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STIMULUS PARAMETERS DETERMINING THE RESPONSES OF CERCAL INTERNEURONS

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Stimulation with linearly changing signals was used to study identified neurons 10-3a and 9-lb of the cereal system of the cricket with the aim of identifying which parameter of the wind stimulus (speed, acceleration, etc.) determined their responses. The results showed that the factor affecting neuron 10-3a was acceleration of the airflow, wile the parameter determining the responses of neuron 9-lb was the derivative of acceleration. Since neuron 10-3a has previously been regarded as sensitive to speed, the present results show the need for reconsidering some earlier data and thus altering the strategy for future studies.

Key words: *Insects, cercal system.*

A method for stimulating the wind-sensitive system of insects was described in [1]; it should be possible to use this method for identifying wind stimulus parameters (active factors) determining the responses of an element of interest, which can be a receptor or a central neuron. In the present report, we present and discuss the first results of studies of this aspect of central neurons in the cereal system of the cricket. This system is very suitable for assessing the use of the method described in [1], as many of its neurons can be identified from their morphological and physiological properties, and have been studied in detail by a number of groups [2]. In terms of making a concrete selection of an experimental system, it was considered appropriate to test both neurons for which the active factor is regarded as having been identified and neurons for which existing data, obtained by traditional methods (sinusoidal stimulation), are inadequate. Thus, verification experiments paid most attention to neurons of types 10-3a and 9-lb, according to the classification described in [3].

Neuron 10-3a is a large neuron, and is one of the best studied neurons of the cereal system of the cricket. Its characteristic features include a tonic response type, in which discharges are synchronized with sound waves; the neuron is very sensitive to the direction of the stimulus [2, 4, 6], Analysis of its frequency characteristics led to the suggestion [4] that it is a detector of the speed of the air flow. All subsequent data [6-8], including studies on the afferentation of neuron 10-3a [7], seemed to support this view. It was of interest to verify this hypothesis using the new method.

Neuron 9-lb has thus far received little study; however, it is known to have very interesting properties [5, 9] which cannot easily be explained using existing concepts: the neuron has high selectivity for frequencies of 30 Hz and below, its responses are inhibited at frequencies of greater than 100 Hz, and it shows no synchronization with sound waves and no habituation. It would be interesting to obtain initial data on this neuron with regard to its active factor.

MATERIALS AND METHODS

Studies were carried out on *Acheta domesticus* crickets, using imagos and pre-imaginal stages. Animals were taken from a laboratory stock.

Insects were prepared by fixing them with melted wax to a plate with the ventral surface uppermost, and bundles were exposed at the output of the terminal abdominal ganglion by cutting a small hole in the cuticle above them. This window was surrounded by a small wall made of waxed paper and the resulting well was filled with physiological saline.

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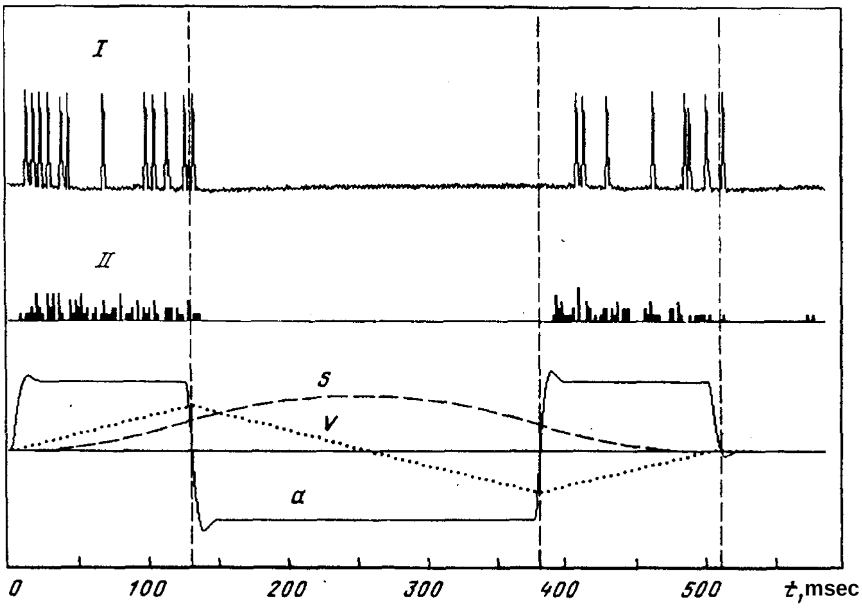

Fig1. An example of the response of neuron 10-3, recorded from the right bundle, above the terminal abdominal ganglion. The rotation angle of the acoustic chamber was 30° relative to the insect. *I*) Oscillogram of the response, in which the spike amplitude is 66-70 m*V*; *II*) histogram showing the distribution of spikes in time, using 10 stimulus presentations and a channel width of 2 msec; *S*, *V*, and *a* are plots of displacement, speed, and acceleration respectively of the air flow. The horizontal axis shows the time from the start of stimulation. The vertical dotted lines show the moment at which the acceleration function was through the zero.

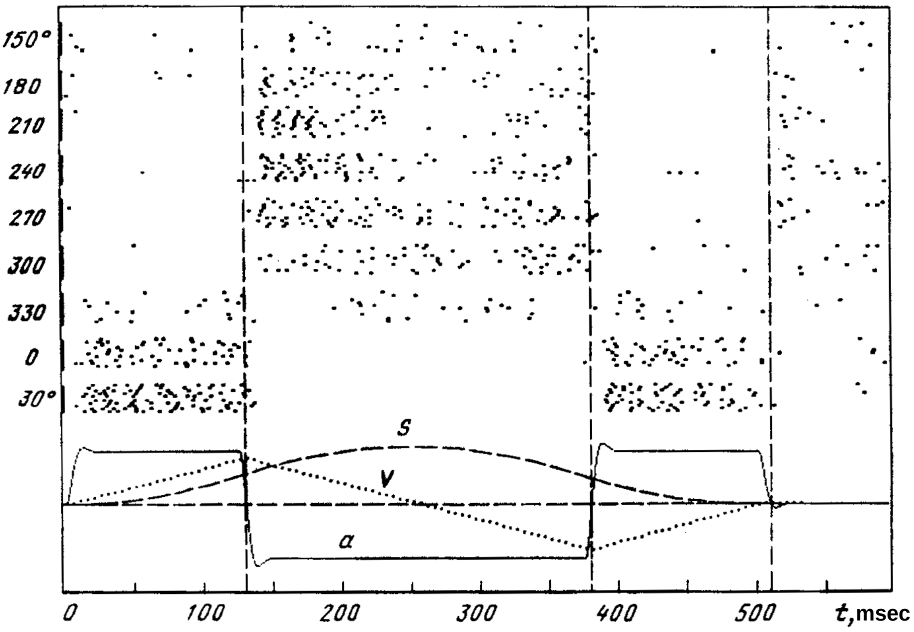

Fig. 2. Relationship between the responses of neuron 10-3a and the rotation angle of the stimulation chamber relative to the long axis of the insect's body. The vertical axis shows the chamber rotation angle, and the horizontal axis shows the time from the start of stimulation. Points show individual spikes in neuron responses. Ten stimuli (ten rows of dots) were presented for each direction, *S*, *V*, and *a* are as in Fig. 1.

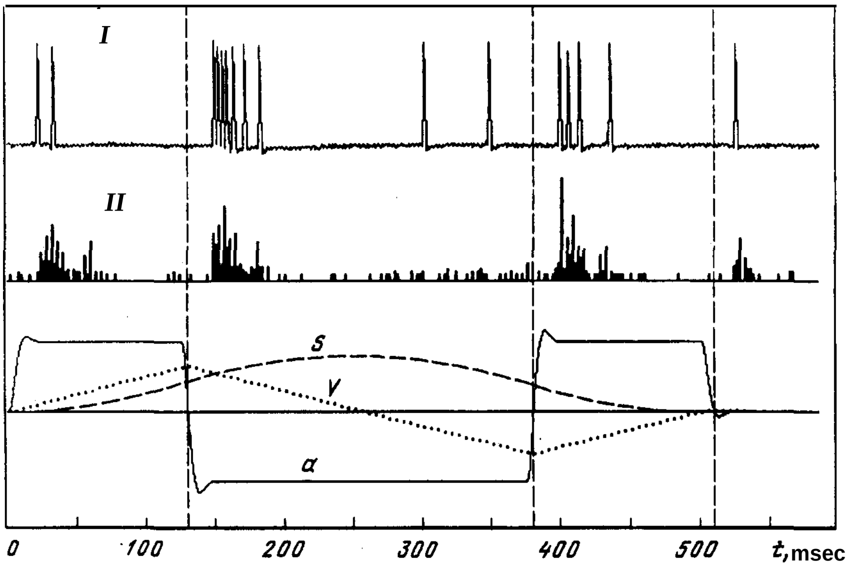

Fig. 3. An example of responses of neuron 9-lb. Recording was from the left bundle. The rotation angle of the acoustic chamber relative to the insect was 30°. *I*) Oscillogram of the response, in which the spike amplitude is 68-77 mV; *II*) histogram showing the distribution of spikes in time, using 70 stimulus presentations and a channel width of 2 msec; *S, V,* and *a* are as in Fig. 1.

Responses of cereal interneurons were recorded using glass microelectrodes (borosilicate glass, tip resistance 50-100 MΩ), laced vertically using a micromanipulator and inserted into the bundles, which had been lifted and fixed to special hooks. The covering of the exposed bundle was initially treated with collagenase to facilitate microelectrode insertion. Morphological identification of cells was performed by injecting the luminescent stain Lucifer yellow from the microelectrode into the axon after experiments.

Wind stimulation was carried out as described in [1]. The main parameters of the stimulus were duration of the driving signal (0.5 sec), peak displacement amplitude (3 mm), maximum speed (23 mm/sec), and periods of constant acceleration (192 mm/sec^2).

Electrophysiological data were analyzed using an electronic "Ékspert" system running on an IBM PC-486 computer.

RESULTS

Neuron 10-3a of the cricket cereal system was studied in three experiments, which gave similar results. Figure 1 shows an example of the responses obtained from neuron 10-3a using a linearly changing wind stimulus. The stimulus direction (30°) was close to the direction regarded as optimal for this neuron [2, 6]. The oscillogram (Fig. 1, *I)* shows that neuron discharges were confined to the positive square-wave parts of the stimulus acceleration function (Fig. la, intervals 0-125 and 380-515 msec). This is also supported by the histogram constructed by accumulating responses to ten presentations (Fig. 1, *II).* The latent period of responses to jumps in acceleration was 10-20 msec.

Figure 2 shows responses of the same neuron to stimuli of different directions. This shows that as the chamber was rotated through 180° relative to the initial position of 30° (which was equivalent to inverting the sign of the signal), spikes in the neuron response showed considerable regrouping (Fig. 2, angle of 210°). At other chamber rotation angles, responses were less pronounced, especially at a direction of 330°. Since the time structure of the response showed clear structural inversion at both sides of this angle (300° and 0°), it appears that the neuron had minimum sensitivity around the angle of 330°. The mean discharge frequency at this angle was similar to the level of spontaneous activity, and was similar to the level seen after the stimulus ended. Analysis of the spike distribution suggested that the neuron was not only excited at positive accelerations, but was also inhibited when the stimulus vector direction was opposed (Fig. 2, angles 0° and 30°, time interval 130-380 msec). At these regions, the spontaneous activity of the neuron was sharply reduced as compared with activity during the post-stimulus period.

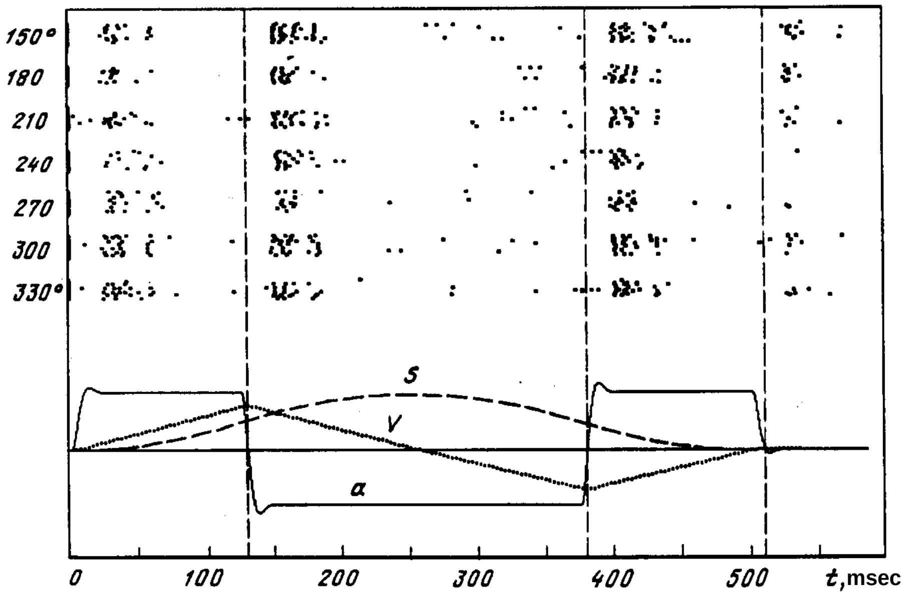

Fig. 4. Relationship between the responses of neuron 9-lb and the rotation angle of the axis of the stimulation chamber relative to the insect's body. For further details see caption to Fig. 2.

Although the pattern of responses of neuron 10-3a was evidently associated with acceleration, it remains possible that the speed component of the signal had some influence on its response. Thus, the first quarter of the stimulus at 30° gave a stronger response than the last quarter (Figs. 1, 2). This difference may result from the fact that in the first case, speed and acceleration were in the same direction, while in the second, speed and acceleration were in opposite directions.

Neuron 9-lb was tested in the same way, using a total of four experiments. An example of its responses is shown in Fig. 3. This shows that maximum responses were seen 12-25 msec after a change in the direction of acceleration. The number of spikes in the discharges increased, on average, proportionally to the modulus of the amplitude jump on the acceleration plot. The responses of this neuron to stimuli of different directions are shown in Fig. 4. Changes in air flow direction had a significant effect on the responses of the neuron, i.e., the direction diagram for acceleration was close to circular.

DISCUSSION

Results obtained using linearly changing signals show that in the case of neuron 10-3a, the active factor was acceleration of the air flow, while for neuron 9-lb the active factor was a derivative of acceleration. For neuron 9-lb, this conclusion does not generally contradict its previously determined properties [5, 9].

It is natural that the nervous system of the cricket can analyze derivatives of stimuli: the differentiation operation eliminates constant or slowly changing components of wind speed, a factor which would provide a limit on the high sensitivity of the cereal system.

The new data on neuron 10-3a were very unexpected, since, as already mentioned, this neuron was considered to be sensitive to speed [6, 7] rather than acceleration. However, our data showed an obvious relationship between the neuron response and acceleration, and the effect of speed, if it has one, is insignificant. This raises the question of how to explain this contradiction. Careful analysis of the concrete data rather than the opinions of other authors led to the preliminary conclusion that the contradiction arises essentially from the interpretation of results. For example, the authors of [4, 7] regarded neuron 10-3a as a speed sensor only on the basis of an analysis of its frequency-threshold curve, measured over a relatively narrow frequency range (2-100 Hz), though this neuron is also sensitive to higher-frequency stimuli [2]. In addition, studies of the relationship of neuron 10-3a with receptors, carried out by lesioning, led the authors of [4, 7] to the conclusion that the main excitatory signal came from speed receptors. However, along with these receptors, the authors incidentally also lesioned acceleration receptors, which was not considered in their final conclusion [7]. Summarizing all the available data suggests that neuron 10-3a is actually associated with acceleration receptors as well as with speed receptors.

The relative contributions of these receptors to the responses of the interneuron could depend on a number of factors: age, physiological state of the preparation, stimulus parameters, etc.

Overall, the results obtained here show that some existing data need to be reconsidered, and that corrections in strategies for future studies are needed.

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