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Energy detection and temporal integration in the noctuid A1 auditory receptor

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Abstract The temporal integration of the A1 auditory receptor of two species of noctuid moths (*Lepidoptera, Noctuidae*) was investigated. Tympanal nerve spikes were recorded while stimulating the ear with broad band clicks. Thresholds were measured for single clicks, pairs of clicks with a separation of 1–20 ms, and trains of up to 8 clicks at separations of 1–2 ms. The average threshold for single clicks was 52.9 dB peSPL (SD 1.7 dB, $n = 40$) for *Noctua pronuba* and 50.1 dB peSPL (SD 4.0 dB, $n = 27$) for *Spodoptera littoralis*.
The thresholds for double clicks with a 1 ms separation were lower than the thresholds for single clicks. The difference decreased as the separation between the clicks was increased. The results were fully consistent with an energy detector model (a leaky integrator with an exponential decay) with a time constant of about 4 ms.
The results are compared to previously published results with pure tone intensity/duration trading. A common underlying mechanism is suggested, based on the passive electric properties of the receptor cell membrane.
It is suggested, that the time constant revealed in the present study characterizes auditory receptors in general, and is related to the short time constants in vertebrate audition.

Key words Insect hearing · Noctuid moth · A1-sensory cell · Leaky integrator · Membrane time constant

Abbreviations *peSPL* peak equivalent sound pressure level · *SD* standard deviation · τ time constant

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Introduction

The threshold for short acoustic signals decreases with increasing duration of the stimulus. This is traditionally ascribed to a temporal integration of the stimulus and has been demonstrated repeatedly in various vertebrates both in psychophysical studies, e.g. in humans (Garner and Miller 1947; Plomp and Bouman 1959), cats (Costalupes 1983), bats (Schmidt and Thaller 1994) and dolphins (Johnson 1968), at lower levels in the auditory pathway as in the 8th nerve in frogs (Dunia and Narins 1989), and in the saccular nerve of goldfish (Fay and Coombs 1983). Several of these studies indicate, that for stimulus durations shorter than a certain value (the integration time), the product of duration and intensity at threshold remains constant (intensity/duration trading) indicating constant threshold energy. The integration time measured this way is around 200 ms for humans (Plomp and Bouman 1959) and 40–200 ms for bottlenosed dolphin (Johnson 1968).

Much shorter time constants of 2–20 ms, however, are found in other types of experiments, eg. gap detection, amplitude modulation and forward and backward masking. This inconsistency has been described as the resolution-integration paradox (de Boer 1985).

A similar paradox is found when comparing intensity/duration trading data with results from experiments with pairs of very short stimuli presented with small intervals (e.g. Au et al. 1988 and Viemeister and Wakefield 1991). These studies revealed integration times of 264 μs for bottlenosed dolphins and around 5 ms for humans, respectively, considerably smaller than the time constants from the duration/intensity trade experiments.

All data on auditory temporal integration in vertebrates have been obtained from higher neural levels than the receptors (the hair cells). Since the basic limitation on any sensory system lies in the receptors, an understanding of temporal resolution and integration

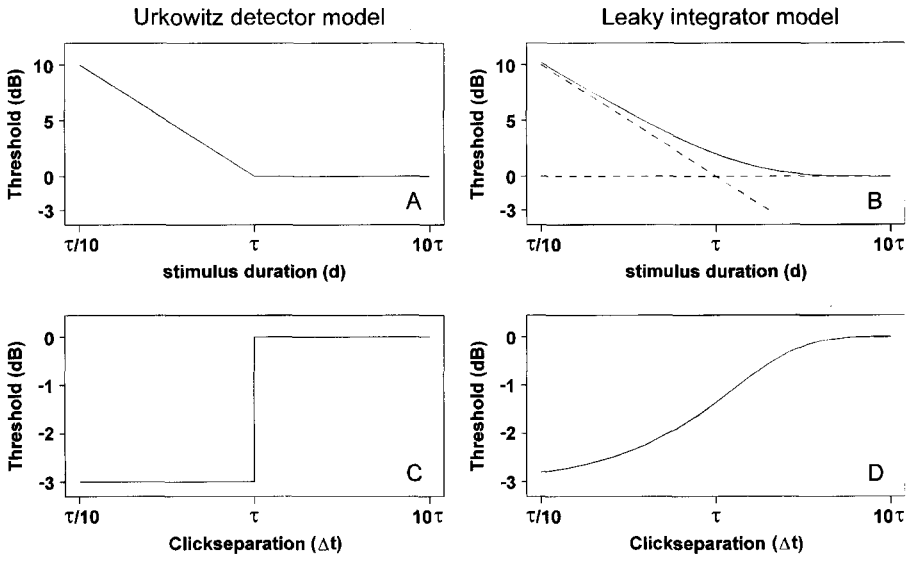

Fig. 1A–D Changes in threshold predicted by the Urkowitz detector model **(A, C)** and the leaky integrator model with an exponential decay **(B, D)**, both with a time constant τ. **A–B** are predictions of the intensity duration trading paradigm and **C–D** of the double click paradigm. Duration of the clicks $\ll \tau$. It is only in the region around τ, that the two models differ significantly in their predictions

Table 1 Predicted threshold changes by the two models in the two different experimental paradigms. Compiled from Plomp and Bouman (1959), Zwislocki (1960) and Au (1988)

	Urkowitz detector model	Leaky integrator model
Dur/int. trade	Threshold $= -10 \log(d/\tau)$ for $d \leq \tau$ Threshold $= 0$ for $d > \tau$	Threshold $= -10 \log(1 - e^{-d/\tau})$
Double click	Threshold $= -3$ for $\Delta t \leq \tau$ Threshold $= 0$ for $\Delta t > \tau$	Threshold $= -10 \log(e^{-\Delta t/\tau} + 1)$

at this level would provide important information for differentiating between the different models and for understanding the phenomena involved. Thus, a study of the temporal integration in the simple primary auditory receptor of the moth ear was conducted.

Temporal integration in an auditory receptor has been studied in noctuid moths (Lepidoptera, Noctuidae) by Adams (1971) and Surlykke et al. (1988). The auditory system of moths presumably evolved in response to the predation from microchiropteran bats (see Hoy 1992 for a review). Each ear consists of an air-filled chamber and a tympanic membrane to which two sensory cells are attached, the A1 and A2 cell. A third cell, the B cell, is not attached directly to the tympanic membrane and seems not to be involved in the reception of sound, although the function is still unclear (Yack 1992). The A1 and A2 cells are broadly tuned to ultrasonic frequencies, being most sensitive around 30 kHz. The A1 cell is the most sensitive (Roeder 1964) and the threshold is approx. 20 dB lower than the threshold of the A2 cell throughout the biological relevant range (5–100 kHz, Roeder and Treat 1957; Surlykke and Miller 1982).

Surlykke et al. (1988) measured the threshold of the A1 cell for pure tone stimuli of different durations. The threshold decreased by 2.5 dB per doubling of stimulus duration in the range 0.2–25 ms and was constant for longer durations indicating an integration time of the moth ear A1 receptor cell of 25 ms.

They (Surlykke et al. 1988) invoked an energy detector model with a time window of the duration τ to explain their results. The detector responds if the energy received during the period τ exceeds a certain threshold. With this type of detector (sometimes referred to as an Urkowitz detector, Au 1988), the threshold will decrease with 3 dB per doubling of stimulus duration (-10 dB per decade) for durations below τ and remain constant for durations larger than τ (Fig. 1A and Table 1). However, does the slope of -2.5 dB per doubling of duration found in the moth ear indicate a deviation from the Urkowitz detector model, thus suggesting that another model is more appropriate?

Several other models have been suggested to describe temporal integration and a characteristic feature of all models is their descriptive nature and this lack of mechanisms led de Boer to describe the models as ad hoc models (de Boer 1985). The model most relevant for the present study is the leaky integrator model with an exponential decay (Plomp and Bouman 1959; Zwislocki 1960). The predictions of the leaky integrator model with respect to duration/intensity trade experiments are outlined in Fig. 1B and Table 1.

In order to distinguish between the two models for energy detection in the receptor, and to investigate

whether different experimental paradigms would give different estimates of the integration time, also at this level of the auditory pathway, a double click experiment was performed on the noctuid A1 receptor.

The double-click experiment is another method to study temporal integration, and the predictions of the Urkowitz detector model and the leaky integrator model with respect to double click stimulations are outlined in Fig. 1C–D and Table 1. The threshold of an energy detector for short clicks will depend on the number of clicks and the interclick interval. Hence, for an Urkowitz detector, a 3 dB decrease in threshold is expected if two clicks arrive at the detector within the integration time τ (Fig. 1C). The threshold improvement of the leaky integrator is nearly 3 dB for very short interclick intervals (separation $\ll \tau$), gradually decreasing to 0 dB as the separation is increased beyond τ (Fig. 1D). Because of this small threshold improvement, the double click paradigm is experimentally more difficult than intensity/duration trading experiments, since thresholds must be determined with a higher degree of accuracy.

Comparing the thresholds for single clicks with thresholds for double clicks is also not trivial. Double click stimuli are likely to have a lower threshold than single click stimuli, since the joint probability of detecting either of the two clicks is larger than the probability of detecting a single click, assuming that the detection of each of the clicks are independent events. This has been demonstrated in humans, where the double click threshold was found to be more than 1 dB lower than the single click threshold, even at interclick intervals of 200 ms (Viemeister and Wakefield 1991). Thus, great care must be taken, when selecting a threshold criterion. The criterion used has to be very robust to random fluctuations in activity in order to measure thresholds with the desired accuracy and at the same time it must be possible to either avoid the statistical artefact mentioned above or at least to assess its importance.

Materials and methods

Preparation

Noctuid moths (*Spodoptera littoralis* and *Noctua pronuba*) were used as experimental animals. They were either caught in light traps (*N. pronuba*) or obtained as pupae (*S. littoralis*) from L. Øgaard, Copenhagen. The animals were kept at 6°C (*N. pronuba*) and 12°C (*S. littoralis*) and were offered a glucose solution *ad libitum*. Experiments were conducted at room temperature (approx. 23°C). The preparation equilibrated to the air temperature during the dissection, which normally lasted 15 min or more. Cold light was used as light source during dissection.

After removal of the head, legs and wings, the moth was fixed with an insect needle on top of a small holder. In a dorsal dissection, the notum was removed together with the flight muscles and tracheas covering the central ganglia. The junction between the auditory nerve, IIIN1b (nerve nomenclature following Nüesch (1957)) and the larger IIIN1-nerve was located, and the nerve IIIN1 cut proximal and distal to the junction with IIIN1b. The proximal cut end of the IIIN1 nerve could then be sucked into a shielded and Ringer-filled glass pipette with a Ag/AgCl wire inside. An indifferent reference electrode (Ag/AgCl) was placed either in the abdomen or in the remaining flight muscles.

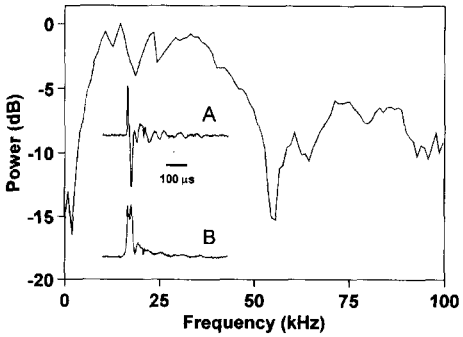

Fig. 2 Spectrum of stimulus. *Insert* shows the waveform of the stimulus (*A*) and the envelope (*B*). The envelope is constructed from a Hilbert-transform of the signal

The level of Ringer solution (the standard insect type of the laboratory) in the thorax of the preparation was adjusted such that the tip of the electrode was just above the surface.

The nerve signal was amplified (Grass P15), band-pass filtered (Krohn-Hite 3550) and fed into a PC-based AD-converter (Data Translation DT2801-A, 12 bit, 10 kHz sampling rate).

Stimulation

The stimulus consisted of broad-band transient clicks produced by presenting sawtooth-like pulses through the loudspeaker. The duration of the click was 35 µs (measured as − 3 dB points on the envelope of the signal, Fig. 2). Rectangular pulses, which generates a two-transient signal were used in the two initial preparations. This signal was longer (100 µs), but the overall bandwidth was the same as for the signal used in all later preparations. The results from sessions using this two-transient signal are considered to be fully comparable to the subsequently obtained results.

The signal intensity was set by a digitally controlled attenuator. The output from the attenuator was fed to a power amplifier (custom build) driving an Kuhl, Schodder and Schroeder type electrostatic loudspeaker (Kuhl et al. 1954, 15 mm Ø, 200 V polarisation voltage, designed by Lee Miller, Odense University), located 40 cm from the preparation. Sound pressures were measured using a B&K 1/4″ microphone (4135, with protection grid on) and a B&K 2606 measuring amplifier connected to an oscilloscope (HP54600A). The microphone was placed at the position of the preparation with the preparation holder in place. The sound pressures were referenced to a standard sound source (B&K 4320) and are given in dB peSPL (which is the rms sound pressure level of the continuous tone having the same amplitude as the transient, Stapells et al. 1982). The experiments were conducted inside an anechoic room (100 m^3, Kremer principle, Reflection coeff. < 0.1 in the range 0.5–100 kHz). No significant reflections were measured at the location of the preparation (i.e. intensity of reflections were less than − 10 dB re. to the stimulus intensity).

Recording

The A/D converter recorded 102 ms long time series of spike activity around the stimulation, beginning 51 ms before stimulus arrival at

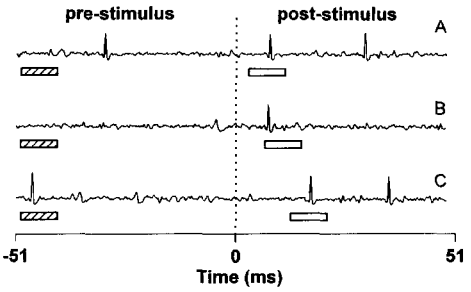

Fig. 3A–C Time windows used for evaluating the response. **A** Windows used for single clicks and double clicks with a 1–2 ms separation. **B** Windows used for 5 ms separation. **C** Windows used for 10 ms separation. *Right bars* (*open*) indicate windows used for evaluating the response, *left bars* (*hatched*) for evaluating the spontaneous (pre-stimulus) activity

the preparation (Fig. 3). The jitter in the timing of the stimulus relative to the beginning of recording was less than 100 μs (i.e. less than one period of the sampling frequency). An on-line peak detection algorithm, which located all peaks in the recording above a preset level, was used to record the spike time-of-occurrence. The experimental data are thus reduced to sets of time-of-occurrences. In preparations where the difference in amplitude between A1 and B-cell spikes was sufficient, the B-cell spikes were excluded by a window discriminating algorithm. The activity of the B-cell was always low and not influenced by the stimulus.

Experimental protocol

For each preparation, a series of experiments (sessions) was performed with different intervals between the clicks. A PC generated the stimulus, controlled stimulus level and recorded the spike response automatically during each session.

An approximate threshold was obtained using an automatic up/down algorithm (see eg. Guilford 1954) in order to locate the intensity range of interest. This was done prior to each data collection session in order to compensate for changes in the threshold during the experiments (which lasted up to several hours). The data thus obtained were not included in the analyses.

The data collection sessions used a method of constant stimuli (e.g. Guilford 1954). Fifty stimulations were repeated at 18 different sound levels for both a single click stimulus and a double click stimulus (conf. Fig. 4). The different sound levels were distributed with approx. one dB intervals symmetrical around the initial threshold estimate. The attenuator was calibrated and sound pressures corrected accordingly. Relative sound pressures are accurate within 0.1 dB. To minimize the effect of fluctuations in the threshold during a session, which took about 15 min. to complete, the stimulations were separated in 90 blocks of 20 stimuli, sound level being constant within each block and the stimulations alternating between single and double clicks. There were consequently five separate blocks at each of the 18 sound levels and these blocks were presented in random order. A session thus consisted of 900 single and 900 double click stimulations. By this method it is possible to measure the difference in thresholds between single and double clicks quite accurately. Since both single and double click thresholds are measured simultaneously, any changes in receptor sensitivity or spontaneous activity during the session affect both threshold measurements in the same way and the fluctuations are thus not reflected in the final threshold difference.

The repetition rate was four stimulations per second. Two subsequent stimulations were thus separated by between 230 and 250 ms and nothing in the data indicated that an adaptation, which could interfere with the response to the subsequent stimulation, occurred.

Six sessions with multiple click stimuli consisting of up to 8 clicks of equal amplitude and interclick interval were also conducted.

Off-line analysis

Every recording (i.e. each 102 ms time segment) was categorized as to whether the receptor responded to the stimulation or not. A response of the receptor was defined as one spike or more following the stimulation. When evaluating the response to single clicks a 10 ms time window starting 4 ms after the time of stimulus arrival at the tympanal membrane was used (Fig. 3A). The size of the window was selected to be large enough to cover the range of latencies of the first elicited spike and short enough to minimize the influence of spontaneous activity.

When stimulation consisted of two clicks separated with 2 ms or less, the response to each of the two clicks merged completely. A 10 ms window as used for single click stimulations, was then used (Fig. 3A). With a click separation of 5 ms or greater a 10 ms time window that began 4 ms after the arrival of the second click to the tympanic membrane was used (conf. Fig. 3B–C). By using this window mainly the response of the receptor to the second click of the pair is recorded, while the response to the first click is excluded, at least at low stimulus intensities, where only one spike is elicited by each click. Thus, for each of the 18 different sound levels, the response is expressed as percent of the stimulations resulting in at least one spike.

The spontaneous activity of the receptor was evaluated by using a similar time window of 10 ms starting 50 ms prior to stimulation (Fig. 3A–C).

The percent of stimulations resulting in a response was plotted against stimulus intensity. This was done separately for each session. A cumulated Gaussian distribution was then fitted to the data using a least squares method. The mean spontaneous activity was set as lower limit and 100% response as the upper limit. The mean of the best fitting Gaussian distribution was defined as the threshold. This value corresponds to the stimulus intensity eliciting a response to 50% of the stimulations, when spontaneous activity is subtracted.

The results from experiments in which more than two clicks were used as stimulus were treated in the same way as described above for up to 4 clicks per stimulus, i.e. they were evaluated using a time window of 4–14 ms. At 8 clicks per stimulus a window of either 4–19 ms (1 ms separation between clicks) or 4–24 ms (2 ms separation) was used.

Data from sessions in which the receptor did not respond to at least 96% of the stimulations (48 out of 50) at the highest sound level of the 18 used were excluded from further analysis. Of the 112 sessions conducted, 46 were discarded in this way. The most common reasons for discharding sessions were either poor or fluctuating signal to noise ratios or contamination of the signal with myopotentials from the flight muscles. This strict procedure was required in order to reduce variability to levels, where thresholds could be measured with the required accuracy.

Results

Spike activity following stimulation

An example of the response of the A1 cell to single click stimulations is shown in Fig. 4A. At sound levels around the threshold, the receptor responds only to a fraction of the 50 stimulations and the timing of the response relative to the stimulus is not very precise. At

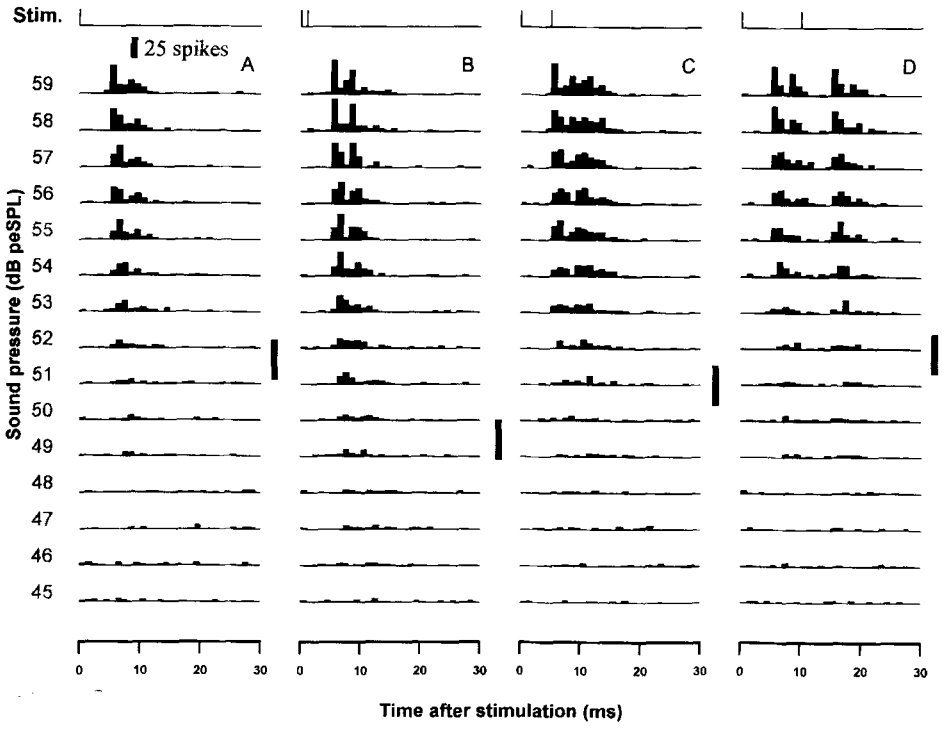

Fig. 4A–D Representative examples of spike activity of the A1 receptor of a *N. pronuba* preparation following stimulation. Each histogram is the summed activity of 50 stimulations distributed in 1 ms bins, at the particular sound level. Time is referenced to the stimulus arrival time at the tympanic membrane. Stimuli are indicated by *vertical lines* at the top. **A** Response to single clicks, **B** double clicks with 1 ms separation, **C** 5 ms and **D** 10 ms separation. Since thresholds differ somewhat between sessions, the sessions cannot be compared at a very detailed level in these histograms. Sound pressures in this figure are rounded to nearest dB. The actual steps in sound pressures, which are given by the digital attenuator are not exactly 1 dB. The 50% threshold determined in the sessions thus cannot be indicated precisely on the figure and are only indicated approximately as vertical bars. The lowest sound levels are omitted

higher intensities, the receptor responds with 1–2 spikes almost every time it is stimulated and the latency time for the first spike is rather constant at 5 ms. When two clicks separated by 1 ms are used (Fig. 4B) the receptor starts responding at a sound level 2–3 dB lower than for single clicks and the overall activity is higher. It is not possible to separate the response to either of the two clicks of the stimulus.

The response to two clicks separated by 5 ms (Fig. 4C) is transitional to the response to clicks with a 10 ms separation (Fig. 4D), where the response is clearly divided into a response to the first click and a response to the second click. The response patterns to each of the two clicks separated by 10 ms are very similar and both resemble the response to single clicks.

The data from sessions with either a 2 ms or 20 ms separation between clicks follow the same patterns as the data from sessions with 1 ms and 10 ms separation respectively (not shown).

Response functions

In Fig. 5A–D the percentage of stimulations resulting in at least one spike at different stimulus levels are shown for four different sessions. The response functions are well described by cumulated Gaussian distributions ($r^2 \geq 0.95$ in 125 of a total 134 threshold determinations). The average threshold for single clicks was 52.9 dB peSPL (SD 1.7 dB, $n = 40$, 5 individuals) for *N. pronuba* and 50.1 dB peSPL (SD 4.0 dB, $n = 27$, 3 individuals) for *S. littoralis*. The threshold for single clicks varied little within the same preparation. Average SD on means of individual preparations was 0.5 dB. This indicates a high stability of the preparation and a robust threshold criterion, allowing threshold determination to within a fraction of a dB.

When the stimulus consisted of pairs of clicks separated by 1 or 2 ms, the shape of the response functions generally remains unchanged, but the curve moved to the left, corresponding to a drop in the threshold by between 2 and 3 dB (Fig. 5A–B).

With a 5 ms separation between clicks the threshold is seen to be approx. 1 dB lower relative to the single click stimulus (Fig. 5C). To test, whether this lower threshold was in fact caused by more spikes being elicited by the second click and not due to "late" spikes elicited by the first click, but occurring with a latency time longer than 9 ms, the response to single clicks was also evaluated using the same 9–19 ms window

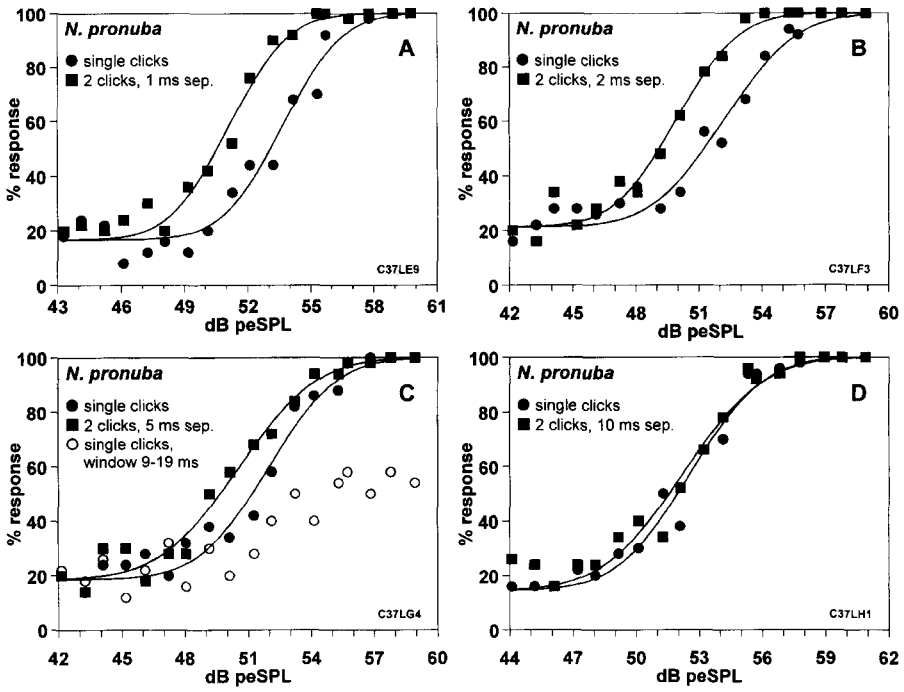

Fig. 5A–D Representative response functions. Each function is based on a single session, i.e. 50 stimulations at each of the 18 different sound levels, using both single clicks (●) and double clicks (■) as stimuli. *Open circles* in **C** represent the response to single clicks evaluated with a 9–19 ms time window (see text). Solid lines represent cumulated Gaussian distributions fitted to the data

(Fig. 5C, open circles). This shows, that only at stimulus levels above the threshold for single clicks does the first click elicit spikes at a time late enough to interfere with the evaluation of the response to the second click of the pair and these spikes will thus not affect the determination of the double click threshold.

At separations between clicks of 10 and 20 ms the response to each of the clicks can be compared directly, since the responses are well separated in time. The response functions to single clicks and to the second click in the pairs appear identical and with equal threshold (Fig. 5D).

Comparison of data and models

The average shift in threshold for double clicks at a separation of 1 ms, relative to single clicks was − 2.5 dB (SD 0.4 dB) for *S. littoralis* and − 2.3 dB (SD 0.3 dB) for *N. pronuba*, decreasing to zero as separation is increased (Fig. 6). The exponential integrator model was fitted to the data using a least squares method and the best fits were obtained with time constants (τ) of 4.1 ms for *S. littoralis* ($r^2 = 0.96$) and 3.4 ms for *N. pronuba* ($r^2 = 0.96$). The Urkowitz detector model produced the best fits with $\tau = 5$ ms ($r^2 = 0.79$ and $r^2 = 0.83$ respectively).

The results of sessions using more than two clicks per stimulation are shown in Fig. 7 together with the predictions of the two integrator models using the time constants found above.

Discussion

Stimulus energy as the threshold determining parameter

The present data show that the auditory threshold of the A1 receptor as determined by click stimuli depend on the number of clicks and their temporal separation. This suggests that the hearing organ integrates the stimulus with time, and thus stimulus energy and not intensity is the threshold determining factor for short stimuli. The average threshold for single clicks is somewhat higher than thresholds reported for pure tone stimuli, which are in the range of 30–45 dB SPL (e.g. Roeder and Treat 1957; Surlykke and Miller 1982; Pérez and Coro 1984), but this is to be expected due to the very short duration of the stimulus (the threshold for a 5 ms stimulus is 19 dB lower than the threshold for a 35 µs stimulus, assuming a leaky integrator with a 4 ms time constant). The threshold of the receptor in an actively flying moth is likely to be somewhat lower, since it is greatly influenced by the temperature, which may reach over 30°C in the thorax of a flying animal (Coro and Pérez 1990). However, this should not affect the interpretation of the present data, since the models operate on relative threshold changes, which are likely to be affected to the same degree by changes in temperature.

From the 5 ms separation data (Fig. 5C) it is evident that the approx. 1 dB lower threshold for double clicks is caused by a temporal integration of the stimulus. At

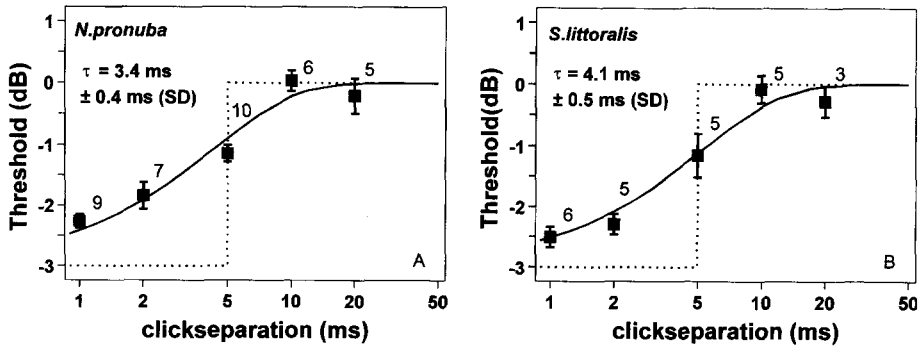

Fig. 6A–B Change in thresholds (± S.D.) for double clicks at different separations in *N. pronuba* and *S. littoralis*. Numbers at datapoints indicate number of sessions at that particular separation. *Solid lines* represent the best fit of the exponential model to the data, *dotted line* the predictions of a Urkowitz detector model with a 5 ms time constant

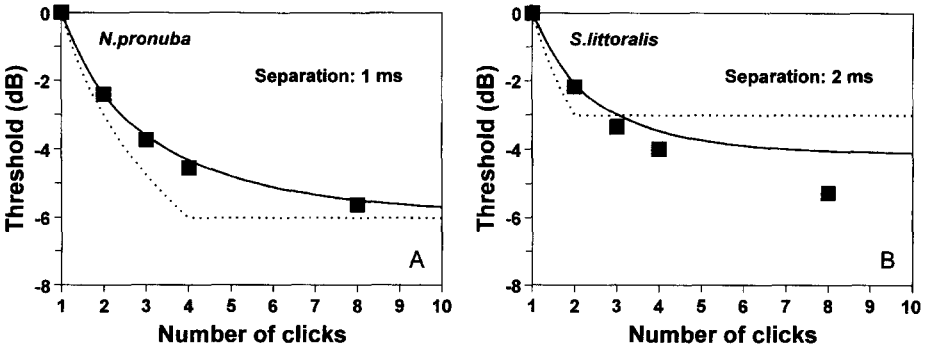

Fig. 7A–B Change in thresholds for different number of clicks in *N. pronuba* and *S. littoralis*. Data for two clicks are means of all sessions with the particular individual, all other points represent one session each. *Solid lines* represent the predicted shifts in thresholds from the exponential model using the time constant found in the double click experiments. *Dotted lines* show the predictions of a Urkowitz detector model with a 5 ms time constant

threshold, more spikes are elicited following the second click of the pair than following the first click. From the figure (open circles) it is seen, that this increased spike activity is in fact linked to the second click. The presence of the first click at sub-threshold levels thus increases the probability of a spike following the second click.

Only in the case of a separation of 5 ms or more between clicks is it possible to separate the responses to each of the two clicks, so the possibility remains, as stated in the introduction, that the lowered threshold with a 1 or 2 ms separation is seen because the probability of eliciting a spike following either the first or the second click is higher than the probability of eliciting a spike to just a single click. This will be the case if the two events (response to first and second click) can be considered *independent*, in which case the joint probability of a response at low stimulus levels will be nearly twice the probability of a response to a single click. However, temporal integration and independence between responses are highly coupled. This is seen in the 5 ms separation data. Due to temporal integration the presence of the first click raises the probability of eliciting a spike following a subsequent click. The second event is thus clearly not independent of the first event. The smaller the interval between the clicks, the more is the probability of a response to the second click raised relative to the probability of a response to the first click, at levels around threshold. Thus, the response of the receptor to the second click is more dependent on the first click the smaller the separation between the clicks is. Since the response of the receptor is inherently stochastic, the two events are however, even at small separations, still partially independent. The effect of independence on the response functions can be seen on the theoretical curves in Fig. 8. The basis for the curves is an idealized response function for single clicks. Spontaneous activity is set at 20% and the standard deviation (σ) of the Gaussian distribution is set to 2.5 dB. These values are the mean values of the present single click data. The integration curve (dotted line) is made by displacing the single click curve by 2.5 dB downwards, thus mimicking the approximate shift in threshold seen when using two clicks separated by 1 ms (conf. Fig. 5A). Some important points appear in this figure. It can be seen, that even when assuming total independence between the responses (broken line), the threshold improvement is only about half of what is actually observed in the 1 ms separation. Thus, the mere increase of probability of a response to two independent clicks cannot alone account for the threshold improvement actually observed. It is also noted, that the slope of the response function assuming total independence is steeper than the single click response function (i.e. σ of the Gaussian distribution is smaller in the double click situation), whereas the response function in case of temporal integration is similar in shape (identical σ), but shifted to the left. This is what is seen

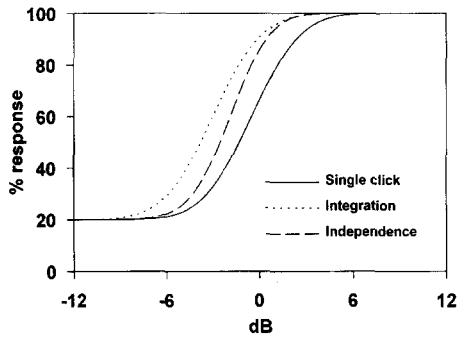

Fig. 8 Theoretical response functions. The response function for single clicks (solid line) is a cumulated Gaussian distribution with a SD of 2.5 dB. Spontaneous activity is 20%. These values are typical for the A1 receptor. The integration curve (*dotted line*) is constructed by assuming a 2.5 dB increase in threshold due to temporal integration (mimicking the 1 ms separation situation) and the independent curve (*broken line*) is calculated from the single click function assuming two independent clicks

in the data (Fig. 4AB). However, some sessions, especially with 2 ms separation seemed to result in response functions with a slightly smaller σ than the corresponding single click curve. The presence of such a change in σ was tested in the 1 ms and 2 ms data using a paired t-test. In case of 1 ms separation there was no significant difference between σ's of single and double click experiments ($p = 0.113$, $n = 15$), whereas a just significant difference ($p = 0.023$, $n = 12$) was found in the 2 ms experiments. This indicates a higher degree of independence in the 2 ms separation situation, and this could lead to some overestimation of the threshold shift caused by integration in the 2 ms separation situation, especially when using more than two clicks. This might explain why the threshold improvements when using more than two clicks with a 2 ms separation are somewhat larger than predicted (Fig. 7B), whereas the data from the experiments using 1 ms separation are in good agreement with the predictions (Fig. 7A).

Because the response to the individual clicks can be separated at separations of 5 ms and more, this possible overestimation will not be present in these data.

Another factor possibly affecting the results, is accommodation of the receptor. If accommodation to the first click of a pair occurs, then the response to the second click will be smaller than predicted. The A1 receptor in *Spodoptera frugiperda*, when stimulated with 45 ms long 20 kHz signals, clearly displays a decrease in firing rate, but it is not evident until at least 5 to 10 ms after stimulus onset (Pérez and Coro 1985). Thus, when considering the very short stimuli used in the present study, it is unlikely that accommodation will affect the data to any significant degree. However, it is possible, that the smaller than predicted change in threshold in the 10 ms separation situation (Fig. 6) is caused by an accommodation to the first of the two clicks. Another likely explanation for this deviation at 10 ms might be that the receptor is refractory to some degree by the time of the second click, provided that a spike was elicited following the first click. The probability of eliciting a spike would then decrease, which would counteract any increase in probability caused by integration.

In the multiple click experiments on the other hand, some accommodation to the stimulus would be expected, especially when using 8 clicks as stimulus.

Comparison with duration/intensity trading data

To test the exponential integrator model further, it was fitted to the intensity/duration trading data from *Agrotis segetum* (Surlykke et al. 1988) using a least squares method. The model was found to fit the data quite well with a time constant τ of 9.8 ms ($r^2 = 0.97$). This time constant is somewhat larger than the time constants used to fit the present double click data, but this difference is not comparable to the 2–3 orders of magnitude in difference as found in dolphins (Johnson 1968; Au et al. 1988) and humans (Plomp and Bouman 1959; Viemeister and Wakefield 1991) when comparing integration times for click detection and pure tones.

The difference in time constants of 9.8 ms from the intensity/duration trade experiment (Surlykke et al. 1988) and around 4 ms from the present double click experiment may be a species specific difference, but this seems unlikely since the two species used in the present study have comparable time constants. It could also be, that differences in the threshold criteria used in the two studies influences the determination of the time constant. In connection to this, statistical effects must also be considered. Everything else being equal, the probability of eliciting a spike to a 100 ms stimulus is twice the probability of eliciting a response to a 50 ms stimulus. Accommodation, especially to the very long stimuli, may also play a role and affect the determination of τ. Finally, it might be that a genuine difference in τ between the two experiments exist. However, more experiments are needed in order to answer these questions.

The passive electric properties of the receptor cell membrane

So far, the exponential decay model has been used only as a description of the data. Does it suggest a possible mechanism? The temporal parameters of the tympanic membrane in noctuids have been studied by laser vibrometry (Schiolten et al. 1981). A time constant of the tympanum at around 60 μs was measured. Thus, the integration time of the tympanal membrane is two orders of magnitude below the one revealed by the present study and the temporal resolution of the membrane is not a limiting factor. This leaves the receptor cell membrane as the most likely location of the memory mechanism required for the integration.

The capacitive properties of the cell membrane means that a stimulus arriving at the ear with a separation to the previous stimulus of less than about five times the time constant of the cell membrane will elicit a generator potential, which is superimposed upon the residual of the generator potential elicited by the first stimulus, thus leading to a larger total magnitude. This will then result in an elevated probability of eliciting an action potential, provided that the receptor is not refractory from a spike elicited by the first stimulus. Furthermore, it can be shown, that provided a proportionality between stimulus intensity and the magnitude of the elicited generator potential exist, then the receptor will behave as predicted by a leaky integrator with an exponential decay and a time constant equal to the time constant of the receptor cell membrane.

No intracellular recordings of generator potentials from noctuid auditory receptors have been made, so no time constant for this part of the cell is available. However, the structure of auditory sensilla is quite conservative across the Arthropoda (Römer and Tautz 1992), and Hill (1983) measured intracellular potentials in receptor cells in the auditory organ of *Locusta migratoria*. He judged the membrane time constant to be in the range of 5–10 ms. This supports the hypothesis of explaining the temporal integration of the A1 receptor by means of the passive electric properties of the receptor cell membrane.

Thus, in conclusion, it is in the present study clearly indicated, that the noctuid A1 receptor can be considered an energy detector. The passive electric properties of the cell membrane seem able to explain the double click integration data and possibly also the intensity/duration trade results.

The time constant found in the present study is compararable to the short time constants found in eg. humans. These short time constants (related to double click detection, gap detection and forward and backward masking) are probably describing the fundamental temporal resolution of the auditory system. It could be possible then, that these time constants are reflecting the time constant of the receptor cell membranes. The longer time constants found in connection with amplitude modulation and especially duration/intensity trading experiments, would thus have their origin at higher levels in the auditory pathway.

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