

AROUSAL SHIFTS IN QUIESCENT LOCUSTS

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Summary

Locusts are usually quiescent at night, but this state can be interrupted by spontaneous periods of motor activity, or arousals, that can also be induced by exposure to light stimuli. To investigate whether repeated arousing stimulation has any lasting effect on behaviour, locusts were confronted at night with a series of 1 s light stimuli. Groups of three stimuli at intervals of 60 s were repeated 11 times at 10 min intervals during the first experimental night, and three stimuli at intervals of 90 s were repeated at 15 min intervals during the next night. Arousals and the effects of stimulation were monitored as changes in the spike activity of muscles in the basal part (the scapus) of the right antenna. In the early part of the night preceding the presentation of the light stimuli, neither 60 s nor 90 s periods were present as significant peaks in spontaneous

changes in spike activity. The initial stimulus of a series evoked an arousal response that habituated on repetition of the stimulus. The end of the series of stimuli was followed by changes in spike activity that tended to have the same periodicity as the preceding stimuli. Furthermore, a single light stimulus at the end of the night evoked changes in spike activity that again tended to have the same periodicity as the preceding entraining stimuli. Repeated stimulation may therefore establish a memory trace for the period of stimulation that can be recalled either spontaneously or by the application of an appropriate external stimulus.

Key words: motor neurone, antennal muscle, arousal, memory, locust, *Schistocerca gregaria*.

Introduction

In honeybees, the sensitising effect of stimulus-evoked arousal represents a form of non-associative short-term memory that might facilitate associative learning during sequential food intake in the course of foraging (Hammer and Menzel, 1995). The effects of stimulus-evoked arousal can be enduring, so that it has to be asked whether there are particular behavioural consequences when stimulation is repeated.

Arousal can be defined in several ways. When used for mammals, the term often relates to the transition from sleep to wakefulness (Steriade, 1993). It can also mean 'to mobilise or incite to action a passive, awake organism' (Lindsley, 1987). In locusts, a behaviourally aroused state can be characterised by increased muscle tone, the assumption of a particular posture, antennal or palp movements, increased ventilatory rate, and movements in response to certain stimuli (Rowell, 1971a). The postulated underlying causes of this change of state include the modification of sensory thresholds, the facilitation of efferent pathways, cardio- and ventilatory acceleration, and activation of energy resources (Corbet, 1991). Thus, arousal typically affects many body systems at the same time. In the locust nervous system, the dishabituating effect of arousal can be demonstrated at the single cell level. The response of an identified visual interneuron (the descending contralateral movement detector, DCMD) to

repeated visual stimuli can be dishabituated (aroused) by the occurrence of certain sensory stimuli (Rowell, 1971b). This effect can also be produced by the activity of particular neurons in the brain that show octopamine-like immunoreactivity or by the application of octopamine itself (Bacon *et al.* 1995; Stern *et al.* 1995).

Arousal episodes can be elicited by external stimulation, but transitions between states of quiescence and arousal also occur spontaneously. Such transitions can best be studied at times when quiescence is pronounced, which is at night both in gregarious, non-migrating adult locusts living in the wild (Uvarov, 1977) and in gregarious adults of *Schistocerca gregaria* kept in rearing cages (Odhiambo, 1966).

Spontaneous changes in arousal may be an expression of a number of underlying rhythms that govern the activity of the body. Initiation of locomotion, or movements of the legs, palps and antennae in locusts that are resting or feeding, can be coupled to an underlying rhythm that has a period of 12.0–16.5 min (Simpson, 1981). A rhythm with a period of 2.0–2.5 h (Edney, 1937) and still longer circadian rhythms may also be involved. In the present study, we ask whether arousal-related rhythms can be set by exposure to patterned light stimuli. Arousing light stimuli were therefore presented periodically to locusts that were in a state of low activity at

night. We demonstrate that subsequent motor activity can reflect the temporal pattern of the arousing stimuli.

Materials and methods

Experiments were performed on adult female locusts, *Schistocerca gregaria* (Forskål), taken from our crowded colony where they were fed daily on wheat and kept under a light:dark regime of 12h:12h (light on at 08:00h and off at 20:00h). The temperature was maintained at 37 °C during the light phase and at 25–27 °C during the dark phase.

In the first series of experiments, two locusts were isolated separately for 36h in a cage in the shape of a half-cylinder (radius 6 cm, height 22 cm) and with a transparent flat side. The temperature was 25–26 °C, and food was available throughout the experiment. A red light (Philips T25, 15 W) positioned in front of the transparent side of the cage gave constant light with a total luminance of 9 cd m⁻² (measured with a Minolta luminance meter LS-110), but only 3.5 cd m⁻² between 380 nm and 600 nm (the weighted spectral power distribution measured with a Monolight System, Rees Instruments). Behavioural activity of these unrestrained locusts was recorded with a video camera that gave a video image of the locust 2.5 times its actual body length. The images taken at a rate of 10 frames s⁻¹ were evaluated in two ways. First, in both locusts, visible movements of the body and appendages, except antennal movements (which were measured separately) and breathing movements, were recorded for 10 s every 5 min, allowing the percentage of intervals in which movements occurred to be calculated for each hour. Second, in one locust, continuous video recordings were made over a period of 17 h to determine whether antennal movements were correlated with other movements of the body.

In the second series of experiments, six locusts were fixed rigidly to a holder by their pronotum beside a vertical treadmill, on which they could crawl, for 3–4 days while their behaviour was video-recorded and extracellular electrical activity was recorded from antennal muscles. Each locust was oriented in a head-up position with its legs contacting the treadmill and its longitudinal body axis perpendicular to the ground, representing the preferred orientation when resting on plants or on other elevated sites. The head was waxed to the pronotum, and pieces of card were glued to the holder so that the legs could not touch the head. The electrical activity of muscles in the proximal part (the scapus) of the right antenna was recorded with two 20 µm diameter steel wires. The right scapus was waxed to the frontal part of the head capsule, but the distal parts (pedicellus and flagellum) of this antenna, and all parts of the left antenna, were allowed to move freely. To subtract any potentials generated in the head capsule, an active indifferent electrode consisting of two 50 µm diameter silver wires was placed between the compound eyes above the lateral ocelli. Spikes of several motor neurons were amplified, bandpass-filtered between 60 and 1000 Hz and fed to a computer interface for on-line analysis with Spike2 data capture software (Cambridge Electronic Design). All spikes

that exceeded a selected threshold were recorded on disc, while the original signals were also recorded on the sound track of the video tapes together with pictures of the locust and a record of absolute time. Movements were recorded with two infrared-sensitive video cameras: one, a Panasonic WV-BP500, was mounted vertically so that it faced the frontal part of the head and showed the two antennae, mouthparts and parts of the forelegs; the other, a Hitachi HV-735K, was mounted horizontally so that it faced the right side of the locust and showed the thorax and its appendages, the abdomen, the treadmill and a grating fixed to the treadmill indicating its movements. Video images were stored on tape with two time-lapse recorders (Mitsubishi HS-5424) operating at 10 frames s⁻¹. Electrical signals recorded on the sound track of video tapes were used to link tape and disc recordings. Absolute time, recorded on tape and calculated from disc recordings, provided an additional, though less precise, link.

The locusts were maintained in the same light/dark regime as in their holding cages. An experimental locust was illuminated continuously by 10 diodes emitting infrared light, invisible to the locust (Bennet *et al.* 1967), so that video images could be taken during the night. Daytime light was provided by a green light-emitting diode (LED) that illuminated a translucent, circular screen (diameter 13 mm) 10 mm above the head of the locust. Luminance at its front surface was 10 cd m⁻². The weighted spectral power distribution of the green LED had its maximum at 560 nm, but power was only 1/100 of the maximum at 525 nm and 625 nm. The temperature was 21–23 °C for locusts 1–4, 25–26 °C for locust 5, and 25–27 °C for locust 6. Each locust was fed before but not during an experiment.

The experimental nights were preceded by one adaptation night in locusts 1, 3, 4 and 5, by two nights in locust 6, but none in locust 2. In the early part of two successive experimental nights (between 20:30 h and 21:32 h), the six locusts were stimulated with a single 1 s light pulse, and then after 10–40 min with series of 1 s light pulses. Later in the night (between 00:60 h and 07:55 h), locusts were stimulated again with a single 1 s test light pulse. During the first experimental night, a series of 11 groups of stimuli each consisting of three light pulses at intervals of 60 s was repeated at intervals of 10 min. During the second experimental night, a test pulse was first delivered to check for any lingering effects of the stimuli given the night before. None was detected. A series of light pulses was then repeated, but the interval between the three pulses within a group was increased to 90 s and the interval between each group to 15 min. The light stimulus was a 5 mm diameter green LED situated 5 mm from the right compound eye that gave a luminance of 200 cd m⁻² at its surface and with a weighted spectral power distribution as above.

Results

Behaviour of unrestrained locusts

Locusts taken from the 12h:12h light:dark regime in their rearing cage continued to show a diurnal rhythm in their motor

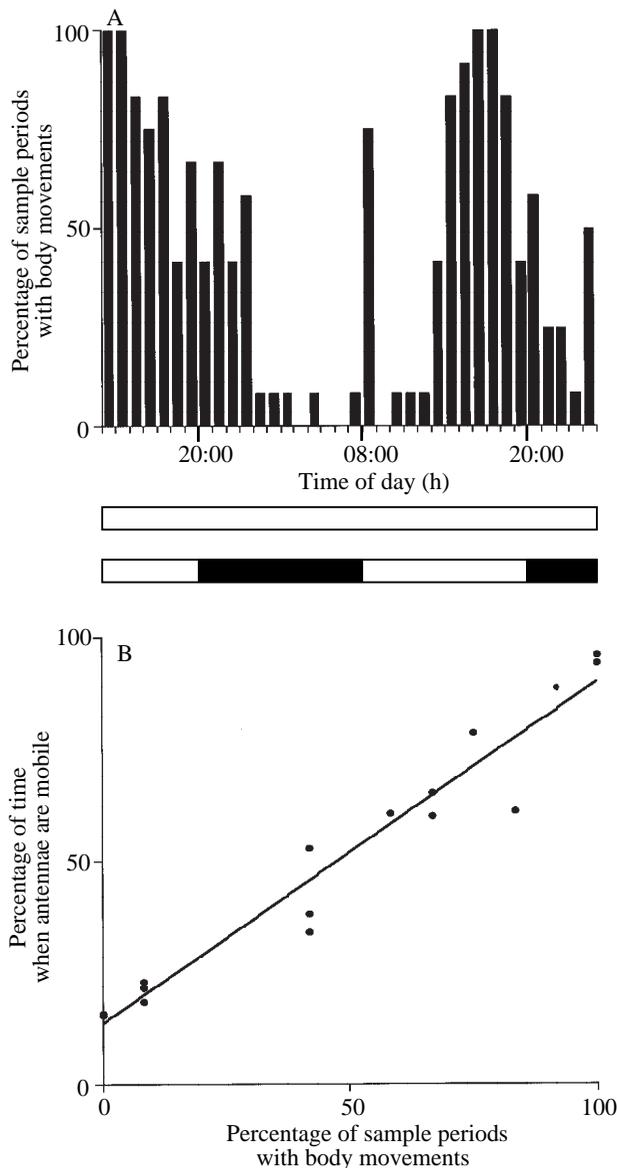


Fig. 1. Movements of an unrestrained locust during a 36h period under continuous red light. (A) Histogram of periods of activity when there were visible movements of the body other than breathing or antennal movements. A 10 s sample was made every 5 min ($12 \text{ samples h}^{-1}$). The percentage of sample periods in which movements occurred is displayed for each hour. The white bar below the histogram indicates the 36h period of continuous red light of the experiment, and the black-and-white bar indicates the preceding light:dark regime in the rearing cage. (B) During the first 17h of the experiment, all times when antennae were moving were measured from video recordings. Times of antennal movement were then summed for each hour. The resulting total time of antennal movement (expressed as a percentage of that 1h period) is correlated (correlation coefficient $r=0.97$, $P \leq 0.01$) with the frequency of body movements each hour. The frequency of body movements per hour was derived from 10 s sample periods made every 5 min, as described in A. Data from locust 7.

activity for the next 36h when they were isolated, but still allowed to move freely, in a small cage illuminated continuously with red light (Fig. 1A). General motor activity,

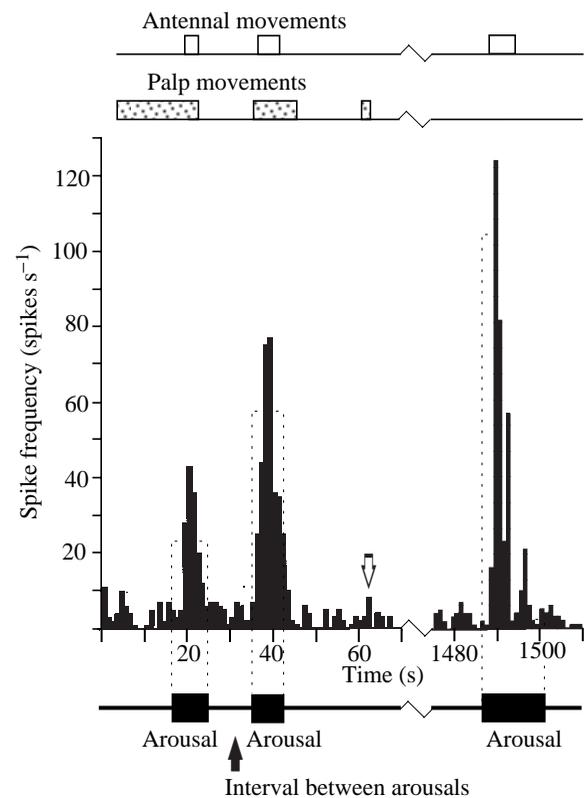


Fig. 2. Transient changes in the spike frequency of antennal motor neurons can indicate arousal. Such shifts in spike rate were identified by an automated analysis: the summed activity of as many as six motor neurons was used to calculate spike rate during an approximately 25 min period during the night (a 12h:12h light:dark regime). In this locust, the threshold level for muscle arousals was set at 19 spikes s^{-1} with a computer program that worked in the following way to give the best average fit to a transient. First, peak times of all transients above 19 spikes s^{-1} were detected. Then, the time at which the spike rate passed through a level (horizontal dashed lines) given by the peak value minus 19 spikes s^{-1} was determined for both the rising and falling phase of each transient. If the falling phase of one transient and the rising phase of the next were within 5 s of each other, the two transients were pooled. The muscle arousal period (black bar) was then defined as the period starting 2.5 s before and extending 2.5 s after these rise and fall times (indicated by vertical dashed lines). The first two arousals were associated with both antennal and palp movements, but only antennal movements accompanied the third. A movement of the palps alone was accompanied by a change in motor activity that fell below the set threshold for an arousal (arrow). Data from locust 6.

defined as any visible body movement other than antennal or breathing movements, was most prevalent during the second half of the projected light phase and least during the projected dark phase. In one locust, the frequency of antennal movements was calculated for each 1h interval during a 17h period beginning at 13:00h and was found to be closely correlated ($r=0.97$) with the frequency of other body movements (Fig. 1B). This suggested that the occurrence of antennal movements could be used as an indicator of arousal. Similarly, most antennal movements (97% in one locust) of

locusts tethered in a head-up position beside a treadwheel were accompanied by palp movements. The simultaneous activity of two different motor systems again suggested that antennal movements were a good indicator of arousal. To avoid the time-consuming evaluation of antennal movements from long-term video recordings, we instead recorded the electrical activity of antennal muscles.

Antennal muscle activity as a measure of arousal

We first checked whether increases in spike activity of antennal muscles reflected movements of the antenna and palps. Pronounced increases usually coincided with movements of both the antenna and palps, whereas smaller increases were often accompanied by palp movements only and not by visible movements of the antenna (Fig. 2). There was no sharp threshold level marking an abrupt transition between the two situations. Thus, to use changes in spike activity as a measure of arousal, we set a threshold level so that more than 50% of the increases of activity were accompanied by both antennal and palp movements. We set this level separately for each locust by comparing movements with spike activity in short segments of the recordings. In the example shown in Fig. 2, two increases in frequency that exceeded the threshold were accompanied by movements of the antenna and palps, and the third was a rare example of a large increase in frequency that was accompanied by an antennal movement only. All three changes were designated as arousals because they exceeded the threshold. Between these arousals were periods of quiescence when muscle activity was low and there were no accompanying movements of the antenna.

Are there periodicities in arousals?

To determine whether spontaneous arousals occurred with particular periodicities, their occurrence was monitored during the whole night in three locusts. The results were pooled and

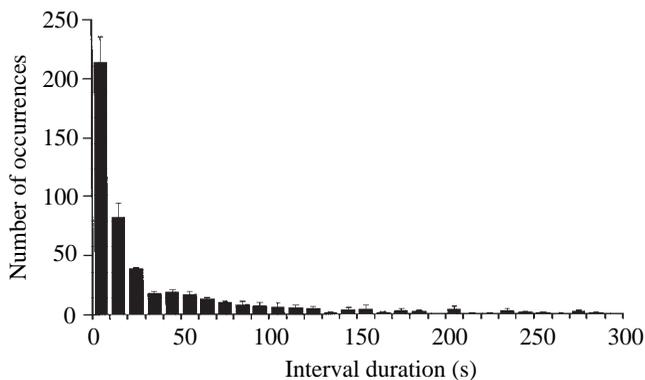


Fig. 3. Frequency distribution of periods between spontaneous arousals. The data were collected throughout the night from three locusts (locusts 1, 5 and 6) and then pooled. The histogram displays the occurrence of intervals between 0 and 300 s. Note that the frequency of intervals declines more-or-less monotonically as interval length increases and that intervals of 60 or 90 s are rare. Error bars are standard errors of the mean.

displayed as histograms of intervals between arousals (Fig. 3). The frequency of intervals declined more-or-less monotonically with increasing length and, in particular, the intervals of 60 s or 90 s that were used in subsequent stimulation regimes did not appear as peaks. Periodic increases in the spike activity in the muscles were, however, occasionally apparent when they were analysed over shorter periods (Fig. 4). In a 400 s period from one locust, large peaks of activity indicating arousals followed each other at irregular intervals, but between these peaks there were smaller transients that suggested a possible underlying periodicity (Fig. 4A). The power spectrum derived from the sequence of all spike transients during this 400 s period showed that frequency components with periods of approximately 15 s were particularly pronounced (Fig. 4B). It is possible, therefore, that the spontaneous arousals are a transient expression of internal rhythms that in long-term recordings are too variable to have

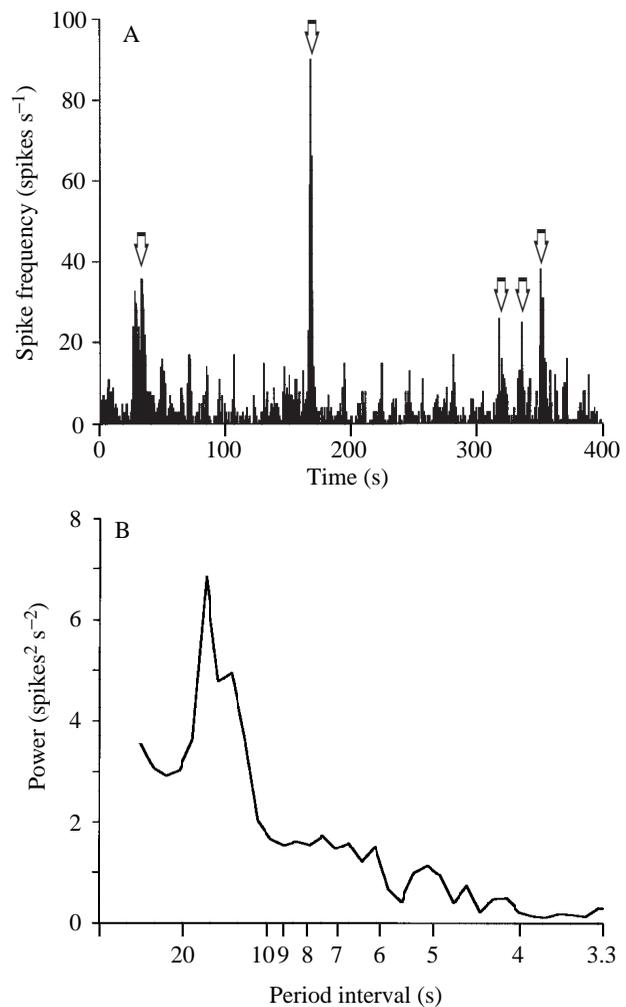


Fig. 4. Periodic variations in the frequency of antennal motor spikes. (A) Frequency of motor spikes during a 400 s period at night displayed as histograms of spike rate (bin width 1 s). The arrows point to large transients that exceeded the threshold level and thus represented arousals. (B) The power spectrum of the spike rate indicates a periodicity of approximately 15 s for the transients. The recording was made in locust 6 from 20:55 h to 21:01:40 h.

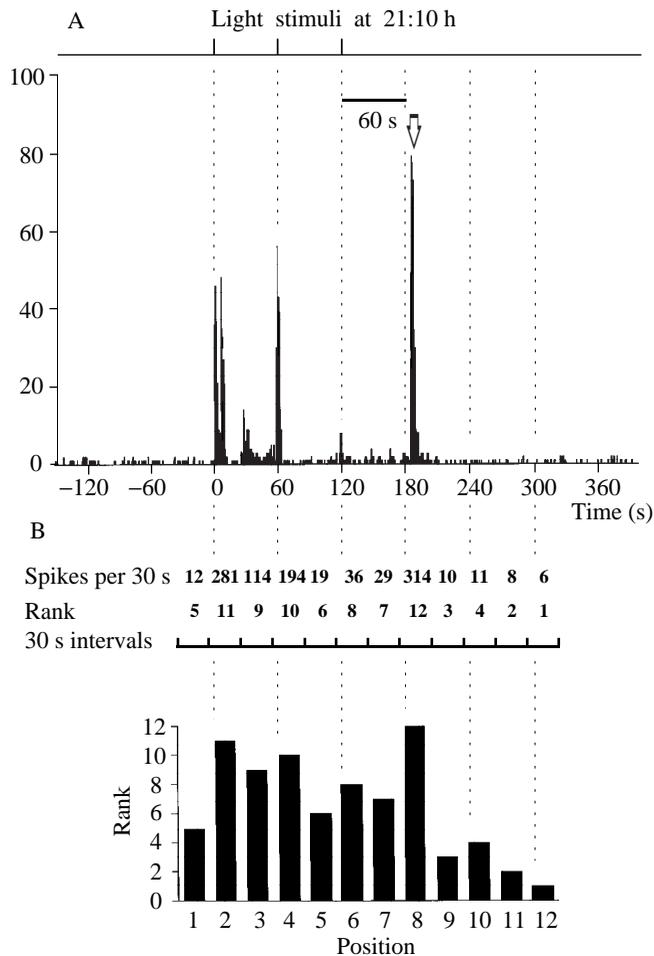


Fig. 5. Arousals evoked by visual stimuli. (A) Three pulses of light at intervals of 60 s each cause transient increases in the frequency of antennal motor spikes, the third response being smaller than the preceding two. These are followed at a similar interval (vertical dashed lines) by a large spontaneous transient (arrow). (B) The method of rank ordering as applied here to the data in A and as used in Fig. 6. The spikes were analysed for a period of 360 s starting 35 s before the first stimulus. The 360 s period was then subdivided into 12 intervals of 30 s, and the number of spikes in each was counted. The interval with the smallest number of spikes was given the rank 1 and that containing the largest, the rank 12. In this way, a ranked time series was generated consisting of 12 values for each recording. Data from locust 1.

an impact on the frequency distribution of intervals between arousals.

Sensory experience and arousals

To determine whether a relationship between arousals and an internal rhythm can be established through exposure to sensory stimuli, we performed the next series of experiments. A locust mounted beside a treadwheel to record its activity was confronted with a series of light stimuli consisting of three pulses at 60 s intervals repeated 11 times at intervals of 10 min (first night) or three pulses at 90 s repeated at intervals of 15 min (second night). The locusts were then tested later in

these nights to see whether the stimulation intervals were reflected in subsequent spontaneous or evoked arousals. The first light pulse of the series always caused a marked increase in the frequency of antennal motor spikes, indicating arousal of the previously quiescent locust (Fig. 5A). The second and third pulses evoked changes that were in general smaller than those in response to the first. Responses to the subsequent groups of three stimuli were usually smaller than those in response to the first group and were more variable. In five of the six locusts, the first group of three stimuli was followed by a spontaneous increase in spike rate at an interval close to that separating the stimuli themselves (Fig. 5A). Occasionally, subsequent groups of three stimuli were also followed by an increase in the spike rate at the same interval.

To quantify these responses in different locusts in which the baseline of their antennal muscle activity was different, a ranking procedure (see Fig. 5B for details) was used that decreased the impact of outliers and allowed data from different locusts to be summed without weighting one more highly than another. From these pooled results, it can be shown that the single light pulse at the beginning of the night always caused a marked increase in antennal muscle activity (Fig. 6A) and a strong behavioural arousal of the previously quiescent locust. Similarly, the first light pulse of the series of three stimuli caused the greatest response, on average, and the second and third light pulses caused slightly smaller responses (Fig. 6B). As in the data from a single locust, the pooled data revealed an increase in the spike rate following the last stimulus at an interval of 60 s. This is apparent in Fig. 6B as a peak at rank position 8 that is significantly greater (one-sided sign test, $N=6$, $P \leq 0.1$) than the preceding or following rank positions. To apply the sign test, ranks of adjacent positions were compared to determine whether there was a change of the rank value from one position to the next. In certain positions, for example, increases of the rank were expected and the one-sided test was used to determine whether there was a change in this direction.

In the second experimental night, the stimulus series consisted of three 1 s light pulses at intervals of 90 s, with each group separated by 15 min. The initial test stimulus at the start of the night caused a large transient response but revealed no lingering effects of the stimuli from the previous night (Fig. 6D). Responses to the repeated stimuli were represented as increases in the spike rate by peaks at rank positions 2, 5 and 8 (Fig. 6E). A further statistically significant (one-sided sign test, $N=6$, $P \leq 0.1$) peak occurred at the end of stimulation at position 11, predicted by the preceding 90 s period of the stimulation (Fig. 6E). As in the first night, the responses to the second and third stimuli were smaller than those to the first, but these could be restored by dishabituating stimuli such as air puffs. To test for dishabituation, all locusts were stimulated repeatedly at the end of the experiment with 1 s light pulses at intervals of 20 s and, occasionally, with an air puff delivered 10 s before a light pulse. In one locust, for example, every seventh light pulse was followed by an air puff and this sequence was repeated five times. Responses to the light pulses

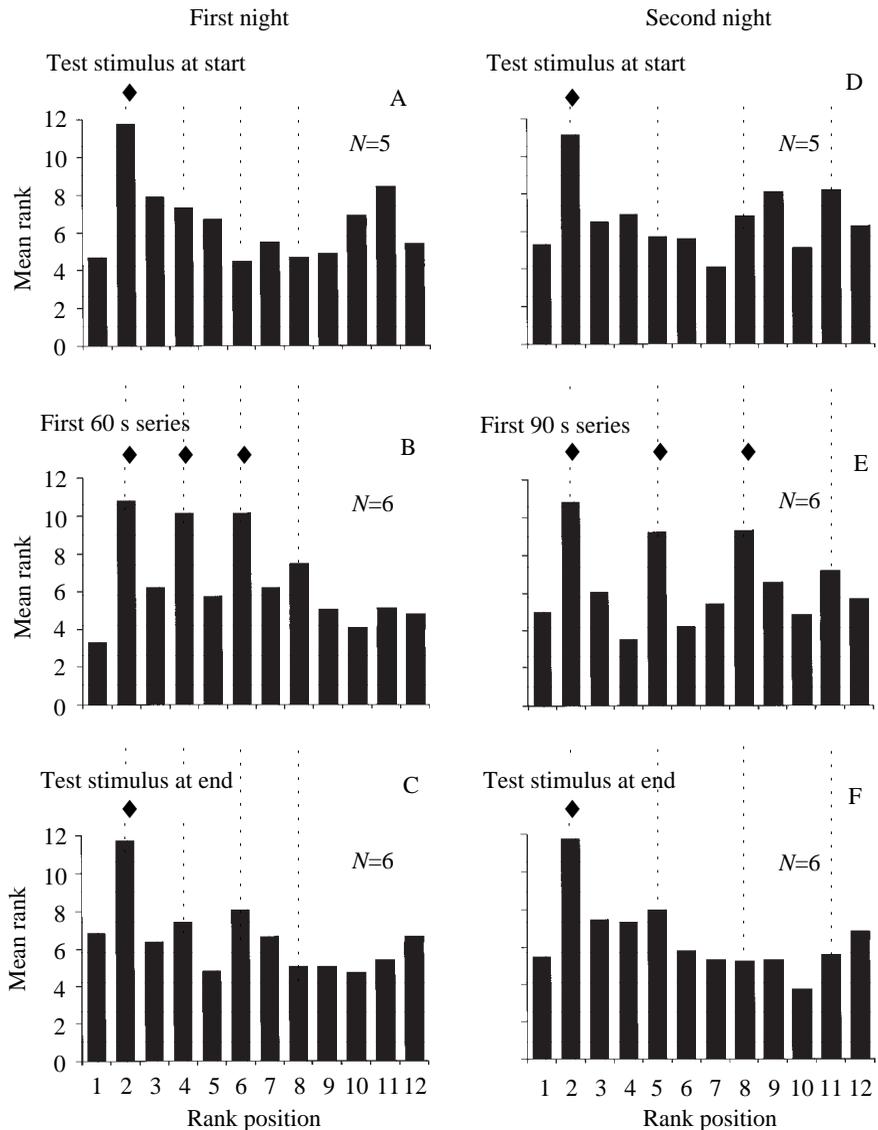


Fig. 6. Rank order analysis of responses to test and repeated stimuli. (A–C) Pooled responses of six locusts exposed at the start of the first night to a single test pulse (solid diamond in A), then to a series of 11 stimuli each consisting of three pulses at 60 s intervals (first three pulses shown in B) and finally to a single test stimulus at the end of the night (C). (D–F) The same six locusts on the second night in which the interval between the stimuli was increased to 90 s. Details of the method for rank ordering are given in Fig. 5.

preceding and following an air puff were then compared. On average ($N=5$), spike rate increased by only 0.4 ± 0.89 spikes s^{-1} (mean \pm S.D.) in response to the light pulse that just preceded an air puff, but by 11.2 ± 8.7 spikes s^{-1} (mean \pm S.D.) to the light pulse that followed it. In the other locusts, dishabituation also occurred with the spike rate in response to a light stimulus increasing after the presentation of an air puff.

To determine whether the repetitive stimuli had a longer-lasting effect, we tested whether a single 1 s light pulse at the end of each of the two experimental nights (between 06:00 h and 07:55 h) would elicit repeated increases of muscle activity that reflected the preceding 60 s or 90 s rhythms. In a locust exposed early in the night to stimuli at intervals of 90 s (Fig. 7A), there was an immediate increase in spike rate following the single test stimulus presented at 07:45 h. At intervals of 90 s and approximately 180 s after the stimulus, there were further peaks of spike rate in the absence of any further light stimuli (Fig. 7B). The data for six locusts exposed to the 60 s and 90 s test regimes were analysed by again creating a rank series of the number of motor spikes

(Fig. 6C,F). If the periodicity of the preceding stimuli were to be reflected in subsequent spontaneous muscle activity, then peaks of activity would be expected in Fig. 6C at positions 4 and 6, at intervals of 60 s following the test stimulus, whereas in Fig. 6F peaks would be expected at positions 5 and 8, at intervals of 90 s. Peaks did, indeed, occur at three of the expected positions (4 and 6 in Fig. 6C, and 5 in Fig. 6F). Only the peak at position 6 in Fig. 6C was significantly different from the preceding rank position (one-sided sign test, $N=6$, $P \leq 0.1$). Nevertheless, because the single test stimulus given at the end of both nights was exactly the same, the responses during the two nights could be compared directly for each individual locust. In position 6 (Fig. 6C), the activity was significantly higher during the first night than during the second, and in position 5 (Fig. 6F), the activity was significantly higher during the second night than during the first (one-sided sign test, $N=6$, $P \leq 0.1$). This result suggests that the timing of the stimuli influenced the periodicity of spike activity elicited by a single test stimulus. Any difference between the two nights in position 4 could have been obscured

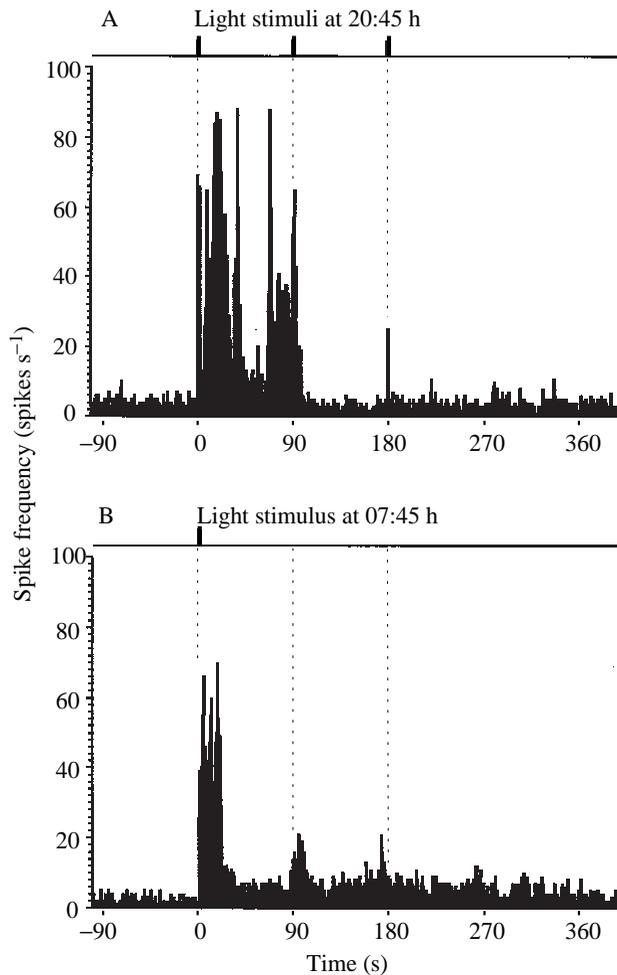


Fig. 7. Repeated visual stimuli influence the periodicity of arousals several hours later. (A) Three stimuli at intervals of 90 s during the second experimental night each led to a transient increase in the frequency of antennal motor spikes and an accompanying arousal. (B) A single test stimulus at the end of the night (07:45 h) was followed by two transient increases in the frequency of spikes at the same intervals as the earlier stimuli. Data from locust 1.

by a prolonged elevation of spike rate caused by the test stimulus at the end of the second night (Fig. 6F).

We also checked whether the periodicity of spontaneous changes in spike rate reflected the preceding stimulation. In three of six locusts, spontaneous arousals that occurred within 30 min of the stimuli did reflect the preceding periodicity of the stimuli. For example, in one of these locusts, three transients occurred within 29 min of the last stimulus at intervals of 60 s, the same as the interval between the preceding stimuli (Fig. 8). To quantify this observation, autocorrelation analysis was performed on recordings from five locusts for 25 min periods (a) just preceding the light stimuli, (b) 5 min after the last stimulus, and (c) 4.5–6.5 h later. During the periods just preceding the light stimuli (Fig. 9A,B) or 4.5–6.5 h following the stimuli, there were no peaks in the autocorrelograms for periodicities of either 60 or 90 s. In

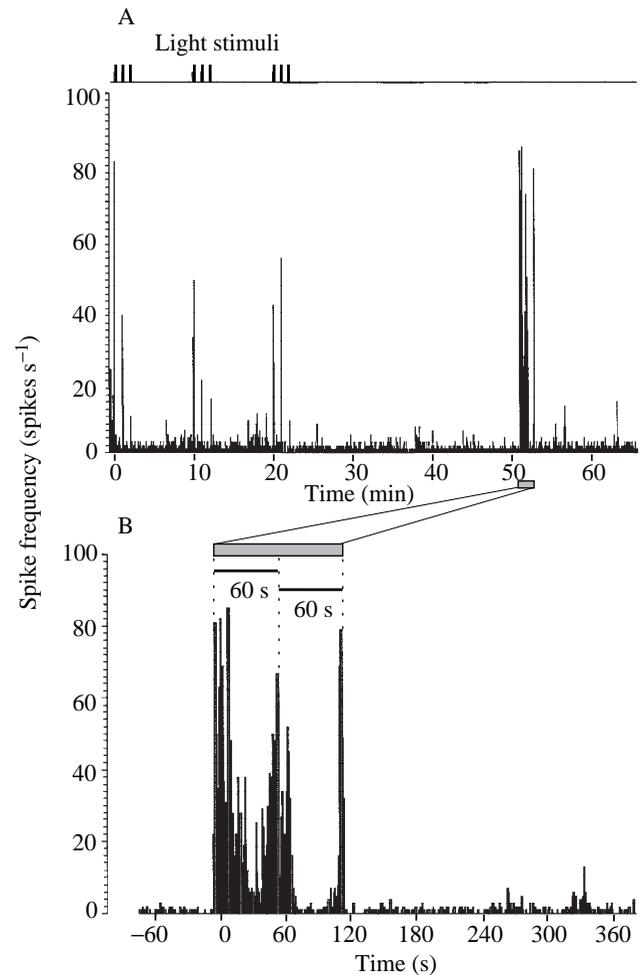


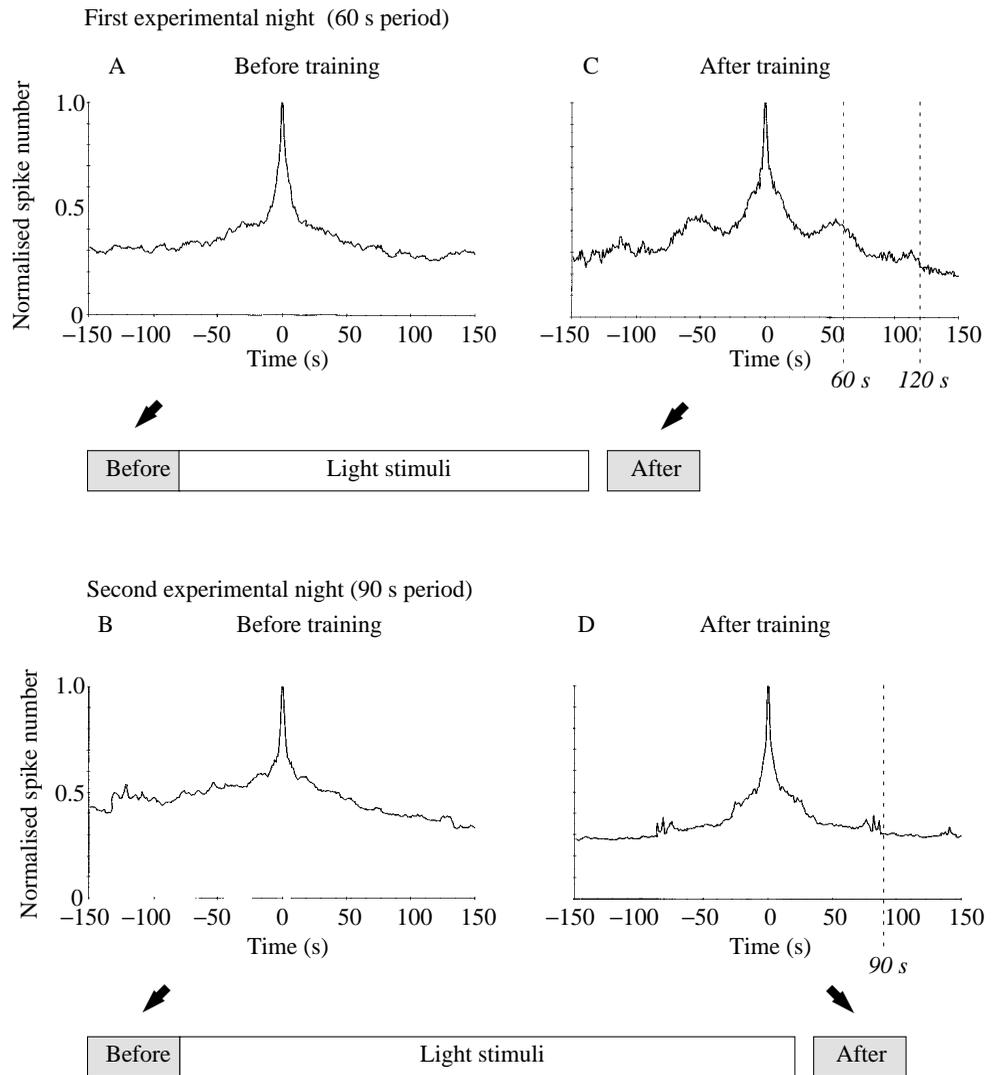
Fig. 8. Spontaneous arousals can occur at the same intervals as the preceding stimuli. (A) The last three groups of stimuli each consisting of three light pulses at intervals of 60 s are followed 29 min later by a large transient increase in the frequency of antennal motor spikes. (B) An expanded view of this spontaneous change shows that it consisted of three transients at intervals of 60 s. Data from locust 1.

contrast, during the first night, peaks at 60 s and 120 s occurred in the 25 min period starting 5 min after the last of the stimuli at 60 s intervals (Fig. 9C). Those peaks resulted from observations in four animals. In the second night during the 25 min period following the stimuli at 90 s intervals, there was a peak in one locust at approximately 80 s that was still apparent in the combined autocorrelogram (Fig. 9D).

Discussion

Locusts kept in a 12 h:12 h light:dark cycle are quiescent by night and active by day (Odhiambo, 1966), and this diurnal rhythm of motor activity can persist even when they are isolated and kept in continuous red light in our experiments. The nightly periods of quiescence are punctuated by arousals that were recognised as increases in antennal muscle activity.

Fig. 9. Autocorrelation analysis of the spike activity in antennal muscles before and after a series of light stimuli. From myographic traces, intervals between each spike and all other spikes within 150 s were calculated and displayed as a histogram (bin width 1 s) representing the number of spikes at each distance from the reference point at time zero. The histogram was normalised by dividing the contents of each bin by the number of spikes falling in the bin at zero time. In the 25 min before the light stimuli in either the first (A) or second (B) experimental night, there was no periodicity in the spike activity. (C) In a 25 min period starting 5 min after the last exposure to light stimuli (at 60 s intervals) in the first night, peaks were present in the autocorrelograms at ± 60 s and ± 120 s. (D) In the second experimental night after exposure to light stimuli (at intervals of 90 s), small peaks were present in correlograms at intervals of approximately ± 80 s, but they were due to the contribution of just one animal. Diagrams show mean values, calculated from locusts 1 and 3–6. Standard deviation at $+60$ s was 0.15 in A, 0.22 in B, 0.37 in C and 0.38 in D. In C,D, the periods analysed started 5 min after the last stimulus to exclude the immediate effects of entrainment (see Fig. 6B,E).



Our experiments were designed to investigate whether repeated stimulation could influence the periodicity of subsequent arousals that occurred spontaneously or following a light stimulus. Our results show that rhythms induced by stimulation were expressed as increases in muscle activity with the same periodicity as the preceding stimuli. These changes in muscle activity, or arousals, occurred at the end of a period of stimulation, as spontaneous changes later at night, and could be elicited following later exposure to a single stimulus.

One explanation of these results could be that the arousals are coupled to an internal pacemaker that is entrained by the stimuli. In our experiments, the activity of such an internal pacemaker might have been reflected transiently in spontaneous rhythmic activity of the antennal muscles. Spontaneous rhythmic activity might have become entrained, even if the stimulus interval was a multiple of the period of the spontaneous rhythmic activity. A predominance of spontaneous rhythms with periods close to those at which light stimuli followed each other was, however, not observed. Thus, if internal rhythms were involved, then they may well have had a period that was a fraction of the stimulus interval; for

example, a period of 15 s similar to the one that is prevalent in the recording shown in Fig. 4. Arousals seen after entrainment may therefore have been coupled to this rhythm, although not to each of its cycles. Coupling of arousals to non-circadian internal rhythms has been described in insects for a wide range of periodicities. In *Locusta migratoria*, there is a preference for rest periods of 2–2.5 h (Edney, 1937), and the alternation of these with bouts of high activity perhaps reflects a hunger rhythm. In *Locusta*, various movements can be coupled to a faster 12–16.5 min oscillation (Simpson, 1981). Arousals can also be coupled to other vegetative rhythms such as the breathing rhythm. In *Periplaneta americana*, slight movements of body appendages are linked to the ventilatory rhythm (Kestler, 1991), and in locusts some flight motor neurons receive a rhythmic synaptic input that is linked to ventilation (Burrows, 1975).

An alternative explanation could be that a new memory was built up representing each interval between stimuli. On this basis, the memory trace would be recalled spontaneously after appropriate internal signals or after the presentation of a single test stimulus at the end of the night. A basic requirement of this

concept is that non-cyclic stimulation (external or internal) is able to elicit cyclic arousals, irrespective of whether they have been entrained before. Human sleep provides an example showing that external stimulation can provoke periodic arousals at frequencies that have not been previously entrained (Terzano and Parrino, 1991). During non-rapid eye movement sleep, sequences of 'cyclic alternating patterns' appear spontaneously and are separated from each other by periods of arousal stability (Terzano and Parrino, 1991). If a stimulus is applied during a period of arousal stability, then this induces the appearance of successive 'cyclic alternating pattern' cycles which, in the electroencephalogram, are characterised by the periodic alternation of two patterns: a heightened and a lesser arousal level. There are clear correlations between arousal cycles seen in the electroencephalogram and periodicities of heart rate, blood pressure, respiration and muscle tone (Coccagna *et al.* 1971; Lugaresi *et al.* 1972; Terzano and Parrino, 1991; Evans, 1992). The generation of 'cyclic alternating patterns' may reflect the synchronised entrainment of various biological rhythms to prevent life-threatening dangers to vital functions during sleep (Terzano and Parrino, 1991).

In locusts, non-cyclic stimulation evoked activities that had been previously established by exposure to stimuli, perhaps by generating internal conditions under which a rhythm in antennal muscle activity again became apparent. In our experiments on locusts, transient changes in the frequency of antennal motor spikes habituated during repeated light stimulation, but could be dishabituated by other stimuli. This observation suggests that a memory trace was built up for the light pulses and became associated with the memory for the rhythm of stimulation. The periodicity could then have been recalled spontaneously or by an external stimulus acting as a cue when presented later that same night. It is possible that the training effect of each stimulation may depend on the long-lasting effect of the preceding one and, thus, on the interval between stimuli. To establish a rhythm, it may also be important that each stimulation has an effect of the same sign. In our experiments, each light stimulus caused an increase in spike activity in the antennal muscles, so that the effects of subsequent stimuli were of the same sign. Under these conditions, the rhythm of stimulation was apparent.

The arousing effects of light stimuli show that they are relevant stimuli to the locust, causing it to raise its level of activity. Our experiments have demonstrated that these arousals occur but we have not addressed their underlying mechanisms. One of a number of possibilities is that octopaminergic neurons might be involved. In the cockroach *Periplaneta americana*, lights flashed at 4 Hz (much higher frequencies than we used) for 15 min increased octopamine levels in the brain and nerve cord (Hirashima and Eto, 1993). In honeybees, injection of octopamine into the region of the brain containing the antennal motor neurons elicited an increase of activity in the flexor muscle moving the distal parts of the antenna (Pribbenow and Erber, 1994). From our experiments, we conclude that periodicities of arousing stimuli are effective after the presentation of several series of stimuli

and then, most likely, kept ready for recall at least until the end of the same night. It may well be, however, that even a single stimulus series has a lasting effect, for it is known from honeybees that the formation of behaviourally significant memory traces which last for days can take place within a single associative learning trial (Menzel, 1968). If arousing stimuli display a certain time structure that is appreciated by the insect, then this time structure could be used as a predictor modulating spontaneous activity of the animal in accordance with earlier experience. In this respect, it would be advantageous for insects to use information about temporal changes of environmental conditions to influence spontaneous behaviour as soon as this information becomes available and to keep this information until it is updated with respect to new experience or with respect to previous memory traces.

In quiescent insects, regular arousals that follow the rhythm of previous disturbing stimuli could help to prepare the animal for action and to cope with potential danger. Transient changes in the excitatory state could also help in the interpretation of the time structure of significant stimuli in active insects. Honeybees, for example, trained to negotiate a maze by following coloured marks, can still find their way when the marks are removed (Zhang *et al.* 1996). The honeybees may acquire a spatial memory of the maze or a sequence of motor commands describing the correct path through it. Such a memory would be particularly effective if there were additional information about the temporal order in which motor commands had to follow each other at a given flight speed. Likewise, temporal and spatial memories could complement each other during navigation if both were associated with the same significant environmental cues. The rules under which memories for temporal patterns are formed have yet to be studied, so that studies in quiescent locusts on arousal shifts that are involved in the formation of temporal memories may have a useful contribution to make.

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References

- BACON, J. P., THOMPSON, K. S. J. AND STERN, M. (1995). Identified octopaminergic neurons provide an arousal mechanism in the locust brain. *J. Neurophysiol.* **74**, 2739–2743.
- BENNET, R. R., TUNSTALL, J. AND HORRIDGE, G. A. (1967). Spectral sensitivity of single retinula cells of the locust. *Z. vergl. Physiol.* **55**, 195–206.
- BURROWS, M. (1975). Co-ordinating interneurons of the locust which convey two patterns of motor commands; their connexions with flight motoneurons. *J. exp. Biol.* **63**, 713–733.
- COCCAGNA, G., MANTOVANI, M., BRIGNANI, F., MANZINI, A. AND LUGARESI, E. (1971). Arterial pressure changes during spontaneous sleep in man. *Electroenceph. clin. Neurophysiol.* **31**, 277–281.
- CORBET, S. A. (1991). A fresh look at the arousal syndrome in insects. *Adv. Insect Physiol.* **23**, 81–116.

- EDNEY, E. B. (1937). A study of spontaneous locomotor activity in *Locusta migratoria migratorioides* (R. & F.) by the actograph method. *Bull. Ent. Res.* **28**, 243–278.
- EVANS, B. M. (1992). Periodic activity in cerebral arousal mechanisms – the relationship to sleep and brain damage. *Electroenceph. clin. Neurophysiol.* **83**, 130–137.
- HAMMER, M. AND MENZEL, R. (1995). Learning and memory in the honeybee. *J. Neurosci.* **15**, 1617–1630.
- HIRASHIMA, A. AND ETO, M. (1993). Effect of stress on levels of octopamine, dopamine and serotonin in the American cockroach (*Periplaneta americana* L.). *Comp. Biochem. Physiol.* **105C**, 279–284.
- KESTLER, P. (1991). Cyclic CO₂ release as a physiological stress indicator in insects. *Comp. Biochem. Physiol.* **100C**, 207–211.
- LINDSLEY, D. B. (1987). Activation, arousal, alertness and attention. In *Encyclopedia of Neuroscience*, vol. I (ed. G. Adelman), pp. 3–6. Boston: Birkhäuser.
- LUGARESI, E., COCCAGNA, G., MANTOVANI, M. AND LEBRUN, R. (1972). Some periodic phenomena arising during drowsiness and sleep in man. *Electroenceph. clin. Neurophysiol.* **32**, 701–705.
- MENZEL, R. (1968). Das Gedächtnis der Honigbiene für Spektralfarben. I. Kurzzeitiges und langzeitiges Behalten. *Z. vergl. Physiol.* **60**, 82–102.
- ODHIAMBO, T. R. (1966). The metabolic effects of the corpus allatum hormone in the male desert locust. *J. exp. Biol.* **45**, 51–63.
- PRIBBENOW, B. AND ERBER, J. (1994). Modulation of antennal scanning in the honeybee by serotonin and octopamine: behaviour and electrophysiology. In *Proceedings 22nd Göttingen Neurobiology Conference* (ed. N. Elsner and H. Breer), p. 272. Thieme, Stuttgart, New York.
- ROWELL, C. H. F. (1971a). Antennal cleaning, arousal and visual interneurone responsiveness in a locust. *J. exp. Biol.* **55**, 749–761.
- ROWELL, C. H. F. (1971b). Variable responsiveness of a visual interneurone in the free-moving locust and its relation to behaviour and arousal. *J. exp. Biol.* **55**, 727–747.
- SIMPSON, S. J. (1981). An oscillation underlying feeding and a number of other behaviours in fifth-instar *Locusta migratoria* nymphs. *Physiol. Ent.* **6**, 315–324.
- STERIADE, M. (1993). Arousal. In *Encyclopedia of Sleep and Dreaming* (ed. M. A. Carskadon, A. Rechtschaffen, G. Richardson, T. Roth and J. Siegel), pp. 50–53. Macmillan: New York.
- STERN, M., THOMPSON, K. S. J., ZHOU, P., WATSON, D. G., MIDGLEY, J. M., GEWECKE, M. AND BACON, J. P. (1995). Octopaminergic neurons in the locust brain: morphological, biochemical and electrophysiological characterisation of potential modulators of the visual system. *J. comp. Physiol. A* **177**, 611–625.
- TERZANO, M. G. AND PARRINO, L. (1991). Functional relationships between micro and macrostructure of sleep. In *Phasic Events and Dynamic Organization of Sleep* (ed. M. G. Terzano, P. L. Halász and A. C. Declerck), pp. 101–119. New York: Raven Press.
- UVAROV, B. (1977). *Grasshoppers and Locusts; A Handbook of General Acridology*, vol. 2. London: Centre For Overseas Pest Research.
- ZHANG, S. W., BARTSCH, K. AND SRINIVASAN, M. V. (1996). Maze learning by honeybees. *Neurobiol. Learning Memory* **66**, 267–282.