Doklady Biological Sciences. Vol. 386, 2002. pp 407-409. *Translated from Doklady Akademii Nauk. Vol. 386, № 3, 2002, pp. 415-417.*
Original Russian Text Copyright © 2002 by *Vorontsov, Lapshin.*

PHYSIOLOGY

Frequency Tuning of the Auditory System of Acoustically Active Noctuids (Noctuidae, Lepidoptera)

D. D. Vorontsov and D. N. Lapshin

Presented by Academician M.A. Ostrovskii April 28, 2002

Received May 16, 2002

The nocturnal moths noctuids (Noctuidae) are the largest lepidopteran family. Noctuids have auditory (tympanic) organs located in the thoracic region, at the level of hind wings. Each tympanic organ (TO) contains two receptor cells responding to evoked vibration of the tympanic membrane (TM). It has long since been discovered that the moths use hearing for detecting the echolocation signals of bats [10]. Having perceived the presence of a bat, the insects can maneuver in time and escape the chasing predator.

According to electrophysiological data, noctuids can perceive ultrasounds in the range from 10 to 100 kHz or even more. The TOs have a peak of sensitivity in the interval 15-25 kHz, which results from the mechanical resonance of the TM [7, 11, 12].

It is generally believed that noctuids are incapable of frequency analysis, although the validity of this notion has never been tested at the behavioral level. The conclusion on the inability of noctuids to distinguish frequencies is only based on the fact that the shapes of the frequency characteristics of both hearing receptors of the TO are similar [13].

To test the results of electrophysiological studies, several series of experiments were performed with intact animals [1,2]. Both the shapes and the absolute thresholds of audiograms obtained under different experimental conditions substantially differed: most of the behavioral audiograms had no distinct maximum of sensitivity in the range 15-25 kHz. To explain the observed discrepancy of the results, we put forward a hypothesis that the frequency of the TM mechanical resonance is not constant in flying moths (in contrast to the results of acute electrophysiological experiments); instead, it cyclically varies within a certain range. To test this hypothesis experimentally, it was necessary, first of all, to learn to determine the moments ("reference points") in which the parameters of the moth's auditory system are similar.

Institute for Information Transmission Problems, Russian, Academy of Sciences, Bol'shoi Karetnyi per. 19. Moscow, 101447 Russia

Noctuids can emit ultrasound clicks while they fly [3]. It was demonstrated in the past decade that these clicks serve as echolocation signals [4,6]. It was logical to assume that, when a noctuid emits the sounds, its auditory system is optimized for the perception of the echo. The first purpose of this study was to test this suggestion. If it were to be confirmed experimentally, the moths' own clicks could be used as the "reference points" when studying the adjustment of the TO acoustic parameters. The second purpose of the study was to show that the main resonance of the TO was actually retuned in intact moths.

Experiments were performed with 14 noctuids (*Enargia paleacea* Esp.) of both sexes, which were caught using a food attractant as a bait. The insects were tested under the tethered-flight conditions. To record the moths' own clicks, we used a B&K 4135 condenser microphone located 5 cm away from the experimental insect. The electric signals at its output were amplified, convened into rectangular pulses, and fed to the input of the regulated delay line. The delayed pulses were used to trigger a generator of acoustic stimuli, which were tonal trains of pulses with a duration of 2.5 ms and a time of the rise and the drop of the envelope amplitude of 0.5 ms. We used two fixed delays of the beginning of the stimulus relative to the moth's own click, 1 ms (the first series of eight experiments) and 15 ms (the second series of six experiments).

The signal amplitude was calibrated by means of a B&K 2235 meter (Brüel and Kjær). An acoustic pressure of 20 μPa was taken as 0 dB.

An insect was considered to respond to the stimulus if the mean spacing frequency of the moth's own clicks was substantially increased (by a factor of two or more) against the background of the interval of stimulation (1.4 s) and during the subsequent 2 s of observation (Fig. 1). The thresholds were determined as follows. At the noise level that we assumed to be subthreshold (1-3 dB below the threshold), the response (increase in the spacing frequency of clicks) was not observed more frequently than once during three successive stimulations; at the threshold level of the stimulus, the response should be repeated two or three times.

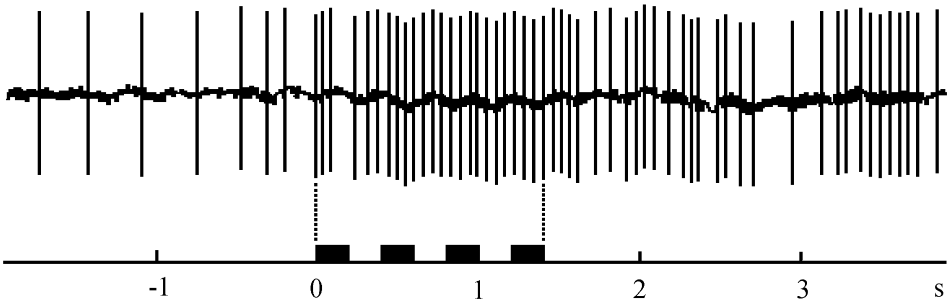

Fig. 1. An example of the response of an experimental noctuid (an increased frequency of click generation) to the above-threshold acoustic stimulation. The solid line shows the moth's movement in the horizontal plain; its rapid oscillations correspond to individual flaps of the insect's wings. The vertical lines against the background of the oscillogram show the moments when the moth emitted clicks. The horizontal axis shows the current time from the record onset; the solid rectangles on the axis indicate the periods of stimulation.

This was the quickest method to study the response thresholds of the moths.

To fulfil the first purpose of our study, we applied the stimuli at a short delay (1 ms) after the emission of the moth's own click. Figure 2 (curve *1*) shows an averaged audiogram constructed based on the results of the first experimental series. If the stimulus carrier frequency was increased from 10 to 40 kHz, the response thresholds monotonically decreased; their minimum values were located in the range 40-50 kHz in all experimental moths and equaled 25 to 30 dB SPL. As the frequency increased within the range 50-70 kHz, the thresholds also increased; however, in the range 60-85 kHz, the thresholds only slightly depended on the carrier frequency.

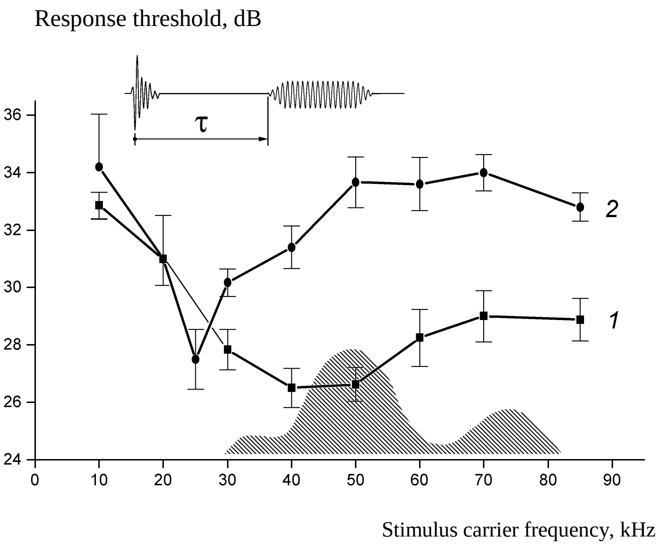

Fig. 2. The frequency-threshold characteristics of noctuids obtained at two values of stimulus delay (τ) relative to the moth's own clicks: (*1*) τ = 1 ms and (*2*) τ = 15 ms. A scheme of the calculation of the delay is shown at the top of the figure; an example of the spectrum of a moth's echolocation click is shown at the bottom.

An example of the spectrum of a noctuid's click is shown in the bottom of Fig. 2. As can be seen from the comparison between the shapes of the spectrum and the audiogram, the TO was "tuned" to the first spectral peak of the moth's own signal.

A different audiogram pattern was observed when the moths were stimulated with tonal pulse trains delayed relative to their own clicks for 15 ms, i.e., the time slightly longer than half the period of wing flapping in this species of noctuids. Under these conditions of stimulation (the second series of experiments), the main minimum of the audiograms was observed in the frequency range 25-30 kHz; i.e., the sensitivity optimum was shifted "downwards" by 20 kHz compared to the preceding series (Fig. 2, curve *2*). The shapes of the frequency-threshold characteristics obtained in this series of experiments were similar to the audiograms constructed based on electrophysiological data.

The possibility of tuning of the main-resonance frequency of the TM was earlier demonstrated experimentally [8]. The authors showed that the moth's auditory system has a feedback from the auditory system to metathorax muscles: muscle contraction changes the tension of the TM and, hence, the frequency of its mechanical resonance. Thus, the specificity of variation in the shape of behavioral audiograms may be explained by the periodic shifts in the TM mechanical resonance.

In our experiments (the first series), the auditory system of noctuids could not be retuned in the range from 25 to 40 kHz during the moth's own click (about 0.1-0.2 ms) and the tonal pulse that followed after a 1-ms delay, because there was no enough time for this process (no more than 4 ms). For comparison, note that the average latent period of the electrophysiological response to an acoustic click at the level of the tympanic nerve is 3.3 ms [5]. Therefore, the "upward" tuning to a frequency of 40 kHz had to precede the moment of acoustic emission; i.e., the resonance may be shifted in noctuids not only in response to acoustic stimulation, but also spontaneously. The close positions of the main spectral peak of moth's own clicks and the region of the maximum sensitivity of the TO (Fig. 2) on the main frequency axis indicate the consistency between the parameters of acoustic emission and perception at the moment of echolocation probing.

The reason for the "downward" TO tuning along the frequency range after the emission of the click remains unclear. In terms of the defense against bats, this behavioral strategy of the moths seems reasonable: a bat having found out the presence of a noctuid due to its echolocation click will immediately emit probing signals towards the presumed location of the prey. An enhanced attention to external sounds in a wide frequency range both before and after the emission of the click is the cost that the moth pays for using its own echolocation. The special attention of noctuids to the low-frequency region of the ultrasound range may he explained by the specificity of echolocation signals of some predatory bats predominantly feeding on tympanate moths [9].

The moth's capacity of quickly tuning the TO allows them to analyze the spectra of sufficiently long sounds. In addition, the insects can effectively discern bats' echolocation signals against the background of other rhythmic sounds, e.g., the calls of abundant grasshopper species.

Thus, we found that:

(1) the mechanical resonance frequency of the TM of noctuids can be tuned within the range of several dozens of kilohertz;

(2) immediately after the emission of an echolocation click, the noctuid TOs have the maximum sensitivity at the frequency that coincides with the main spectral maximum of the emitted signal; and

(3) the shift of the TM resonance to the high-frequency region makes the sensitivity of the noctuid auditory system in the ultrasound range above 30 kHz considerably higher than that estimated from electrophysiological data.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project № 02-04-48256).

REFERENCES

1. Zhantiev, R.D. and Fedorova, M.V., Zool. *Zh.,* 1988, vol. 67, № 7, pp.995-1001.
2. Lapshin, D.N. and Vorontsov, D.D., *Sensor. Syst.,* 2000, vol. 14, № 4, pp.213-321.
3. Lapshin, D.N. and Vorontsov, D.D., Zoo/. *Zh.,* 2000, vol.79, № 10, pp. 1189-1202.
4. Lapshin, D.N. and Vorontsov, D.D., *Sensor. Syst.,* 2000, vol.14, № 2, pp.156-166.
5. Lapshin, D.N. and Fedorova, M.V, *Sensor. Syst.,* 1996, vol.10,no.1,pp.5-17.
6. Lapshin, D.N., Fedorova, M.V, and Zhantiev, R.D., Zool. *Zh.,* 1993, vol. 72, № 9, pp. 93-105.
7. Adams, W.B., *J. Exp. Biol.,* 1972, vol. 57, pp. 297-304.
8. Callahan, P.S. and Carlysle, T.C., *Ann. Entomol. Soc. Amer..* 1972, vol. 65, № 4, pp. 918-925.
9. Rydell, J. and Arlettaz, R., *Proc. R. Soc. London, Ser. B: Biol. Sd.,* 1994. vol. 257, № 1349, pp. 175-178.
10. Schaller, F. and Timm, C., Z. *Vergl. Phvsiol.,* 1950, vol. 32, pp. 468-481.
11. Schiolten, P., Larsen, O.N., and Michelsen, A., *J. Comp. Physiol.,* 1981, vol. 143, pp. 289-295.
12. Surlykke, A., *Phvsiol. Entomol.,* 1986, vol. 11, pp.221-225.
13. Suga, N., *Jap. J. Physiol.,* 1961, vol. 11, pp. 666-677.

D.D. VORONTSOV and D.N. LAPSHIN

FREQUENCY TUNING OF THE AUDITORY SYSTEM OF ACOUSTICALLY ACTIVE NOCTUIDS (NOCTUIDAE, LEPIDOPTERA)

We investigated the frequency threshold curves (audiograms) depending on the delay between moths own click and the following short (2.5 ms) tonal stimulus. An insect was considered to respond to the stimulus if the mean spacing frequency of the its own clicks was substantially increased. Moths (*Enargia paleacea* Esp.) were tested under the tethered-flight conditions. At a delay of 1 ms the minimal thresholds were observed in the frequency range from 40 to 50 kHz, whereas increasing of stimulus delay to 15 ms caused the sensitivity optimum to shift down to 25 kHz. This shift of the sensitivity optimum can be explained supposing that there exists dynamic readjustment of the tympanic membrane mechanical resonance caused by longitudinal tension of the membrane towards the conjunctiva. The moth's ability of quickly tuning the tympanic organs allows it to analyze the spectra of sufficiently long sounds (sequential frequency analysis).