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ACTIVITY IN THE LOCUST NERVE CORD IN RESPONSE TO WING-NERVE STIMULATION

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INTRODUCTION

Control of forewing twisting in locusts depends upon transfer of nervous activity between pterothoracic segments. Thus, destruction of campaniform sensilla on the wings abolish regulation of forewing twisting (Gettrup & Wilson, 1964) and cutting the connectives with recurrent nerves between the ganglia in the pterothorax interferes with and stops forewing twisting (Wilson, 1961). Precise interruption of part of the afferent discharge from hindwing campaniform sensilla affects the degree of active forewing twisting. Certain parts of the sensory discharge were found to be more essential than other parts in this respect, and the reflex developed slowly during 100–150 wing cycles (Gettrup, 1966). The effect of peripheral afferent stimulation upon activity of flight motor units was studied by Waldron (1967), who concluded that reflex action responsible for changes in 'average burst length' (average number of spikes per burst per flight period) of certain motor units developed during 10–20 wing cycles.

Direct recordings from the central nervous system during stimulation of the sensory nerves $1C_1$ and $1D_2$ (Campbell, 1961; IA and IBb of Ewer, 1953, 1954) from the pterothorax have shown that a depolarization of 3–4 mV., which lasted approximately 10 msec., developed in some motoneurones in the pterothoracic ganglia (Kendig, 1968); the latency of the motoneurone potential was found to be 7–8 msec. or more. Also, Iwasaki & Wilson (1966) found compound, graded responses in the mesothoracic ganglion, lasting 20–30 msec., when mesothoracic nerves $1C_1$ and 1D (1A and 1B) were stimulated.

In this work processing of afferent activity in pathways possibly concerned with modulation of flight activity was investigated employing de-afferentated locust preparations.

METHODS

Males of the desert locust, *Schistocerca gregaria* Forskål, were mounted on a wax block, ventral side up. The thorax was opened ventrally, and a few air sacs were removed, so that the ventral surfaces of the two pterothoracic ganglia were exposed. Preparations were selectively de-afferentated; except for some scolopophorous organs ventrally in the thorax probably all sensilla of scolopophorous type within the head, thorax, abdomen, and legs were eliminated. Also visual organs, stretch receptors and hairplates (in particular the tegulae) were removed or cauterized.

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Electrodes for stimulation were hooked on the nerve branches $1C_1$ and 1D just distal to the bifurcation where $1C_1$ or 1D leave the main nerve 1 (Nerve branches 1A and 1B of Ewer, 1953, 1954; nerves are numbered according to Campbell, 1961, cf. Fig. 1). Recording electrodes were placed on ipsilateral and contralateral connectives anterior to the mesothoracic ganglion, posterior to the ganglion in the metathorax or between the mesothoracic ganglion and the ganglion in the metathorax. The ventral side of the thorax and the electrodes were covered with petroleum jelly to avoid spread of stimulus current.

Extracellular recordings were also made with glass capillary microelectrodes filled with 3 M-KCl (less than 10 MΩ) from the anterior parts of the second and third ganglion within the thorax, in particular from the regions anterior and lateral to the entrances of nerves 3 and their ipsilateral connectives.

A neutralized-input-capacitance amplifier (Bioelectric Instruments), a Tektronix pre-amplifier 122, a Tektronix oscilloscope with mounted Grass camera and an Ampex tape recorder made up the recording system. Electric stimuli were provided by a laboratory stimulator, American Electronic Laboratories.

Experiments were carried out at room temperature, 20–25° C.

RESULTS

Numerous central units were active in the preparations investigated. Records indicated that ascending as well as descending activity may occur in unstimulated preparations. Some of this activity is due to respiratory units. The spontaneous cord activity differ from preparation to preparation and must be evaluated separately in each case. Activity in either direction may be increased by means of electrical stimulation of the nerve cord or the peripheral nerves. Stimulation of mesothoracic and metathoracic nerves $1C_1$ and 1D does occasionally cause motoneurones to fire, depending on the level of spontaneous activity in the cord and on extra inputs from head, legs, etc. (cf. Guthrie, 1964; Wilson & Wyman, 1965), but usually the response of motoneurones to stimulation of wing-nerve branches is sub-threshold, amounting to only a few mV. (Kendig, 1968).

It is characteristic that the discharges in the cord have some firings in common with the less well defined and longer spontaneous discharges in non-stimulated preparations. A discharge (a burst) in response to stimulation is characterized by number of spikes, length of interspike intervals, number of spikes per unit time, spike types and by comparison to spontaneous activity. During repetitive stimulation of nerves 1, ipsilateral discharges recorded in the nerve cord anterior to the mesothoracic ganglion are delayed 4–7 msec. with respect to the stimulus and delays are almost identical for the mesothoracic and metathoracic nerves 1, differing by 1–3 msec. (Fig. 2). The distance between the recording electrodes and the entrance of nerve 1 in the meta-thorax was approximately 3–4 mm., whereas the distance between the entrances of nerves 1 in mesothorax and meta-thorax was 1·6–1·9 mm.

From records it can be seen that the first few pulses vary extensively from one stimulus cycle to the next, both with respect to size and duration. A direct action of the electric field around the stimulating electrodes probably does not affect units in the ganglia, since the degree of synchronization is low. The central fibres involved, and their conduction velocities, are not known; conduction velocities for unknown

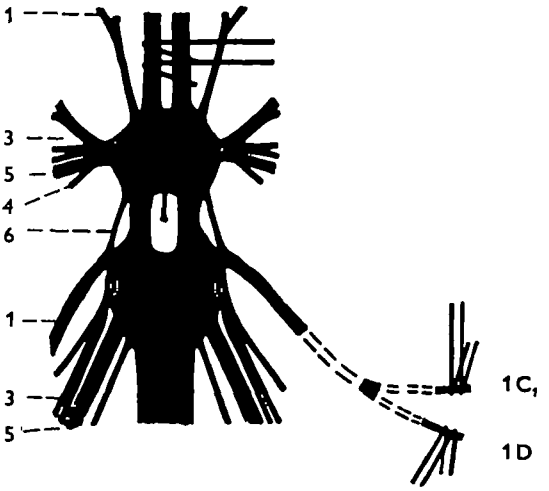

Fig. 1. The ganglia of the pterothorax in ventral view. Nerves are numbered according to Campbell (1961). Many nerve branches radiate from the posterior ganglion, which is a fusion of several segmental ganglia (the metathoracic ganglion plus abdominal ganglia). Recording electrodes are shown at the connectives between prothoracic and mesothoracic ganglia. Hooked on nerve 1 in the metathorax are shown electrodes for stimulation of either $1C_1$ whilst recording from the anterior connectives or 1D during recording from the anterior connectives.

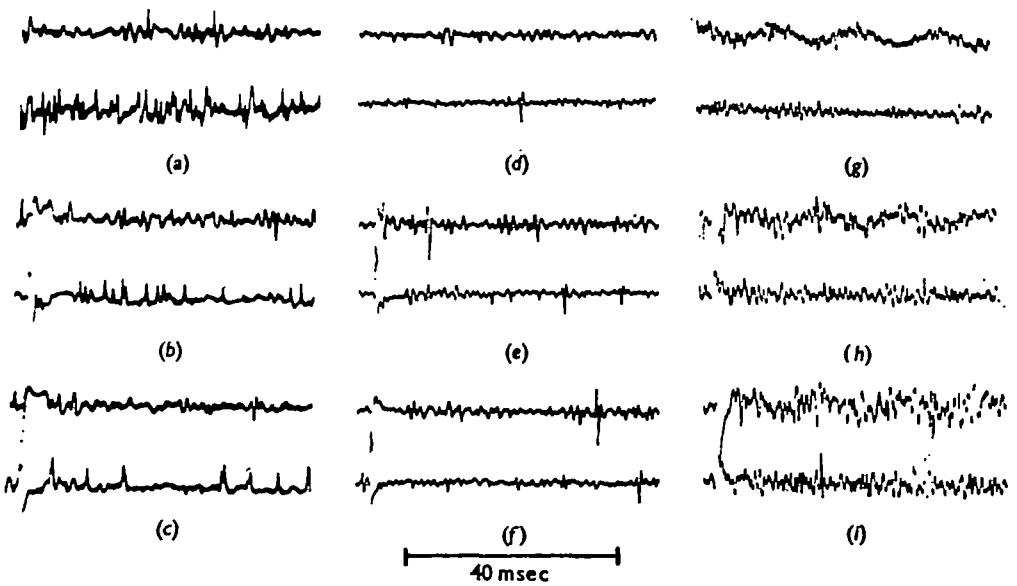

Fig. 2. Cord activity in response to stimulation of forewing nerve $1C_1$ (*a*, *b*, *c*), hindwing nerve $1C_1$ (*d*, *e*, *f*), and hindwing nerve 1D (*g*, *h*, *i*). (*a*), (*d*), (*g*), resting discharge; (*b*), (*e*), (*h*), ipsi- and contralateral discharges at start of repetitive stimulation; (*c*), (*f*), (*i*), discharges ipsi- and contralaterally after 10 cycles of repetitive stimulation. The stimulus period is 62 msec. (*b*), (*c*); 61 msec. (*e*), (*f*); and 61 msec (*h*), (*i*). Upper records ipsilateral; lower records contralateral. Horizontal scale: 40 msec. Spikes retouched.

units firing in the connectives were roughly estimated in a few experiments; units conducting at 1·7–1·8 msec. were recorded when stimulating and recording across the two last ganglia in the thorax. In another experiment the metathoracic nerve $1C_1$ was stimulated and microelectrode recordings were made from a different central unit. The time for conduction through a distance of approximately 3 mm. was found to be 8 msec. (Conduction velocities for $1C_1$ fibres may be within the range 0·5–1·5 msec.; Wilson, 1961.)

In 10 experiments nerves $1C_1$ and 1D in the mesothorax a metathorax were stimulated repetitively at frequencies from 9·6 to 32·9 cyc./sec. Recordings were made from the connectives between the prothoracic and mesothoracic ganglia (Fig. 1). The duration of the stimulus was approximately 0·5 msec., the stimulus voltage constant, and under these conditions a discharge of firings lasting a few msec., comprising potentials from most if not all excitable fibres within the nerve, may occur (Neville, 1963; and previous work by the author).

Activity in the cord induced by repetitive stimulation of $1C_1$ consists of irregular discharges 10–20 msec. or more long on the ipsilateral side (Fig. 2) and of contralateral discharges at least 10–20 msec. long, which often divide into two or more poorly defined sub-discharges. At the start there is some adaptation of activity within a period corresponding to 3–6 stimuli or more at 17 stimuli/sec. During repetitive stimulation estimates of discharge delays based on 50–100 measurements from a single experiment are 7 msec., s.d. 2 msec., ipsilaterally and 8 msec., s.d. 3·4 msec., contralaterally. Also, stimulation of the mixed nerve 1D induces ipsilateral and contralateral discharges both delayed 5 msec., s.d. 1·4 msec., which adapt within 5–6 stimulus cycles (Fig. 2). If the positions of the external electrodes at the connectives between prothoracic and mesothoracic ganglia are more or less the same in the experiments mentioned this could imply that some fibres in nerve 1 of the metathorax may proceed to the anterior ganglion or participate in synapses different from those of the $1C_1$ fibres, involving other central units.

Microelectrode recordings were made from the ganglia in the thorax, in particular the ganglion in the metathorax. A number of different units were revealed by means of their extracellular potential in response to stimulation of connectives and peripheral nerves. Similar potentials may be recorded at different electrode positions when a unit (or units) is 'spontaneously' active in the ganglion.

The approach was to activate branches of nerves 1 of the mesothorax and metathorax and to record extracellularly from within the pterothoracic ganglia (Fig. 1). Occasionally nerves 3, 4, 5 and the connectives were stimulated too. When stimulating $1C_1$ of the hindwings central units from the ganglion in the metathorax were recorded, which could be induced to fire within an interval of 10 msec. or more, in response both to repetitive stimulation and single stimuli (Fig. 3). The precision of time of occurrence of firing increased with stimulus intensity. Within this region of the metathoracic ganglion, activity of units could be recorded when the cord was stimulated between the prothoracic ganglion and the mesothoracic ganglion. Characteristically, the precision of the time of occurrence of units increased as for $1C_1$ stimulation (a few msec. compared to 10 msec. or more). The mixed 1D may time the central activity; however, units timed by $1C_1$ or 1D were not activated, at least not specifically, by stimulating the mixed nerves 3, 4 and 5 (cf. Svidersky, 1967).

The extracellularly recorded potentials, in particular from the ganglion in the metathorax, representing units responding to wing-nerve stimulation, had amplitudes of 0·5–1·0 mV. and lasted up to 5 or 6 msec. The potentials had their maxima 2–2·5 msec. from the point of initial deflexion of the base line (Fig. 3 A). Occasionally, potentials of longer duration, a 4–6 msec. spike superimposed on a potential of less than half amplitude lasting 30–40 msec., were found (Fig. 3 B). 'Synaptic' potentials lasting 30–40 msec. were only recorded when stimulating the cord between prothoracic and mesothoracic ganglia.

Within the metathoracic ganglion a spontaneous active unit, timed by stimulation of $1C_1$, was recorded; this unit fired after a delay of 4–5 msec. with respect to the stimulus, a relatively short delay when compared to similar latency records of 5, 7, 8, or more msec. It could not be decided in this type of recording whether the potentials were propagated and in which direction. Activity recorded from whole connectives anterior to the mesothoracic ganglion was delayed 4–7 msec. and usually lasted more than 10 or 20 msec. It is therefore not possible to exclude that activity recorded in the cord anterior to the mesothoracic ganglion, induced by stimulation of $1C_1$ in the metathorax, may have its origin at synapses within the metathoracic and mesothoracic ganglia.

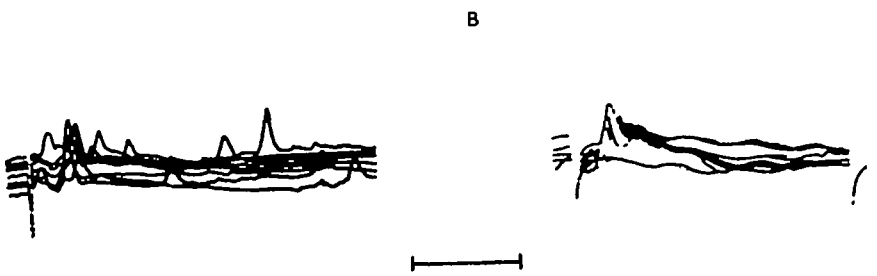

Fig. 3. (A) Extracellularly recorded activity in the metathoracic ganglion, adjusted by stimulation of the hindwing nerve $1C_1$. (B) Tracings of 'synaptic potentials' recorded from the metathoracic ganglion in response to stimulation of the nerve cord anterior to the mesothoracic ganglion. Time scale: 30 msec.

DISCUSSION

In flying locusts, destruction of the metathoracic campaniform sensilla affects the control of mesothoracic wing twisting. Direct pathways between sense organs and control depressors of the front segment have not been established, and the control mechanism seems by no means simple. Thus, the reflex for intersegmental control was found to be slow, 100–150 cyc./sec., but faster reflexes may be involved too (Gettrup, 1966).

There is no evidence so far as to whether central interneurones participate in this reflex or as to whether the parts involved may function in non-flying intact animals or in dissected preparations. It was found in this work that motoneurone activity does not necessarily accompany activity of other parts of CNS during wing-nerve stimulation. This is true, however, only for this type of preparation and experiment.

The antidromically activated central activity (stimulation of motoneurones innervating leg and flight muscles) was not found to be affected by $1C_1$ stimulation at electrode positions at which activity timed by stimulation of $1C_1$ was also recorded. This does not exclude that activity in motoneurones cannot reach first or second order

$1C_1$ units or vice versa; lack of central stimulation from anterior ganglia, de-afferentation and the 'unnatural' experimental conditions may seriously distort the findings. It is characteristic that timing of potentials in the ganglion in response to $1C_1$ stimulation is more precise when antidromic stimulation occurs. This increase in precision may also come about by stimulation of other mixed nerves, or sense organs located at the surface of the body.

Fibres may cross over at several levels in the nerve cord; Guthrie (1964) has shown that some fibres of the dorsal tegumentary nerve cross over to the opposite side in the posterior region of the suboesophageal ganglion. During investigations of central inhibition of a prothoracic reflex (cleaning or grooming movement of the front leg) Rowell (1964) found that crossing of inhibitory 'influence' from one side to the other occurred between the metathoracic and prothoracic ganglia, but not between the mesothoracic and prothoracic ganglia. Inhibition may vary with 'the level of activity' within the ganglia considered and this 'level of activity' is not constant, a fact which limits conclusions to be drawn on basis of recordings of electric activity in the nerve cord.

There is no evidence yet for occurrence of crossing to the opposite side of the ganglia of fibres from nerves $1C_1$ and 1D, which only occasionally were found to make synapses at the same 'loci' within the ganglion (Iwasaki & Wilson, 1966, and present work). Previously it has been mentioned that the time of arrival for central burst patterns induced by stimulation of $1C_1$ may differ at ipsilateral and contralateral sides by 6 msec. or more, whereas the corresponding difference in case of the mixed nerve 1D may be considerably less. Contralateral firings are less uniformly distributed within the discharge period than ipsilateral firings. The tendency for occurrence of several contralateral sub-discharges may originate within the ganglion stimulated, since it was found to occur in response to stimulation of mesothoracic as well as metathoracic wing nerves.

Synapses between some afferents and central 'rhythm generators' were suggested by Svidersky (1967). Interaction between motoneurones of the flight system was investigated by Kendig (1968), who described several electrotonically and chemically transmitted excitatory potentials possibly originating from the mutual interaction between two motoneurones. With respect to amplitude these potentials were more or less of same order of magnitude as sensory-induced subthreshold activity in the motoneurones. No inhibitory interaction was observed unless most of the flight muscles were activated. However, motoneurones within the prothoracic ganglion (connected to dorsal longitudinal muscles of the mesothorax) seemed unable to produce an alternating pattern, neither in response to 'wind or head' alone nor in response to 'wind on head' and electric activation of four motor units of one muscle of the pair.

SUMMARY

1. Nerve cord activity in response to repetitive stimulation of wing nerves $1C_1$ and 1D was investigated in preparations of locusts. Firings of single central units occurred at latencies defined with an accuracy of few milliseconds. Various recordings of latencies show that it is not possible to exclude ganglionic synapses within the pathways considered.

2. Records from the connectives anterior to the mesothoracic ganglion or from the abdominal connectives show an increase in activity during repetitive stimulation. When recorded between prothoracic and mesothoracic ganglia a response was found in ipsilateral as well as contralateral connectives.

3. The ipsilateral and contralateral responses were delayed differently with respect to the stimulus. When nerve $1C_1$ of metathorax was activated the ipsilateral delay amounted to 7 msec., s.d. 2 msec., whereas the contralateral delay was found to be 8 msec., s.d. 3·4 msec. Ipsi- and contralateral latencies during stimulation of 1D of the metathorax were 5 msec., s.d. 1·4 msec.

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