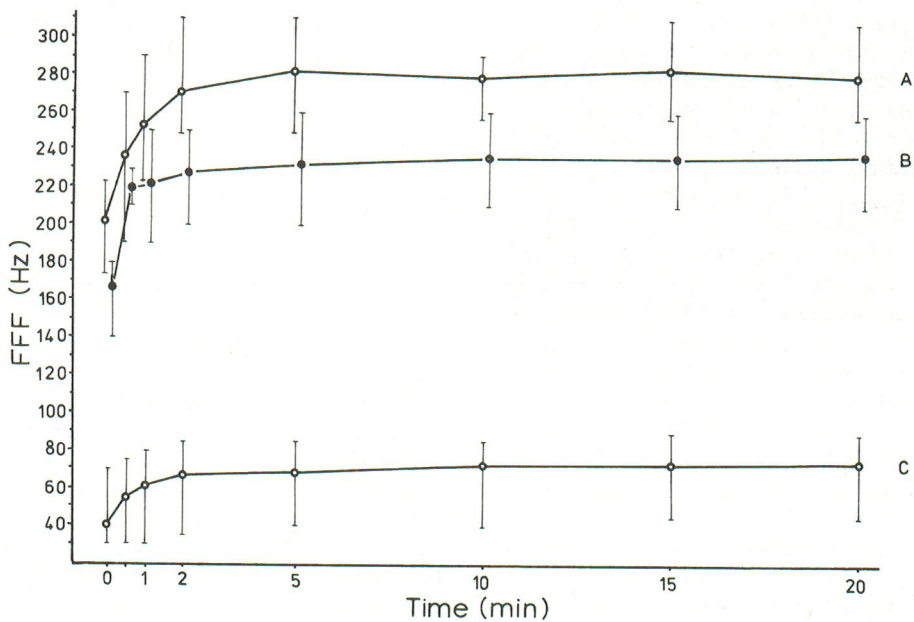

FIG. 4. Increase in flicker-fusion frequency during exposure to light of 300 lx; FFF against time after light-on. Circles indicate mean values; bars, ranges. (A) *Glossina* (male, $n = 5$); (B) *Glossina* (female, $n = 5$); (C) *Locusta* nymphs (sex unknown, $n = 10$).

source, as this was itself necessitated by the high FFFs recorded with the original light-source.

The FFFs of both sexes increased rapidly when the insects were light-exposed, reaching 285–308 Hz (mean 290.0 Hz, $n = 5$) in the males, and 220–270 Hz (mean 248.0 Hz, $n = 5$) in the females. As in the locust the process was almost complete within 2 min (Fig. 4). The sex difference in FFF cannot be attributed to changing experimental conditions, as the two sexes were used in overlapping sessions of trials.

FFF increases under varying light intensities

The effect of light intensity on the FFF was tested in one locust nymph, and in one male and one female tsetse fly. Each was dark-adapted for 30 min and its FFF determined as usual; further FFF determinations were then made during a 20-min light-exposure, as before, to light of a known intensity. This entire process (dark-adaptation and subsequent light-exposure) was repeated five times using different light intensities, with stimulus and adapting lights being reduced together by neutral density filters.

The results (Fig. 5) show that light intensity had two effects on the change in FFF during light-adaptation. First, as the intensity was increased, the overall change within the 20-min adaptation period also increased. In the locust this rise was 15 Hz under c. 0.6 lx, and 50 Hz under 300 lx, representing increases of 33% and 111%, respectively. In the male tsetse the rise was 30 Hz (14%) under 8 lx and 60 Hz (26%) under 300 lx. In the female it was 30 Hz (17%) and 80 Hz (42%), under the same intensities. Second, as the intensity increased, the speed of the adaptation process was also increased, and it seems probable that at 70 lx and below, adaptation was not complete within the 20 min allowed. It is likely that these phenomena are partly, but not wholly, due to the reduced intensity of the stimulating light, reducing the ripple-response magnitude of the ERG.

ERG responses under 100 Hz and 300 Hz fluorescent lights

A total of six male *Glossina*, with light-adapted FFFs ranging from 270 to 300 Hz,
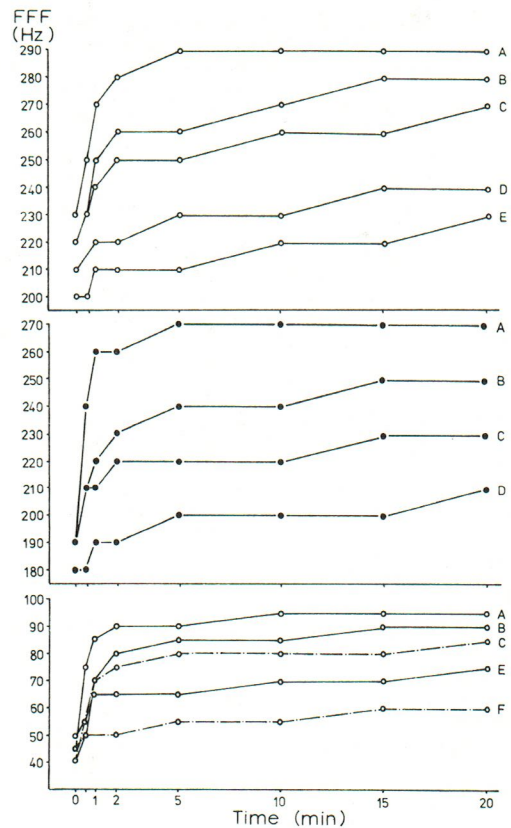

FIG. 5. Increase in flicker fusion frequency during exposure to light of varying intensities; FFF against time after light-on. Top: *Glossina* (male, $n = 1$); middle: *Glossina* (female, $n = 1$), bottom: *Locusta* nymphs (sex unknown, $n = 1$). Light intensities: (A) 300 lx; (B) 70 lx; (C) 25 lx; (D) 7.5 lx; (E) 3 lx; (F) 0.6 lx.

were tested without dark-adaptation, under both 100 and 300 Hz conditions. Because the former was achieved by cutting out two-phases of a three-phase lighting system, the light intensity at the insect's eye was reduced from 180 lx (at 300 Hz) to 70 lx (at 100 Hz).

The single-phase lighting had an intensity ripple of ±56% of the mean intensity. All six flies gave strong 100 Hz ERG ripple-responses to this (Fig. 6a). Because the fluorescent tubes used on the three phases were not perfectly matched, in colour or intensity, a low amplitude 100 Hz component of ±6% of the mean intensity over-laid the 300 Hz ripple emitted (Fig. 6b). The ERGs also showed this 100 Hz component but apparently did not respond to
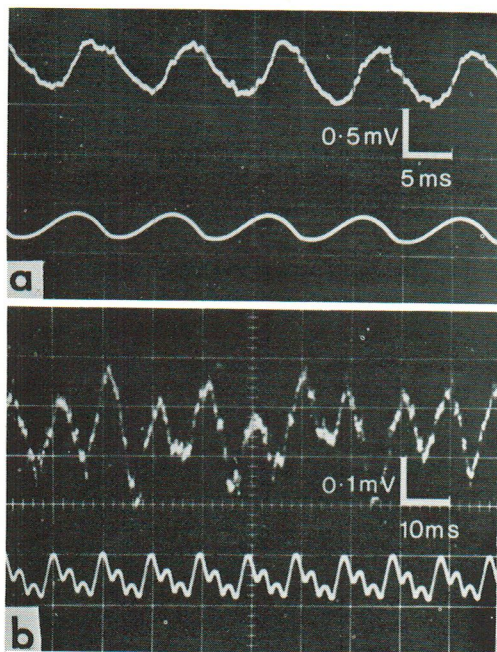
FIG. 6. Ripple responses of male *Glossina* to fluorescent lighting at (a) 100 Hz and (b) 300 Hz. Top trace: ERG response; bottom trace: photocell response.

the 300 Hz component (Fig. 6b). Two individuals with high light-adapted FFFs (290 and 300 Hz) were left under the 300 Hz lighting for over 1 h, but still no 300 Hz components were detected in their response. The possibility cannot be excluded, however, that there was a small 300 Hz component to these responses hidden in the noise. This was high owing to the difficulty of shielding the apparatus in this room.

Two locust nymphs (with light-adapted FFFs of 85 and 90 Hz) were similarly tested, but neither showed any ripple-responses to the 100 Hz lighting. The 90 Hz individual was left for over 1 h under the fluorescent lighting, but still no ripple was detectable.

Conclusions

From these findings it seems probable that those insects classified by Antrum (1950) as 'fast-eyed', e.g. honeybees, dragonflies, house-flies and most diurnal, fast-flying insects, can resolve mains-driven lighting as flickering. From the work of Renner (1957)

and Van Praagh (1975), it is also likely that this ability is reflected in some behavioural changes, either when studied under 100 Hz lighting or as a result of being reared under such lighting systems. Although the 'slow-eyed' insects, such as locusts, stick-insects, cockroaches and moths are generally thought to have FFFs of 40–60 Hz (Bullock & Horridge, 1965), these values are considerably raised by light-adaptation. It therefore seems likely that at least some individuals can resolve the flicker of mains-driven lights, if allowed even 2 min exposure to relatively dim conditions (300 lx). It also seems apparent that if three-phase lighting is adopted to avoid these problems, care must be taken to ensure that each phase is of equal intensity and colour, since tsetse flies, at least, can resolve even a 6% 100 Hz ripple.

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