EFFECT OF VISUAL STIMULI ON THE DYNAMICS OF ACOUSTIC EMISSIONS BY NOCTUID MOTHS

(*Amphipyra perflua:* Noctuidae)

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Visual stimuli imitating approaching obstacles were presented on a projection screen to nocturnal cutworm moths. Acoustic and movement activity of experimental insects were recorded simultaneously. Visual stimuli elicited increases in the frequency of click emissions in cutworm moths. The optomotor responses of the moths were weak. Possible interactions between the visual and echolocation systems in nocturnal lepidoptera are discussed.

Noctuid moths are most active at twilight and at night; their vision is adapted to the perception of weak light sources [2, 8]. After data indicating that echolocation may be used by nocturnal moths [5, 7] were obtained, the question of interactions between vision and echolocation was raised; these represent sensory systems of two modalities based on completely different physical principles but having similar tasks. Their combined use may provide the insect with a wider range of abilities in terms of orientation in space.

Analysis of noctuid moth flight trajectories around model obstacles demonstrated that in the time between two sequential probe clicks, the moths flew distances which were often greater than the radius of action of their echolocation system [5]. These observations cast doubt on the possibility that the insects can orientate themselves completely using only the echolocation system.

On the other hand, it was noted that noctuid moths more frequently and regularly emit ultrasound in response to light [7]. It is possible that vision in moths in particular conditions initiates the emission of clicks, and instability of acoustic emission results from a lack of adequate light levels in behavioral experiments [5, 7].

In [5, 7] is was shown that noctuid moths, having detected an adjacent obstacle, could turn both towards the source of the echo and away from it. If the variability in the responses of moths to echoes is determined by their internal motivation, it is possible that this would also occur on presentation of moving images imitating obstacles approaching the moths.

The aim of the present work was to study the effect of vision on the dynamics of ultrasound click emission and to identify the specific features of phototaxis in moths in response to visual stimuli imitating moving obstacles (tree trunks).

MATERIALS AND METHODS

Studies were carried out using noctuid moths *Amphipyra perflua* F., of both sexes, caught in the light of a mercury luminescent lamp in the surroundings of Moscow in June-August, 1994. Each experiment, of which there were a total of 21, used a single moth.

Visual stimuli imitating a tree trunk (4 cm in diameter) moving towards the moth at a speed of 1 m/sec were presented to the experimental moths on a projection screen. Acoustic clicks emitted by the noctuid moths were recorded simultaneously with movements of the insect in the horizontal plane while attempting to carry out maneuvers.
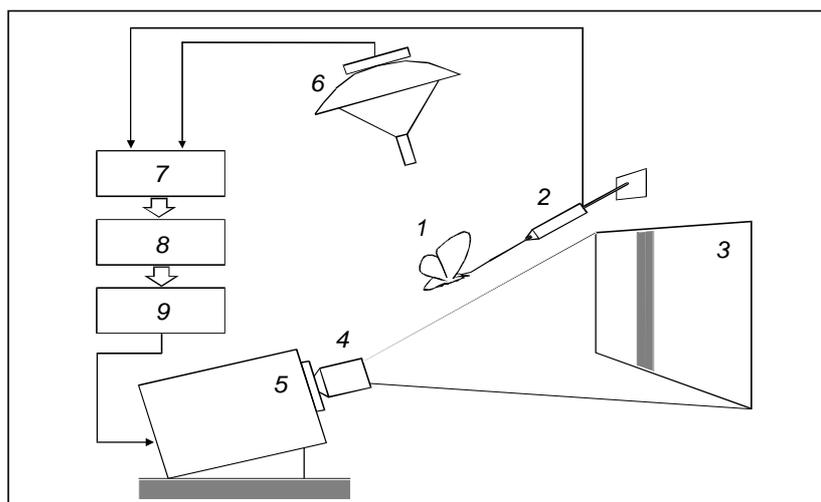

Fig. 1. Diagram of the experimental apparatus. Moth (1) were attached to the horizontal movement sensor (2) in front of a screen (3). Images were projected onto the screen via the objective (4) of an oscilloscope (5). Clicks emitted by the insects were recorded using an acoustic transducer (6). Signals from the sensory and acoustic transducer were fed into an analog-to-digital converter (7), which was connected to a computer (8). The computer drove a video adapter (9) to produce the control signals for the oscilloscope.

Echolocation contact of noctuid moths with any kind of obstacle can stimulate the emission of probe impulses. This effect is well known in bats [1]. Special measures were taken to avoid this possibility in the current experiments- the projection screen was placed beyond the range of the echolocation system of the moths, and the movement recorder was streamlined in shape to reduce reflections of sound towards the experimental insects.

A diagram of the experimental apparatus is shown in Fig. 1. The moth, attached by a filament to the horizontal movement detector probe, was placed at a distance of 50 cm from the projection screen, which was 1 m wide and 70 cm high. The projector was an oscilloscope with an objective lens attached in front of its screen. The oscilloscope screen luminophore emitted green light. The movement and brightness of the oscilloscope beam were controlled by a computer connected via a special video adapter. This system produced on the oscilloscope screen a rectangular light area of size 4 x 3 cm, with a dark band on this background. The positions of the edges of this band and its rate of movement relative to the center of the light area were controlled by the computer using a special program. The raster picture sweep frequency was 700 Hz and the line frequency was 179.2 kHz. At this rate of picture change, noctuid moths should be able to perceive a fused image without flickering [2].

The background raster illumination of the projection screen corresponded to the natural light level in the presence of a full moon (0.2-0.5 Lux).

At the start of the experiment, the moth was kept for 10 min without illumination (dark adaptation), and stimuli were then presented sequentially; stimuli corresponded to three versions of obstacle movement: to a shift of 15 mm to the left of the outer edge of the insect's wing (L); a similar movement but to the right (/?); and a movement directly towards the moth (F). Stimuli in the form of narrow dark bands appeared on the screen suddenly. Plots of changes in the angular positions of the right and left edges of the stimuli relative to the center of the screen are shown in Fig. 2. Calculations were performed for a range of changes in the distance from 62 to 2 cm from the obstacle to the insect. At the last stage, presentation of the edges of the stimulus was shifted beyond the limits of the raster. At this point in the *F* regime, the screen was completely dark for 10 msec.
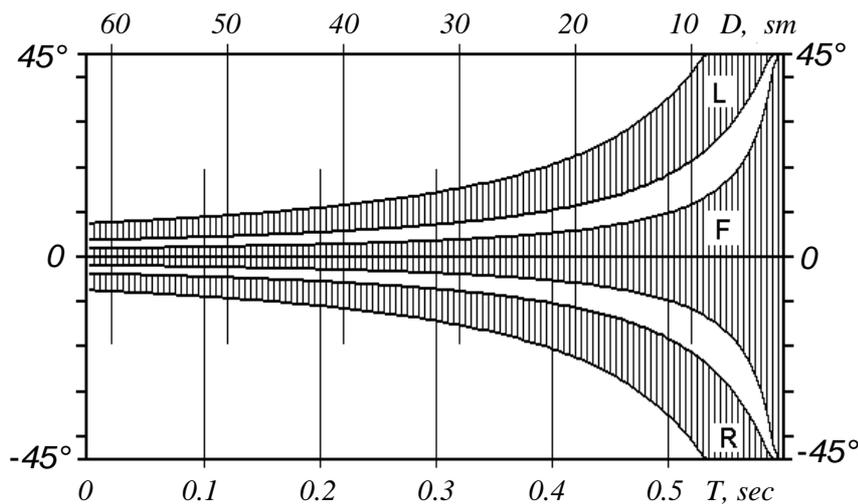
Fig. 2. Plots of the angular position of the edge of the stimulus in the field of vision of the experimental moth. L is the region in which shifts of the obstacle to the left of the insect were imitated; R indicates shift of the obstacle to the right; F indicates shift directly towards the moth. The upper horizontal axis indicates the calculated distance to the obstacle; the lower horizontal axis shows time from the start of signal presentation; the vertical axis indicates the angle of visualization.

In initial experiments, insects were also presented with images inverted in terms of brightness, i.e., a light band on a dark background; however, the moths made almost no response to this type of stimulus, and this regime was not used in subsequent experiments.

Ultrasound clicks emitted by the experimental insects were recorded using a condenser microphone attached to a parabolic reflector located at a distance of 1 m from the experimental insect.

Experiments were carried out in laboratory conditions in the evening and night at temperatures of 20-22°C.

RESULTS

In flight, noctuid moths can generate two acoustic impulses per wing beat, at the moments of lifting and lowering the wing [3], though this transmission regime was not seen in the current experiments. The interval between individual clicks was either close to the wingbeat period (T_w) or shorter.

The wingbeat period T_w, measured as the minimum interval between clicks, varied from moth to moth within the range 27-33 msec with a mean value of $T_w = 31 \pm 1.8$ msec. Stimulation conditions had no significant effect on the value of T_w.

Presentation of stimuli to noctuid months generally led to an increase in the frequency of clicks (Fig. 3, a, *b*). If the moths showed no background acoustic activity before the experiment, stimulation did not initiate ultrasound emissions. The significance of the increase in acoustic activity at various time points, compared to the background level, was assessed using the criterion of signs Z [4] for each version of stimulation. It is of note that four presentations (out of 63) resulted in reductions in acoustic activity on stimulation, and in three of these cases the stimuli consisted of imitation of the movement of the obstacle directly towards the insects (Fig. 3, c).

Histograms showing the total distribution of clicks with time (Fig. 4) show that on average the acoustic activity of noctuid moths increased as the obstacle became closer and remained at a more or less constant level for 0.5-2 sec after the image disappeared from the screen.
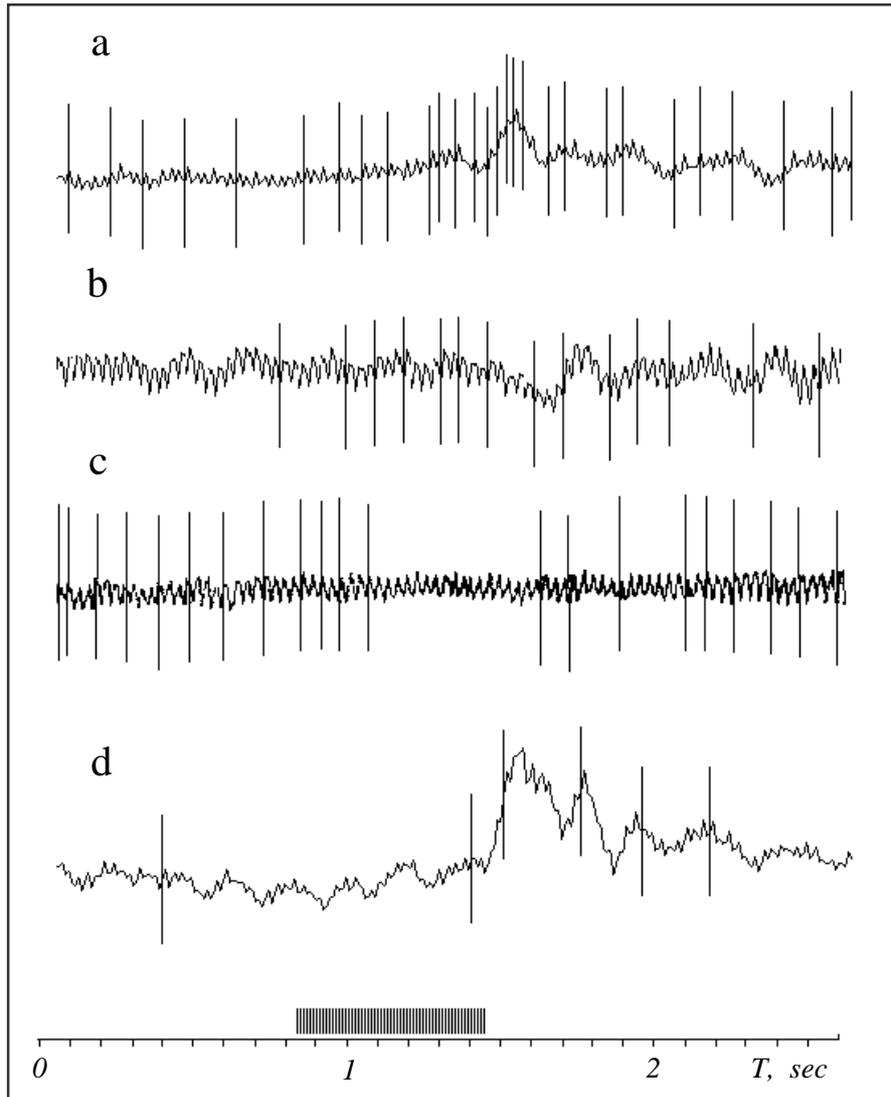
Fig. 3. Responses of noctuid moths to visual stimuli: a - movement of the image to the right of the moth (R); b - movement of the image to the left of the moth (L); c, d - movement of the image directly towards the moth (F). Continuous lines indicate movement of the experimental insect in the horizontal plane. Upward deviations of the line show movements of the moth to the left, and rapid oscillations result from individual wingbeat movements. Vertical lines show the moments at which clicks were recorded. The horizontal axis shows time from the start of recording. The dark rectangle (below) shows the time of stimulus presentation.

Plots of L (movement of the image to the left of the moth) and F (movement directly towards the moth) had a first peak characterized by rapid increases in acoustic activity by experimental moths over a period of 1.3-1.4 sec, i.e., in the last 100 msec of the presence of the stimulus. This increase in the amplitude of the distribution resulted from a sharp increase in the clicks rate (Fig. 3, a). Additionally, it is possible that at an initially low click frequency at this time point, there was an increase in the probability that single clicks would be emitted.
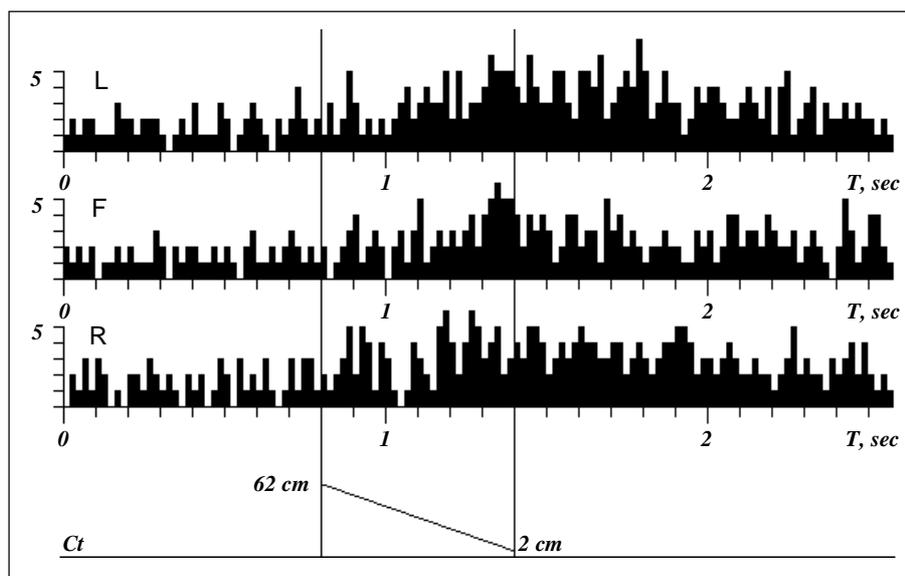
Fig. 4. Histograms of the distribution of noctuid moth acoustic impulses. The horizontal axis shows time from the start of recording, in sec; the vertical axis shows the number of impulses in each recording channel. The quantization step was 20 msec. Vertical lines indicate the points of starting (0.8 sec) and ending (1.4 sec) the visual stimulus imitating the movement of an obstacle to the left (L), right (R) relative to the insect, and directly towards the insect (F). The diagram below shows changes in the calculated distance (*Ct*) between the obstacle and the insect. Each histogram was constructed using the results of 21 stimulus presentations.
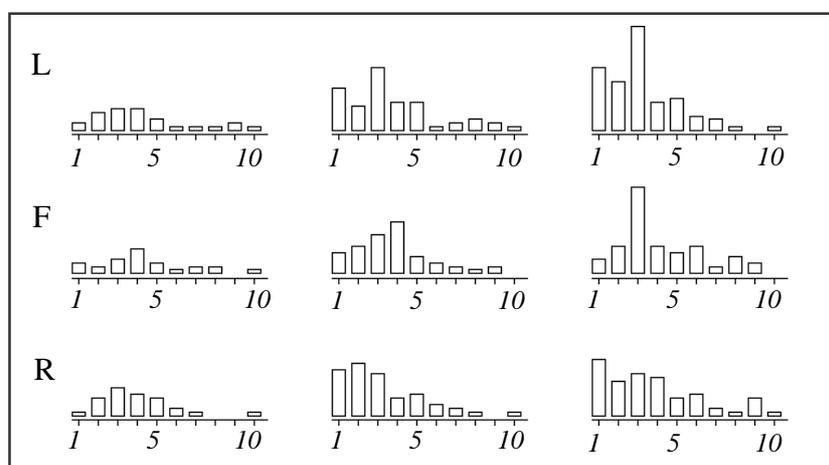

Fig. 5. Histograms of interimpulse intervals. The left-most plots show the 0.6 sec before stimulation; the middle plots show during stimulation, and the right-most plots show the 0.6 sec after stimulation. The horizontal axes show the intervals between clicks; the vertical axes show the number of intervals in each recording channel. The quantization step was one beat period (31 msec). Each histogram was constructed using data from 21 identical stimulus presentation.
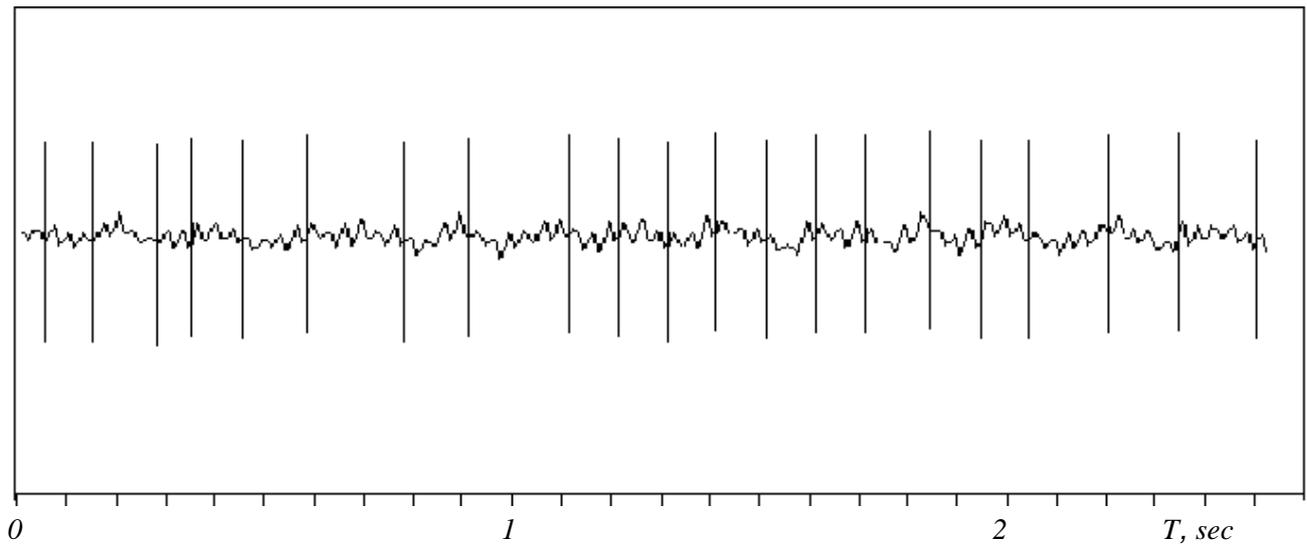
Fig. 6. Fragment of a trace of spontaneous activity of a noctuid moth

To verily the latter possibility, further analysis was carried out using the results of those experiments in which moths emitted a single click during the stimulus (Fig. 3, d). There were six such experiments in the L regime. In four of these, the acoustic impulse was recorded in the last 100 msec of image presentation. In the F regime, there were five such experiments, in four of which the acoustic impulse was emitted in the last 100 msec, and in the R regime this occurred in four of seven experiments. The probabilities that this pattern would be random, as calculated by Bernoulli's equation [4], were $p = 0.8\%$ for the L regime, $p = 0.3\%$ for the F regime, and $p = 1.6\%$ for the R regime. This leads to the conclusion that the emission of clicks over this interval of time could in fact be synchronized by the visual stimulus. However, the question of which of the parameters of the image projected on the screen (the angular rate of edge movement, the distance to the "obstacle," etc.) launched the acoustic impulse generator in the experiment insects still remains open.

In the histogram in Fig. 4, R, the total distribution has a number of sharp peaks when the stimulus was acting. The irregularity of the distribution can be explained by the synchronizing action of the moment at which the image appeared on the screen, and the shift of the major peaks to the left can be explained in terms of a reduction in the threshold for excitation of the acoustic generator.

The effect of visual stimulation on the distribution of intervals between clicks was also studied in terms of inter-click intervals before, during, and after stimulus presentation (Fig. 5). For convenience of the analysis, intervals are presented as whole numbers of wingbeat periods, such that 1 indicates clicks immediately after each beat; 2 indicates clicks after every second beat, and so on. The upper and lower rows correspond to movements of the obstacle image to the left (L) and right (R) of the moth respectively. It is clear that with and after the stimulus, there were increases in the numbers of short intervals ($1-3T_w$). In the case of imitation of obstacle movement directly towards the moth (middle row, F), the proportion of shortest intervals was relatively small. Subsequently, the distribution tended to retain the same form for each modification of the stimulus.

Attention should be drawn to the relatively high probability of seeing intervals close to $3T_w$: five histograms out of nine (Fig. 5) showed maximum amplitude peaks corresponding to this value. Evidently the tendency of noctuid moths to emit one click every two to four beats is not directly associated with the conditions of stimulation. Figure 6 shows a fragment from the trace showing spontaneous activity from a moth; a series of seven impulses is seen at the interval 1.1-1.8 sec, with a period of $3T_w$.

There was no relationship between the direction of movement of the stimulus and movement of the moth in the horizontal plane. In most cases (45 stimulus presentations), noctuid moths gave no motor response to stimuli. When such responses were obtained (Fig. 3, a, d), there was a constant direction which was specific for each individual insect, which was independent of the direction of movement of the image on the screen.

DISCUSSION

The functional relationship detected in noctuid moths between vision and the emission of ultrasound suggests a defined role for echolocation in the orientational system of these insects.

When moths undertake straight-line flight in dense vegetation, the rate of change of the angular position of particular elements in the field of vision increases sharply with increases in the visualization angle φ, reaching a maximum at $\varphi = 90°$ (270°). In conditions of low illumination, there is a reduction in the visual ability of noctuid moths to perceive rapid changes [2], i.e., a reduction in the efficiency of the visual system in evaluating spatial obstructions should be expected at target aspects close to 90° (270°). In the same conditions, there are increases in the probability of detecting an obstacle using echolocation, because of the reduction in the mean distances to the surface being probed, the enhancement of binaural differences at large angles [6], and the concentration of acoustic energy reflected from surrounding leaves [7].

Maximum acoustic activity was seen in noctuid moths subjected to visual stimulation at the point before the moment of maximal closeness to the "obstruction". This suggests that the main task of the echolocation system in moths is to estimate the position of surrounding obstacles within the work area of the wing, i.e., a those target aspects at which visual ability appears to be reduced. At small visualization angles (less than 45°), the ability of noctuid moths to respond to echoes is significantly limited [5]. This point also indirectly indicates that the abilities of the visual and echolocation systems complement each other.

The efficiency of orientation by echolocation (without vision) depends partially on the distance flown by the moth in the time between two sequential probe impulses. When this distance is greater than the radius of sensitivity of the echolocation system, there is a sharp reduction in the probability that the insect will detect the obstacle in time. When the visual and echolocation systems are working in coordination, the stability requirement of the acoustic emissions become less acute because the moment of emission of identical clicks or a series of clicks can be determined by the central nervous system on the basis of visual information, i.e., when the obstruction has already been seen.

The relatively high level of acoustic activity seen in noctuid moths after disappearance of the image from the screen can be explained by the fact that the moths did not receive confirmation of the closeness of the obstacle from echolocation contacts, i.e., they were unable to detect the point to which the obstacles disappeared after action of the stimulus. While the task of following an approaching object is performed sequentially in the central nervous system in terms of vision followed by the echolocation system, the absence of an echo after disappearance of the image from the insect's field of vision indicated an error in orientation, and, consequently, the need for repeated verification of the information, resulting in an increase in the frequency of emissions. The absence of echolocation support also explains the low level of movement activity of the insects at the point of coming close to the "obstacle."

During moth flight in an open space, the efficiency of the echolocation system must decrease sharply because of its small radius of action. Since the signals emitted by noctuid moths represent a strong demasking factor, these are the conditions in which the insects are most susceptible to bats. Contrarily, among dense vegetation, an increase in the efficiency of the moth echolocation system should be expected, along with a decreased probability of suffering predation because of the difficulty of maneuvering. It is perfectly possible that the low frequency of spontaneous probe impulses in noctuid moths is the direct result of pressure applied by predators using the same frequency range in their own echolocation systems [1, 3].

REFERENCES

1. E. Sh. Airapet'yants and A. I. Konstantinov, Echolocation in Nature [in Russian], Nauka, Leningrad (1970).
2. N. V. Antakova, A. V. Kalinina, L. K. Len'shina, A. N. Malakhov, E. A. Russina, V. P. Ryabokon, M. Yu. Ul'yanov, and B. M. Chudinovich, "The frequency characteristics and absolute sensitivity thresholds of photoreceptors in a number of insects", in: Bionics [in Russian], Nauka, Moscow (1965), pp. 100-106.
3. R.D. Zhantiev, D. N. Lapshin, and M. V. Fyodorova, "The emission and perception of ultrasound in noctuid moths", Zool. Zh., 72, №3, 76-85 (1993).
4. G. F. Lakin, Biometrics [in Russian], Vysshaya Shkola, Moscow (1990).
5. D. N. Lapshin, "Echolocational orientation in noctuid moths (Noctuidae: *Amphipyra perflua*)", Sensor. Sistemy, 9, № 4, 76-83 (1995).

6. D. N. Lapshin and M. V. Fyodorova, "The responses of the tympanic organs of cutworms *(Amphipyra perflua:* Noctuidae) on pulsed ultrasound signals", Sensor. Sistemy, 10**,** №1, 5-17 (1996).

7. D. N. Lapshin, M. V. Fyodorova, and R. D. Zhantiev, "Echolocation in noctuid moths (Lepidoptera, Noctuidae)", Zool Zh., 72, №9, 93-105 (1993).

8. G. A. Mazokhin-Porshnyakov, Insect Vision [in Russian], Nauka, Moscow (1965).