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RECEPTOR CELL HABITUATION IN THE A_1 AUDITORY RECEPTOR OF FOUR NOCTUOID MOTHS

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Summary

Moths of both sexes of *Empyreuma affinis* (=*pugione*) and *Syntomeida epilais* (Arctiidae, Ctenuchinae), *Maenas jussiae* (Arctiidae, Arctiinae) and *Spodoptera frugiperda* (Noctuidae, Amphipyrinae) were studied. Spike activity in the A_1 cell was recorded using a stainless-steel hook electrode from the tympanic nerve in the mesothorax. Acoustic stimuli consisting of 25 and 100 ms pulses at the best frequency for the species and at intensities that evoke A_1 cell saturation response were used at repetition rates of 0.5 and 5 Hz for 100 ms stimuli, and between 2 and 20 Hz for 25 ms stimuli. Stimuli at a repetition rate corresponding to a duty cycle of 5 % (25 ms at 2 Hz and 100 ms at 0.5 Hz) did not evoke monotonic changes in the responses of the A_1 cell. With 25 ms pulses, rates above 5 Hz evoked an exponential decrease in the number of spikes and an increase in the latency of the responses of all the 37 specimens tested. The response duration showed no apparent change with stimulus repetition rates even at the highest duty cycle used (50 %), i.e. 25 ms at 20 Hz and 100 ms at 5 Hz. The higher the rate of stimulus repetition, the more marked were the changes in the A_1 cell responses. In 16 of 17 preparations from two species, habituation had no effect on the adaptation rate in each response, while in seven of eight specimens of another species, the adaptation rate decreased with stimulus repetition. These results, and those from another mechanoreceptor cell, indicate that receptor cell adaptation (changes evoked in the response by a stimulus of constant intensity) and habituation (changes in the responses due to stimulus repetition rate) are two distinctive phenomena. The A_1 cell in its habituated state showed an increase in its response to incremental increases in stimulus intensity of 10 dB. This result supports the idea that receptor cell habituation does not seem to be due to fatigue, i.e. to a temporary loss of the ability to respond to stimulation induced in a sensory receptor by continued stimulation.

Key words: noctuoid moth, moth, auditory receptor, repetitive stimulus, adaptation, habituation, *Empyreuma affinis* (=*pugione*), *Syntomeida epilais*, *Maenas jussiae*, *Spodoptera frugiperda*.

Introduction

Mechanical and acoustic signals emitted by animals show a certain temporal pattern, usually expressed by a given value for the rate of stimulus repetition, which may convey information relevant to the way in which the animal behaves. The use of repetitive stimulation in invertebrate mechanoreceptor organs has shown that monotonic changes may occur in the physiological features of some receptor cells (Bush and Pasztor, 1983; Pasztor and Bush, 1983, 1987, 1989; Corfas and Dudai, 1990*a*,*b*; Pasztor and Macmillan, 1990; Engel and Wu, 1994), while other mechanoreceptor cells show response stability under such stimulation (Zucker, 1972; Byrne *et al.* 1978). Changes in some physiological characteristics induced by repetitive acoustic stimuli have also been described in the auditory receptor cells of orthopterans and lepidopterans (Esch *et al.* 1980; Sippel and Breckow, 1984; Coro *et al.* 1995; Waters, 1996). Among these changes are a decrease in the number of spikes per stimulus and an increase in latency during a stimulus series (Esch *et al.* 1980; Pasztor and Bush, 1983, 1987, 1989; Sippel and Brechow, 1984; Corfas and Dudai, 1990*a*; Engel and Wu, 1994). The receptor-cell response decrement has been given various names: receptor fatigue (Zucker, 1972), fatigue (Byrne *et al.* 1978; Waters, 1996), sensory habituation (Bush and Pasztor, 1983; Pasztor and Bush, 1983, 1989), adaptation (Sippel and Breckow, 1984), sensory fatigue (Corfas and Dudai, 1990*a*,*b*) and cumulative adaptation (Engel and Wu, 1994). We consider that none of these names adequately describes the physiological process evoked by repetitive stimulation in

mechanosensory receptor cells; we shall discuss these names and propose a new one.

The tympanic organ of most noctuoid moths has only two auditory receptor cells, which differ in their sensitivity to acoustic stimuli; the more sensitive is the A_1 cell, while the less sensitive is the A_2 receptor (Roeder, 1964*a*, 1974; Coro and Pérez, 1984; Pérez and Coro, 1984). The behavioural significance of this auditory system has mainly been associated with the evasive behaviour that these moths show in the presence of their predators, insectivorous bats (Roeder and Treat, 1961; Roeder, 1964*b*; Miller, 1982; Fullard, 1987; Surlykke, 1988). These bat species change the pulse repetition rate of their echolocation signals during their search for, and approach to, their prey (Miller, 1982; Neuweiler, 1984); thus, the repetition rate of the acoustic stimuli in nature may convey important information to enable the moth to take evasive action (Fullard, 1984; Surlykke, 1984; Waters, 1996). In addition, in some Arctiidae that possess sound-emission organs, acoustic signals play a role in mating behaviour (Peter, 1912; Conner, 1987; Portilla *et al.* 1987; Krasnoff and Yager, 1988; Sanderford and Conner, 1990; Simmons and Conner, 1996; Sanderford *et al.* 1998). In two arctiid-ctenuchine moths, *Syntomeida epilais* (Sanderford and Conner, 1990) and *Empyreuma affinis* (=*pugione*) (Sanderford *et al.* 1998), there is sexual dimorphism in the repetition rate of the modulation cycle (*sensu* Fullard and Fenton, 1977) of the acoustic signals; thus, in nature, their tympanic organ has to deal with repetitive stimuli that convey information about the sex of the emitting source (Sanderford and Conner, 1995).

In this paper, we describe the response of the A_1 cell to acoustic stimuli of 25 and 100 ms duration at different repetition rates (from 0.5 to 20 Hz) in four noctuoid moths: three Arctiidae and one Noctuidae. Our aims are (1) to show that stimulus repetition rate may evoke monotonic changes in the number of spikes per pulse and in the latency of the responses and that there is a method to quantify these changes; (2) to analyse whether receptor cell habituation (changes in the responses due to stimulus repetition rate) affects the adaptation phenomenon (changes evoked in the response by a stimulus of constant intensity); and (3) to study whether a habituated receptor cell responds to changes in stimulus intensity.

Materials and methods

Animals

Moths of both sexes of the following species were used: *Empyreuma affinis* (Roths.) (=*pugione*) (L.) (Arctiidae, Ctenuchinae); *Syntomeida epilais* (Walker) (Arctiidae, Ctenuchinae); *Spodoptera frugiperda* (Smith and Abbott) (Noctuidae, Amphipyrinae); and *Maenas jussiae* (Poey) (Arctiidae, Arctiinae).

Specimens of *E. affinis* and *M. jussiae* were collected as last-instar larvae in Havana City, Cuba, and kept in the laboratory under the same temperature, humidity and photoperiod conditions as those of the environment. Larvae of *E. affinis* were fed with fresh leaves of *Nerium oleander*, and those of *M. jussiae* with *Piper auritum* leaves, their normal food plants. Specimens of *Spodoptera frugiperda* were reared from eggs, and the larvae were fed with fresh leaves of *Ricinus comunis*. Specimens of *Syntomeida epilais* were collected in Indian River and Sarasota counties, Florida, USA, during July 1995. Larvae were housed in cages, which were kept in a screened building where the insects were exposed to the temperatures and photoperiod typical of Florida in mid-July. The larvae were fed with fresh leaves of *N. oleander*, their food plant. Moths of both sexes were used 2 weeks after emergence in electrophysiological experiments. Moths were fed with a 30 % sucrose solution.

Electrophysiological recording

Moths were placed ventral side up and dissected, so that spike activity could be recorded with a stainless-steel hook electrode from the tympanic nerve at the position where it joins the alar nerve in the mesothorax. The alar nerve was sectioned from its central and peripheral connections, except from the tympanic nerve (for details of the dissection technique, see Coro and Pérez, 1984, 1993). In many preparations, the entire thoracic central nervous system was destroyed and disconnected from the cephalic and abdominal portions of the system. Nerve activity was amplified, filtered (200 or 300 Hz high-pass and 1000 Hz low-pass filters) and stored on an AM tape recorder (TEAC or AKAI), together with information from a stimulus monitor. The electrophysiological recordings were obtained inside a Faraday cage at temperatures between 22 and 29 °C. The preparations typically lasted more than 1 h, without evident loss of A_1 cell sensitivity to acoustic stimuli.

Acoustic stimulation

The equipment used to produce acoustic stimuli consisted of a sine-wave generator, an electronic stimulator, a modulator that mixes and attenuates the signals from this equipment, and an amplifier that sends the signals to a loudspeaker placed in front of the electrophysiological preparation (0 °), at the same horizontal plane and at a distance of 25–30 cm. The loudspeaker was calibrated periodically.

Acoustic stimuli consisted of 25 and 100 ms duration pulses, with a 1 ms rise-and-fall time, at different carrier frequencies, depending on the moth species, and at an intensity that evokes a saturation response in the A_1 cell (between 50 and 70 dB SPL). The carrier frequencies used were as follows: 35 kHz for *E. affinis*, 32 kHz for *Syntomeida epilais*, 20 or 40 kHz for *Spodoptera frugiperda* and 55 kHz for *M. jussiae*, all of which correspond to the best frequency for the A_1 cell in each species (Coro and Barro, 1997). With 25 ms pulses, eight different repetition rates were used, which varied between 2 stimuli s^{-1} (Hz), corresponding to a duty cycle of 5 %, and 20 stimuli s^{-1} corresponding to a duty cycle of 50 %. Each stimulus rate was applied for the time needed to produce 50 acoustic pulses, e.g. 25 s for 2 Hz and 2.5 s for 20 Hz. After stimuli had been applied at each rate, at least 3 min elapsed before application of the next rate, thus ensuring that the A_1 cell recovered completely from the stimuli applied previously. The stimulus rates were presented in the following order: 20, 2, 13, 4, 10, 5, 8 and 7 Hz. The moths used for this experimental series were *E. affinis*

(eight females and six males), *Syntomeida epilais* (five females and seven males), *Spodoptera frugiperda* (two females and four males) and *M. jussiae* (one female and four males).

A stimulus duration of 100 ms at an intensity that evokes a saturation response in the A_1 cell produces adaptation in this receptor, the rate of which may be quantified for comparative purposes (Coro *et al.* 1994). As a consequence, this stimulus duration was selected to compare adaptation and habituation in the same preparation. The 100 ms pulses were presented at two repetition rates: 0.5 Hz (5 % duty cycle), and 4 or 5 Hz (40 % or 50 % duty cycle). In this experimental series, A_1 cell responses to 20 acoustic pulses at 4 or 5 Hz (depending on the preparation), and another 20 pulses at 0.5 Hz, applied with a 5 min interval between the two stimulation rates, were analysed in six females and seven males of *E. affinis*, four moths of each sex of *Syntomeida epilais*, and three of each sex of *Spodoptera frugiperda*. In addition, in seven *E. affinis* preparations (two females and five males), 100 ms pulses at 4 Hz were used to study the effects of variations in step intensity: stimulus intensity was increased abruptly in 10 dB steps every 16 pulses, from threshold up to 30 dB above this value.

Data collection

The spike activity evoked in the A_1 receptor cell by the acoustic stimuli described above was analysed using the technique described by Coro *et al.* (1994). Briefly, this consisted of discriminating electronically the spikes of the A_1 cell together with the signal synchronised with each acoustic pulse using two custom-made window discriminators. Both discriminated signals were fed to the LPT1 port of a computer. The latency of each response and the interspike intervals were measured using software especially designed for this purpose. The time resolution of the method is 0.1 ms. The analysis times were 40 ms for responses to the 25 ms pulses and 130 ms for those to the 100 ms stimuli, thus including the afterdischarge of this auditory receptor (Pérez and Coro, 1986). The physiological features of the A_1 cell that were studied included: latency, interspike interval (ISI), instantaneous discharge frequency, number of action potentials per pulse and response duration per pulse. This last feature was measured by summing the latency and all the ISI values during the time analysed after the application of each acoustic pulse. Since the A_1 cell in noctuoid moths becomes silent after it has shown a response, particularly at stimulus intensities that evoke its saturation response (Pérez and Coro, 1986), there is almost no possibility of including in the response duration an ISI corresponding to the spontaneous activity of this receptor cell.

Results

Mean responses

In the four noctuoid moth species studied, the responses of A_1 cells to 25 ms duration acoustic pulses were strongly dependent on the stimulus repetition rate (SRR). These effects may be observed by plotting the mean values of the number of spikes per pulse and the latency against the SRR: the number of action potentials per pulse decreases monotonically, while the latency increases (Fig. 1A). Linear regression analyses between the mean number of action potentials per pulse and SRR, and between mean latency and SRR, showed highly significant correlation coefficients, greater than 0.95 (N=8), in each of the 37 specimens studied.

The variations observed in the mean number of action potentials per pulse and the latency evoked by the SRR cannot be explained by deterioration of the preparation, since these

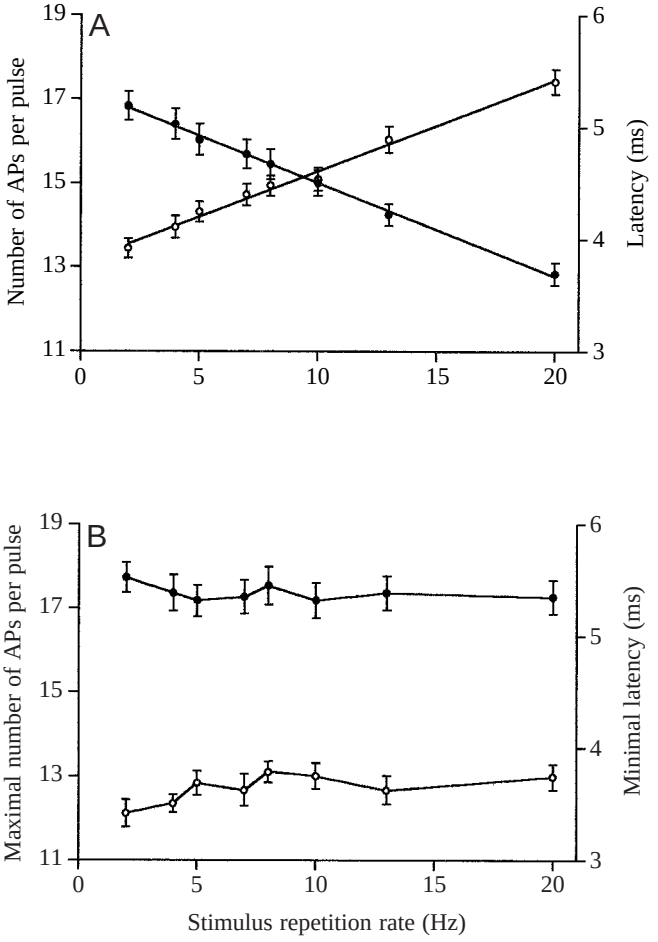

Fig. 1. The effects of changing the repetition rate of pulses with a duration of 25 ms on some physiological features of the A_1 cell in *Empyreuma affinis*. (A) The number of action potentials (APs) per pulse (filled circles) decreases and the response latency (open circles) increases with increased stimulus repetition rate. Each data point reflects the mean ± S.E.M. of the averaged mean values in 11 specimens, in each of which 50 responses were averaged at each repetition rate. The correlation coefficient of both linear regression analyses is 0.99 (N=8), which is statistically highly significant (P<0.001). (B) Stimulus repetition rate does not affect either the maximal number of action potentials per pulse (filled circles) or the minimal latency (open circles) of the responses to the first stimulus of a series. Each data point is the mean ± S.E.M. of the values of these features in the same 11 specimens as in A. For the maximal number of action potentials per pulse (r=0.40, N=8) and for the minimal latency (r=0.60, N=8), and there is no linear relationship between the plotted variables.

pulses were applied with enough time without stimulation between them to allow complete recovery of the A_1 cell. Moreover, the frequencies were alternated in such a way that a high SRR, i.e. 20 Hz, was followed by a low one, 2 Hz, and the responses to the latter stimuli served as controls for the former. Keeping the intensity and duration of the pulses constant and changing only their repetition rate, we observed that, at the SRRs that evoke changes in the A_1-cell responses, the maximal number of action potentials per pulse and the minimal latency are attained in the response to the first stimulus, where there is no influence from responses to previous stimuli. In none of the 37 specimens studied was there a statistically significant (P<0.05) correlation between the maximal number of action potentials per pulse and SRR (Fig. 1B). The minimal latency is not affected by the SRR (Fig. 1B). The stability of the maximal numbers of action potentials per pulse and the minimal latencies demonstrates that the sensitivity of the A_1 cell does not change throughout the course of the experiment.

The above experiments on the influence of the SRR on the response of the A_1 cell do not allow detailed analysis of the changes evoked by successive stimuli in a given series. Thus, it is necessary to analyse each A_1 response of the stimulation series.

Single responses

Stimulation with 25 ms pulses at 2 Hz (5 % duty cycle) did not evoke a monotonic decrease in the number of action potentials per pulse, while higher SRRs did (Fig. 2A). In all the 37 experiments, this decrease seemed to follow an exponential function. To test this possibility, linear regression analyses were performed using the stimulus number as the independent variable for all the SRRs for the 14 specimens of *E. affinis*. The correlation coefficient was transformed to a z value in the regressions in which r^2 exceeded 0.50, and these values were compared by pairs for the following relationships: linear *versus* semilogarithmic; semilogarithmic with smoothing by weighted averaging of three successive values *versus* no smoothing; and semilogarithmic with smoothing every three *versus* every five successive data points. The results of the 150 comparisons made showed that the best goodness-of-fit was attained with the semilogarithmic condition with smoothing every three successive values of number of action potentials per pulse. This result demonstrates that the number of action potentials per pulse decreases exponentially with stimulus repetition. Thus, the rate of this response decrease, which demonstrates the habituation of the receptor cell, may be quantified by the theoretical straight-line slope obtained from linear regression analyses (Fig. 2B).

To determine whether the effect of the SRR on the number of action potentials per pulse was graded, we plotted the slope value of the theoretical straight line obtained from the linear regression analysis between the logarithm of the stimulus number and the number of action potentials per pulse, smoothed every three successive values, against the SRR

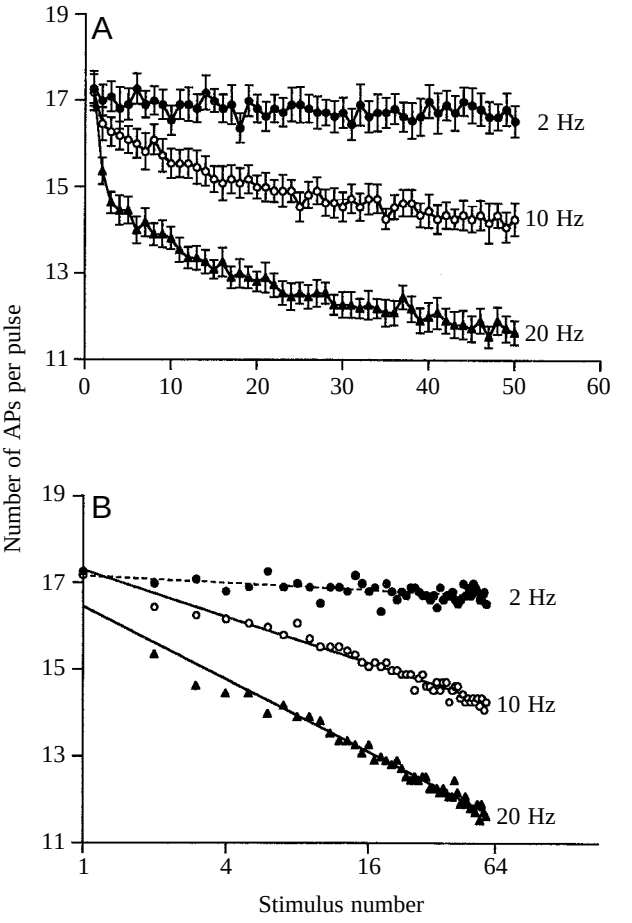

Fig. 2. Relationship between the number of action potentials (APs) per pulse in the A_1 cell and the stimulus number for 25 ms acoustic pulses at three repetition rates. Each data point corresponds to the mean value of 11 specimens in *Empyreuma affinis*. (A) Both axes are on a linear scale. The mean ± S.E.M. of the response to each stimulus at each repetition rate is shown. Note that the number of spikes per pulse in the response to the first stimulus is the same in each repetition rate series. (B) Semilogarithmic plot showing the data points and the theoretical straight lines of the corresponding linear regression analyses. The r^2 value for the 2 Hz series is 0.30, and the straight line (shown as a broken line) is drawn only for comparative purposes. The r^2 value for the 10 Hz series is 0.96, and the theoretical straight-line slope value, with the 95 % CI, is [−0.77 (0.04)]. The r^2 value for the 20 Hz series is 0.97, with a slope value of [−1.22 (0.06)]. The slope values are expressed as the number of action potentials per pulse per logarithmic unit of stimulus number.

(shown for *E. affinis* only in Fig. 3). Linear regression analyses between these two variables showed that in 13 of 14 *E.* affinis, 9 of 12 *Syntomieda epilais*, 5 of 6 *Spodoptera frugiperda* and 4 of 5 *M. jussiae* there was a statistically significant (P<0.05) relationship; thus, these results show that in 31 of 37 specimens the effect of SRR on the number of action potentials per pulse is graded.

An analysis of variance test of the regression coefficients of the responses from the four species to SRRs of 7, 10 and 20 Hz showed that they do not differ (F<1.3, N=37) in their decrease

in the response of the A_1 cell. The same test applied to the relationship between the slope value and SRR showed that there were no statistically significant differences (F=1.0, N=31) between these four species.

When single responses to repetitive stimuli above a certain SRR value are analysed, the latency shows a monotonic increase with a tendency to follow an exponential relationship (Fig. 4A). An analysis such as that described for the number of action potentials per pulse gave similar results: the latency increase may be quantified by the theoretical straight-line slope value of the linear regression analysis between the latency data, smoothed every three successive points, and the logarithm of the stimulus number (Fig. 4B).

Does the repetition rate affect all physiological features of single responses in the A_1 cell? Fig. 5 indicates that it does not, since the duration of each response does not show any monotonic change throughout the whole stimulation time, even at SRRs that evoke a significant change in other physiological features of the A_1 cell (compare Fig. 5B with Figs 2A and 4A).

Internal structure of the responses

We also analysed the fine temporal structure of single responses, for which the value of the first interspike interval (ISI) for each response was plotted against the stimulus number (Fig. 6A). For SRRs that do not evoke a decrease in receptor-cell response, there was no tendency for the first ISI to show a monotonic change with stimulus repetition; for SRRs that evoke habituation, there was an increase in the first ISI at higher stimulus numbers.

The increase in the ISI values follows an exponential relationship, the rate of which was quantified by the regression coefficient of the linear regression analysis between the ISI value and the logarithm of the stimulus number (Fig. 6B). The application of this method, with the ISI values smoothed every three successive data points, to the 20 Hz SRR series in the four species showed that r^2 is greater than 0.50 in 322 of the 336 analyses. Comparing the regression coefficients of the first and last ISI values in the responses by their 95 % confidence intervals (CIs), we found that, in 12 of 14 specimens of *E. affinis*, 11 of 12 *Syntomeida epilais*, 5 of 6 *Spodoptera frugiperda* and 4 of 5 *M. jussiae*, the rate of increase in the ISI with stimulus repetition was higher in the last one. In these experiments, we performed linear regression analyses between the ISI number during the response and its regression coefficient. In 10 of 12 experiments in *E. affinis*, 8

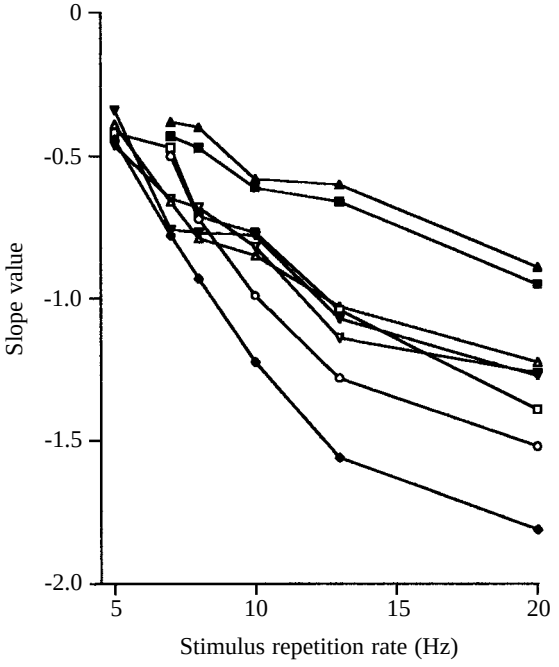

Fig. 3. Relationship between the slope value, expressed as the number of action potentials per pulse per logarithmic unit of stimulus number, and the stimulus repetition rate in eight specimens of *Empyreuma affinis*.

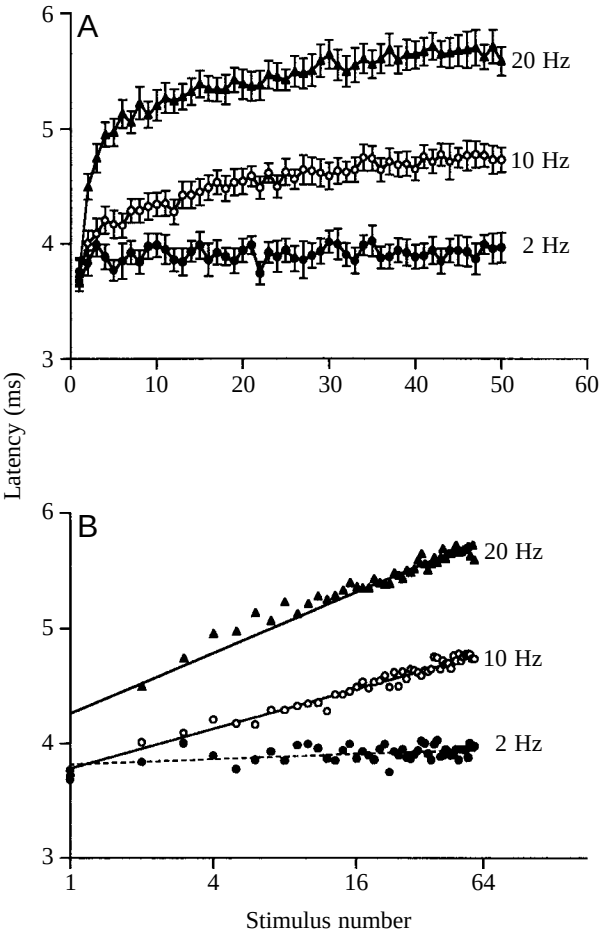

Fig. 4. Relationship between the stimulus number for 25 ms acoustic pulses at three repetition rates and the A_1-cell latency in the same 11 specimens of *Empyreuma affinis* as shown in Fig. 2. (A) The latency of the response increases monotonically with stimulus number, and the latencies of the responses to the first stimulus in each repetition rate series are very similar. (B) For the 2 Hz series, (r^2=0.17), the straight line (shown as a broken line) is given only for comparative purposes. For the 10 Hz series (r^2=97), the slope value, with 95 % CI, was [0.25 (0.02)]; for the 20 Hz series (r^2=0.92), the slope value was [0.38 (0.03)]. The slope values are expressed in milliseconds per logarithmic unit of stimulus number.

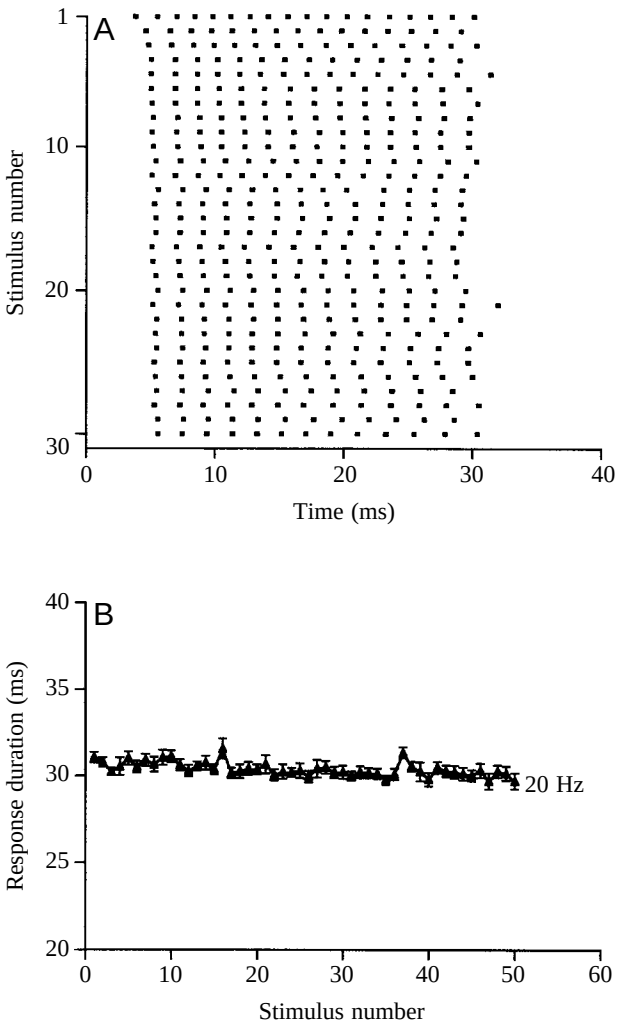

Fig. 5. Effect of the repetition rate of 25 ms pulses on the A_1-cell response duration. (A) Responses to the first 30 stimuli at 20 Hz in one experiment for *Empyreuma affinis*. Each row shows the response to an acoustic pulse, and each dot represents an A_1-cell spike. The decrease in the number of action potentials per pulse, from 18 to 12 action potentials per pulse in this specimen, evoked by stimulus repetition is not associated with a change in response duration, but with an increase in the value of the interspike intervals. (B) Relationship between A_1-cell response duration and stimulus number at 20 Hz; each point represents the mean ± S.E.M. obtained from the same 11 specimens of *E. affinis* used to illustrate Figs 2 and 4.

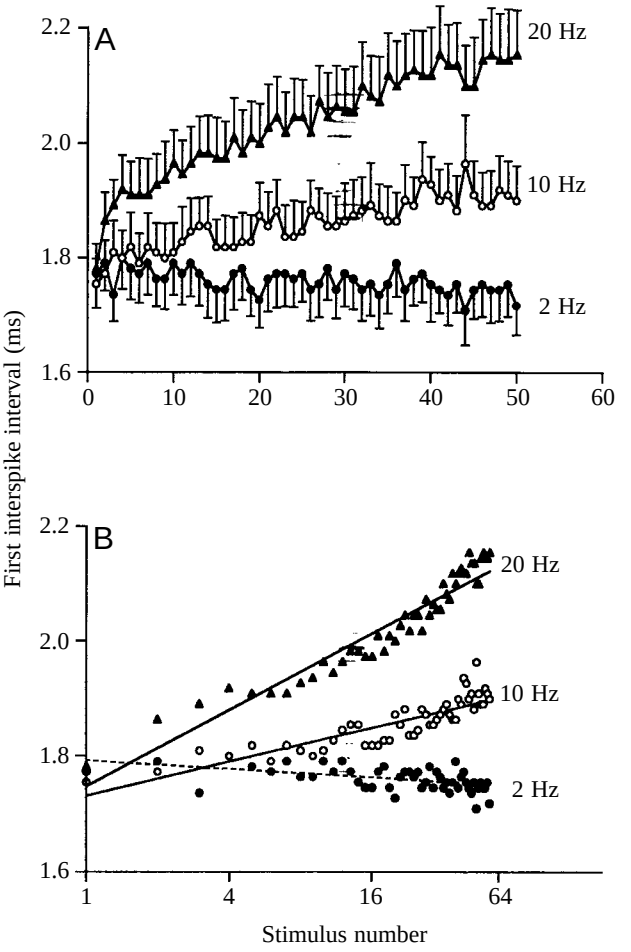

Fig. 6. Relationship between the value of the first interspike interval (ISI) in each response and the stimulus number at three repetition rates. Each data point corresponds to the mean value of 11 specimens of *Empyreuma affinis*. (A) Both axes are plotted on a linear scale. Mean values ± S.E.M. are plotted. Note that the value of the ISI is the same in response to the first stimulus in each repetition rate series. (B) Semilogarithmic plot showing the data points and the theoretical straight lines of the corresponding linear regression analyses. For the 2 Hz series (r^2=0.23), the straight line (shown as a broken line) is given only for comparative purposes. For the 10 Hz series (r^2=0.75), the slope value, with its 95 % CI, was [0.04 (0.01)]; for the 20 Hz series (r^2=0.91), the slope value was [0.10 (0.01)]. The slope values are given in milliseconds per logarithmic unit of stimulus number.

of 11 in *Syntomeida epilais* and 3 of 5 in *Spodoptera frugiperda*, r^2 was greater than 0.50, a result demonstrating that the stimulus repetition effects are stronger towards the end of the response. In addition, in *E. affinis* and *Syntomieda epilais*, we calculated the mean ± 1S.D. value of the determination and regression coefficients of these linear regression analyses. Both species showed the same r^2 value (0.78±0.14), while the regression coefficients were statistically different (Student's *t*-test, $P<0.05$): 0.79±0.22 for *E. affinis* and 1.74±0.48 for *Syntomeida epilais*. This result shows that, with 25 ms pulses at 20 Hz, the effect of the stimulus repetition rate on the increase in the ISI is greater as each response proceeds in *Syntomeida epilais* than in *E. affinis* (Fig. 7).

Habituation and adaptation

To analyse whether receptor cell habituation (changes in the responses due to stimulus repetition rate) and adaptation (the progressive decrease in instantaneous firing rate during the response to a stimulus of constant intensity) are two different physiological processes, we used 100 ms acoustic pulses presented at two different SRRs in each experiment: 4 or 5 Hz,

which evokes both phenomena; and 0.5 Hz, which produces only adaptation.

In each of the 27 specimens studied, adaptation was initially measured as the ratio between the minimal and maximal discharge frequency during the averaged response to the 20 stimuli presented at 0.5 Hz. This value, expressed as a percentage and subtracted from 100 %, showed the magnitude of the decrease in spike frequency during the response. In the 13 specimens of *E. affinis* studied, this decrease showed a median value of 31 % (range 26–43 %); in the eight experiments with *Syntomeida epilais*, its value was 37 % (range 30–41 %); and in six specimens of *Spodoptera frugiperda* its value was 34 % (range 32–40 %). In these same 27 specimens, habituation was measured by linear regression analyses between the number of action potentials per pulse and the logarithm of the stimulus number. The r^2 values of these analyses in the responses to the 4 Hz series were all greater than 0.55 (N=20), with the following means ± 1S.D.: *E. affinis*, 0.89±0.11; *Syntomeida epilais*, 0.94±0.02; and *Spodoptera frugiperda*, 0.93±0.06. These results show that this stimulation series evokes habituation in the A_1 cell of these species.

The next step was to quantify the adaptation rate of each response with the two SRRs used in such a way that they could be compared statistically. For this purpose, we used the method described by Coro *et al.* (1994), in which the adaptation rate is measured by the theoretical straight-line slope value obtained from the linear regression analysis between the instantaneous discharge frequency and the logarithm of time. The spike frequency was smoothed using the weighted average of every five successive values, and the analysis began at the maximal instantaneous discharge frequency and ended at the last ISI of each response. A comparison of the pairs of values of the regression coefficients of these analyses was made using their 95 % CIs. The results can be divided in three categories. (1) The adaptation rate of the responses to stimuli that evoke habituation and to those that do not evoke it does not differ significantly in 95 of 120 responses analysed from *Spodoptera frugiperda*, in 121 of 220 from *E. affinis* and in 38 of 160 from *Syntomeida epilais*. (2) The adaptation rate of the responses to stimuli that do not evoke habituation is faster than that to stimuli that do evoke it in 16 of 120 responses analysed from *Spodoptera frugiperda*, in 93 of 220 from *E. affinis* and in 121 of 160 from *Syntomeida epilais*. (3) The adaptation rate of the responses to stimuli that evoke habituation is faster than to stimuli that do not evoke it in 9 of 120 responses from *Spodoptera frugiperda*, in 8 of 220 from *E. affinis* and in 1 of 160 from *Syntomeida epilais*.

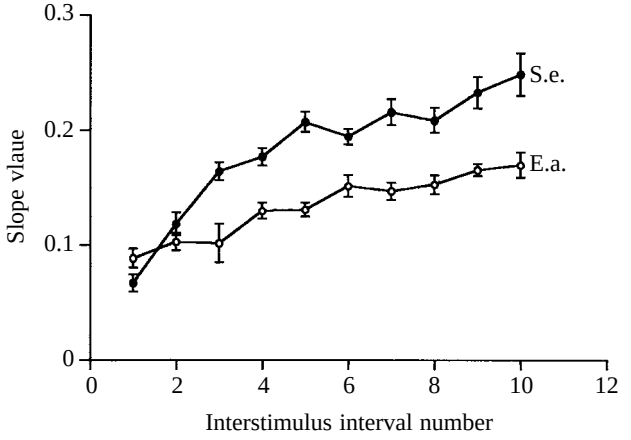

Fig. 7. Relationship between the slope of the increment in the interstimulus interval (ISI) duration of the A_1 cell and the ISI number in responses to 25 ms pulses at 20 Hz in *Empyreuma affinis* (E.a.) and *Syntomeida epilais* (S.e.). Each data point is the mean ± S.E.M. of the slope value from seven specimens of each species. The slope value is that of the theoretical straight line calculated from the linear regression analysis between the ISI values, smoothed by the weighted average of every three successive values, and the logarithm of the stimulus number.

We also analysed whether the changes that occur in the adaptation rate are associated with the stimulus number. If there is an association, the adaptation rates to the first stimulus in each of the stimulation series should not differ significantly, and from then on, there should be a significant difference in the adaptation rate in at least half the responses. Most importantly, these should occur in a systematic manner. Considering these restrictions, in the six specimens of *Spodoptera frugiperda* and in 10 of 11 experiments on *E. affinis*, there were no significant differences between the adaptation rates in the responses to the same stimulus number of each stimulation series. In contrast, in 7 of 8 specimens of *Syntomeida epilais* and in 1 of 11 specimens of *E. affinis*, the adaptation rate of the responses to the 4 Hz series is statistically significantly slower than that of the corresponding response to the 0.5 Hz series in more than 12 of the 20 responses analysed.

Finally, we pooled the adaptation rate values in the responses to each stimulus series from five specimens in each species (Fig. 8) and compared them using a Student's *t*-test. In the responses to all the stimuli in *Spodoptera frugiperda* and in 17 of 20 in *E. affinis*, there is no statistically significant difference (P<0.05) between the adaptation rate of the responses in the habituated state (4 Hz) and those that are not habituated (0.5 Hz). In *Syntomeida epilais*, however, starting from the response to the second stimulus, the adaptation rate in the responses to the 4 Hz series is significantly (P<0.05) slower.

In summary, these results show that, in the A_1 cell of these three noctuoid moths, habituation may not affect the adaptation rate of each response, as in *Spodoptera frugiperda* and in most specimens of *E. affinis*, or the adaptation rate may become slower in the presence of habituation as in the case of *Syntomeida epilais*.

Habituation and step-intensity stimulation

To analyse the possible effect of habituation on the response to a sudden increase in stimulus intensity, we used

100 ms pulses at 4 Hz presented every 16 stimuli at intensities which increased in 10 dB steps from threshold up to 30 dB above threshold. In the seven specimens of *E. affinis* tested, the A_1 cell in its habituated state showed an increase in its response, expressed as an increment in the number of action potentials per pulse (Fig. 9), and a decrease in its latency to the increase in stimulus intensity in 10 dB steps. It is also noteworthy that, at every new and higher stimulus intensity, habituation seems to start again, since there is always a decrement in the number of action potentials per pulse with stimulus repetition (Fig. 9) and an increase in the latency.

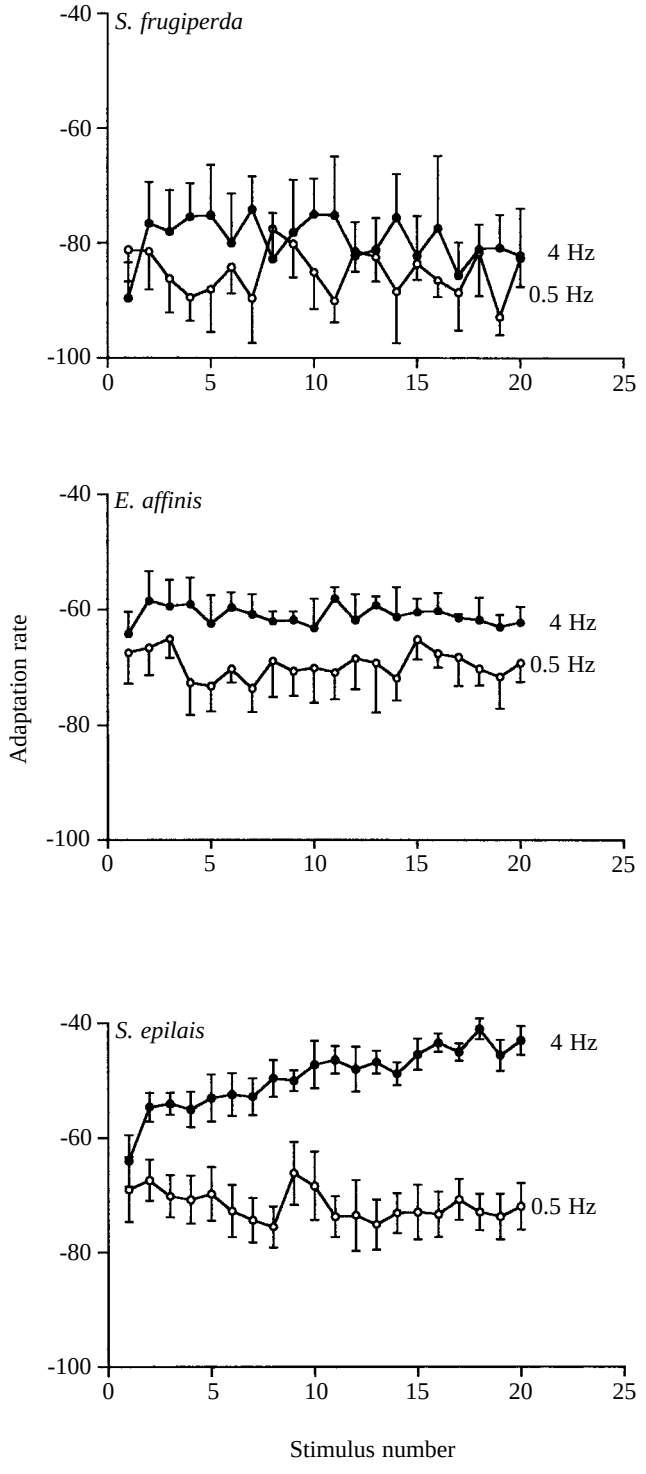

Fig. 8. A_1-cell adaptation rate in responses to 100 ms pulses at two different repetition rates: 0.5 Hz, which does not evoke receptor cell habituation; and 4 Hz, which evokes a decrease in response with stimulus repetition. The adaptation rate of each response was calculated from the slope of the theoretical straight line from the linear regression analysis between the instantaneous discharge frequency and the logarithm of the time elapsed since the beginning of the stimulus. The adaptation rate is expressed as the spike discharge frequency (action potentials per second) per logarithmic unit of time. Since adaptation implies a decrease in spike frequency with time, the adaptation rate has negative values. The spike frequency was smoothed using the weighted average of every five successive data points, and the analysis started at the maximal instantaneous discharge frequency and finished with the last interstimulus interval of each response. Each data point represents the mean ± S.E.M. of the responses in five specimens of each species.

Discussion

Features of the acoustic stimuli used

The duration (25 ms) of the acoustic pulses used to analyse the effects of different repetition rates on the responses of the A_1 cell in the noctuoid moth species studied is common to interspecific (bat–moth) and intraspecific (moth–moth) behavioural interactions. During their prey-searching phase, different insectivorous bat species are known to emit ultrasonic pulses with durations ranging from 5 to 100 ms (Miller, 1983; Neuweiler, 1984). Thus, 25 ms pulses at different repetition rates are stimuli that may be present at night, when both *Spodoptera frugiperda* and *M. jussiae* are known to be actively flying. Surlykke *et al.* (1988) showed that the A_1-cell integration time is 25 ms in *Agrotis segetum* and *Noctua pronuba* (Noctuidae). The integration time is the time beyond which the duration of the stimulus has little or no effect on threshold intensity. In some arctiid species that use sound communication during mating behaviour, the duration of the acoustic signals (=modulation cycles) emitted is between 18 and 30 ms: for *Pyrrharctia isabella* females it is approximately 32 ms (Krasnoff and Yager, 1988); for both sexes of *Syntomeida epilais* it is approximately 25 ms (Sanderford and Conner, 1990); for both sexes of *Euchaetes egle* it is approximately 30 ms (Simmons and Conner, 1996); and for females of *E. affinis*, it is approximately 18 ms (Sanderford *et al.* 1998).

An explanation of the term 'receptor cell habituation'

As mentioned in the Introduction, the changes evoked in mechanoreceptor cells by repetitive stimulation have been given various names by different authors. Here, we suggest a new term 'receptor cell habituation', to describe this phenomenon which we support by the following considerations.

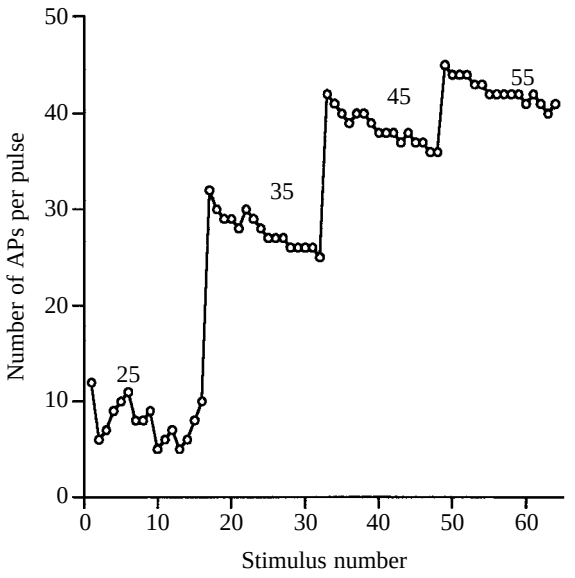

Fig. 9. Effect of step increases in stimulus intensity on a habituated receptor cell. The relationship between the number of A_1-cell spikes per pulse and the stimulus number in one experiment with *Empyreuma affinis* is shown. Acoustic stimuli consisted of 100 ms pulses at 4 Hz and at intensities between 25 and 55 dB SPL, which varied in 10 dB steps every 16 stimuli. The A_1-cell threshold response in this specimen is at 25 dB SPL. The A_1 cell habituated to a stimulus of a particular intensity and responded with an increase in the number of action potentials per pulse to stimuli of increased intensity.

First, we believe that the term adaptation should be used strictly to describe the changes evoked in the receptor cells by a single stimulus of constant intensity. This belief is based on the results obtained in the anteronotopleural bristle of *Drosophila melanogaster* (Corfas and Dudai, 1990*a*,*b*) and in the noctuoid A_1 auditory cell. Corfas and Dudai (1990*a*,*b*) showed that two *Drosophila melanogaster* mutants had alterations in their responses to repetitive stimuli, while their adaptation rate did not differ from that of the wild type with stimuli that do not evoke habituation. These authors also showed that memory mutations which impair the cyclic AMP cascade affect the responses to repetitive stimuli, but not the adaptation rate, and they suggested that adaptation and sensory fatigue, as they called the decrease in response evoked by stimulus repetition, were mediated by different mechanisms. Our results show that, in the A_1-cell responses to 100 ms acoustic pulses at 40–50 % duty cycle in all the specimens of *Spodoptera frugiperda* and in most specimens of *E. affinis*, the habituation phenomenon did not affect the adaptation rate, a result similar to that described for the *Drosophila melanogaster* mutant *rutabaga* (*rut*) (Fig. 5c in Corfas and Dudai, 1990*a*). In most specimens of *Syntomeida epilais*, however, the adaptation rate decreases with stimulus repetition. This result differs from those described by Corfas and Dudai (1990*a*) in the *Drosophila melanogaster* wild type and the mutant *dunce* (*dnc*), in which the firing frequency during each response decayed more rapidly when the stimuli were repetitive than when they were isolated. The data from the noctuoid A_1 auditory cell and those from the anteronotopleural bristle in wild-type and (*rut*) and (*dnc*) *Drosophila melanogaster* mutants show that habituation in these mechanoreceptor cells may be accompanied by no change, by a decrease or by an increase in the adaptation rate of each response to the repetitive stimuli. Considered as a whole, all these results, obtained from different biological species and different mechanoreceptor cells, indicate that receptor cell adaptation and habituation are two distinct phenomena.

Another term used for the changes evoked by repetitive stimuli in mechanoreceptor cells is fatigue, which is defined in Webster's dictionary as 'the temporary loss of power to respond induced in a sensory receptor by continued stimulation'. Our results with step-intensity stimulation are in agreement with those of Pasztor and Bush (1983) from the lobster oval organ in the sense that, even though habituation leads to diminished responses during repetitive stimulation, the receptor cells are still capable of responding to an increase in stimulus intensity. We consider that these results support the idea that the habituation phenomenon does not seem to be due to fatigue of the receptor cell.

The term sensory habituation has also been proposed for the response decrement evoked by repetitive stimuli in mechanoreceptor cells. The problem with this term is that it may also be used for the habituation phenomenon known to occur in higher-level neurones of the system. To avoid this problem, we propose the term receptor cell habituation to emphasise that the changes evoked by repetitive stimuli occur at the receptor cells, probably without any influence from the central nervous system (CNS). This proposal is based on the following considerations. The results of Corfas and Dudai (1990*a*) were obtained in fruit flies, in which the notum was completely separated from the CNS. Engel and Wu (1994) worked with decapitated electrophysiological preparations of other *Drosophila melanogaster* mutants that also showed changes in a mechanoreceptor cell with repetitive stimulation. They state that it is likely that the effect described in the mutant is due to action at the mechanoreceptor cell rather than feedback from the CNS. In corroboration, in many preparations of the four moth species studied, we destroyed the entire thoracic nervous system, and the habituation phenomenon was still observed. In the remainder of the preparations, the alar nerve was sectioned from its central connections, so that, although the CNS was maintained intact, it had no direct contact with the tympanic organ from which the A_1-cell spikes were recorded. In all the noctuoid moth preparations, there was A_1-cell habituation at an appropriate repetition rate, which depended on stimulus duration.

Comparison of A_1-cell habituation with that in other mechanoreceptors

The results obtained by other authors in different mechanoreceptor cells suggest that the decrease in the number of spikes per pulse (Fig. 5 in Esch *et al.* 1980; Fig. 1 in Sippel and Breckow, 1984; Figs 6–9 in Corfas and Dudai, 1990*a*) and

the increase in the latency (Fig. 5 in Esch *et al.* 1980) produced by repetitive stimulation follow an exponential relationship. We have now demonstrated that the variations in these physiological features in the A_1-cell responses in four noctuoid moths also follow an exponential relationship. This result allows us to quantify, and thus to compare, the habituation phenomenon in different receptor cells using the theoretical straight-line slope value obtained from the linear regression analyses between the logarithm of the stimulus number and the value of the physiological feature under study. This quantitative approach is similar to that proposed by Chapman and Smith (1963) to describe the adaptation phenomenon and which has been used by other authors (Corfas and Dudai, 1990*a*; Engel and Wu, 1994; Coro *et al.* 1994) to compare the adaptation rate of the receptor cell under different circumstances.

We have demonstrated quantitatively the graded character of habituation of the A_1 cell in the four noctuoid species studied with duty cycles of up to 50 %, a feature suggested previously in other mechanoreceptor cells (Fig. 1 in Sippel and Breckow, 1984; Fig. 9 in Corfas and Dudai, 1990*a*). This is a point to consider when describing receptor cell habituation, since the *Drosophila melanogaster* mutant *dnc*, characterized by reduced cyclic AMP hydrolysis, showed saturation of habituation at a 20 % duty cycle, while the wild type maintained the graded character of this phenomenon up to a 40 % duty cycle.

Although there are common features in the receptor cell habituation phenomenon described in different mechanoreceptors, there are other characteristics in which they differ. One of these characteristics is the effect of stimulus repetition on the temporal pattern of the spike discharge from the receptor. In the lobster oval organ (Pasztor and Bush, 1983), spiking often ceased altogether during repetitive stimuli; thus, there is complete habituation. In the anteronotopleural bristle of *Drosophila melanogaster*, Corfas and Dudai (1990*a*) describe how, in many cases, neurones that responded to the first stimulus of the series with a long burst of spikes, responded after several stimuli with a short burst and then remained silent for the rest of the stimulus. Thus, there is a change from a phasic–tonic pattern to a purely phasic response. In the A_1 cell of the four noctuoid species studied, the repetitive stimuli, even at a 50 % duty cycle, do not evoke a decrease in the response duration, which maintains its tonic pattern throughout the stimulation series. These differences could be explained either by differences in the physiological features of these cells or by the stimulus duration, which was different in each case, although the maximal duty cycle used was similar in all of them.

Is there a behavioural correlate to A_1-cell habituation?

Habituation of the A_1 cell in the four noctuoid species studied shows two features that accord with the parametric characteristics proposed by Thompson and Spencer (1966) to define behavioural habituation. These are (1) a decrease in the response to repetitive stimulation (habituation) is usually a negative exponential function of the number of stimulus presentations; and (2) the more rapid the frequency of stimulation, the more rapid and/or more pronounced is habituation. These authors state, however, that strong stimuli may yield no significant habituation. All the results of A_1-cell habituation have been obtained at intensities 20–30 dB above threshold, which represents a rather strong stimulus. Judging only by these features, it is difficult to correlate habituation directly at the behavioural and receptor cell levels.

The behavioural results described by Fullard (1984) in *Cycnia tenera* (Arctiidae) show that the optimal rates for evoking a behavioural response (acoustic emission to a bat-like stimulus) are between 30 and 50 Hz for 5 ms pulses. The electrophysiological results described in this same species, in which the number of nerve impulses elicited per pulse decreases as the stimulus repetition rate increases, including at the optimal rate values obtained for the behavioural responses, suggest that the receptor cell phenomenon is not necessarily correlated with behavioural habituation. In morphologically identified thoracic auditory interneurons in noctuid moths, Boyan and Fullard (1986) describe little decrement (habituation) of the response over the range of stimulus rates tested, which was between 2 and 20 Hz for 10 ms pulses, i.e. from a 2 % to a 20 % duty cycle. This result may mean that, although the A_1 cell in noctuoid moths shows habituation, the auditory receptor response to repetitive stimuli is great enough to ensure that second-order neurones of the system show no significant decrements in their responses to repetitive stimuli.

Of the four noctuoid species studied, two, *Syntomeida epilais* (Sanderford and Conner, 1990) and *E. affinis* (Sanderford *et al.* 1998), use acoustic communication during mating behaviour, while the other two, *Spodoptera frugiperda* (Pérez *et al.* 1988) and *M. jussiae* (F. Coro and A. Barro, unpublished results), do not. Although the auditory systems of these four species participate in different behavioural interactions, their A_1-cell habituation shows more similarities than differences. In fact, the differences that are observed are between the two species that use acoustic signals for intraspecific communication, which may indicate that these species have a more specialised auditory system, starting from their receptor cells, than those species using this sensory system as an antipredatory device.

Finally, if in noctuoid moths, as in *Galleria mellonella* (Pyralidoidea, Galleriidae), an intensity increment of 10 dB evokes dishabituation in a behaviour pattern elicited by an acoustic stimulus (Skals and Surlykke, 1995), this result could be explained by the fact that the A_1 cell in the habituated state is still capable of responding to a 10 dB step increase in stimulus intensity. Of course, all these speculations would have to be tested at different levels of the moth auditory system. In any case, the fact that the A_1 auditory receptor, at least in the four noctuoid moth species studied, shows receptor cell habituation has to be kept in mind when describing the responses of auditory interneurones to repetitive acoustic stimuli.

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