PHASIC STIMULATION OF A THORACIC STRETCH RECEPTOR IN LOCUSTS

By ERIK GETTRUP

*Zoophysiological Laboratory B, Juliane Maries Vej 36,
University of Copenhagen, Denmark*

INTRODUCTION

The co-ordination of activity in muscles necessary for flight in insects depends upon a complicated interaction between the central nervous system and the peripheral sense organs. In the desert locust, *Schistocerca gregaria* Forskål, there is, built into the central nervous system, a cyclic pattern of firing of different motoneurons used during flight. Sensory inflow is important in modifying the motor output both with respect to details of pattern and as to frequency of repetition (Wilson, 1961). Simple cauterization experiments have shown that the frequency is modified significantly by the input from stretch receptors suspended inside the thorax and connected with the moving parts of the flight system (Gettrup, 1962). In order to reach a better understanding of the way in which the flight rhythm is controlled by the periphery, it was decided to investigate the responses of the stretch receptors when stimulated phasically, in particular by sinusoidal stretching. This should answer questions concerning the kind of information contained in the response produced in the sense organ by deformations approximating those during flight.

Phasic stimulation has been used in a number of investigations on mechanoreceptors but in most cases the interest has been focused on impulse formation and other basic receptor mechanisms (Katz, 1950; Eyzaguirre & Kuffler, 1955). More closely related to the present study are papers by Lippold, Redfearn & Vučo (1958) and by Lowenstein & Finlayson (1960). They give detailed accounts of the relations between stimulus parameters and frequency of response but are directed primarily towards the connexion between extent of deformation and impulse frequency. The main point of the present paper is to investigate the timing between the phase of deformation and the first sensory impulse, and to suggest how this information may be used by the animal.

A sinusoidally varying stimulus was chosen since it represents a fairly good approximation to the natural wing movement and makes the calculation simple. The investigation was extended to include experiments in which the velocity of deformation was constant.

The stretch receptor concerned belongs to the group of phasic-tonic receptors, but the response to a constant deformation will not be dealt with here since it has been studied by Pabst & Schwartzkopff (1962) in the corresponding sense organ of *Locusta migratoria*.

ANATOMY

The thoracic stretch receptors of *Schistocerca gregaria* have been described else-where (Gettrup, 1962). There are four unicellular receptors, one for each wing. Each receptor is closely attached to a small bundle of scolopoforous sensilla located at the body wall near to the wing hinge. In the mesothorax the stretch receptor of the fore-wing is suspended between the pleuron and the phragma in the plane of the latter. The point of attachment to the pleuron is posterior to the subalar sclerite and just beneath the wing hinge. The anterior insertion in the metathorax is also within the region of the subalar sclerite but here the sense organ originates from the anterior edge of the metapostnotum. In this segment both attachments can be identified in the intact animal, which makes possible direct estimates of the deformation during flight. Observations on flying animals and manipulated imitations of normal wing movements in anaesthetized animals showed that the sense organ alters in length by 100–$200\,\mu$ per wing stroke, depending on the size of the animal.

A stretch receptor consists of a single neuron associated with connective tissue. Muscle cells are absent so that the animal is unable to adjust the length of the sense organ. Within a few hundred microns from its origin the axon from the stretch receptor joins the axons from the scolopoforous organ and form part of a posterior branch of the first nerve of the ganglion.

MATERIALS AND METHODS

Full grown adults of the desert locust, *Schistocerca gregaria* Forskål, were used in the experiments. The locusts were provided by the Anti-Locust Research Centre, London.

The gut was removed and the thorax plus the first abdominal segment were isolated. The latero-dorsal part of one side was removed and the preparation was fastened by means of wax (a mixture of 10 g. beeswax and 4·5 g. resin) to a Perspex plate which was clamped to the bottom of a saline bath. The temperature was 34° C., i.e. close to that within flying locusts, but the effect of temperature on firing is probably not very important; in abdominal stretch receptors of *Astacus leptodactylus* a Q_{10} of about 1·5 has been found for the tonic response and, although the value increases with the degree of stretch, a Q_{10} above 2 has not been observed (Burkhardt, 1959). Since the preparation was totally immersed in warm saline, special precautions had to be taken in order to provide it with sufficient oxygen. In most cases the lower end of a short bent glass tube was waxed on to the pleuron over the second thoracic spiracle, letting the upper end extend above the surface. The spiracle was kept open by means of a piece of copper wire.

Only methathoracic sense organs were used. In order to stretch the receptor along its long axis, the posterior insertion was fixed by means of wax to a short piece of wood which, in turn, was fastened to the Perspex plate (see Fig. 1 A, *g*). A piece of celluloid (*e*), a few centimetres long and about 1 mm. wide, was fixed to the wing base just above the anterior insertion of the stretch receptor (*f*) and in the direction of the long axis of the latter. The celluloid piece provided the connexion to a steel rod of the phasic stimulator.

The mechanical stimulator consisted of (1) a sine wave generator (Packard model 200 CD/CDR wide range oscillator) or a generator for signals with constant rate of change (the signal is produced by cutting the discharge of a condenser by means of a Zener diode), (2) a DC-amplifier and (3) a loudspeaker unit. The amplified signal was fed into the loudspeaker (Peerless, E 35 M with a resonance frequency of 250 cyc./sec). A steel rod was glued with Araldite to the centre of the reinforced loudspeaker membrane and further supported a few centimetres away by a Teflon bushing. High-vacuum silicone grease was used as a damping agent to avoid vibrations due to abrupt changes in velocity of displacement. The mechanical displacements were recorded by means of a vane (glued to the loudspeaker rod) which intercepted a light beam to the
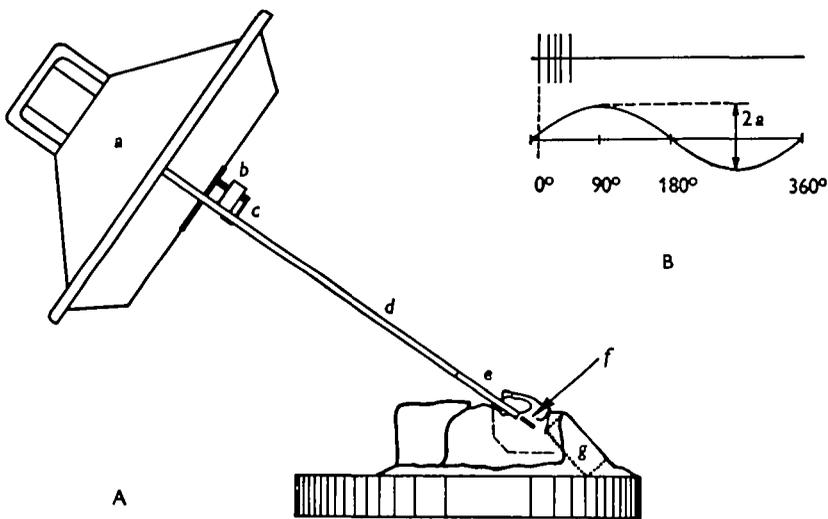

Fig. 1. (A) Arrangement for mechanical stimulation of the wing-hinge receptor. The arrow points to the metathoracic sense organ (*f*). The steel rod (*d*) of the loudspeaker (*a*) is connected to the wing base via the celluloid rod (*e*). Before the experiment, the length of the sense organ was adjusted to that in the intact animal (with unfolded wings in horizontal position). Mechanical displacements were recorded by means of a vane-phototransistor system (*b*) and (*c*). (B) Scheme of a photographic record. The upper trace is from the stretch receptor, and the mechanical deformation is displayed below. The rising phase of the sine wave represents stretching. In this paper the term 'maximum displacement' refers exclusively to the peak-to-peak distance (2*a*). The phase angle of a potential is the abscissa of the corresponding point on the sine curve (as shown by the vertical line).

phototransistor TP 50 (Siemens). The voltage across the phototransistor varied linearly with displacement within the proper amplitude range (0–1000 μ) and was fed directly into one channel of a Tektronix type 502 oscilloscope. The electrical activity in the nerve from the stretch receptor was recorded with a hooked platinum-iridium electrode (0·25 mm. in diameter) insulated to near the tip by means of cellulose lacquer. Petroleum jelly was used to insulate the immersed electrode from the surrounding medium. A platinum-iridium wire placed in the saline bath was used as a reference electrode. A Tektronix type 122 preamplifier and a camera formed the rest of the recording apparatus.

RESULTS

The material presented here derives from a few selected experiments, but the stimulus-response relations described were found in all cases. In the case of sinusoidal deformations the following variables of the response were measured (see Fig. 1 B): phase angle of first-action potential (spike), time between first and second spike, shortest duration between spikes and number of spikes during a deformation period. (The phase angle of a point on the sine curve refers to the time axis; when the
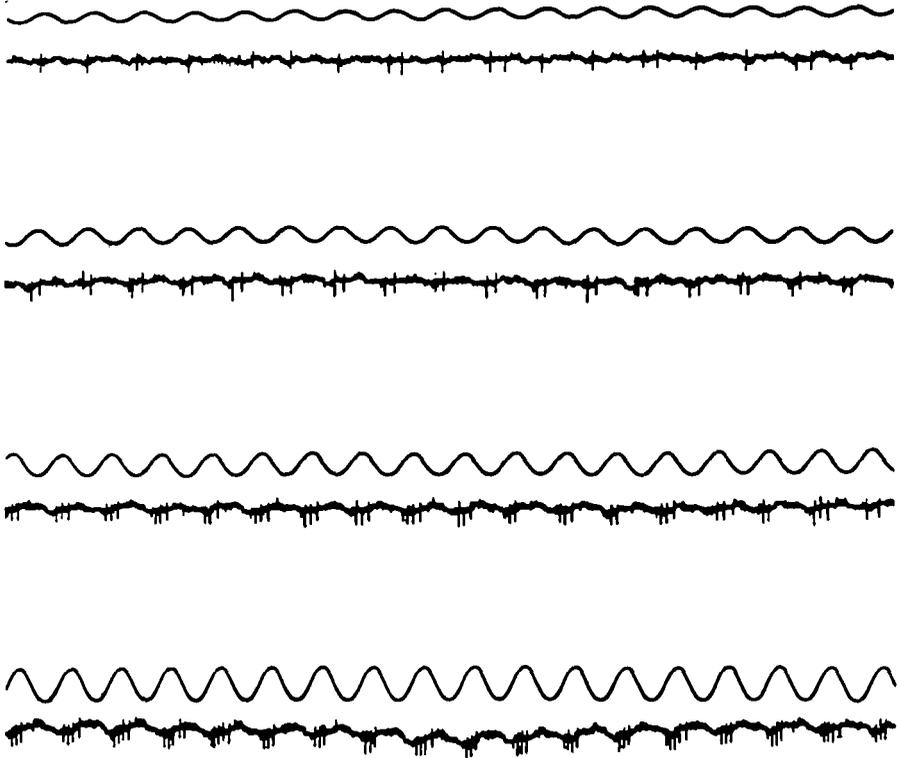

Fig. 2. Sinusoidal stretching and shortening at constant frequency (20 cyc./sec.). The maximum displacements are 120, 230, 370 and 500 μ. The rising phase represents stretching. The delay due to the time for spike conduction along the nerve from sense organ to recording electrode is insignificant (about 1 msec.).

stretch velocity is maximum, the phase angle is 0° (360°) and when the stretch is maximum the phase angle is 90°.) The only stimulus parameter which had to be measured on the recorded curve was the maximum velocity of stretch, since both stimulus and maximum displacement were read directly during the experiment. Stimulus frequencies between 5 and 50 cyc./sec. were used. The wing-stroke frequency during normal flight is about 17 cyc./sec. but since the upstroke, during which the receptor is stretched, amounts to 40% of the total wing-stroke period, a stimulus frequency of 22 cyc./sec. represents a better approximation to natural flight. The maximum displacement of the apparatus does not correspond exactly to the displace₁

▮hent between the two suspension points of the stretch receptor because some deformation of the soft cuticle at the anterior suspension is unavoidable. However, direct measurements showed that the deformations of the sense organ are proportional to the excursions of the steel rod, the factor of proportionality being about o·8.

The response to sinusoidal deformations at constant frequency and constant displacement is shown in Figs. 2 and 3 respectively. The burst of large potentials within the stretching phase derives from the stretch receptor, whereas the smaller potentials originate from vibration-sensitive scolopoforous units. Some of the scolopoforous
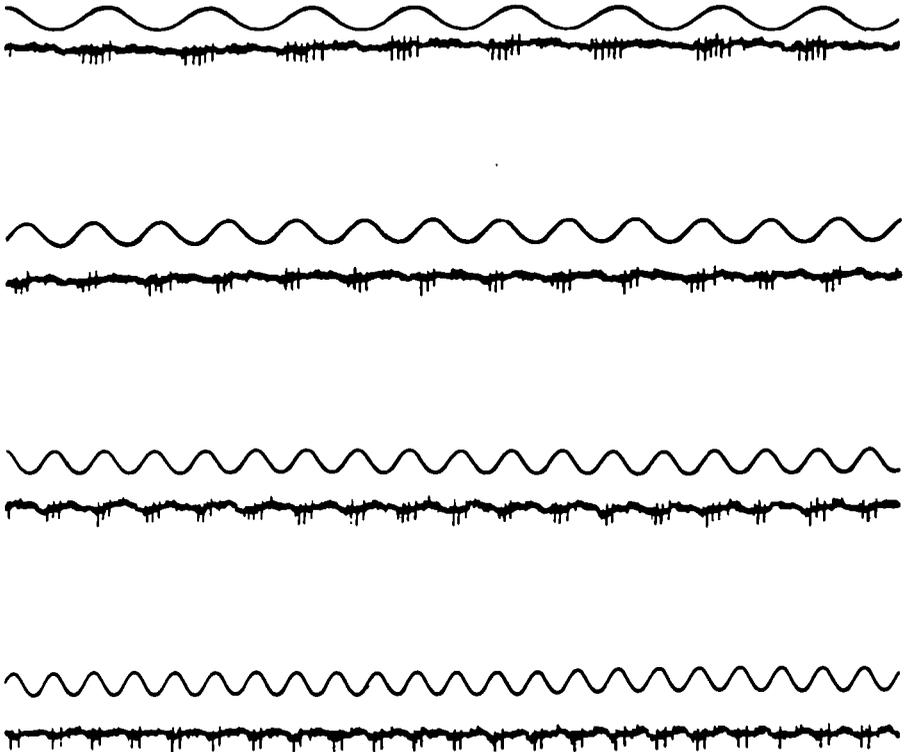
Fig. 3. Sinusoidal stimulation at different frequencies (10, 15, 20 and 25 cyc./sec.), but with constant displacement (370 μ).

sensilla, which have a low adaptation rate, fire almost continuously and make the record look as if it was amplified to near the noise level. Other sensilla with thicker axons, higher threshold and faster adaptation fire only at certain phases of the stimulus cycle, but since the spikes amount to maximally half the spikes from the stretch receptor, there should be no misinterpretations.

To a constant stimulus the stretch receptor responds with a tonic discharge whose impulse frequency increases with the amount of stretching. Also, after an extreme shortening some activity persists but the discharge is abolished during the period of shortening and immediately afterwards. During stretching, phasic discharges are superimposed upon the tonic response and, at low frequencies and/or small displace-▮ents, this results in small variation in the number of potentials per period, as seen
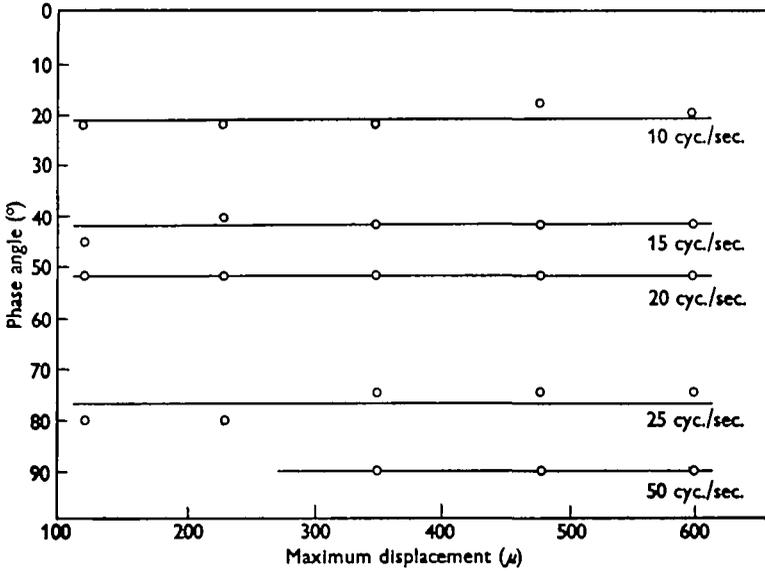
Fig. 4. Phase angle of the first potential of the response as a function of the maximum displacement at different frequencies of stimulation. The graph is based upon a single, but typical experiment.
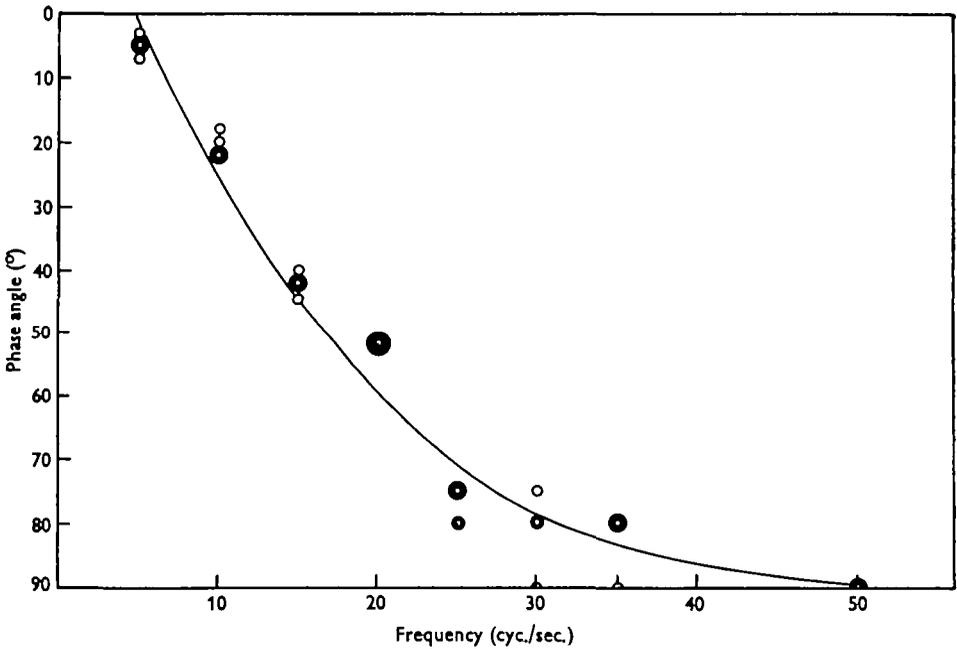

Fig. 5. Phase angle of the first potential as a function of frequency of stimulation. Concentric circles indicate that the values coincide. Data from a single experiment.

from the upper tracing of Fig. 2. The displacement at which the receptor fires at least once per stimulus cycle was not determined by adequate methods, but some information was gained by the application of trains of damped harmonic oscillations to the sense organ. At flight frequency, it was found that the maximum displacement must exceed 45 μ.

(a) Relation between phase angle of first potential and maximum displacement

During normal flight the response of the stretch receptor often consists of a single potential, so that the timing of the first potential may be especially important. In Fig. 4 the phase angle of the first potential is plotted as a function of the displacement
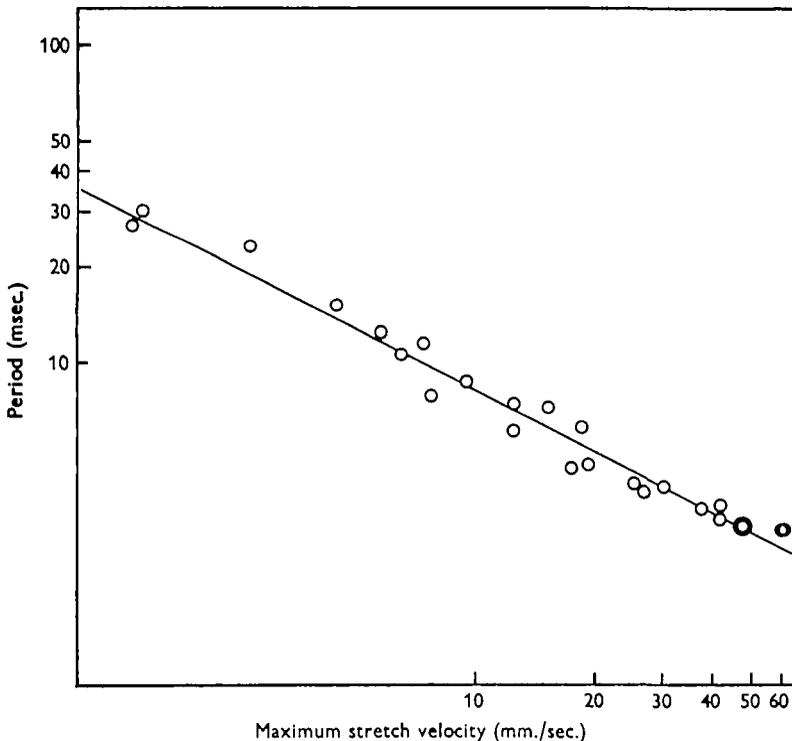

Fig. 6. Time between the two first potentials as a function of maximum velocity of stretch. Both scales are logarithmic.

at different frequencies. The curves clearly demonstrate that this angle is independent of deformation at any given frequency. At deformations smaller than 100 μ, the lines in Fig. 4 decline and approach 90° at about 50 μ below which stimulation is inadequate. It should be noticed that, in terms of time, the deviations in Fig. 4 are less than 1·6 msec., and in most cases 1·0 msec., i.e. less than twice the standard deviation. The non-linear relationship between phase angle and frequency is seen in Fig. 5. It shows that the time interval between the beginning of the stretching phase and firing is independent of the displacement, the velocity of stretch and the acceleration of stretch, whereas it depends on the duration of the period of stimulation. During

normal flight the independence between phase angle and deformation implies that as long as the frequency is constant, the stretch receptors fire at a certain position of the upwardly moving wing, this position being defined within 1 msec.
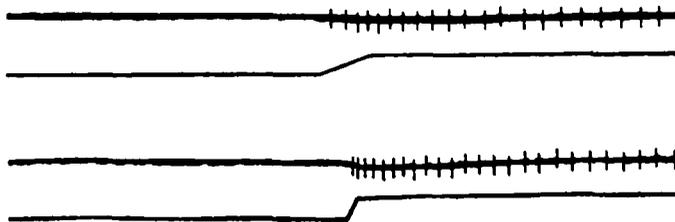

Fig. 7. Records of the response to stretching at constant velocity. The rising time of the stimulus is 40 and 10 msec. respectively. The deformation is 400 μ.
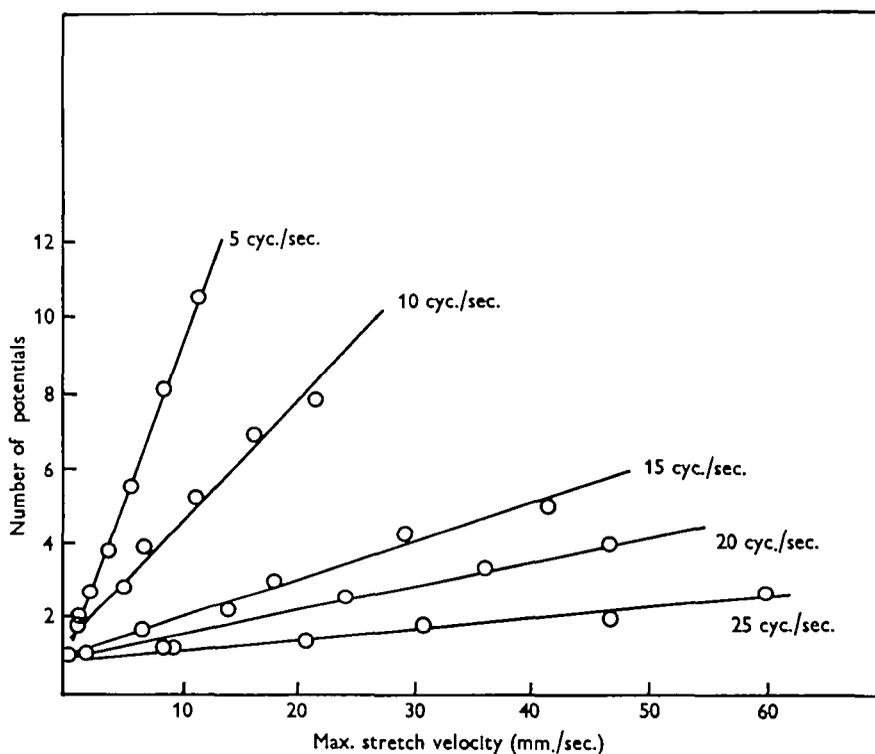

Fig. 8. The number of potentials per stimulus cycle plotted against the maximum velocity of stretch. The ordinate of each point represents the mean of 10 counts.

(b) *Period and number of successive potentials as a function of maximum velocity and displacement*

Fig. 6 shows the duration of the first period of response as a function of maximum velocity. This is the most relevant period from a biological point of view because there are usually only two action potentials per upstroke. Both axes are logarithmic, the straight line indicating that the period–velocity dependence has the form of

ower function. The graph is based on material from the entire spectrum of frequencies and deformations. The same kind of curve was obtained by using constant-velocity deformations (see Fig. 7). The only difference was a small change in inclination of the straight line, showing that the period is going into the power function with a slightly different index. This may be due to the fact that the tonic discharge at rest length differed in the two kinds of experiments and, also, that the velocity in the sinusoidal case is not constant between the first and the second potential. The observation that velocity has an effect on the frequency of response has been made in many other mechanoreceptors and was not analysed further.

When the total number of potentials during a sinusoidal stretching phase is plotted against maximum velocity, a straight line appears for each frequency of stimulation (Fig. 8). In other words, when the frequency is constant, the number of potentials provides a simple measure of velocity and, consequently, also of amplitude. Even a few firings per cycle therefore offer precise information about the movement.

DISCUSSION

It must be emphasized that the relations found are valid only within the range investigated but, in all cases, the range extends beyond the natural one. In the extreme regions, which are not shown in the graphs, the stimulus-response relationship often changed towards one of higher complexity.

The justification for using the behaviour of the sense organ under experimental conditions as a model for the response during normal flight can be questioned. For several reasons the metathoracic sense organs were used in the experiments. Thus, the connexion to the stimulating apparatus was easier to establish in this segment, and it is only in the metathorax that one can be certain that the deformations during flight are almost linearly related to the wing movements. The movements of the mesothoracic stretch receptor, which inserts on the phragma, are distorted by the mutual action of the two dorsal longitudinal muscles. Also, the metathorax is the more relevant segment when considering the basic mechanisms in flight co-ordination because the mesothoracic motor output is more labile and more dependent upon changes in the outside world than is the metathoracic output (Wilson & Weis-Fogh, 1962).

In other stretch receptors one has tried to use the highest firing frequency as a measure of the excitation of the organ and found that the excitation at low frequencies is greatest about the end of the stretching phase but occurs earlier when the frequency increases (Lippold *et al.* 1958; Finlayson & Lowenstein, 1960). In the present case the same tendency was apparent, but due to the relatively high frequencies (5–50 cyc./ sec.) and the small number of firings per cycle the relationship was not analysed further.

From a behavioural point of view, one of the most remarkable results is the independence of the phase angle of the first potential and the deformation. This simple relationship has not been emphasized before but may also be important in many other systems. The first and often the only signal which the central nervous system receives during each upstroke is, in other words, timed to a point of the mechanical cycle depending only on the wing-stroke frequency. It is interesting to relate this observation to the behaviour of flying locusts. Destruction of all thoracic stretch receptors

results in a reduction of the wing-stroke frequency to about half its normal value and it is also observed that small fluctuations occur in the duration of a cycle. Furthermore, small irregularities were noticed in the timing of the different muscle contractions. (It should be noticed that some of these irregularities could have been caused by damage to the scolopoforous sensilla of the dorsal part of the thorax). The stretch receptors could therefore have a dual function where one is related to an accurate timing of the motor impulses and the other is related to a general and more prolonged change in the excitation of the central nervous system. The latter tonic effect is discussed in detail by Wilson & Gettrup (1963).

As to the time relationship between the sensory response and the motor impulses, the following *hypothesis* may account for all observations made so far: the changes in excitation of a neuron within the flight system, in relation to the wing cycle, depend on the balance between its own activity and the presynaptic activity. Changes in the latter can be caused directly by sensory inflow, but may also originate within the central nervous system due, for instance, to reciprocal interaction between neurons (Maynard, 1955). The range within which firing can occur may be small compared with the duration of the wing-stroke cycle. A sudden rise in the level of excitation to near or above threshold caused by the stretch receptor signal would tend to fix the firing in time within the possible phase range. In this way the stretch receptor, by a strong influence on a *discrete* part of the neuron pool, under normal conditions could set the activity cycle precisely relative to the mechanical events. On the other hand, the coupling between this neuron group and the rest of the pool is supposed to be loose in order to account both for the changed phasing of the stretch receptor signal at high and low frequencies and for the tonic effect.

SUMMARY

1. Phasic sinusoidal and constant-velocity deformation was applied to a stretch receptor from the thorax of the desert locust, *Schistocerca gregaria* Forskål. The response was analysed with respect to the main parameters of the stimulus.

2. The stretch receptors respond to the flight movements with 1–3 potentials per wing-stroke cycle. Special interest was therefore given to the first potentials of the response. At constant frequency of stimulation the phase angle of the first potential of the response was found to be independent of changes in length and length–time parameters. The period between the two first potentials was found to be related to velocity in a rather complex way. At constant frequency, the number of impulses is a linear function of maximum displacement and/or velocity.

3. The functional significance of the relations is discussed in relation to co-ordination of flight and a hypothesis is proposed which explains the constancy of the wing-stroke period as the result of a stabilizing influence of the first potential of the response.

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