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PHYSIOLOGY

Sensitivity of the Noctuid Moth *Enargia paleacea* Esp. (Lepidoptera, Noctuidae) to Echo-like Stimuli

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Among insects, the pulse echolocation capacity has been so far demonstrated only in the group of noctuid moths (Noctuidae) [7]. The probing signals of the noctuid moths are short (25-190 μs) ultrasonic clicks, the main spectral maximums of the signals being located above 30 kHz [4]. Auditory (tympanal) organs of noctuid moths are paired organs located in the thoracic segment. The tympanal organ (TO) contains two receptor cells responding to the tympanal membrane vibration. According to the results of the electrophysiological and behavioral studies, the moths are capable of detecting ultrasound within the frequency range from 10 to more than 100 kHz (1,9, 10].

Pulse echolocation can be implemented only if the auditory receptors do not respond to the insect's own probing ultrasonic click. Otherwise, the moment of the echo-signal detection coincides either with the moment of the generation of the action potential or with the refractory period of the receptor [2]. This requirement pertains to all pulse location systems without exception. This requirement may be formulated as follows: the insect's own acoustic signals should suppress the response activity of the TO receptors.

In addition, the time of the subsequent recovery of the hearing sensitivity should be comparable with or longer than the damping time of the tympanal membrane free oscillations and the time of ringing of wing structures excited by the echolocation click. Theoretical calculations showed that physical restrictions to echo detection cease 200-300 μs after the generation of the ultrasonic click [6]. The distance traveled by the sound wave during this time interval is equal to the distance from the moth body to the wing tips and back.

It should be noted that certain stimuli following the moth's own ultrasonic click are similar to the echo-signals reflected from the actual target. The process of recovery of the hearing system sensitivity can be represented by the dependence of the threshold reaction of the insect on the delay time of these echo-like stimuli.

Experimental measurement of this dependence was the goal of this work.

The method of determination of thresholds was based on the principle of retranslation of echo-like stimuli to an intact moth from a virtual (nonexistent) target. The essence of the method can be described as follows. The clicks emitted by a moth were detected with a microphone. The output electric pulses of the microphone were amplified and used for triggering an echo-like stimulus, which was emitted back towards the moth, after a programmable delay time relative to the initial signal had passed [3, 5]. The temporal and spectral characteristics of the click stimuli emitted by the system were similar to those of the noctuid moth's own signals. After retranslation, the repeated triggering of the system was automatically blocked for about 6 ms. This suppressed the results of the direct effect of the loud-speaker on the microphone, preventing thereby the possibility of generation of signals irrelevant directly to the activity of the insect.

Thirty-six specimens of the noctuid moth *Enargia paleacea* Esp. (both males and females) were used. Before measurements, the experimental moth was glued with warm wax to one end of a fine leash of a mechano-optical sensor of horizontal shift movement (the so-called tethered flight). These settings allowed the insect maneuvers in the horizontal plane to be recorded. The noctuid moths were tested at four fixed values of the stimulus delay relative to the intrinsic clicks of the insect: 0.2, 0.3, 0.5, and 1 ms. The delay times included the total time of acoustic signal propagation from the moth to the microphone and from the loudspeaker to the moth. Nine identical experiments were performed at each delay-time value. One moth was taken for each experiment.

To simulate natural changes in the echolocation situation near the flying insect, the interval of the readiness to retranslation was divided into four sequential parts, 0.2 s each. During three intermediate parts of the interval, the experimental device did not emit the echo

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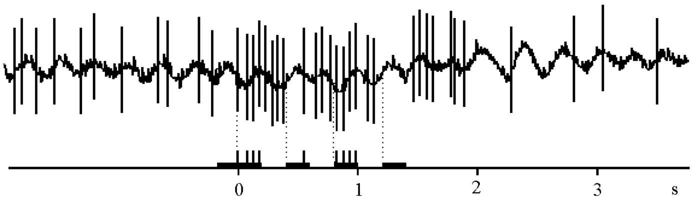


Fig. 1. Response of a noctuid moth induced by retranslated echo signals. The oscillogram shows the moth's shift in the horizontal plane. The vertical lines superimposed on the oscillogram mark the moments of emission of echolocational clicks by the moth. The abscissa is the lime from the moment of the first echolocational contact; black rectangles on the time axis indicate the periods of readiness of stimulation devices for retranslation; vertical lines superimposed on the periods of readiness indicate the moments of generation of retranslated echo-like stimuli.

signal to the insect. This cycle of stimulation approximately corresponded to the dynamics of the flight of a locating moth along four plane objects.

A cylindrical condenser loudspeaker was used as a source of echolike clicks. The loudspeaker was placed at a distance of 35 mm by the side of the insect. The outer diameter of the loudspeaker was 7 mm, and the length of the cylinder generatrix was 15 mm. Attenuation of the passive echo reflected from the loudspeaker surface at the site of moth location was 17 dB.

A B&K 4135 microphone in combination with a B&K 2235 amplifier (Brüel and Kjær) was used to measure the peak amplitude of the stimuli. The sound pressure level (SPL) of 20 μPa was taken to be 0 dB SPL.

The effect of the increase in the repetition frequency of the emission of the insect's own clicks (Fig. 1) was used as a criterion of the response to retranslation. Because the motor reactions of noctuid moths (sidewise jerks) were unstable, they were not used for measuring thresholds.

The histograms of the distribution of clicks are shown in Fig. 2. The histograms were measured at different amplitudes of stimuli at subthreshold (Fig. 2a) and threshold (Fig. 2b) levels. The threshold level was taken to be equal to the sound pressure level (dB SPL) at which the total number of moth's clicks recorded per stimulation interval (0-1.4 s) was significantly larger than the sum of clicks recorded within the same interval of time immediately preceding the stimulation period (-1.4 - 0 s). The confidence level of the difference was assessed from the results of ten successive presentations using Wilcockson's paired test at $p < 0.05$. When comparing two samples, the clicks of the first echolocational contacts were attributed to the background activity during the time interval preceding the stimulation period.

The responses of insects observed at the subthreshold level of stimulation (usually, 2 dB below the threshold level determined earlier) were studied similarly.

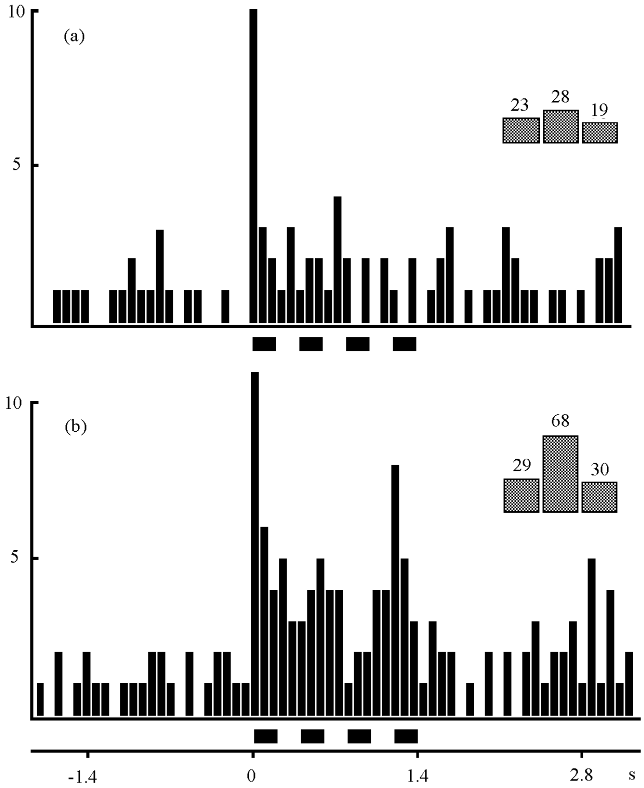


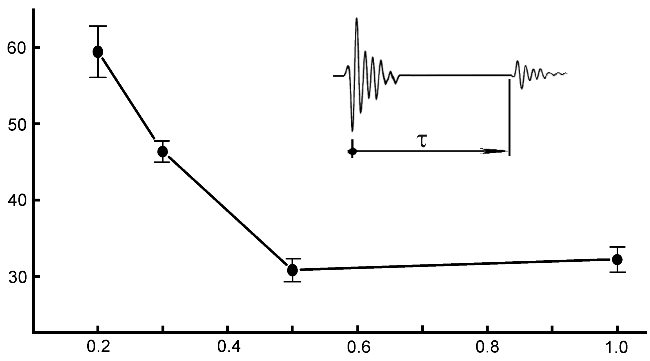
Fig. 2. Histograms of the distribution of intrinsic clicks of noctuid moth plotted from the results of one experiment: (a) subthreshold (29 dB SPL) and (b) threshold (32 dB SPL) levels of stimulation. The delay time of stimulation signals relative to the moth's own clicks was 1 ms. The zero point on the horizontal time axis corresponds to the moment of the first echolocational contact between the insect and stimulator in each presentation; four black rectangles (from 0 to 1.4 s) indicate the periods of stimulation. The vertical axis is the count of pulses per 80-ms - wide data accumulation channel. Each histogram was plotted from the results often presentations. The heights of shaded bars in the upper right comer of each figure correspond to the total number of clicks per time interval: -1.4 s- 0, the background activity; 0-1.4 s, the activity measured against the background of stimulation; 1.4 - 2.8 s, the aftereffect activity.

The stimulation-induced increase in the acoustic activity of moths observed in these experiments is expected to be statistically nonsignificant (according to the statistical test described above).

The curve of the dependence of the mean values of the behavioral thresholds on the stimulus delay time is shown in Fig. 3. It follows from Fig. 3 that the minimum thresholds (an average of 31 dB SPL) were observed at a delay time of 0.5 ms.

An increase in the delay time to 1 ms was accompanied by only insignificant changes in the moth sensitivity to retranslated stimuli, whereas the threshold level rose with an increase in the slope upon decreasing the delay time: the change in the delay time from 0.5 to 0.3 or 0.2 ms was accompanied by a 15 or 29 dB (i.e., almost 30-fold) increase in the threshold value.

Reaction threshold, dB



Delay time of echo-like stimuli, ms

Fig. 3. Dependence of the threshold of the behavioral reactions ofnoctuid moths on the delay time of echo-like stimuli. The error of the mean value is shown. The scheme of the delay time (τ) calculation is shown at the top.

The experimental data obtained in this work provide additional evidence for the echolocation capacity of noctuid moths, because the dependence of physiological characteristics upon the echo delay is generally believed to be a very important criterion of location systems [8].

The minimum location distance can be assessed from the dynamics of recovery of the hearing sensitivity of moths (Fig. 3): based on the accepted value of the speed of sound of 333 m/s, the time interval of 0.3-0.5 ms corresponds to the distance of 5-8 cm to the reflecting surface. This estimate is approximately four times shorter than the maximum operating radius of the echolocator calculated from the moth click amplitude and the moth auditory system sensitivity [2]. Therefore, the echolocation system of noctuid moths is designed to detect opposing obstacles in the immediate proximity of a flying insect. The moth can use echolocation to detect opposing obstacles when flying in dense vegetation.

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D.N. LAPSHIN, D.D. VORONTSOV

THE SENSITIVITY OF MOTHS *ENARGIA PALEACEA* ESP. (LEPIDOPTERA, NOCTUIDAE) TO THE ECHO-LIKE STIMULI

The auditory system of noctuoid moths has long been a model for anti-predator studies in neuroethology. Another suggested function for ultrasonic audition in moths along with their capability to emit loud ultrasonic clicks was an impulse echolocation. However, it seemed impossible to have temporal resolution sufficient for echolocator being realised by a simple auditory system and, thus, echolocation in moths looked rather incredible. Here we present an evidence of capability to perceive an echo following moth's own click with a very short delay. The behavioural responses of moths to the acoustic pulses imitating echoes of their own clicks were investigated under conditions of tethered flight. It has been found that such echo-like stimulation evokes an increase in average emission rate of own acoustic signals in moths. Auditory thresholds were measured in noctuid moths *Enargia paleacea* Esp. at four values of the stimulus delay: 0.2, 0.3, 0.5, and 1 ms in relation to the respective moth's clicks. Our findings demonstrate the ability of these moths to perceive echoes of their own signals, thus suggesting a possibility for use of impulse echolocation.