Sensory Systems, Vol. 11, No. 2, 1997

APPARATUS FOR STUDIES OF THE WIND-SENSITIVE SYSTEM IN INSECTS

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An apparatus for stimulation of the wind-sensitive systems of insects is described; the apparatus allows convenient assessment of the active component of the signal (displacement of air particles, their speed, acceleration, etc.) as well as convenient measurement of speed thresholds and latent periods of responses.

Key words: *Insects, cercal system.*

INTRODUCTION

Studies of the wind-sensitive systems of insects (such as the cercal system) generally make use of stimuli consisting of tonal bursts in which the amplitude envelopes have smooth rise and decay fronts. The use of this type of test signal provides the most direct means of assessing the frequency properties of the structures of interest. Additionally, the electronic apparatus used for generating the sinusoidal bursts is relatively simple, which has also promoted the wide use of these methods. However, this stimulation system also has a number of disadvantages.

Firstly, experimental results do not allow easy identification of the active parameter of the stimulus (displacement of air masses, rates of airflow, acceleration, etc.). These assessments are generally carried out by analyzing the slopes of wind frequency-threshold characteristic curves. However, since audiogram shapes are generally determined by a number of elements acting in concert (in the case of the cereal system, these include the sensitive fibers, adjacent structures, and the time characteristics of receptors and interneurons) [4, 5], assessment of the active parameters of the stimulus can only be regarded as preliminary.

Secondly, there is no stable relationship between electrical signals exciting the loudspeaker and real stimuli, because of the phase distortion of the loudspeaker. In general, data presented as examples in reports on wind-sensitive systems generally consist of oscillograms of the electrical signals driving the loudspeakers. The active stimuli are usually significantly different in shape as compared to the loudspeaker-driving signals in that they are proportional to the first or second derivatives of the displacement function (i.e., the speed or acceleration) of the loudspeaker membrane. Displacement itself also has a phase shift relative to the input current, which changes at different frequencies of acoustic bursts over the range 0 to π . All these phase shifts, taken together, make it very difficult to measure the latent period of the response of the neuron under study.

Thirdly, calibration of the amplitude of the wind stimulus is a fairly complex procedure, for which the only satisfactory solution found thus far involves use of a laser anemometer [3].

Fourthly, at low frequencies, many neurons of the cereal system generate trains of impulses synchronously with sinusoidal signals [1], i.e., the number of spikes in the response depends on the stimulus frequency. At intensities close to the threshold, the probability that a given neuron will produce a discharge increases in proportion to the frequency, which makes measurement of the threshold more difficult.

Thus, it is clear that traditional methods for testing need to be improved and perfected, particularly by using contemporary computerized generators which can be used to produce electrical signals of virtually any form. Here we describe a version of a system which, in principle, makes it possible to define the active factor of a wind stimulus.

Institute for Information Transmission Problems of the Russian Academy of Sciences, 19 B. Karetnyi per., 127994 Moscow. Translated from Sensornye Sistemy, Vol. 11, No. 2, pp. 168-174, April-June, 1997. Original article submitted February 10, 1997.

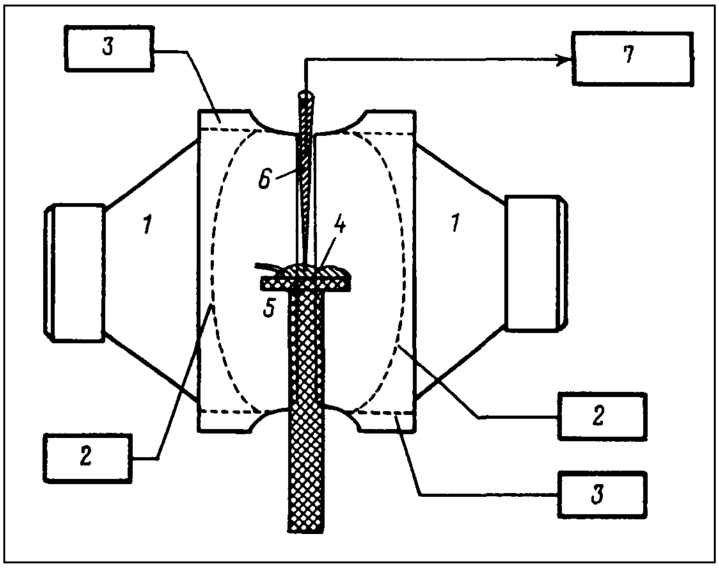

Fig. 1. Diagram of the experimental apparatus. *1*) Loudspeaker; *2*) mesh screen; *3*) plastic tube; *4*) experimental insect; *5*) platform; *6*) electrode; *7*) amplifier.

Fundamental Principles of the Stimulation System. The source of the wind stimulus consisted of a synchronized pair of loudspeakers. The shape of the controlling electrical signal was selected such that the amplitude and time characteristics of displacement, speed, and acceleration of the air flow could be varied radically.

Construction of the Stimulatory Apparatus. The general layout of the stimulatory apparatus was similar to that published previously [2]. The acoustic chamber consisted of two symmetrical parts, each of which in turn consisted of a transparent plastic tube of internal diameter 120 mm and length 45 mm (Fig. 1). WS 13 BF (1045) loudspeakers (from Visaton) were attached in the front parts of the tube, facing each other and electrically connected in parallel and in opposite phase. The major parameters of the loudspeakers were: the first resonance frequency of the diffuser F_o was 40 Hz; the quality factor of the vibrating system Q was 0.5-0.6; the length of working travel of the diffuser was 6 mm; the peak power was 60 W; the electrical resistance at constant current was 6 Ω. One half of the acoustic chamber was fixed to a stand, and the other was attached to the first half during experiments, after the platform and insect were placed in the center of the chamber. A microelectrode was attached to the preparation from above through an appropriate opening.

Since the vast majority of wind receptors are sensitive to the direction of the signal, it was considered necessary to provide the ability to change the relative orientation of the preparation and the stimulus source during the experiments. For this purpose, the entire chamber could be rotated.

In order to reduce the electrical interference with the microelectrode and preparation, metal meshwork screens were placed in front of the loudspeakers. These were concave in shape, to prevent any possible break-up of its components during stimulation.

At low frequencies, when the length of the acoustic waves was much greater than the distance between the diffusers, the rate of air flow within the regulated chamber was virtually equal to the rate of loudspeaker movement. The better the symmetry of the acoustic parameters of the loudspeakers, the more accurately the air flow rate reflects the electrical current driving the loudspeakers. Unavoidable variations in loudspeaker performance lead to increases in the sound pressure within the tube. This pressure can easily be detected with a microphone inserted through the upper opening in the wall of the chamber (in place of the microelectrode), perpendicular to its long axis.

The driving signal was produced using an Ékspert electronic system in an IBM PC-486 computer. The sampling frequency was 20 kHz. The electrical signal from the output of the digital-to-analog converter was filtered (low-frequency filtration with a cutoff at 1 kHz) and was fed into a power amplifier and then to the loudspeaker.

General Description of the Stimulus. The signal driving the loudspeaker was composed of three sequential fragments of the parabolic functions

$$U(t) = kt^2 \qquad 0 < t < T_0, \qquad (1)$$
$$U(t) = U_m - k(t - 2T_0)^2 \qquad T_0 \le t \le 3T_0,$$
$$U(t) = k(t - 4T_0)^2 \qquad 3T_0 \le t \le 4T_0,$$
$$U(t) = 0 \qquad t \ge 4T_0,$$

where U_m = 2.5 V (maximum voltage), T_0 = 0.125 sec; $k = U_m/2T_0^2$ = 80 V/sec^2.

The components of the driving signal were selected in such a way that it and its first derivatives would meet the condition of smoothness for the resulting function (i.e. without breaks or sharp jumps) during the exposure time. The total duration of the signal was $4T_0$ = 0.5 sec. For comparison, the period of the intrinsic vibration of the loudspeaker diffusers was $1/F_0$ = 0.025 sec, i.e. 20 times smaller. Resonant systems (the diffuser and elements of the support formed a resonant system) are known to follow a slow forcing action with some delay. Computer calculations performed by the Duamel method for the loudspeaker parameters gave a delay time for the response of the order r = 4 msec. This is small in comparison with the duration of the driving signal and, to a first approximation, the function of displacement of the diffusers from the equilibrium position $S(t)$ can be taken as being proportional to $U(t - \tau)$ (Fig. 2, plot "*S*"):

$$S(t) = gU(t - \tau), \text{ where } t > \tau. \qquad (2)$$

The displacement function was also determined by statistical transfer of the loudspeaker characteristic *g* (mm/V), which can be measured easily in laboratory conditions, as it is numerically equal to the linear displacement of the diffuser when a constant voltage of 1 V is applied to the loudspeaker. For the loudspeakers used in this apparatus, *g* = 1.2 mm/V, and

$$S_m = gU_m = 3 \text{ (mm)}, \qquad (3)$$

where S_m is the amplitude at maximum displacement. This value must not exceed one half of the length of the working travel of the loudspeaker diffuser.

As already mentioned, the rate of air flow at low frequencies is in fact equal to the speed of the diffusers, i.e., the first derivative of their displacement function:

$$V(t) = dS(t)/\,dt$$

The speed increased linearly over the range $\tau < t < T_0$; the speed also decreased linearly over the interval $T_0 + 3\tau < t < 3T_0$, becoming negative after the point $2T_0 + \tau$ and returning to null over the range $3T_0 + \tau < t < 4T_0 + 3\tau$ (Fig. 2, plot "*V*"). The maximum speed V_m of the diffusers was developed close to the flexure point of the function $S(t)$ (at the points $T_0 + \tau$ and $3T_0 + \tau$, allowing for the delay):

$$V_m = 0.965 S_m/T_0 = 0.965 g U_m/T_0 = 23 \text{ (mm/sec)}. \qquad (4)$$

It follows from Eqs. (3) and (4) that stimulus calibration leads to determination, with the required level of accuracy, of either *g* or S_m. The problem of measuring linear displacement of solid objects, such as the loudspeaker diffuser, is technically much simpler than measuring air flow rates in small volumes. The time parameters of the signals were determined by the digital-to-analog converted, which was very stable, so the effects of errors in the values of *t* and T_0 at the input signal were ignored.

Diffuser acceleration was determined by differentiating the speed function:

$$a(t) = dV(t)/dt.$$

Since the speed changes were for most of the time linear in this case, the plot of acceleration was a stepped function with jumps from one level to another, due to the inertia of the moving parts of the loudspeakers (Fig.2, plot "*a*").

The magnitude of the acceleration (the step height) was calculated using

$$a_m = S_m/T_0^2 = gU_m/T_0^2 = 192 \text{ (mm/sec}^2\text{)}. \quad (5)$$

This plot has four regions at which the levels change, around points 0, T_0 , $3T_0$, and $4T_0$; after points T_0 and $3T_0$, the amplitude of the jumps was $2a_m$. The calculated duration of the transition processes was essentially equal to 3τ (12 msec). The ratio of S_m to a_m was strictly maintained, i.e. the accuracy of measurement of the maximum acceleration depended only on the error in measurement of S_m . The intrinsic frequency F_0 and the quality Q of the moving parts of the loudspeaker had no effect on the amplitude of acceleration a_m at stationary intervals.

Thus, the function for displacement of air particles near the preparation *S(t)* has no points at which sign inversion occurs, the speed function *(V(t))* has only one such point ($2T_0 + \tau$) , and the acceleration function ($a(t)$) has two points ($T_0 + 2\tau$, $3T_0 + 2\tau$); the points of sign inversion for speed and acceleration do not coincide.

The directions of displacement, speed, and acceleration vectors over the range $0 – T_0$ are positive when air particles move from the insect's head to its cercus.

Naturally, selection of the value of U_m and, consequently, of S_m , V_m , and a_m , are determined by the particular requirements of an experiment, and depend on preliminary data on the properties of the neurons of interest and consideration of the limitations resulting from loudspeaker linearity.

Selection of Loudspeaker Type for the Stimulation Chamber. Equations (3)-(5) provide general guidance as to the type of loudspeaker to be selected for the apparatus. Calculations showed that the delay in the mechanical response of the diffuser relative to the driving signal is determined primarily by the intrinsic resonance frequency (F_0). As an approximation, the time required for transfer processes is half the period of the intrinsic vibrations of the loudspeaker ($3\tau \approx 0.5/F_0$). Thus, reduction of time distortion requires a loudspeaker with the highest possible intrinsic resonance frequency. However, at a given rated power, a loudspeaker with a higher resonance frequency generally has a smaller reserve for the diffuser's working travel, with the result that the calculated displacement amplitude S_m can fall beyond the limits of the linear range. The length of the working travel of the diffuser also limits the maximum air flow speed, especially for slow processes (not only for the signals described here, but also for tonal bursts in the frequency range below F_0). On average, the range of vibration which the diffuser can achieve increases with the loudspeaker's power rating and, consequently, its size. For the present studies we selected a loudspeaker with the greatest diffuser working travel available for a convenient size.

Methods Used for Neuron Studies. Assuming that the element under study (for example, a receptor or an interneuron of the insect's cereal system) functions as a probe for determining air speed. At the first part of the range ($0 – T_0$) of stimulus speed *V(t)*, responses are expected after the point at which the speed of the air flow is greater than the physiological threshold of the neuron. In fact, latency has the effect that the response is seen a little later, at time point t_1 (Fig. 2) after the start of the driving signal. The threshold speed V_T is related to time t_1 as follows:

$$V_T = 1.04V_m(t_1 - \tau - l)/T_0, \quad (6)$$

where l is the latent period of the neuron response.

The value of l can be determined by two methods: a) by stimulating the neuron with short clicks whose amplitude is close to the threshold level, and b) by measuring t_1 and t_2 at two maximum speeds V_{m1} and V_{m2} , which differ by a factor of 1.5-2. The value of t_2 has the same sense as t_1 in Eq. (6). In method b, the resulting data are used to make easy calculations of the latent period of the neuron response and its sensitivity threshold using a system of two equations with two unknown parameters:

$$V_T = 1.04V_{m1}(t_1 - \tau - l)/T_0, \quad (7)$$
$$V_T = 1.04V_{m2}(t_2 - \tau - l)/T_0.$$

If the neuron of interest responds tonically to the stimulus, the instantaneous spike frequency at suprathreshold levels will correspond to its amplitude characteristics. Assessment of the nature of responses at reduced speeds (T_0 - $2T_0$) also allows measurement of the symmetry of the neuron's characteristics. Inversion of the air flow rate occurs during the signal presentation period (at time $2T_0 + \tau$). Analyzing the distribution of spikes in responses at positive and negative speeds immediately provides two points on the directional sensitivity diagram.

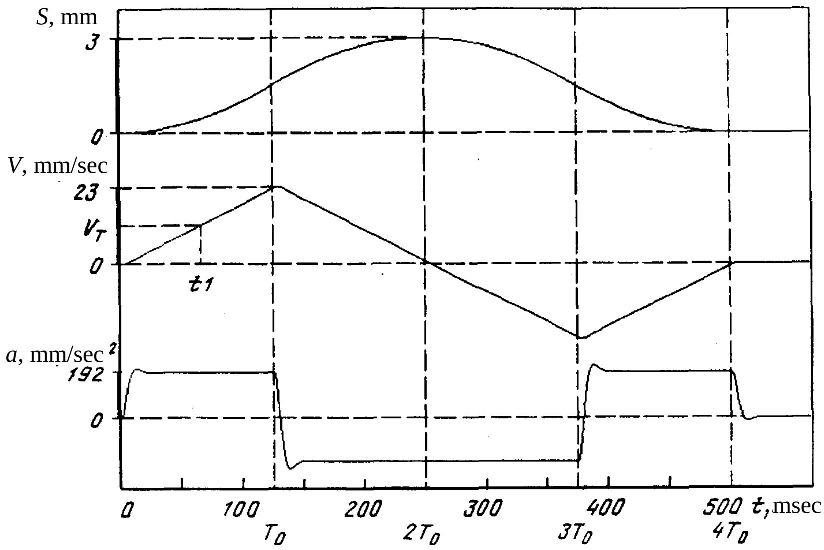

Fig. 2. Plots of the displacement functions *S(t)* (*"S"*) of the loudspeaker diffuser, its rate *V(t)* ("V"), and its acceleration *a(t)* (*"a"*). The horizontal axis shows time from the point of applying the driving voltage to the loudspeaker. Calculations were made using Q = 0.6.

All these calculations apply only in cases in which the active parameter for the neuron of interest is the rate of air flow. This is not usually known in advance, so a preliminary analysis is made of the distribution of spikes during the stimulus and a period of time following the end of the stimulus. If the element under study is sensitive to air flow acceleration, its discharge pattern will undergo highly significant changes immediately after time points 0, T_0 , $3T_0$, and $4T_0$, when the active parameter undergoes sharp jumps, including sign inversion. If two factors act simultaneously on the neuron, i.e., speed and acceleration, the pattern will be complex. In this case, it is helpful to carry out a comparative analysis of responses at regions in which the speed and acceleration vectors act out of phase (T_0 - $2T_0$; $3T_0$ - $4T_0$) and at regions in which the vectors are in the same direction (0 – T_0 ; $2T_0$ - $3T_0$).

Analysis of the active factor may reveal situations of ambiguity when the time response of the cell of interest is limited to the period of 0 - T_0, i.e., the first quarter of the stimulus. All three functions act at this region — displacement, speed, and acceleration, and are positive and increasing. One method of solving this problem is to repeat the experiment with the stimulating tube turned through 180° relative to its initial position.

In conclusion, the type of stimulus proposed here is only a partial case of nonsinusoidal influence. For example, if the neuron of interest is sensitive to acceleration, it is logical to use a driving signal of a form for which the plot of the acceleration function *a(t)* is similar to the speed plot *V(t)* used here. It is easy to see that the driving signal will in this case be a sequential combination of cubic parabolas, but without return to zero at the end of the stimulus duration.

The authors would like to thank V. Shul and G. Kämper for assisting production of the experimental apparatus, and A. K. Panyutin for developing the computer programs and for technical assistance. Studies were performed with support from the International Volkswagen — Stiftung Fund, Gram No. Az.I/69911.

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