THE CENTRAL NERVOUS CONTROL OF FLIGHT IN A LOCUST

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INTRODUCTION

That proprioceptive feedback is important in the regulation of locomotory patterns has been widely demonstrated. In fact, reflexes based on such peripheral loops have been suggested as adequate to produce the pattern and rhythm of movements in arthropod walking and flight. The present study characterizes some of the sensory input during flight of the locust which could be expected to be involved in these reflexes. However, it will be shown that this input is unnecessary for the establishment of a patterned oscillation in the thoracic ganglia which appears to be the same as that which produces the flight movements. The feedback from the moving parts of the flight system modifies considerably the frequency and details of ordering of a pattern which, nevertheless, is inherent in the ganglia. The elicitation and maintenance of this pattern depends on some source of excitatory input which need not be phased to the flight movements.

The desert locust *Schistocerca gregaria* Forskål is a particularly favourable object for such studies for a number of reasons. It can be induced to fly in the laboratory for extended periods of time with almost constant, cyclical and strictly co-ordinated movements of its two pairs of wings (Weis-Fogh, 1956a). The fundamental oscillation of each wing is up and down, but superimposed upon this is an active twisting of the wing surface, the pronation, starting at the beginning of the downstroke. The reverse supination is mainly passive-elastic and prevails during the upstroke. Each wing is moved up by the contraction of a medial row of vertical elevators and moved down partly by elasticity (Weis-Fogh, 1959), partly by a dorsal longitudinal depressor muscle and, finally, by a lateral row of vertical depressor muscles which also control the twisting (two basalar and one subalar on each side in each segment). Each of these muscles consists of one to four fast-type motor units which are activated zero, one, or two times per wing stroke (Weis-Fogh & Wilson, 1961). The typical motor output then consists of a rather simple score of nerve impulses strictly correlated in time with the observed wing movements and repeated at the same frequency (Weis-Fogh & Wilson, 1961). It is this remarkable quantitative reduction in number of units and activities of the wing system that makes it worthy of study.

The studies of Weis-Fogh (1956b) on sensory mechanisms in locust flight and the hypothesis on the role of these in the production of the motor command formed the basis for the experiments described here. Since the behavioural experiments showed

the necessity of some control inputs and the temperature independence of the frequency of output suggested a mechanical timing step, it seemed possible that these were both necessary and sufficient for the operation of the flight control system. However, the results of the search for such all-important inputs were negative; the approach was therefore reversed and an attempt was made to make the system operate without input from the moving parts.

Anatomy. The musculature and pertinent aspects of locust physiology are described elsewhere (Weis-Fogh & Wilson, 1961). The thoracic nervous system is described by Ewer (1953, 1954) and his nomenclature is used here. Fig. 1 depicts the main structures as they appear in a dissection similar to that of some of the preparations. The
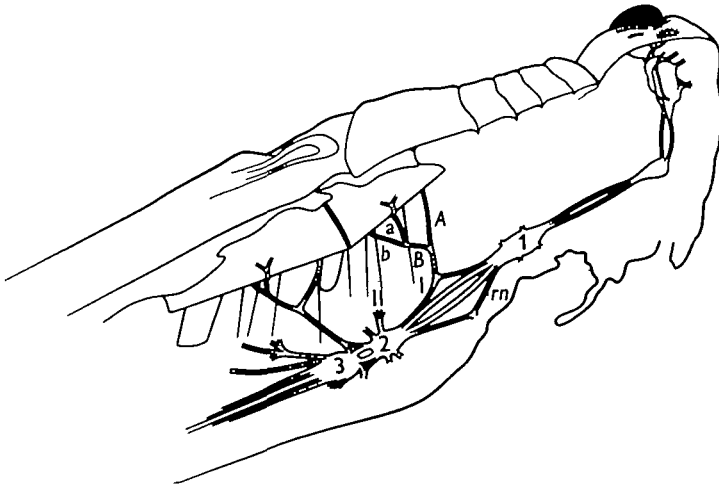

Fig. 1. Diagram of a dissection of *Schistocerca*. The dorsal tegmentary nerve supplying the head hairs is indicated as a solid line The nerves and ganglia are numbered as in Ewer (1953, 1954). *rn* indicates recurrent nerve. The positions of the dorsal longitudinal muscles and elevator muscles are indicated. The controller depressors lie behind the elevators in this view. Many non-flight structures are omitted.

thoracic ganglia are not fused but are connected both by the main cords and more lateral recurrent nerves. The first two pairs of peripheral nerves of the pterothoracic segments are concerned in basic flight mechanisms. Nerve I connects to the recurrent nerve of the next anterior segment. Its anterior (I*A*) and posterior branches (I*Bb*) are wing sensory nerves and the middle branch (I*Ba*), innervates the dorsal longitudinal musculature. The second pair of nerves innervates the vertical flight muscles.

Wing movements. The wing-stroke frequency is about 17 cyc./sec. and is nearly independent of temperature over the normal flight range. The upstroke occupies about 41 % of the cycle in standard flight. The two segments are 6–8 msec. out of synchrony with the hindwings leading. In general terms the contractions must follow the sequence: metathoracic elevators, mesothoracic elevators, metathoracic depressors and controller depressors, mesothoracic depressors and controller depressors, metathoracic elevators, ...

Sensory inputs. The behavioural evidence on the roles of various input sources has been summarized elsewhere (Weis-Fogh, 1956*b*). Wind-sensitive hairs on the head

discharge into the cord (Haskell, 1958) and can initiate and maintain flight (Weis-Fogh, 1956b). Sensation from wind on the moving wings can maintain flight and also must be involved in some control measures including the control of lift against imposed changes in pitch. Loss of tarsal contact and pinching the abdomen may start flight but are insufficient to maintain it.

MATERIALS AND METHODS

Adults of *Schistocerca gregaria* Forskål, phasis *gregaria* or *transiens* were used in all experiments. These were bred at the Anti-Locust Research Centre, London. Large females at least 1 week past last moult, but not yet egg-laden were chosen for most operations. Smaller males were sometimes used with the easier techniques.

Experiments with flying animals made use of a wind tunnel and several animal suspensions already described by Weis-Fogh (1956a), including an aerodynamic balance. In most experiments the animal was *tethered*; that is, could not choose its own wind speed. In cases where a servo-mechanism was used to adjust wind speed to the thrust of the animal the term *suspended flight* will be used.

For electrical recordings contact with the animal was via $40\,\mu$ or $100\,\mu$ platinum or platinum-iridium wires, either bare or insulated to near the tip. All recordings were extracellular. Both mono- and bipolar recording technique was used dependent upon each special case. Tektronix type 122 preamplifiers and a Tektronix type 502 oscilloscope were used to amplify and display electric signals. Films of the oscilloscope were taken with a Shackman type no. AC 2/25 Oscilloscope Recording Camera. Electrical stimulation was accomplished with a two channel Disa Multistim. For repeated trains of stimuli the Multistim was driven by the synchronizing output of a stroboscope, General Radio Corp. Strobotac, also used for visual observations of wing and muscle movements.

Operations from which recovery was necessary, such as cutting of sensory nerves and central connectives, were carried out after CO_2 anaesthesia. Effort was made to reduce locomotory and respiratory movements without abolishing the latter. For longer operations a mixture of CO_2 and O_2 gases was used.

Acute preparations were made without anaesthesia. In highly dissected preparations some of the damage to the respiratory system was compensated by aerating the main tracheal supply to the central nervous system. This was accomplished by introducing moistened air at a pressure not greater than 20 cm. of H_2O through a cannula inserted into the mandible. Air in one mandibular air sac reached both longitudinal ventral trunks. Escape of air from each longitudinal ventral trunk cut posterior to the thoracic nervous system was ascertained by watching bubbling of air or eddying of liquid surfaces near the cut ends. This flow of air was found necessary for the maintenance of the nervous responses to be described.

The locust saline of Weis-Fogh (1956c) was used saturated with air but without penicillin. In those experiments requiring saline solution, damage to the nerve sheath was either peripheral or at some distance along the cord from the pterothoracic ganglia and the ganglia under consideration were probably not exposed to any unusual ionic medium.

The temperature of the room during all experiments was about 25–27° C.

RESULTS

I. *Sections of the nerve cord and recurrent nerves*

Destruction of the supra-oesophageal ganglia or removal of the abdominal nervous system had the same effects as previously reported (Weis-Fogh, 1956*b*). In good preparations normal flight occurred. Complete decapitation, removing the sub-oesophageal ganglia, resulted in an animal which could give an apparently normal tethered performance. The same effect was produced in another animal by a careful operation in which the connectives were cut in front of thoracic ganglion 1 and the wound sealed with wax. On the next day no response could be obtained to wind on head. The animal could walk and jump well. Once started to fly by tarsal reflex it could generate lift greater than body weight and maintain flight in wind. Post-mortem examination showed the connectives to be completely severed. The basic co-ordinating system for flight must be independent of these higher centres.

Results of cuts between thoracic ganglia 1 and 2 on four animals are not so clear. In some cases control operations in which the cord was exposed but not cut resulted in weak fliers. In all four animals some flight movements could be elicited by pinching the abdomen or by tarsal reflex. These were usually only weak flutterings of the wings. The best result was obtained in one animal by stimulating the abdominal cord electrically. This resulted in somewhat low-amplitude wing beating at 13–14/sec. The phasing between fore- and hindwings was normal and wing twisting occurred as usual. The flight continued in wind for a few minutes after a long stimulation. The recurrent nerves were cut along with the connectives in these operations. The success of the operations was checked by observing the walking pattern. The forelegs were no longer co-ordinated with the hindlegs and stimuli on either side of the cut caused the legs in that side only to respond. Post-mortem examinations were also confirmatory.

Section of the connectives and recurrent nerves between thoracic ganglia 2 and 3 in three animals gave the expected effects on leg reflexes. Animals walked with the front two pairs, dragging the third. Ten Cate (1936) reports co-ordinated movements after such a cut in *Locusta*. This is true only to a slight extent in *Schistocerca*. A hind-leg will occasionally step when it is dragged. Pinching the abdomen gave good jumping responses. Blowing on the head of a suspended animal resulted in flight posture of the forelegs and flapping or weak trembling of the forewings. Often the forewings could not unfold but if placed open would flap. The amplitude was low and in the one case measurable the frequency was about 12/sec. Wing twisting did not occur. Probably only a few muscles were active and not the main pronators. Perhaps only the elevators were active and downstroke was due only to elasticity. It will be shown later that the mesothoracic depressors are not exclusively excited through the third ganglion since they may be caused to oscillate with the elevators even when the metathorax is quiet if the anterior cord is stimulated electrically. It is not yet possible to decide whether or not the main excitation of the mesothorax comes from the metathoracic ganglion. In the same preparations the hindwings flapped weakly when the abdomen was pinched. There appeared to be some coupling between the wing pairs. When either pair was flapping some very weak twitching movements were noticeable in the other. These were not entirely passive mechanical movements since they were not alway

present, and therefore probably indicate reflex activation of some muscles in the adjacent segment.

In one animal only the recurrent nerves between 2 and 3 were cut. The post-mortem dissection gave a clear check on this. In a wind of 2·5 m./sec. the wings moved in proper phasing, twisted a little weakly, with wing beats of rather low amplitude and frequency of about 13/sec. Blocking the wind to the head did not stop flight, but did lower the frequency a little. This damage seems to have weakened the flight but not to have reduced co-ordination. If the effect is not just a general one due to the operation itself, it would seem to indicate a sensory disturbance rather than a co-ordinative one.

During recovery from operations between ganglia 2 and 3 spontaneous twitchings in the wings and legs were seen for an hour or two. Muscle recordings from these operated animals will be described in a later section.

The main flight-control pattern is established in the pterothorax with possibly some necessity for the prothoracic ganglion. Subdivision of the thoracic nervous system results in partial flight patterns in which the number of components active and the frequency of operation are reduced.

II. *Recordings from sensory nerves*

Sensory recordings were made in three types of preparations representing differing degrees of dissection. In the least dissected animals the legs, the pronotal shield, and the last two segments of the abdomen were amputated. The soft cuticle between the prothorax and pterothorax was cut except ventrally. The gut was pulled out through this anterior hole. The thoracic air sacs were cleaned away and electrodes fixed to the cuticle so that they extended into the half-empty thorax. A nerve was then lifted over the electrodes and thereby suspended in the moist air of the thoracic cavity. The anterior part of the body was waxed back into place so that wind did not enter the cavity and air-stream stimulation would be near normal. These preparations gave good tethered performances for at least 1 hr. One animal treated similarly, except for placing electrodes, was released by throwing it into the air. It flew away in level flight indicating that the structures removed, including much of the sympathetic nervous system, are not necessary for controlled flight and that this amount of general damage to the animal does not destroy flight reactions.

In a second recording situation the isolated pterothorax was mounted and electrodes brought into the thoracic cavity and placed under various nerves. The wings were moved from the elastic equilibrium position passively by pushing down on the notum or pulling up on the wings with attached threads or actively by stimulating muscles electrically.

Other recordings were from sagittally split thoraces in which the central nervous system was removed. In these it was easier to pick up the nerve desired. Confusion due to reflex effects and motor discharge was not possible. These preparations were also mounted so that the wings could be moved through their normal range.

The anterior branch of the first pair of nerves in each flight segment (nerve I*A*) is apparently purely sensory and innervates the wing and the anterior two-thirds of the wing-base cuticle. Included in it are fibres responding to tactile stimulation of the

wing surface. Sotavalta (1954) has reported these in a lepidopteran. If the wing is cauterized near the base these responses disappear, but there are still present numerous units responding to wing movements, especially downward movements. It is not unreasonable to suppose that these fibres innervate the campaniform sensilla on the basal regions of the large veins, since in the forewing at least no other route has been found over which these may connect to the ganglia. Organs in the wing hinge may also contribute to the discharge in this nerve, but no evidence is available on this.

The in-flight discharge through nerve IA of the forewings (mesothorax) is shown in Fig. 2. The number of responsive units cannot be counted. Most of the response occurs during the middle of the downstroke. The conduction distance from the sense organs is about 4–5 mm. The fibres can be judged from the amplitude of the recorded response to be much smaller than the motor fibres in nerve IBa (to the dorsal longi- tudinal depressor) which conduct at about 3 m./sec. From an estimation of probable velocity of 0.5–1.5 m./sec. it is expected that the main response in nerve IA begins near to top wing position and is maximal during mid downstroke.

Fig. 2. Sensory discharge in mesothoracic nerve IA during flight. The arrows indicate the approximate time of the top of the forewing stroke.

The posterior branch of nerve I (nerve IBb), according to Ewer (1953), has its main ending(s) on the soft cuticle just below the subalare. Electrical recordings from this nerve in the mesothorax show one large unit together with a few relatively very small ones. The recorded amplitudes of the smaller spikes were only a few per cent (under 10 %) of the amplitude of the conspicuous unit. The small units responded to touching and moving the wing, but the response to movement was not orderly.

The single large unit in the mesothoracic nerve IBb gave a proprioceptive response which was some function of wing position, rate of movement, and direction of move- ment. In the wing-down or folded position the unit was spontaneous at a low frequency, at most a few times per second. This rate depended on activity minutes before. After a period of flight or manipulation of the wing the unit was often silent for a minute or two. When the wing was elevated either muscularly, or by pushing down on the notum, or by pulling directly on the wing, the frequency increased and adapted to a new higher level dependent upon the degree of elevation. In a high position the discharge might remain at about 40/sec. for minutes. During upward movement the frequency was higher, up to 350/sec. for an extreme pull. Downward movement abolished all response. When downward movement was stopped the tonic frequency for that position gradually returned. The recorded frequencies at different positions varied from animal to animal but the qualitative relationships were always the same and this unit could be identified in every preparation.

The discharge of this unit during flight, is, of course, phasic, but it is possible that some averaging of tonic effect over several cycles might influence the frequency o

the phasic response. During tethered flight the unit fires one to several times toward the end of upstroke with occasional firings just after the up-position. Fig. 3*a* shows this response in a preparation in which the central connexion of nerve I*B* is cut, leaving the dorsal longitudinal muscle of that side denervated, but eliminating the possibility of confusion with motor impulses. Fig. 3*b* shows the response with the nerve intact. The upper trace is a record of muscle action potentials in the first basalar muscle which has only one motor unit. A variable elevator muscle unit potential shows up in the lower trace. The large mainly downward spikes are motor impulses
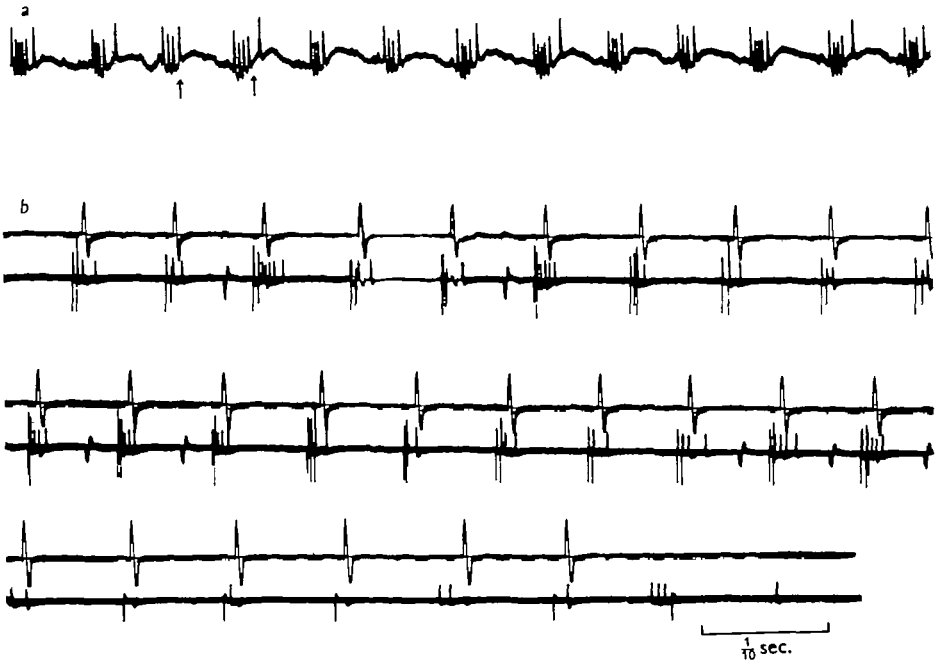

Fig. 3. (a) Sensory discharge in mesothoracic nerve I*Bb* during flight. The arrows indicate the approximate time of the top of the forewing stroke. (b) Upper trace, electrical record from the first basalar muscle of the mesothorax. Lower trace, sensory and motor discharge in mesothoracic nerve I*B* and action potential from one elevator muscle unit. Nerve spikes beginning downward are motor. The smaller mainly upward spike is the sensory unit. The three strips are from parts of the same flight, the last showing cessation of flight.

to the dorsal longitudinal muscle. The smaller mainly upward spikes are from the sense organ. If a few milliseconds are allowed for conduction, the record indicates firing of the sensory unit mostly just before activity in the basalar muscle which fires 2–3 msec. before the top position (Weis-Fogh & Wilson). In Fig. 3*b* a correlation is seen between number of firings of the sensory unit and activity in one elevator muscle. Activity in the sensory unit is greatest at the beginning and end of flight (Fig. 4). The records show also that elevator muscle activity predominates over depressor activity

these times. The time relationships indicate that the increased activity in the elevators causes more sensory firings and not vice versa. An effect of the increased sensory firings is not discernible in the records even though three differently acting muscles can be observed and frequency and other time relationships measured. If there is a discrete cycle-by-cycle effect it remains obscured.

Recordings from the nerves of the hindwing were made only in non-flying preparations. The results were similar except that the metathoracic nerve I *Bb* contains some additional large units including one which responded in a way opposite to that above, in other words a *down* unit.

Discharge due to wind on the head has been recorded from the whole cord (Haskell, 1958). This discharge consists of many asynchronous units which adapt quickly to a maintained level. The discharge appears continuous rather than temporally patterned. There is certainly no large burst formation at a frequency near that of flight. Single units have not been followed.

In summary, there are both tonic and phasic inputs to the thoracic central nervous system during flight. These have at least the potential capacity for indicating wing position and magnitude of wing movement. The behavioural evidence shows that wing forces must be measured as well. Presumably sufficient information is carried centrally to allow of a pure reflex integration of the flight pattern.

Fig. 4. Upper trace, muscle action-potentials from an elevator muscle (downward) and the overlying first basalar muscle of the mesothorax. Lower trace, record from mesothoracic nerve I *Bb* plus some elevator muscle activity. The first strip shows the beginning of flight, the second the end, including an extra burst. Vertical amplitude was increased in the lower trace of the second strip.

III. *Sensory reduction*

The various sources of input described were eliminated operatively in an attempt to determine their precise roles in flight control. It was shown by Weis-Fogh (1956 *b*) that the wind-sensitive exteroceptors on the head are sufficient to initiate and maintain flight at least when the thoracic proprioceptors are intact, but that if the wings move in wind, head input is not necessary. In the following experiments the head hairs were stimulated by wind except in specifically stated cases.

A. Whole wings or portions of wings may be amputated in various combinations without changing the basic pattern of wing movements. Slight changes in the over-all frequency and in amplitude of beat of the remaining members may occur, but a simple regularity of response has not been detected in the few dozen cases tested. Decreasing the length of all four wings does not result in increased frequency as it does in the myogenically controlled insect flight systems. There is a tendency rather to decreased frequency. If the wings are all cut off as near as possible to their bases and the frequency measured by stroboscopic observation of the nota or wing stumps

it is found that the rhythm is no longer smooth but changes often and quickly over a rather wide range. The measurement is consequently difficult, but values from 13 to 20/sec. certainly occur in the same animal within a few minutes. In some cases it is necessary to increase the wind velocity to unnatural values in order to keep the flight going. The decreased input due to the amputation can apparently be compensated in some ways by increased input via another route. The unsteadiness of the frequency of response may be due to near-threshold condition of a few important units.

B. A branch of nerve I *A* enters the tegula. In three animals all four tegulae were severely cauterized. Destruction of sense endings therein should have been quite complete. All three animals still flew in a flight balance with lifts from 70 to 90 % of body weight and at wing-beat frequencies of 16–18/sec. at first, with both lift and frequency gradually decreasing. One animal was thrown high in the air. It flew nearly straight, in good balance, but slowly lost altitude. No specific damage to the flight system is indicated in this set of experiments although the performances were a little weak.

C. On seven animals an attempt was made to cut the whole of each nerve I *A* of meso- and metathorax. This was done by removing the soft membrane surrounding the tegula, locating the nerve and lifting it with forceps, and cutting as far centrally as possible (*ca.* 2 mm. ventral to wing hinge). Tests of the success of the operation were of a negative kind. In one case electrical recording was attempted on a dissected animal. No response was found in the nerve with large passive wing movements. Dissection was twice attempted to check for continuity of the nerves, also with negative results. It is possible, however, that some fine twigs have remained intact in some preparations, but this is highly unlikely for them all. The results were fairly similar in all animals at the beginning. The lasting qualities of the preparations were more variable, with some deteriorating rapidly. In all these animals the posture, wing twisting, and wing phasing appeared normal. All gave frequencies between 15 and 17/sec. at first, going down to minima of 13–15/sec. Lift was measured in three. Before the operation this was equal to or greater than body weight in each, but later was only one-half to three-quarters body weight in long runs. For short bursts it was higher, as when starting or when stimulated with light flashes. Preferred wind velocities were under 3·0 m./sec.

The head hairs of three of these animals were painted with celluloselac. After this dried no response was obtained with air-puff stimuli. One animal now could fly for only a few tenths of a second when started by loss of tarsal contact in wind. Another maintained flight for a few minutes at a time. The third flew for long periods in a wind of 3 m./sec. However, it would not fly with a speed greater than 2·5 m./sec., so free flight would have stopped. The effect was reversed by removal of the paint.

Again no specific flight damage can be pointed to. The animals flew quite weakly but comparably to ones in which other types of operation have been made not involving sensory reduction in the flight system. The nerves I *A* may play a role in maintenance, but not an exclusive one. They have at most a small function in frequency control. They could be involved in lift control, but this was not studied.

D. More striking results were obtained when each entire nerve I was cut. In order to do this the abdomen was removed, the prothorax loosened except ventrally, and the gut taken out. The five animals could still fly when tethered in wind. The

frequency before nerve section was at the low end of the normal range. After nerve section the frequency dropped and the rhythm became a little unsteady, making stroboscopic observations difficult. The dorsal longitudinal muscles are, of course, denervated in this operation and the downstroke is due to elastic forces plus'the activity of the controller depressor muscles. Electrical recordings of muscle action-potentials were combined with approximate stroboscopic frequency values. Three values were 8, 9, and 11/sec.; the other two were crudely estimated as at about one-half normal frequency. The flights were of short duration. The decrease in frequency is probably not due only to inactivation of the dorsal longitudinal muscles. In another type of preparation described in the first paragraph of § II recordings from the motor nerve to one anterior dorsal longitudinal muscle show that it can become completely silent without out the flight frequency being abnormally reduced (Fig. 5). The damage to that animal was not of the sort that would cause one to expect that only that dorsal longitudinal
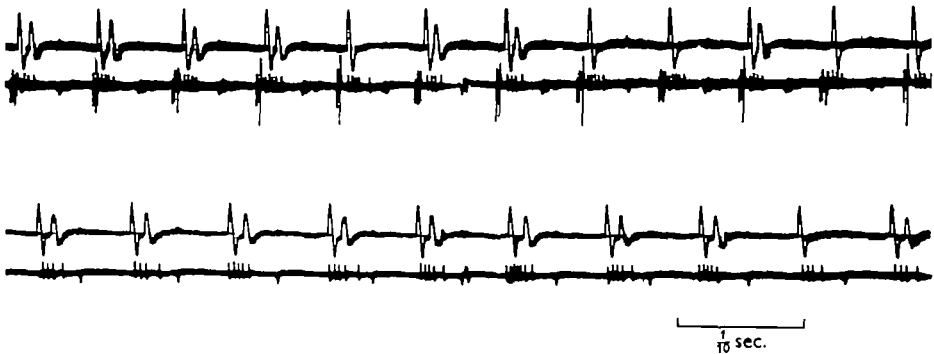

Fig. 5. Recordings the same as in Fig. 3b. In the second strip the motor axons to the dorsal longitudinal muscle are silent. The strips are from the same flight.

muscle was silent. The wing movements were approximately symmetrical. Even if it were mainly lack of activity of the dorsal longitudinal muscles which results in lowered frequencies this effect would depend on lack of, or later, sensory input. It is probable that the information for maintenance of the normal frequency does originate in the movement of the wing and is carried in nerve I. No evidence suggests that the flight muscles contain proprioceptors. Nerve IA does not carry any specific frequency-setting information. The large unit of nerve IBb of the mesothorax could over several cycles have an influence but it hardly does so in the period of one or two cycles (Fig. 3b). The unanalysed units of the metathoracic nerve IBb remain as possible cycle-by-cycle frequency setters.

Eliminating sensation from the wing and motor innervation of the dorsal longitudinal muscles has the definite effect of lowering the frequency of the wing-beat cycle. No measurements have been possible yet on the effect this produces on the ability to make control adjustments. This reduction of input did not, however, upset the basic pattern of wing movements including wing twisting and segmental phase differences. This surprising result led to the hypothesis of a built-in central pattern which is not dependent upon peripheral feedback loops for its basic operation, but which is modified by such input. This input apparently increases frequency as well as affecting small changes in pattern which control flight.

IV. *Semi-isolated nervous preparations*

A series of experiments was carried out in which the possibilities for feedback were further and further reduced, the final form consisting only of head, thoracic nerve cord with tracheae, and a ventral cuticular strip. More than forty diverse preparations were made. Three were of the last type.

All the operations had some features in common. The wings and legs were removed, the last few segments of the abdomen cut off and the prothoracic–mesothoracic junction opened dorsally and laterally. The gut was then pulled out forward. The body was waxed into a Perspex chamber with the head projecting through a hole. The ventral tracheae were ventilated. The lateral half or the dorsal half of the thorax and abdomen were removed. The whole preparation was put at the opening of a wind tunnel and electrodes were placed as necessary. The chamber was covered with moist paper or sealed with cellophane and grease. Good preparations remained responsive for 1 or 2 hr. at 27–28° C. If placed in a refrigerator they could be kept overnight but then deteriorated rapidly when warmed up.

On nearly any locust in 'flight condition' the following observation can be made. The animal is quickly cut down to head and ventral half of thorax with the gut removed. Here all the wing sensory nerves are cut, the dorsal longitudinal muscles are removed and all other flight muscles bisected transversely. When the head is placed in wind it can be seen directly that these muscles contract in a rhythmic, patterned way, the cut elevators and depressors resembling reciprocating pistons, and opposite sides in phase. The fact that such bisected muscle stumps lengthen passively and rapidly after each contraction is unusual and is probably due to the strong passive-elastic system present in locust flight-muscle (Buchthal, Weis-Fogh & Rosenfalck, 1957). The frequency is obviously lower than normal flight frequency.

Recordings of muscle action-potentials showed the frequencies of eleven more careful preparations to be between 4/sec. and 12/sec., the mode being about 7/sec.

The frequency of cycling was measured in one lateral half-animal before and after cutting the wing sensory nerves of that side. The elimination of input from the other side and the derangement of the mechanical conditions for the intact side resulted in an immediate decrease in frequency to about 7/sec. Cutting the four sensory nerve branches caused an extra small drop to about 6/sec.

In three of the best preparations the latencies from elevator response to adjacent controller-depressor response were, 30, 30 and 40 msec. (Fig. 6a). The elevator-to-depressor interval was 25–35 % of the whole cycle, compared to 41 % for upstroke in the intact animal, measured from observations of the moving wings. (In intact animals the elevators and vertical depressors normally fire a few milliseconds prior to the bottom and top of the wing stroke respectively (Weis-Fogh & Wilson, 1961).) In once case the interval was 40 % of the whole cycle at 7/sec. but the same preparation shifted slowly to a less even distribution. Most of the decrease in frequency is due to increased depressor-to-elevator interval. The intersegmental lag was always small. The difference between apparently homologous elevators was up to 10 msec. with the metathoracic one leading, i.e. much as in a normal animal. Fig. 6b shows a typical case.

In three lateral half-animals the time relationships between the dorsal longitudinal

muscles were observed with all wing sensory nerves cut. In each case the phasing varied over long periods so that first one then the other led by up to 10 msec. The phasing was constant, however, for hundreds of cycles at a time. Under some conditions, one being excessive wind speed, the mesothoracic dorsal longitudinal muscle sometimes fired excessively often and irregularly while the metathoracic dorsal longitudinal muscle continued a neat rhythm.

In three preparations lacking any thoracic muscles and with the abdomen removed recordings were made from the motor nerves. (Similar recordings were also made in preparations still having flight muscles. These have no extra interest except that they show definitely that the nerve potentials recorded are related to activity of the flight muscles.) Nerve I contains motor axons to the dorsal longitudinal muscles and apparently no others. The recordings show the same results as found in the above
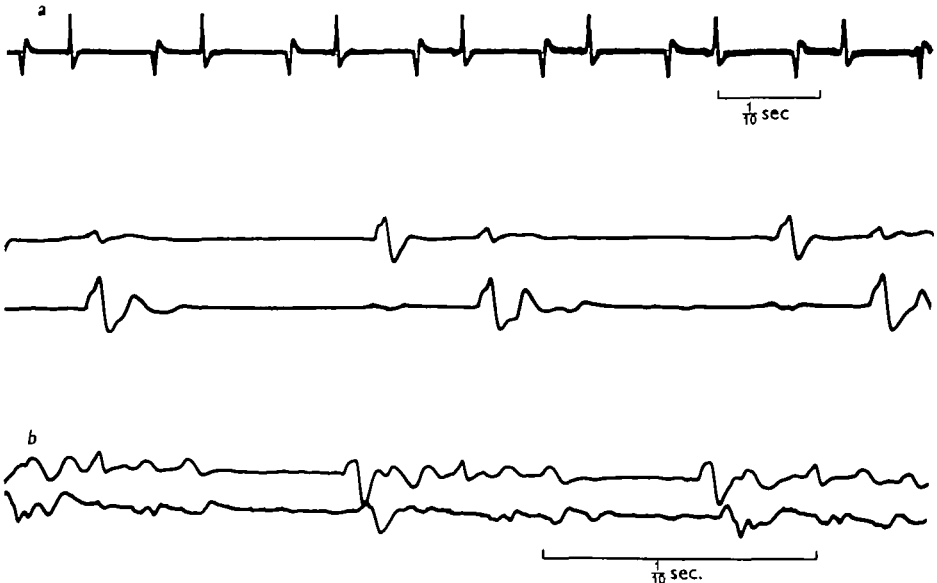

Fig. 6. (a) Muscle action-potentials from adjacent elevator and depressor muscles of a head and ventral half-thorax preparation. First strip: elevator spikes downward, depressor upward. Second strip: upper trace, elevator muscle; lower trace, depressor muscle. (b) Action potentials from elevator muscles of the same preparation. Upper trace, metathoracic muscles. Lower trace, mesothoracic muscles.

muscle recordings. A small burst of spikes was repeated rhythmically at frequencies between 4 and 10/sec. in different preparations. The two segments were sometimes in phase or either one led by up to 10 msec., this condition changing slowly after hundreds of cycles. In one animal the mesothoracic nerve I was higher in frequency and erratic while the metathoracic nerve I appeared normal (see Fig. 7).

Recordings from nerve II were not so clear, there being many more units, both elevator and depressor. They do, however, show bursts of spikes repeating rhythmically at 5–10/sec. The single long burst seemed to contain both the elevator and depressor activity, the distinction between them not being clear. The responses in the two segments were nearly in phase, the bursts overlapping in time. The metathoracic spikes began a few milliseconds earlier than the earliest mesothoracic ones.

The results show that the thoracic nervous system of this locust when stimulated continuously by way of the wind-sensitive head hairs can produce an output in the flight motor axons which resembles the basic flight pattern but at lower frequency.

The possibility for phasing feedback is limited in the most extreme preparations to muscles in the head and to non-moving structures of the head or thorax. That the response of the isolated cord system is really a flight response is suggested by several facts.

(a) The sensory input used to elicit the response was one which normally produced flight.

(b) Many of the muscles from whose axons recordings were made can move the wings only. They are normally quiet when the wings are not moving in flight (Weis-Fogh & Wilson, 1961).

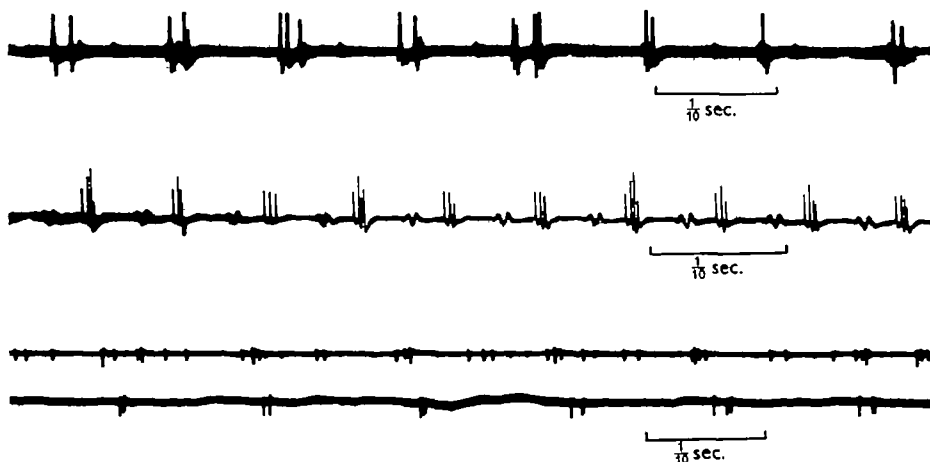(c) The gross pattern of firing was like that in flight.

Fig. 7. First strip, discharge in the central stump of metathoracic nerve I of a head and ventral cord preparation during response to wind on head. Second strip, discharge in the central stump of mesothoracic nerve I during flight of a nearly whole animal. Third strip, discharge in the central stumps of the mesothoracic (upper trace) and metathoracic (lower trace) nerves I of a head and ventral cord preparation during response to wind on head.

(d) The frequency of the cycling was like that of whole flying animals in which the sensory wing nerves had been cut (and also the motor nerve for the dorsal longitudinal muscles). Further reductions in possible input sources had no further effects.

When some muscle stumps were left connected other details of the normal pattern could be recognized since more specific identification of output could be made.

V. *Responses to electrical stimulation*

Evidence for a thoracic oscillator.

Stimulation of the central nervous system anywhere from the brain to the abdominal cord could produce movements of the flight muscles. These experiments were performed on ventral half preparations. The tracheae were usually not aerated. The responses usually degenerated rapidly, but even the animals in bad condition were

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useful in that they gave results on parts of the whole system. Shocks to the ptero-thoracic ganglia caused one-to-one responses, probably due to direct effects on the motor neurons. Elsewhere response usually required several shocks and the number required was inversely related to the stimulus intensity. Apparently synapses are involved in which both temporal and spatial summation or facilitation are effective. Depending on the stimulus parameters and the condition (mainly age) of the preparation the response varied in magnitude from a single muscle unit to the whole set of fast flight muscles. In the latter cases the response was either co-ordinated or irregular.
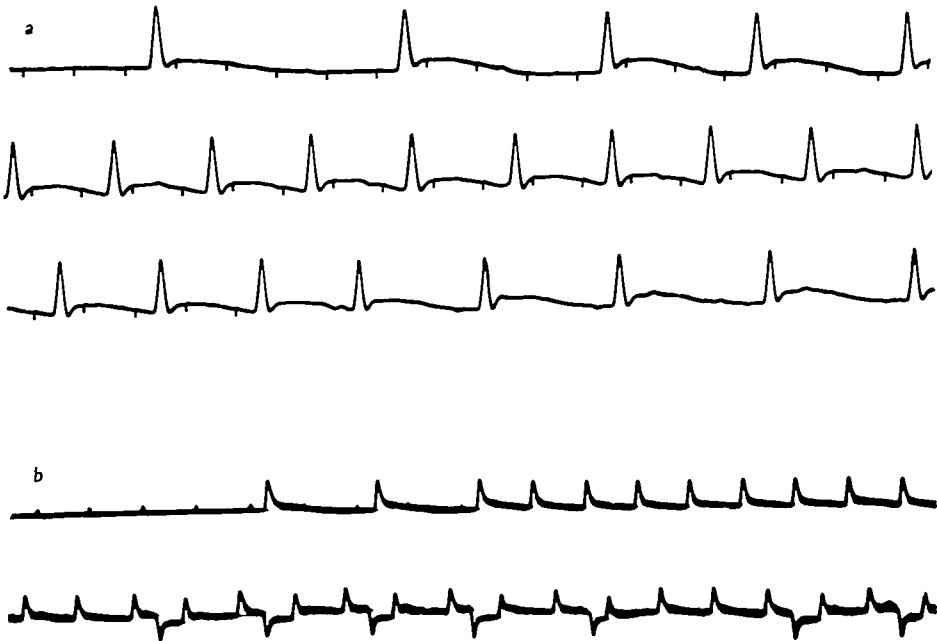

Fig. 8. (a) Portions of the response of one elevator muscle unit to stimulation of the anterior nerve cord. Stimulation was at 50/sec. Seventy facilitating shocks preceded the first response, sixty-four responses at exactly one-half stimulus frequency occurred before stimulation ended and fourteen after-discharges followed. (b) Similar to (a) but with the other electrode on the adjacent depressor muscle and at stimulus frequency of 25/sec.

In the case of response of only one unit the response was in phase with the stimulus but did not usually follow every stimulus (Fig. 8a). At stimulation frequencies between 25 and 100/sec the response followed at a fraction of the stimulus frequency which could within limits be set by intensity of the stimulus. Most often the response followed exactly every nth stimulus. By careful adjustment of the stimulus parameters an unstable condition could be achieved in which the response fluctuated between every nth and $(n+1)$th stimulus. This choosing of stimuli by the follower cell indicates an oscillation within it. This oscillation manifests itself after the end of stimulation, in many cases, by after-discharge in the form of a few to many extra firings at gradually decreasing frequency (Fig. 8a). By varying stimulation different units have been driven at frequencies over the range of 5–10/sec. Cyclic after-discharge following a long stimulus train sometimes lasted hundreds of cycles. In rare cases the preparation became silent immediately on cessation of stimulation and after a fraction of a second

pause started again for a few cycles. Similar behaviour can be seen occasionally in intact animals when the wind is stopped. In this case there may remain some external stimulation. Such a case is recorded in Fig. 4 where only elevators are seen to respond.

In fresh preparations although the response may begin in only one unit it usually spreads to others. A limited case of such spread is shown in Fig. 8*b*. An elevator unit begins firing after many facilitating stimuli and it follows in phase with the stimulus at 25/sec. After many cycles the adjacent depressor joins the response in a nearly constant phase relationship, but not synchronous, with the elevator and at a lower rate. It appears that an intermediate driving step is involved which requires greater facilitation. This may be by way of the elevator itself. In nearly all cases of stimulation of the cord elevator muscles showed the lower threshold.

When the spread of excitation is great the response may lose its phase relationship with the stimulus, and the co-ordination between elevator and depressor muscles may also be upset. In some cases, however, the whole system of fast flight muscles is activated in what appears to be the normal co-ordination. Measurements have been made from not more than two muscles at a time, but these and visual observations reveal a high degree of co-ordination. In cases both of wind and electrical stimulation one or more elevators often began firing at an excessively high frequency but when the depressors were added a more normal frequency was established. The same phenomenon occurs at the beginning of normal flight (Fig. 4). In these more co-ordinated preparations adjacent elevator-depressor pairs were observed under a wide variety of stimulating conditions. After an ordinary train of facilitating stimuli the stimulus frequency was decreased and, if necessary, the intensity increased to find the minimum possible frequency for maintained response. At a frequency as low as 5/sec. each shock may produce an elevator followed by a depressor response. The responses of the two muscles are a few tens of milliseconds apart with a long silence until the next stimulus. If at a frequency of 5/sec. the stimulus intensity is increased greatly the response rate may jump to twice that of the stimulus frequency (or some other small whole number multiple). The pairs of responses are nearly evenly spaced and not grouped right after the stimulus. By means of high frequency, high intensity, short bursts of stimulation via preganglionic fibres the flight motor neurons were stimulated at even lower overall frequencies. When bursts of stimulation, at 300/sec. and lasting 1/10 sec. were repeated at 2/sec. a general tetanus occurred during the burst, but in the interval between there were 4 to 5 cycles of elevator depressor activity in a smooth rhythm at around 10 cycles/sec.

The above preparations lacked wing sense organs and all the responding muscles were transversely bisected. The head could be removed without effect. Stimulation anterior or posterior to the prothoracic ganglion was similar in effect and in some cases that ganglion as well was removed.

Multiplicity of oscillators and input pathways

One of the previously mentioned animals, in which the connectives and recurrent nerves between thoracic ganglia 2 and 3 were cut, was prepared for electrical stimulation. Stimulation of the anterior part had an effect similar to wind on the head. It

produced rhythmic movements of the flight muscles of the mesothorax. Stimulation of the abdominal cord did the same in the metathorax, presumably through fibres from the anterior which have responded antidromically. The two could be excited simultaneously but independently. Separate oscillators must exist in the two segments.

If only one anterior connective is stimulated (behind the head) the response begins on the same side in the elevator muscles. After a few cycles the adjacent depressors begin. The contralateral muscles begin still later, but perfectly in phase. If the other connective is stimulated the sequence starts on that side.

High-frequency stimulation of the supra-oesophageal ganglion of one animal caused the two most posterior elevator muscles to fire at independent rates with no steady phase relationship. Similar cases have been observed between other muscles. Several oscillators in each segment become necessary to explain this abnormal type of behaviour. In the very worst preparations chaos results when these oscillators are not acting together. In these cases no sign of co-ordination is apparent and muscles fire at diverse rates.

Systematic study of the effects of stimulating the peripheral nerves has not been undertaken. A few observations show that muscular activity can be elicited by stimulating the sensory branches of nerve I. These reflex effects probably account for the after-discharge in Voskresenskaya's nerve-muscle preparations (Ewer & Ripley, 1953; Chadwick, 1953). In numerous cases stimulation of the stumps of the wing sensory nerves of quiet preparations caused activity in one or several flight motor units. A co-ordinated whole response was not evident. In five preparations an attempt was made by means of stimulation of these nerves during the ordinary response to wind on the head to gain control of the timing of the oscillations. The results were all negative so that no specific timing signal from the wings has been found as yet. Small changes in frequency could be produced in this way.

The frequency of discharge is affected by normal types of stimulation as well. Increasing wind speed increases frequency, but this usually adapts unless the system is near to threshold at first. Increasing light intensity, when the eyes are connected, may cause either increase or decrease in frequency. The conditions determining the sign of the reaction are unknown. This change also adapts. The effects of wind and light and of electrical stimulation of the sensory nerves are additive.

Only one case in which a mesothoracic depressor muscle fired at lower threshold than any elevator during stimulation of the anterior cord has been observed, but this case is sufficient to prove that at least some parallel paths exist from the head to the antagonistic pairs. In this case the elevator muscle responded later and as in more normal cases the two never fired together. Specific input from the head to the depressor muscles is certainly to be expected if these are the only controller muscles. Stimulating nerve IA can result in selective depressor responses also.

Stimulation across the supra-oesophageal ganglion of intact or dissected animals has given various fragmentary responses from firing of a single unit to massive response of the elevators like that in the beginning of flight. A means of stimulation which would keep flight going without wind has not been found although this could be expected from appropriate brain stimulation. In one case flight could be abruptly inhibited by shocks at 100/sec. As long as the stimulation continued the animal

maintained a resting posture with the wings folded. Flight was resumed immediately after the stimulus was turned off.

Excitability of the peripheral motor axon

Features of the ganglionic (synaptic) transmission to the flight motor axon can be mimicked by a peripheral nerve preparation of axons to the dorsal longitudinal muscle. Ewer & Ripley (1953) found in a nerve-muscle preparation that the axons would respond to only a fraction of near-threshold stimuli. This result has been duplicated on isolated nerve under conditions in which relative refractoriness could be separated from late supernormality. Slight refractoriness can be detected for up to 100 msec. This is about 10 times as long as figures given for central axons in *Periplaneta* (Boistel, 1960). At 30 msec. the threshold was about 110% of resting threshold. The axons could not be caused to fire repetitively to single shocks applied externally, although stimulus intensities were increased to those which caused irreversible damage and durations up to 1 sec. were used.

DISCUSSION

The specific hypothesis of Weis-Fogh (1956b) concerning the origin of the flight co-ordination in locusts and the opinion expressed by Pringle (1957) suggest a reflex explanation of insect flight movements. The evidence presented here supports strongly the notion that there is an innate central pattern for the production of flight movements in the locust. In some of these experiments the only source of feedback from muscle activity was from head structures. In others the head was removed but portions of the thoracic muscles remained. In all cases where input was deranged there was no further effect on frequency and co-ordination beyond that produced by cutting the main sensory nerves from the wings. Either a normal flight-inducing stimulus or special electric shocks could produce a pattern of contractions like those in flight. The existence of an innate pattern does not preclude the possibility that peripheral loops are *also sufficient* to produce the same pattern, but important as those feedbacks must be for the setting of the frequency of cycling, for accurate timing of contraction cycle and movement cycle, and for fine adjustments for control manœuvres they act on top of what is already determined by the central nervous structure and function. It is possible that the inherent and the reflex mechanisms operate in parallel upon the motor neurons, but it is also possible, and seems more probable to the author, that the two operate through the same elements. Indeed, the evidence does not yet indicate internuncial integrative cells. In unco-ordinated preparations there seem to be as many pacemakers as there are motor units. The hypothesis which emerges is that many pacemakers, which could be the motor neurons themselves, are each capable of independent oscillation over a wide frequency range but that when many act at once they are coupled into a stable pattern with a single dominating frequency which is dependent on total quantity of input. Specific changes in input to a certain motor unit may change the position of that unit in the over-all pattern.

The nature of oscillation of the individual pacemakers is not clear. The similarity of the relative refractory period of the motor axon to the interval at the lower limit for rhythmic oscillation of the flight system may be only a coincidence, but it appears

a possible mechanism for oscillation and at least if not of basic importance an indication that many factors are working together to provide a fool-proof system. The main argument against a refractory type or spontaneous relaxation oscillator is the fact that many, if not all, of the motor units can fire not once but twice in rapid succession during a single cycle. This double firing may be due to a particularly massive input capable of exciting even a rather refractory neuron. It has already been shown that some of the inputs do operate discontinuously and we have also found that some of the double firing must be due to specific input to certain cells since the controller depressor muscles show antagonism in this respect as part of their flight control mechanism (Weis-Fogh & Wilson, 1961). The pacemakers having no fixed frequencies, it should be possible to set not only the phase of each but also its frequency. This could be done by resetting the phase on each cycle. Transganglionic stimulus-recording experiments showed that this can occur when few units are active. Although a unit may discharge rhythmically by itself during pauses in stimulation it usually follows in a fixed phase relationship during stimulation.

Until the several pacemakers are individually better understood it is futile to discuss how they might be coupled. It is interesting, however, that if the above hypothesis is correct in principle, if not in detail, then the vague concept of a 'flight control centre' is supported neither in the anatomical sense nor in the sense of a group of hypothetical neurons which are uniquely concerned with integrating input with a certain pattern and passing this on to 'motor centres'.

The animals with reduced input never gave co-ordinated responses at a frequency greater than normal. Although all parts of the cycle slowed somewhat, the greatest part of the reduction in frequency was due to a longer interval between depressor action and elevator action. This and the fact that elevator action normally starts before depressor action in most preparations suggest that the sequence of events is an open chain or one with a very weak link, and that a triggering occurs at the metathoracic elevators. This triggering may be due to the pacemaker activity in the case of lower frequency deafferented preparations. In normal animals the higher frequency may be due only to an increased pacemaker activity because of an increased quantity of input but, after flight has started, the system may also be triggered by specific timing input. Excitatory connexions between successively acting sets of muscles seem likely. The elevators discharge at a high frequency at the beginning of flight and at a lower rate as soon as the depressors begin, suggesting an inhibitory relationship between them. Some of the preparations indicate the same thing.

Comparisons with other systems

The ventilation and spiracular movements in the same animal have been found by Miller (1960 a, b) and Hoyle (1959) to be controlled in a manner quite similar to that described here. The rhythmic output comes from several autonomous centres which can be influenced by input. A certain CO_2 level (input) seems to be necessary for cycling, but the cycling is not due to build-up and release of CO_2. The rhythm of the fastest centre sets that of the others. Thus in both flight and respiration several pacemakers, each dependent on some level of input, are able to oscillate at some frequency of their own, but are ordinarily dependent on each other in such a way that a single

pattern emerges. In both cases this pattern can be modified by input. That functions so different in purpose and time course are organized similarly, in a way that is not the only imaginable organization and not even considered the most probable one in flight, may indicate that there is a basic structure of the nervous system from which the two functions evolved. The copulatory movements of mantids and roaches are also under central control modified by input (Roeder, Tozian & Weiant, 1960). Here the output is spontaneous but ordinarily inhibited by higher centres. It is, however, not known to be a patterned output. A remarkably similar situation has been described for the control of certain crustacean appendages. Hughes & Wiersma (1960) found that the isolated abdominal nerve cord produces intermittent bursts in the first nerve roots grossly similar to those which are normally associated with the swimmeret beat but differing in pattern, frequency, and phasing. Proprioceptive and descending central nervous pathways influence the pattern but are not alone responsible for it. The crayfish and locust seem to differ in the degree of perfection of the central co-ordination for these activities, but there is no suggestion of different basic mechanisms.

The experiments on vertebrates, mainly of Gray & Lissmann (1946), Lissmann (1946) and Weiss (1950), have shown that some degree of innate central patterning must exist. The patterned outputs are apparently not spontaneous but depend on some input from proprioceptors. It is not decided, however, whether the pace-setting is dependent on the timing of the input or on a central pacemaker which needs only to be sufficiently excited.

The existence of proprioceptive leg reflexes (Pringle, 1940) and close observations of normal walking and the effects of amputations (Hughes, 1952, 1957) in cockroaches have led to a reflex hypothesis for locomotion here as well. Pure reflex mechanisms seem likely in some centipedes (von Holst, 1935). However, experiments which seem to demonstrate the sufficiency of feedback loops do not prove the lack of a central pattern. In the case of locust flight the mechanism could have been a pure reflex system. It seems not too early to conclude that central oscillators in arthropods are of such fundamental importance that they are used even where other mechanisms might suffice.

SUMMARY

1. The co-ordination of the flight movements of *Schistocerca gregaria* Forskål was examined in order to determine the extent of central patterning and reflex control.

2. Electrical recordings from wing sensory nerves showed many units which responded to wing movements of various kinds. During flight the sensory discharge was timed to certain phases of the wing-beat cycle.

3. Surgical removal of the sources of timed input did not abolish patterned output, which resembled that during flight, but the frequency of cycling was considerably reduced. Either electrical stimulation of the nerve cord or continuous wind on the head could elicit the pattern.

4. A multiplicity of oscillators in the flight control system was demonstrated.

5. It is suggested that the basic co-ordination of flight is an inherent function of the central nervous system but that peripheral feedback loops influence the frequency of operation and details of pattern.

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