MOTOR OUTPUT PATTERNS DURING RANDOM AND RHYTHMIC STIMULATION OF LOCUST THORACIC GANGLIA

D. M. WILSON *and* R. J. WYMAN
From the Department of Zoology, University of California, Berkeley

ABSTRACT This paper employs new statistical techniques to further analyze the flight control system of grasshoppers. The quantitative results confirm some hypotheses which arise from previous studies of this system. After decapitation and ablation of wing proprioceptors, stimulation of the nerve cord at random intervals can elicit a coordinated response closely resembling the normal flight motor output pattern. The coordinated response begins only after many stimuli and there are usually many cycles of after-discharge. The frequency of the cyclic output is rather low and may be increased only slightly by large increases in stimulus frequency. Input from the stretch receptors is necessary to attain normal wingstroke frequency. Frequency of wingbeat rises with a time constant of about 2 seconds (or about 25 wingbeats) when stretch receptor stimulation is initiated. Frequency decay after cessation of stimulation has about the same time constant. No special phase relationship between stimulation and output is necessary for the increase in frequency or maintenance of normal pattern. When input frequency is adjusted as closely as possible to output frequency it is still not possible to force the output to maintain a particular phase with respect to the stimulation, all phase relationships still occur. In some animals all phases occurred with equal probability; in others a particular phase was preferred. When there was a strong phase preference the normal output pattern was disrupted.

INTRODUCTION

The flight control system of grasshoppers has now been the subject of considerable descriptive and experimental analysis. Two hypotheses which are generated by the earlier work are tested here. These tests have become possible because of the development of new techniques associated with parallel studies on other flying insects.

The flight control system is known to be composed partially of a central nervous pattern generator which requires excitatory input. It has been shown that the input pattern may be very different from the output pattern (Wilson, 1961) and the suggestion has arisen that the input might even be a random one; *i.e.*, contain no information other than that which says, "there is an input above threshold value." This has been found to be true. Stimulation at random intervals can trigger the highly coordinated output.

The output is always of less than normal frequency, however, unless certain receptors are active. Normal frequency requires excitatory feedback from sense organs which register wing movements, but it is not necessary that the feedback bear a special phase relationship to the output (Wilson and Gettrup, 1963). The earlier experiments indicate a complete phase insensitivity, in fact. It seemed worthwhile to confirm or modify this surprising result by statistical analysis of large data samples.

A third purpose of the present study was to accumulate more knowledge about the properties of the constituent neurons of the flight control system. This accumulation is a prerequisite to building models of the whole system.

MATERIAL

All the experiments were upon male *Schistocerca gregaria* (Forskål). The animals were obtained as young adults from the Anti-Locust Research Centre, London. They were used between 1 and 4 weeks of adult age.

METHODS

Records of single unit muscle action potentials were obtained by positioning 100 μ diameter wires, (either platinum or copper), insulated except at the tip, within the body of individual muscles and comparing the potential to a reference in the abdomen. The main locust flight muscles are of the fast type (having non-facilitating muscle action potentials). Each muscle used here has one or two motor units. An electrode within a muscle records all units in the muscle at much higher voltage than units of other adjacent muscles. The muscle action potentials accurately follow, after a few milliseconds delay, the nerve impulses leaving the central nervous system. Thus records from the muscles are perfectly correlated with the ganglionic output, even though a synapse, the neuromuscular junction, intervenes. It is possible to record the muscle action potentials either in dissected preparations or in intact, flying animals.

The nerve cord of highly dissected animals was stimulated either anterior or posterior to the thorax *via* a pair of hooked wire electrodes which were used to lift the cord into insulating medium (air or oil). Stimulation of the sensory nerve branches containing the stretch receptor axons was accomplished in similar manner except that two nerves were stimulated simultaneously through two electrode pairs connected in parallel.

The recording and stimulation techniques are described in greater detail elsewhere (Wilson and Weis-Fogh, 1962; Wilson and Gettrup, 1963).

The random stimulator consisted of a noise generator, a constant pulse generator, and an ordinary biological stimulator connected serially. The noise generator was a very high gain transistor amplifier with no input but a noisy first element. The output was clipped from below; *i.e.*, only voltages greater than a certain (but variable) value were passed. The resulting potentials triggered a constant pulse multivibrator. Average frequency could be set either by the amplifier gain or the clipping level. The pulses triggered the stimulator, which was used to set stimulus duration and voltage. The stimulator had an

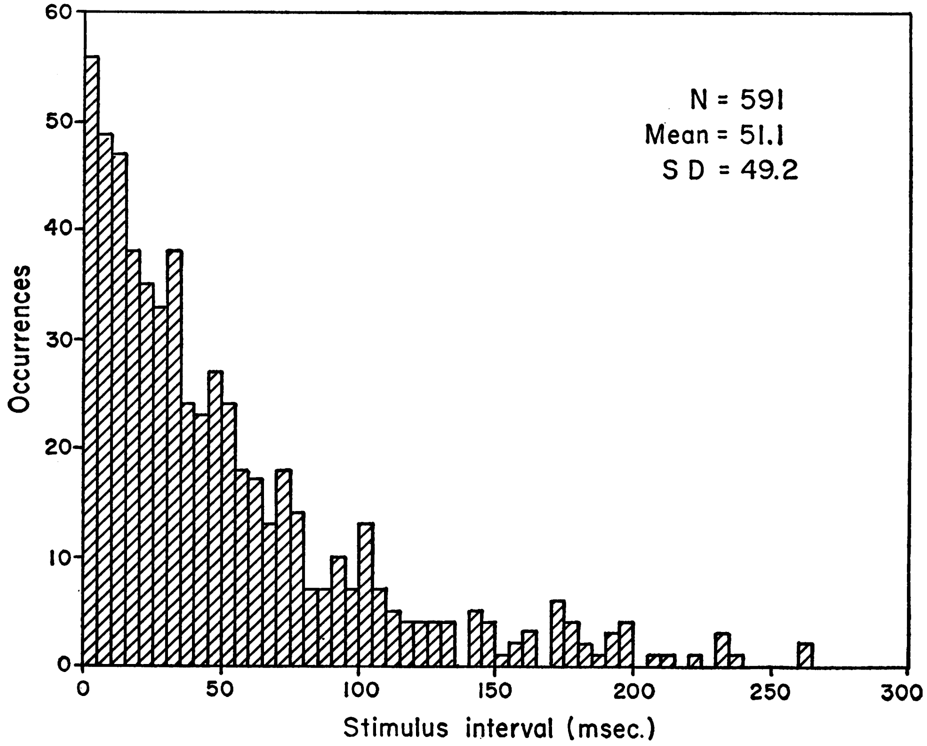


FIGURE 1*a*

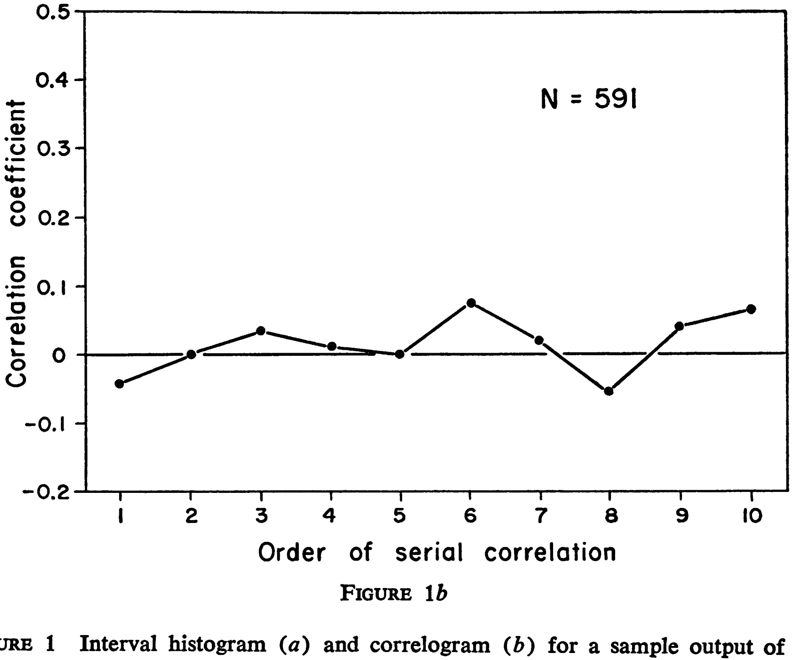


FIGURE 1*b*

FIGURE 1 Interval histogram (*a*) and correlogram (*b*) for a sample output of the random interval pulse generator.

upper frequency limit of about 5000 cps. Owing to this factor, at least, the pulse interval distribution was not truly random, but it did not differ significantly from random in several important respects. Fig. 1*a* shows the interval histogram which is approximately exponential. X^2 test for fit to an exponential distribution shows no significant deviation from theoretical values. The mean of the distribution equals its standard deviation within the expected limits of sampling error. The first ten orders of serial correlation coefficients are all insignificantly small and show no trend (Fig. 1*b*).

Data are presented as interval histograms (time between impulses), time lag or latency histograms (time from stimulus or impulse to next impulse), and phase histograms (relative time of an impulse in stimulus interval or another pulse train interval). Serial correlation coefficients were calculated according to the method of Wyman (1964). Autocorrelations were used to indicate short term frequency trending. Cross-correlations were used to indicate frequency correlations between responding units or between stimuli and unit response. Computations were made with the aid of a general purpose digital computer.

RESULTS

Random Input to Nerve Cord

General. Random input to the nerve cord of locust preparations may cause a variety of qualities of response. The mechanisms of the differences are not understood, but they could be due to the uncontrolled sampling of different input units due to electrode arrangement, or to differences in the state of the thoracic system itself which result from the previous handling the preparation has received. Several of the kinds of response relate to the flight control system. Even after decapitation and ablation of the wing sense organs, random stimulation can cause a co-ordinated response closely resembling the normal flight motor output pattern. When flight is elicited *via* normal stimuli, such as wind blowing on the sensory hair fields on the head, it sometimes happens that random stimulation of the abdominal cord *inhibits* flight, but this is uncommon. Occasionally, long-lasting stimulation of the abdomen of a quiescent preparation results in the generation of a few cycles of the flight pattern only *after* the stimulus ceases, apparently owing to a rebound. Descending inhibitory effects have been seen also during brain stimulation.

However, most often random stimulation of even the abdominal cord has an excitatory effect on the flight control system. When the random stimulation does have an excitatory effect it may cause either co-ordinated or chaotic responses, but the response is never random as to temporal sequences in the individual units. Since uncoordinated responses can be elicited in similar preparations by means of several experimentally patterned stimuli and normal flight stimuli it is possible that coordination or lack of coordination is due to the properties of the thoracic nervous system and not to the nature of the stimulus pattern (Wilson, 1961). Rhythmicity in the individual units during random stimulation is at least partly due to long lasting and accumulating refractoriness (Wilson, 1964) which gives the system low pass filter qualities. As is found in other systems (*e.g.*, Hagiwara, 1954; Bullock and Diecke,

1956) rhythmicity increases with output frequency (Fig. 2*d*). Output frequency also increases with input frequency. Increasing stimulus intensity recruits more input fibers in the cord and increases output frequency of individual motor units. The latter indicates spatial summation of input pathways upon the output unit.

Under different conditions different numbers of units may respond. At low stimulus intensities and in deteriorated preparations fewer units respond and the response is less coordinated. We presume that under these conditions there is less coupling between the units and it may be possible to determine the properties of the individual units. When only a single unit responds to the stimulus it may show both a frequency and a latency dependence upon the stimulus. If several motor units respond they may be quite independent of each other in phase even though they show a positive frequency correlation (due to independent correlation with the input?), or they may exhibit a temporal pattern. When many or all flight motor neurons respond the pattern is stronger, the individual unit rhythmicity is more perfect, even though of low frequency, and the output frequency is relatively independent (within limits) of the input frequency.

A few experiments will now be examined in detail in order to illustrate some of the above points.

Unit Responses. We will begin by describing the results of experiments in which the abdominal nerve cord was stimulated with the random interval generator and only a single unit could be observed to respond. It is possible that slow muscles and muscles which were not part of the flight system responded also, since this could not be seen by our technique. Fig. 1*a* and *b* show the characteristics of the input. These have been described already in the Methods section. The output characteristics are shown in Fig. 2 *a, b, c,* and *d.* The difference between input and output distributions can be considered due to filtering at several points in the nervous pathways. Certainly some high frequency filtering due to refractoriness occurs at the site of stimulation; that is, during the stimulation of the presynaptic fibers. Simple refractoriness will give an output distribution which follows exactly the input distribution, but which leaves out intervals less than the functional refractory period. However, these input fibers are capable of responding at much shorter intervals than those found in the ganglionic output, and we presume that additional filtering or integration is synaptic. The output interval distribution indicates that the timing of successive impulses is is not independent. Serial correlation coefficients for successive intervals shows a dependence here as well. The degree of dependence varies from unit to unit and from time to time, but qualitatively the dependence is similar (except that in some cases the values for r are insignificant). In general the first few orders of autocorrelation are significantly positive, with successive orders decreasing in value but remaining positive. The correlogram suggests that there are short term (3 or 4 cycle) frequency trends. In some cases all the values are individually insignificant, but even then the first few values are all positive. In other cases all the 10 calculated coefficients are

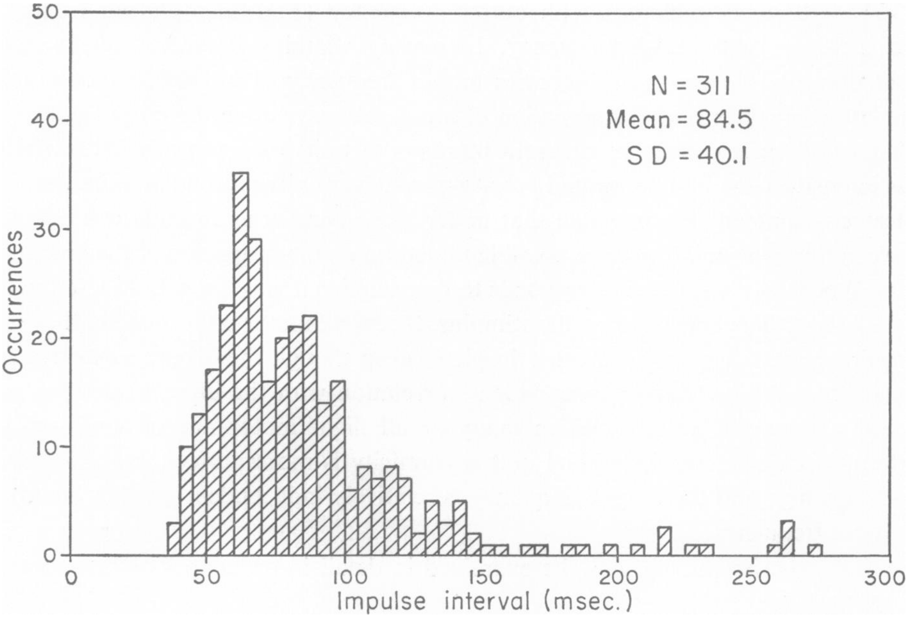


FIGURE 2*a*

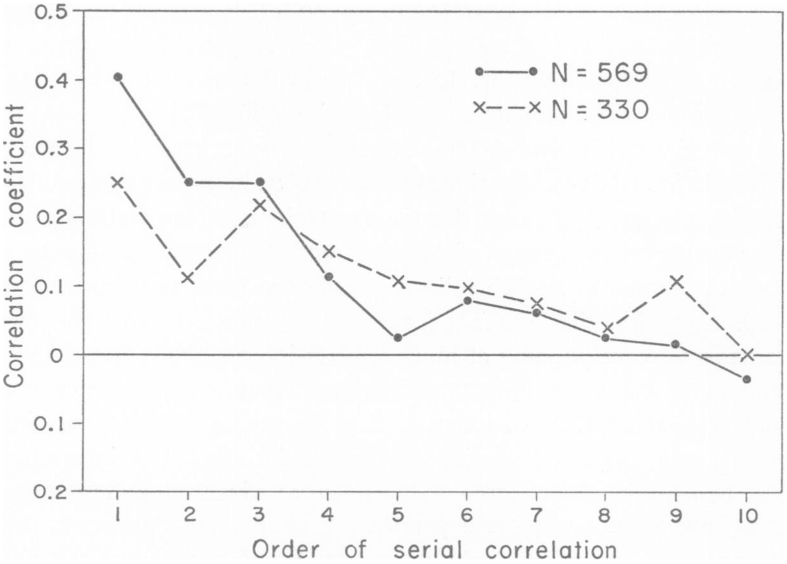


FIGURE 2*b*

FIGURES 2*a* to *d* Response of a single unit to random presynaptic stimulation. (*a*) The interval histogram. (*b*) The first ten orders of serial correlation coefficients. (*c*) A plot of interval length (ordinate) *versus* time of occurrence of impulse (abscissa) for stimulus □ and response ○. (*d*) Records at different input frequencies showing frequency dependence of the output. The small pips and dots indicate the stimulus times. The spike amplitude varies due to summation with noisy baseline and to relative refractoriness.

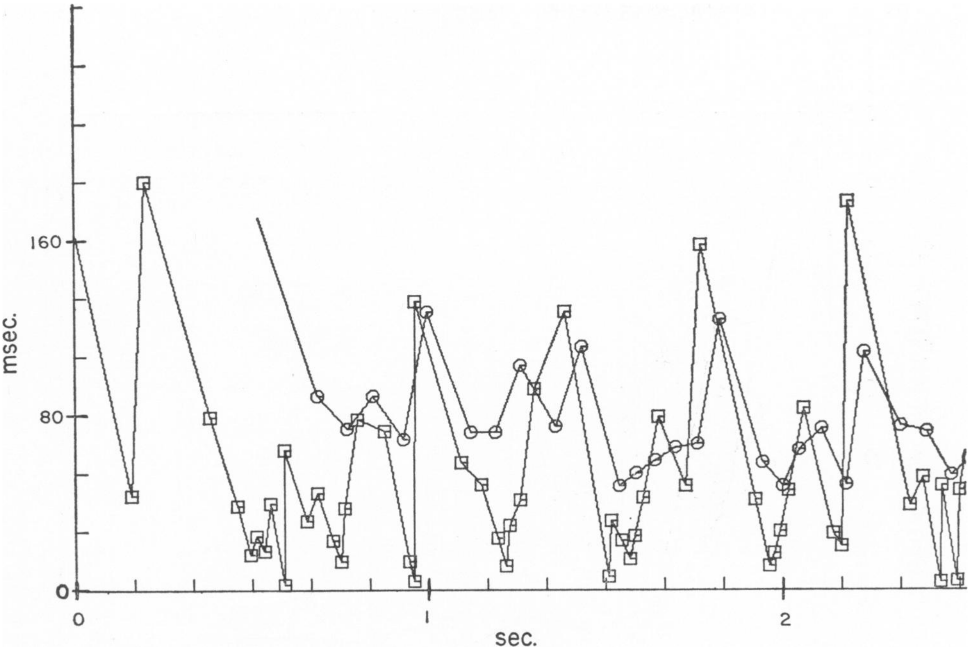


FIGURE 2c

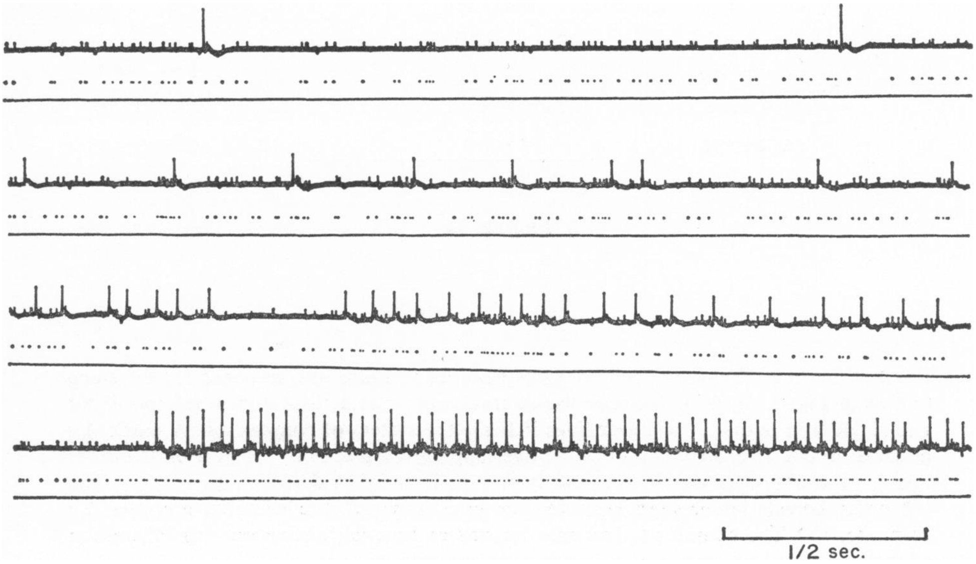


FIGURE 2d

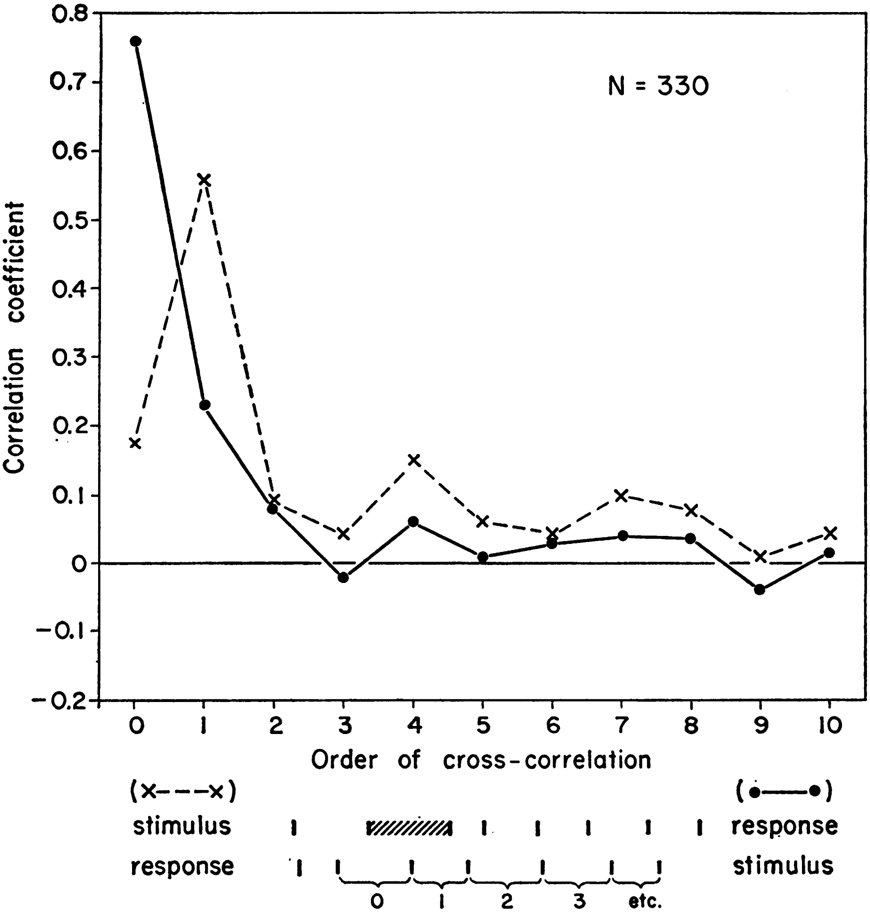
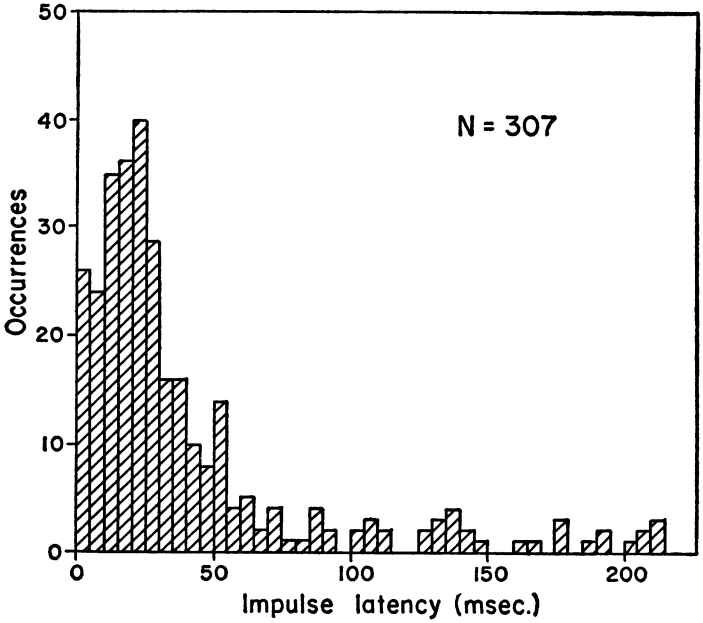
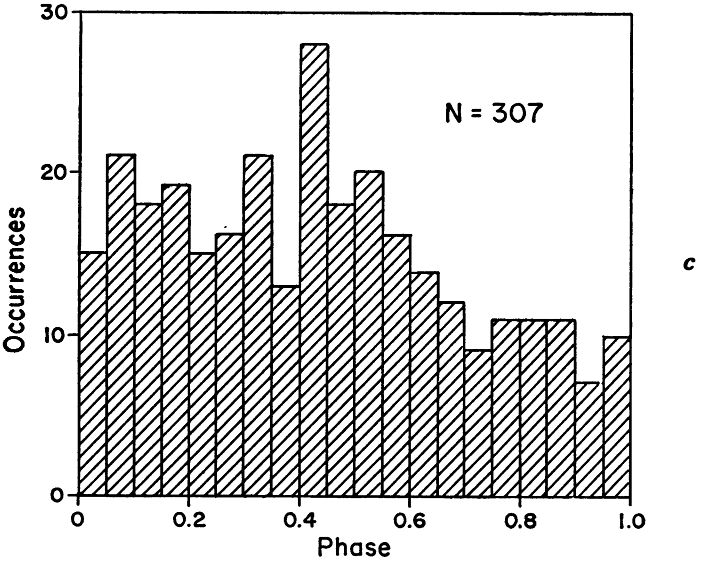


FIGURE 3a

FIGURES 3a to c Temporal relationships between stimulus and response for the same unit as in Fig. 2. (a) Stimulus-response interval cross-correlations during random input and single unit output. The lower lines indicate the system of comparison. A particular interval on one line is compared to the overlapping but preceding interval on the other line for the 0th order correlation and then with later intervals for higher orders. The 0th order correlation is high only for the case in which the stimulus precedes the response. (b) The stimulus-to-response latency or time-lag histogram. (c) Histogram of the phase of the muscle spikes in the stimulus intervals.





FIGURES 3*b* and *c*

large, indicating either longer term cycles of change within the sample or non-stationary statistical conditions.

The response intervals show a correlation with the preceding stimulus intervals. The cross-correlation may be strong for overlapping stimulus and response intervals when the stimulus is precedent, and it decreases to insignificance within the first few successive responses (Fig. 3*a*). In other cases the correlation is weaker but longer lasting. Here the response seems to follow a summing of several previous stimuli which arrive indeterminately. There is also a statistically significant, but weak tendency for the response to follow after a latency of about 20 msec. (Figs. 3*b* and *c*).

Multiunit Responses. In the next described experiment records were taken from the two units of a single muscle, the only active muscle in the preparation. In flight these two units of the right mesothoracic tergosternal muscle fire synchronously whenever both are active. This muscle can move only the wing and the units are therefore presumably never normally used asynchronously. Fig. 4 shows a sample of the electrical record. Our first impression was that the units showed a tendency toward synchrony, but the phase histogram (Fig. 5*c*) shows that this was largely illusory. Both units responded to random input in a manner similar to the isolated units described in the last section. The higher frequency unit shows high serial correlation coefficients for up to four intervals; *i.e.,* it is rhythmic with rapid frequency trends. The lower frequency unit is relatively arrhythmic, as indicated by both the correlogram (Fig. 5*d*) and the large standard deviation. In Fig. 6 the short term frequency trends may be seen. It is interesting that the two units show a short term positive frequency correlation even though they are otherwise temporally independent. This effect has been found to occur normally in the motor control of fly wings (Wyman, 1964).

Coordinated Responses. Some preparations respond with all the flight muscles when the nerve cord is stimulated randomly. When this is the case, then most often the response is highly coordinated and similar to that during normal flight, except that the repetition rate of the cyclic output is rather low. The coordinated response does not occur until after many input stimuli and there are usually many cycles of after-discharge. The frequency of response is dependent upon average input frequency, but only over a narrow range of output frequency. The output does not appear to be phased by the input, but in the present experiments the input-output ratio was so large (*ca* 5:1) that one can imagine that there was nearly always an available triggering stimulus. However, the after-discharge and the occasional long periods during which there was no stimulus, but normal responses, show that a triggering stimulus was not necessary. Fig. 7 shows a typical example. However, not in all cases does the over-all cycle divide into ⅓ upstroke, ⅔ downstroke as it does in Fig. 7. The cause of variation in upstroke-downstroke ratio is still unknown.

Discussion. The individual motor neurons of the flight control system

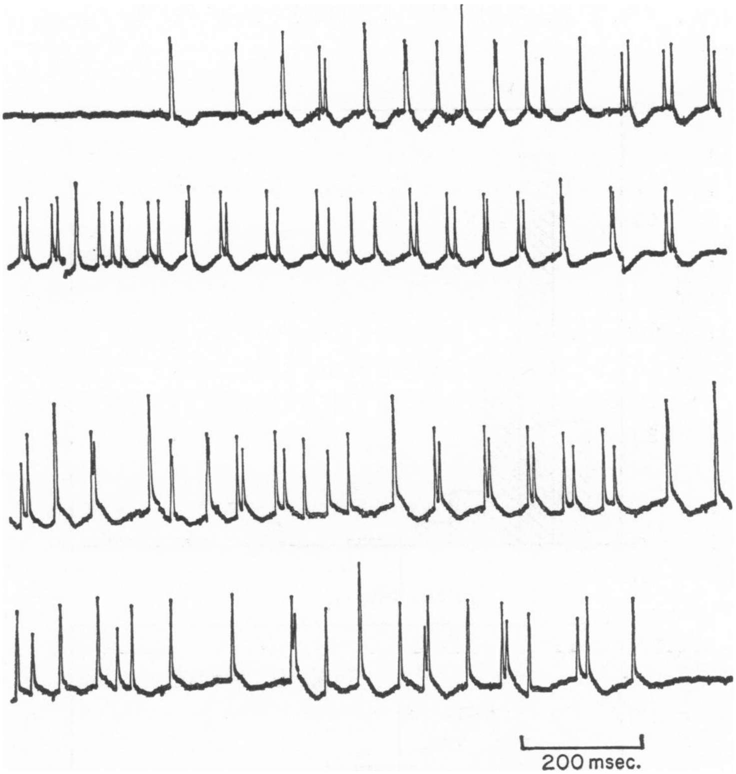
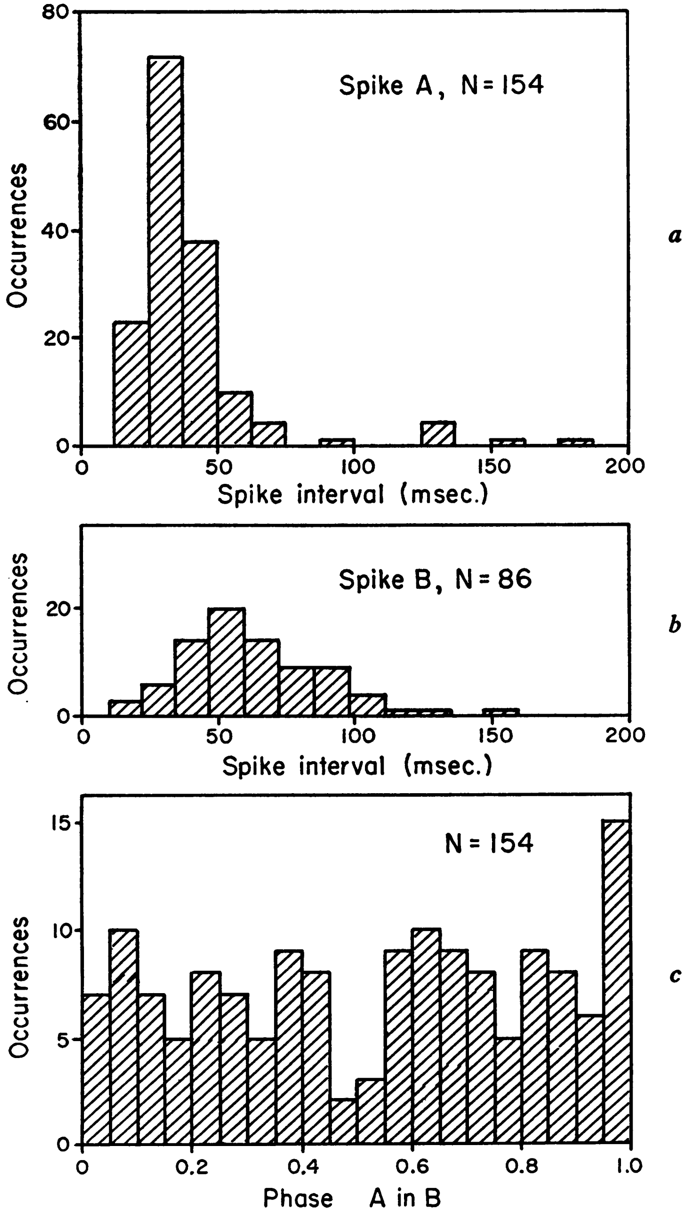


FIGURE 4 Records from the two units of a tergosternal muscle during random stimulation of the preganglionic pathways. The units may be distinguished by amplitude. They are only occasionally synchronous.

seem to respond alike to preganglionic input. There is no reason yet to doubt that at least the separate groups of elevator and depressor motor neurons form small populations of similar units.

The motor system is capable of responding to an unpatterned input with a patterned output. The several orders of positive autocorrelation indicate that the rhythmicity is not due simply to relative refractoriness, for a refractoriness which only blocked the next impulse during a random input train would result in no significant correlation, while refractoriness outlasting the next impulse (which it may, Wilson, 1964) would give a negative first order correlation (Kuffler *et al.,* 1957).

A plausible interpretation of the positive autocorrelations is that the unit sums input over several input intervals and stores the resulting excited state through several output cycles. We do not have enough data to express an exact time constant for the decay of the integration, but the correlation and after discharge data suggest that it is of the order of at least a few tenths of a second. Possibly this integration constant varies with the physiological state of the preparations. That the decay constant



FIGURES 5*a* to *d* Analysis of records of the same two units as in Fig. 4. (*a*) and (*b*) interval histograms for the two units. (*c*) Phase histogram for spike A in the intervals of B. (*d*) Serial correlations between intervals for the two units.

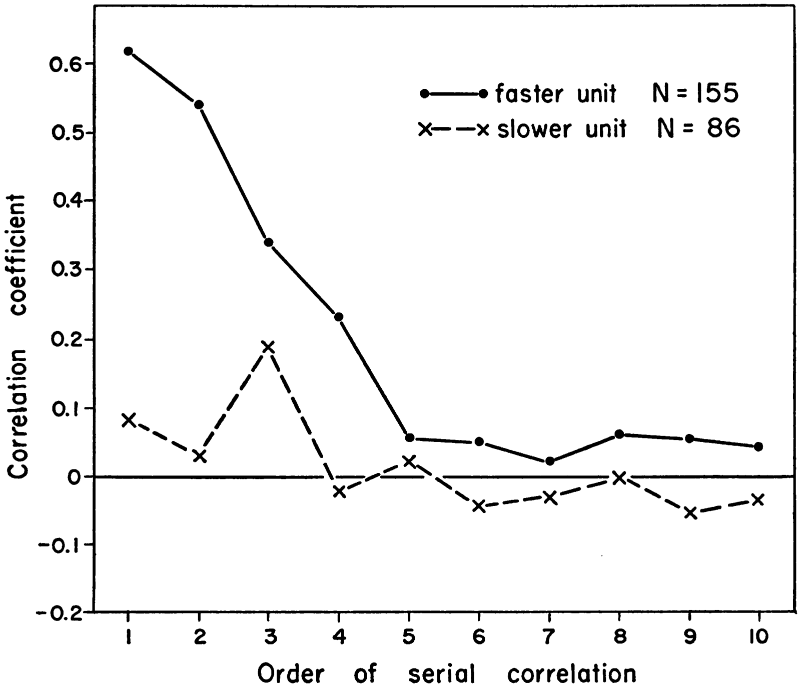


FIGURE 5*d*

is significantly long is indicated also by the weak latency and phase dependences of the response upon the stimulus. If the excitation due to a single input waned rapidly relative to input frequency then a temporal dependence would be a necessary condition of firing, since the neurons are not spontaneously active. In fact, in the present experiment with random input the units show only a small tendency to be phased by the stimulus. Earlier experiments with lower frequency rhythmic inputs produced stricter time dependences (Wilson; 1961, 1963).

Even when more than one unit responds to the stimulus it is possible for each to behave independently in the above described way. In other cases the units become temporally coupled and even less dependent upon input timing. The most interesting problem for the future regards the nature of this intraganglionic coupling.

Rhythmic Input via the Stretch Receptors

Four stretch receptors, one at the base of each wing, normally respond during flight with one or a few impulses just before the top of each wingstroke. This activity is necessary for the maintenance of normal wingstroke frequency. If only two of the receptors are stimulated electrically it is possible to drive the output to a frequency intermediate between those of the normal and the fully deafferented preparations, even when the stimulation is not phased correctly with respect to the output. Indeed, it has not been possible to entrain the output even when input and output frequencies differ only slightly. By entrainment we mean a fixed temporal relationship between input and output, with output following input with a constant latency or in constant phase. However, it seemed possible that there might be a loose phase coupling even if phase locking (equals entrainment) was highly improbable. Such a loose coupling should be demonstrable as phase preference in the long run. In the present experiments phase preference was either lacking or present in different cases. When there was a strong phase preference the normal output pattern was disrupted. Several experiments will now be described to illustrate these points.

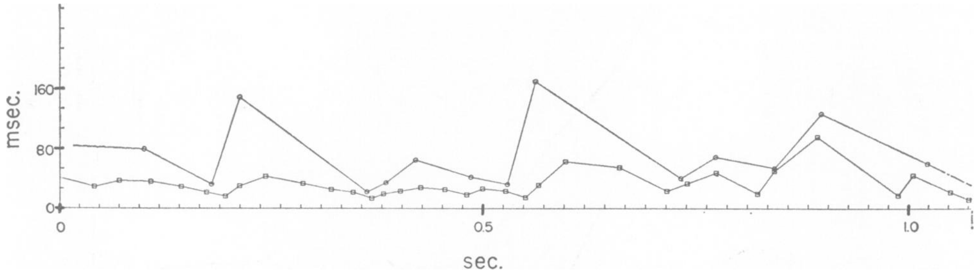


Figure 6 Interval *versus* time of occurrence plot for the two units of Figs. 4 and 5.

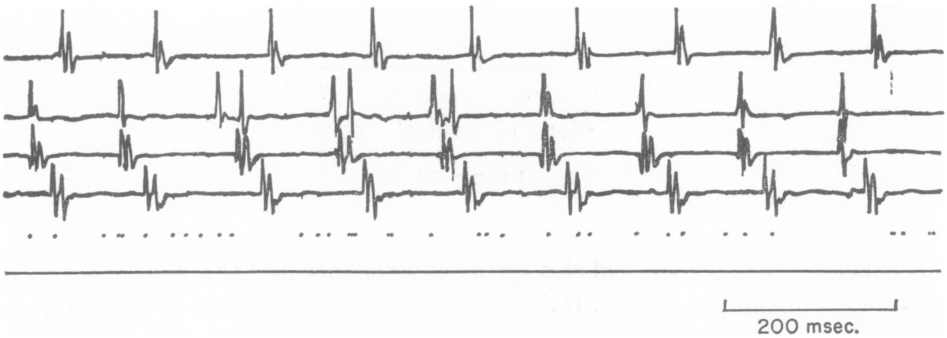


Figure 7 The coordinated response of four flight muscles during random stimulation of a wingless preparation. The response remains rhythmic and well-patterned even when stimuli are absent during whole cycles of output. From top to bottom the traces represent activity in a depressor muscle, an elevator muscle, an elevator, another depressor, and the stimulator.

Fig. 8 illustrates the two effects that stimulation of the stretch receptors has on

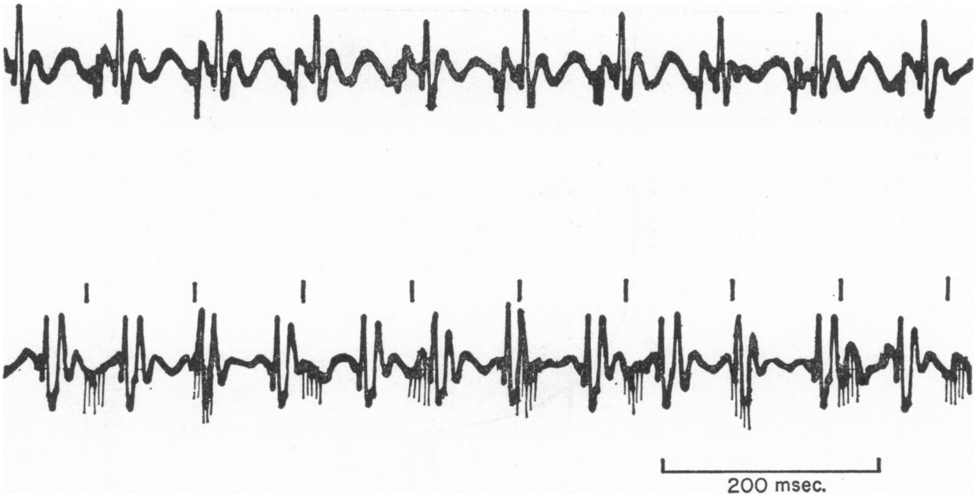


FIGURE 8 Response of the deafferented flight system monitored at a single muscle unit. Upper line, stimulated by wind blowing on the head only. The wavy baseline is an artifact due to low fidelity tape recording. Lower line, stimulation by wind on head *and* electrical stimulation of two of the stretch receptor axons. The electrical stimulation is in high frequency bursts of five pulses, each burst beginning at the time of the marking dash.

the flight motor pattern. The upper line is the response to wind on the head when the wing sensory nerves are cut. The frequency is about 11 CPS. In the second line the stimulus artifacts mark the time of stretch receptor firing. The fundamental output frequency is raised by over 30 per cent and the motor unit fires twice during each cycle; there appears to be a general increase in excitation, but no phase locking. The effect of stimulation is not immediate but requires many cycles to develop. Long periods of stimulation alternating with periods of no input except wind on the head hairs give rise to reversible changes. Fig. 9 shows the time course of the increase and decrease in frequencies at the beginning and end of stimulation. The interval variation between successive intervals in this figure is within the expected measurement error and may have no biological meaning. The smoothed curve shows that the induced changes in frequency have a time constant of about 20 to 30 cycles, or about 2 seconds. This figure agrees roughly with that arrived at by another technique by Wilson and Gettrup (1963).

The phase dependences for three consecutive bouts of stimulation are illustrated in Fig. 10. The first histogram shows no significant dependence at all. The others do deviate slightly but just significantly from random (X^2, P *ca.* 0.05).

A random phase dependence is illustrated in a rather different experiment in Figs. 11 *a* and *b*. In this preparation wind on the head failed to elicit a flight response, but additional stimulation of two stretch receptors did produce the usual low fre-

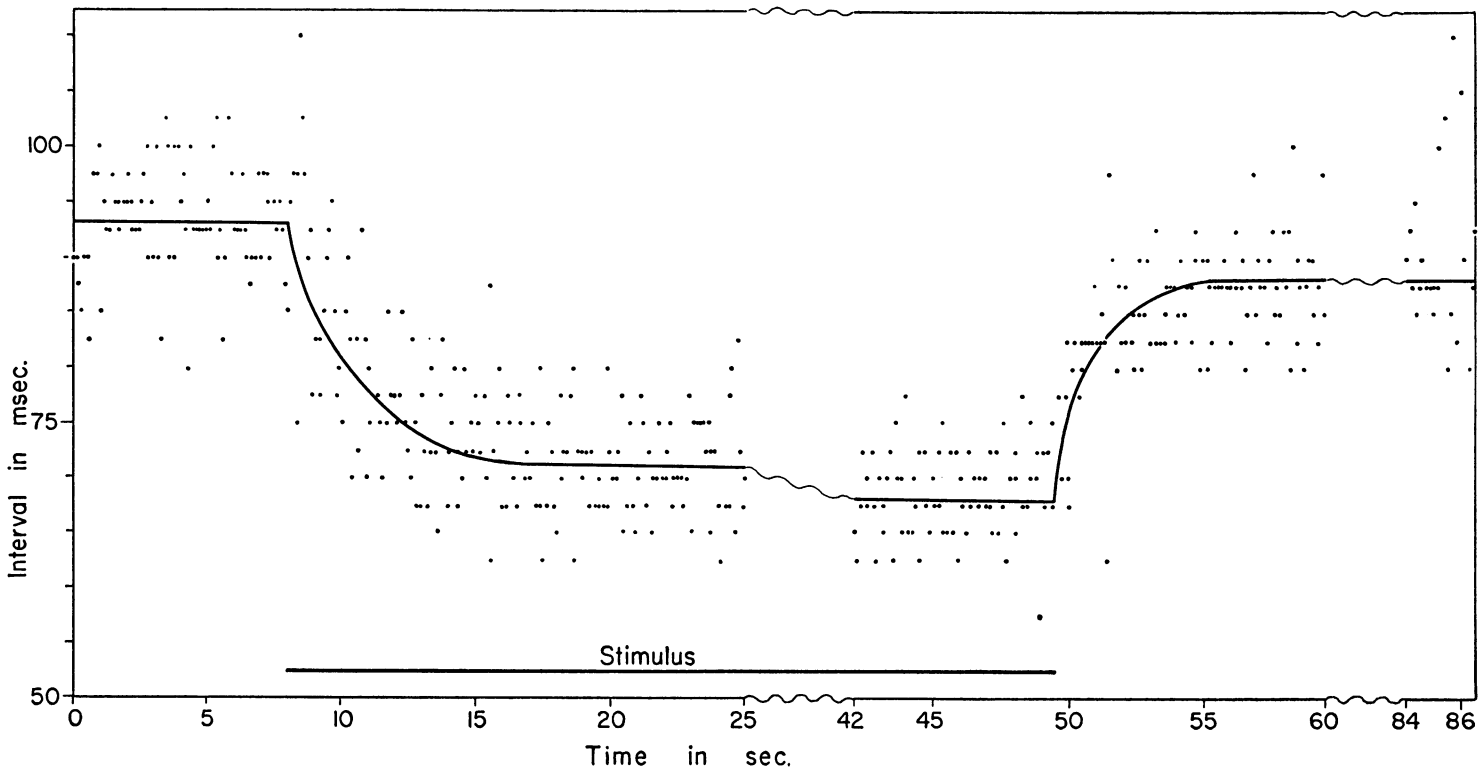


Figure 9 Output frequency of the flight system during stimulation by wind on the head before, during, and after a long bout of stimulation of two stretch receptors.

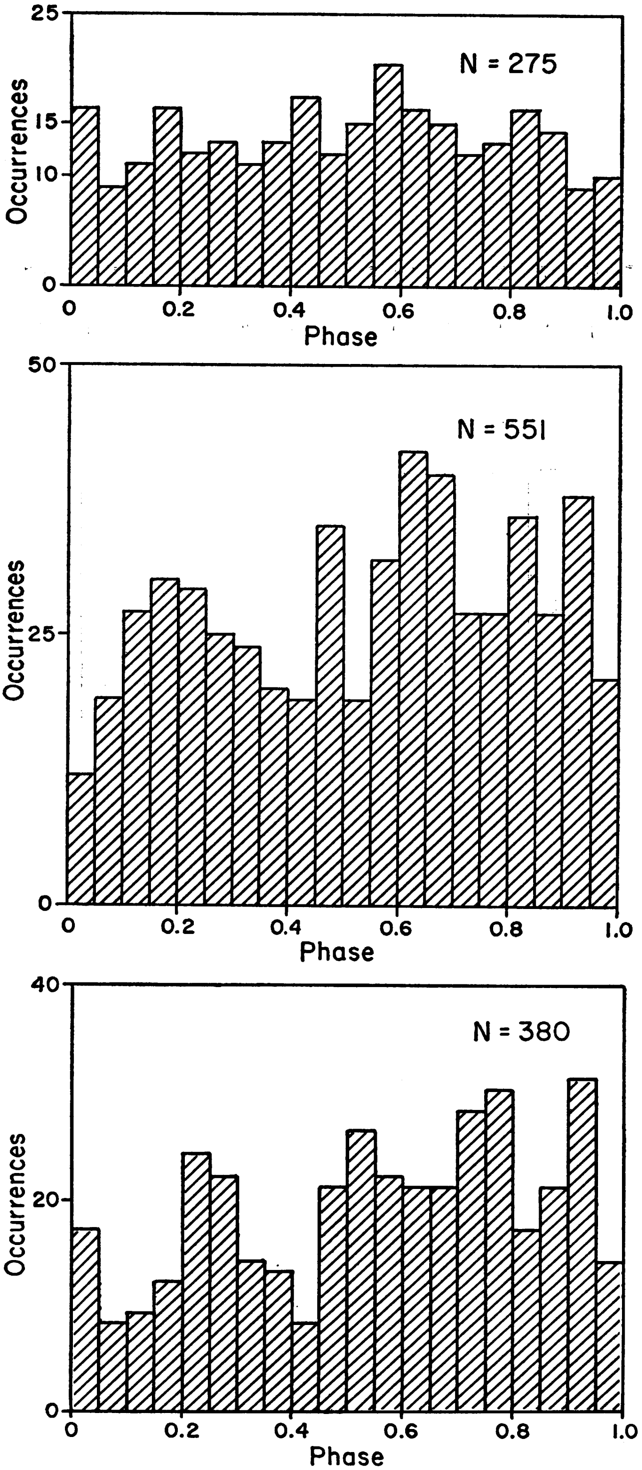


FIGURE 10 Histogram of the phase of an output impulse in the stimulus interval for three successive bouts of stretch receptor stimulation. Conditions as in Fig. 9.

quency output. Even in this case in which the stretch receptor input was *necessary,* the output was phase-independent. It should be pointed out here that the stretch reflex by itself produces no output under any circumstances, and in this experiment it was adding to a barely subthreshold stimulation from another source.

In another experiment the system responded with the usual low frequency rhythmic output when stimulated by wind on the head only, and when the stretch receptors were stimulated the frequency was increased. However, at the same time, irregularities in the flight pattern appeared and a fairly strong phase preference was exhibited (Figs. 12*a, b,* and *c*). The irregularities consisted of extra impulses which did not occur at the normal time, and associated with these extra impulses there were large perturbations in the fundamental interval. The two interval histograms (Figs. 12*d* and *e*) show that the response differed obviously from the normal flight pattern. The irregular portions of the response recurred periodically, apparently as the input and output rhythms shifted through the various phase relationships. Apparently strong phase coupling between the input and output is not consistent with normal operation even when the two frequencies differ by a small amount. The result of the phase coupling to an input of improper frequency is not to entrain the output, but to disorganize it.

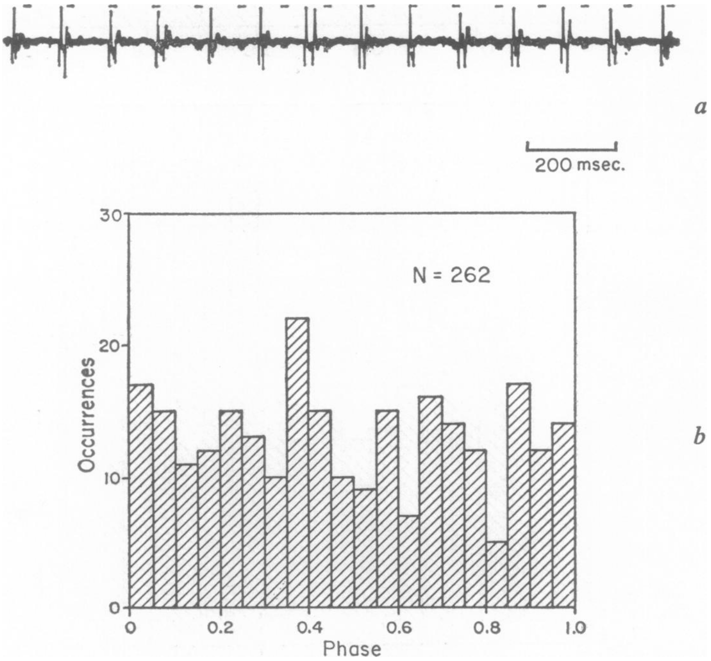
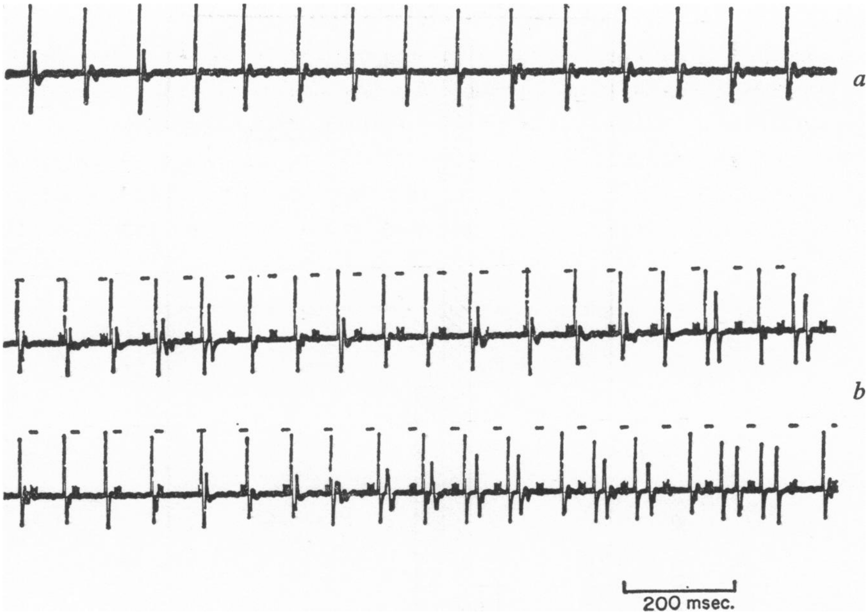


FIGURE 11 An experiment in which wind on the head was insufficient to elicit a response but stimulation of the stretch receptors was also necessary. (*a*) Sample of the electrical record. (*b*) The phase histogram.



FIGURES 12*a* and *b*

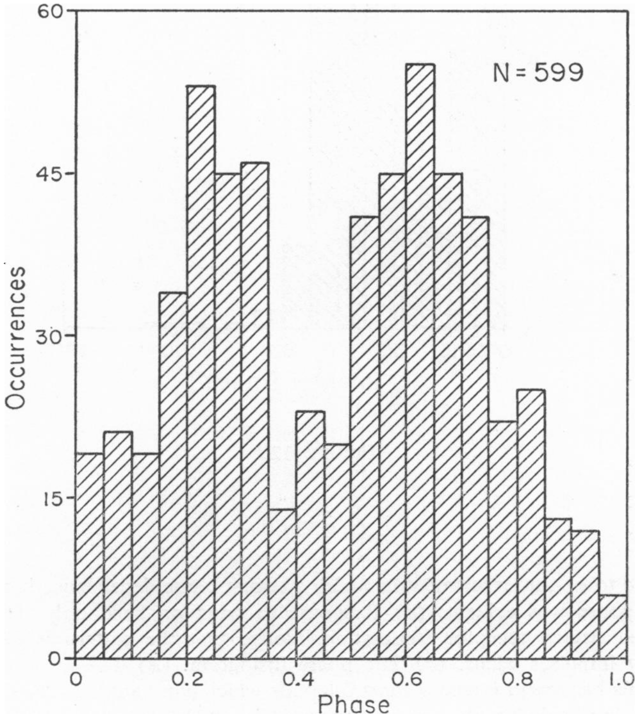


FIGURE 12*c*

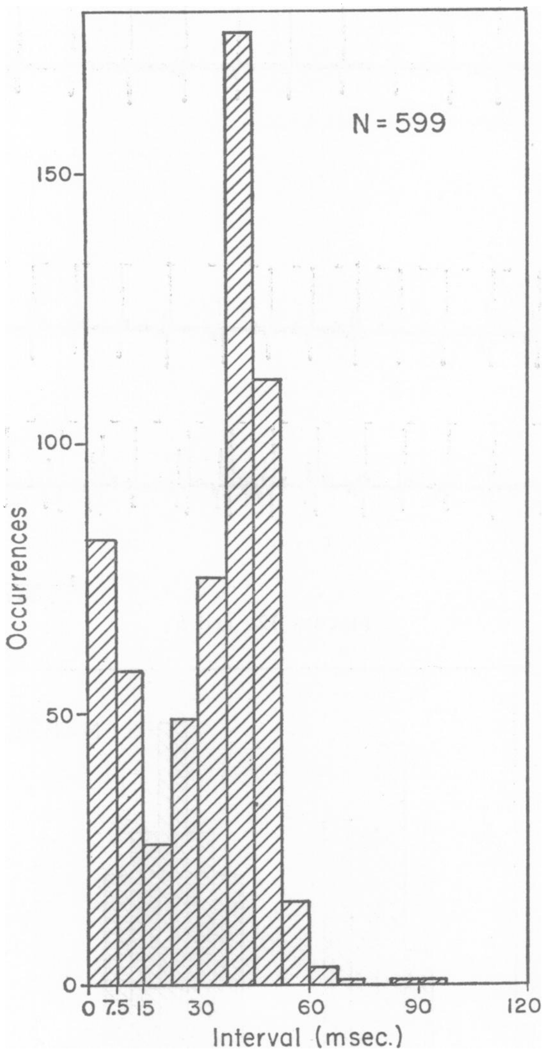


Figure 12*d*

Figures 12*a* to *e* An experiment in which a strong phase dependence between stimulus and response was evident. (*a*) Response to wind on head only. (*b*) Effect of superimposed stretch receptor stimulation. Notice that the pattern is disrupted and extra output impulses occur. (*c*) The phase histogram. (*d*) The interval histogram. Note that this histogram includes short intervals which can be due to the normal firing in doublets, but includes also many abnormal intervals. (*e*) An interval histogram from a normal flying animal, for comparison with (*d*).

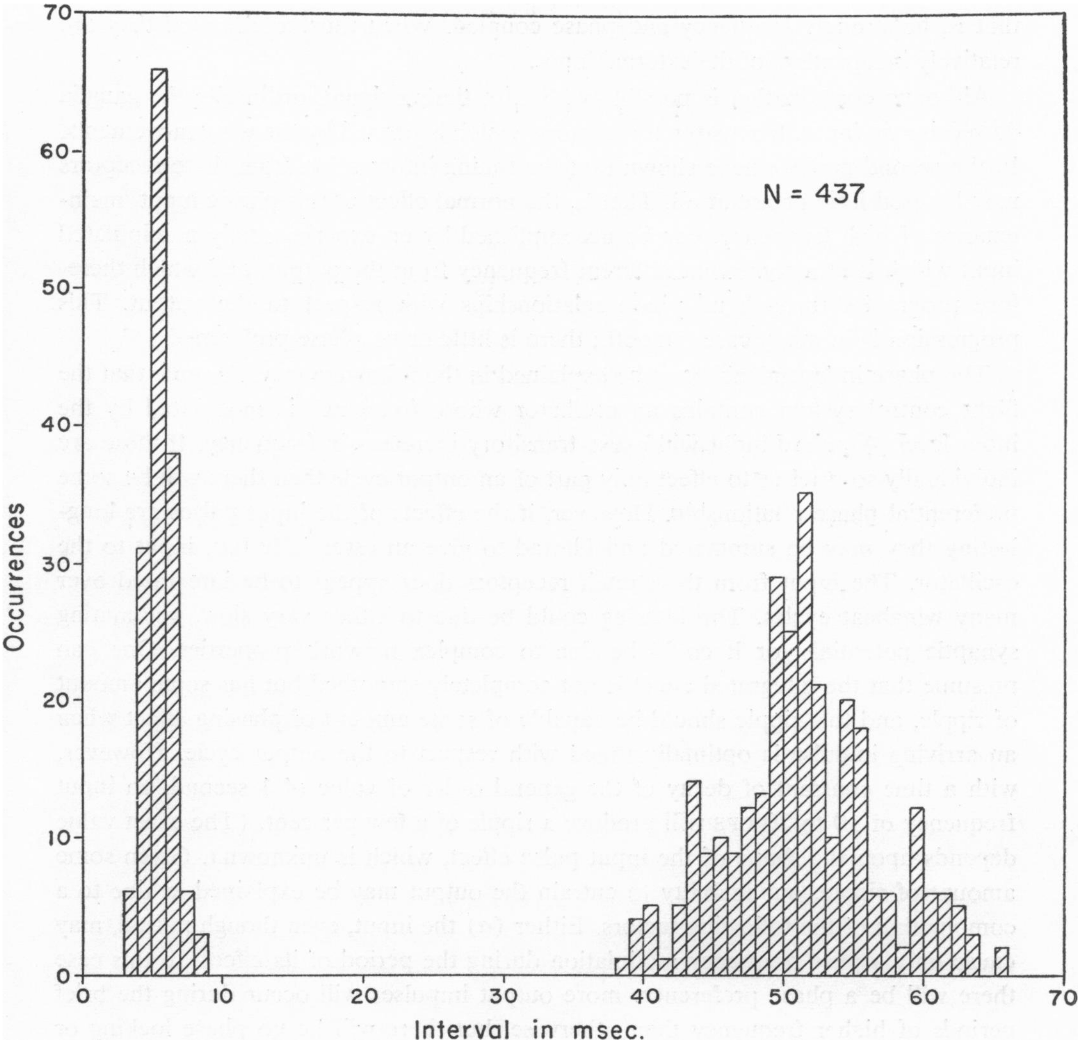


FIGURE 12*e*

DISCUSSION

In the first part of this paper we show that a random input to the thoracic ganglia of locusts can elicit, in the absence of feedback from moving parts, a fully coordinated output pattern resembling the one which occurs during normal flight. This input, being random, cannot contain the information which controls the timing of the units. When only one or a few units respond the output shows short-term frequency correlation and slight latency dependence upon the input. The units may be rather independent of each other. When more units respond they may be well coordinated, that is, be strongly frequency and phase coupled. When thus coordinated they are relatively independent of the external input.

Although coordination is possible without a timing signal, ordinarily the ganglia do receive an input, from stretch receptors, which is phased by the wing movements. In the second part we have shown that the timing information from these receptors may be used little or not at all. That is, the normal effect of this phasic input, maintenance of high frequency, can be accomplished by an experimentally manipulated input which is at a somewhat different frequency from the output, and which therefore progresses through all phase relationships with respect to the output. This progression is in many cases smooth; there is little or no phase preference.

The phase independence may be explained in the following way. Assume that the flight control system contains an oscillator whose frequency is modulated by the input level. A pulsed input will cause transitory increases in frequency. If these are individually so brief as to effect only part of an output cycle then there will be some preferential phase relationship. However, if the effects of the input pulses are long-lasting they may be summated and filtered to give an essentially D.C. input to the oscillator. The input from the stretch receptors does appear to be integrated over many wingbeat cycles. The filtering could be due to either very slow, summating synaptic potentials, or it could be due to complex network properties. One can presume that the integrated effect is not completely smoothed but has some amount of ripple, and this ripple should be capable of some amount of phasing effect when an arriving impulse is optimally timed with respect to the output cycle. However, with a time constant of decay of the general order of value of 1 second, an input frequency of 10 to 20 CPS will produce a ripple of a few per cent. (The exact value depends upon the shape of the input pulse effect, which is unknown). Given some amount of ripple, our inability to entrain the output may be explained as due to a combination of the following factors. Either (*a*) the input, even though pulsed, may cause only a pure frequency modulation during the period of its effect. In this case there will be a phase preference, more output impulses will occur during the brief periods of higher frequency than otherwise, but there will be no phase locking or entrainment; or (*b*) the phase setting effect of the input is weak relative to the effect on frequency; *i.e.*, the ripple is small. If the latter (*b*) is true, then other factors enter. (*c*) The output frequency changes when the input frequency is changed; therefore the experimental attempt to adjust the input frequency drives the output frequency away. (*d*) The output frequency naturally drifts somewhat. (*e*) There are noise sources not under experimental control. The last factor may obscure even a tendency to phase locking only for short periods of observation. However, if in the intact animal the stretch receptor input were needed to time the CNS with respect to the wings, then the necessary information ought to be rather immediately available and not require detection by means of analysis of hundreds of cycles.

If one considers the system from the point of view of the effect of motor output on the sensory input instead, a different picture emerges. In the intact animal the motor output always bears a specific relationship in time to the wing movements. It is not possible in locusts for it to be otherwise as long as the muscles are causing the movements in response to the nervous command. (This is not the case in all insects; see Wyman, 1964.) The movements themselves precisely control the timing of the stretch receptors. Therefore the ganglionic output influences very strongly the timing of its input while in the opposite sense the influence is weak or nil.

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REFERENCES

BULLOCK, T. H., and DIECKE, F. P. J., 1956, Properties of an infrared receptor, *J. Physiol.*, **134,** 47.

HAGIWARA, S., 1954, Analysis of interval fluctuation of the sensory nerve impulse, *Japan. J. Physiol.*, **4,** 234.

KUFFLER, S. W., FITZHUGH, R. and BARLOW, H. B., 1957, Maintained activity in the cat's retina in light and darkness, *J. Gen. Physiol.*, **40,** 683.

WILSON, D. M., 1961, The central nervous control of flight in a locust, *J. Exp. Biol.*, **38,** 471.

WILSON, D. M., 1964, Relative refractoriness and patterned discharge of locust flight motor neurons, *J. Exp. Biol.*, **41,** 191.

WILSON, D. M., and GETTRUP, E., 1963, A stretch reflex controlling wingbeat frequency in grasshoppers, *J. Exp. Biol.*, **40,** 171.

WILSON, D. M., and WEIS-FOGH, T. 1962, Patterned activity in co-ordinated motor units, studied in flying locusts, *J. Exp. Biol.* **39,** 643.

WYMAN, R. J., 1964, Probabilistic characterization of simultaneous nerve impulse sequences, in preparation.