J. exp. Biol. **122**, 387–411 (1986)
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OVIPOSITION DIGGING IN THE GRASSHOPPER

I. FUNCTIONAL ANATOMY AND THE MOTOR PROGRAMME

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Accepted 1 January 1986

SUMMARY

1. The ovipositor appendages of acridid insects (grasshoppers and locusts) consist of two pairs of shovel-shaped valves that are used to dig a deep chamber in the ground for egg burial, to manipulate the eggs, and to assist in capping the egg-pod with froth.

2. During oviposition the valves undergo cyclical opening, closing, retraction and protraction movements. These movements are produced by the contractions of ten pairs of muscles. The eighth and ninth segmental nerves of the terminal abdominal ganglion supply the ovipositor muscles.

3. Rhythmical ovipositor movements are produced by the severed abdomen of sexually mature female grasshoppers. By comparing this activity to the activity underlying the natural behaviour, it was determined that the isolated abdomen produced the digging portion of the oviposition motor programme.

4. Electrical recordings from the ovipositor nerves in the isolated nervous system showed spontaneous rhythmical bursting activity. This activity corresponds to the neural correlate of digging behaviour and indicates the presence of a central pattern generator for oviposition digging in the terminal abdominal ganglion of females.

INTRODUCTION

In grasshoppers the ovipositor is a highly specialized structure comprising heavy cuticular appendages, hinges and large muscles. It extends beyond the tip of the female abdomen, where it appears externally as two pairs of shovel-shaped structures called ovipositor valves. The ovipositor valves are swung open and closed about the hinges by intrinsic muscle contraction, and the contractions of extrinsic muscles cause the entire ovipositor to be pushed or pulled in and out of the abdomen tip. Snodgrass (1935) has shown that the four valves are hinged at their bases to each other and to a prominent pair of internal apodemes, and that only ten pairs of muscles are involved in ovipositor movements. The ovipositor muscles derive from abdominal segments eight and nine (Nel, 1929).

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Key words: oviposition, motor programme, central pattern generator, grasshopper, terminal abdominal ganglion.

In the grasshopper embryo, the abdominal segments all have pairs of ventral appendages. Most of these disappear by hatching, but the pairs on segments eight and nine of females are retained. These are modified throughout larval and adult development to become the ventral and dorsal ovipositor valves, respectively (Nel, 1929; Thompson & Schabtach, 1983). In addition, this segmental organization has been shown to be preserved in the innervation of the ovipositor (Seabrooke, 1968). Thus, the ventral pair of valves, muscles and nerves are derived from the eighth abdominal segment and dorsal valves, nerves and muscles from segment nine. Acridids are rare among insects in having ovipositor valves derived from appendages and are the only insects whose ovipositor works by opening and closing movements rather than by sliding valves upon each other (Snodgrass, 1935; Matsuda, 1976).

During oviposition behaviour the ovipositor expresses several different sequences of rhythmical movements. The first part of the behaviour consists of digging a deep hole in the ground. To accomplish this, the animal begins by pressing the abdomen tip into the ground while making sweeping opening and closing valve movements. Once the ovipositor has become engaged in the substrate, the female simply stands on the surface while the ovipositor burrows beneath her. The result is that the abdomen becomes enormously extended into the ground. To allow this, the soft cuticle between some abdominal segments and the intersegmental muscles are modified permitting about 10-fold length changes without damage (Vincent, 1975*b*; Jorgensen & Rice, 1982). After the hole has been dug, the abdomen tip retracts slightly from the bottom, and pauses. Soon, very rapid opening and closing movements of the valves ensue, and then the first egg slowly emerges during a sustained gaping posture assumed by the valves. Once the egg has emerged the valves close, whereupon the egg drops to the bottom of the hole. The closing of the valves reverses the initial orientation of the egg (50–100 eggs in all), which assures that when the future embryo develops the head will be positioned upwards. Following the deposition of each egg, rapid opening and closing movements are repeated. These movements are accompanied by the secretion of a small amount of white frothy substance from the accessory glands. The egg-laying process continues until all eggs are laid forming an 'egg-pod' while the abdomen slowly retracts from the bottom of the hole. The final portion of oviposition behaviour is the capping of the egg-pod with a large amount of the frothy substance. The froth then hardens and darkens.

The occurrence of oviposition behaviour is related to the cycle of maturation of the eggs, and is expressed once each 7–10 days, but females can postpone egg-laying for several days if a suitable site is not available. Copulation and fertilization are not necessary requisites for the behaviour because quite successful oviposition and hatching can occur parthenogenically in these animals (Hamilton, 1955). The infrequent occurrence of oviposition in an individual grasshopper, as well as the problem of the ovipositor being buried underground during oviposition, suggested that it might be difficult to analyse the behaviour. Fortunately, it was found that continuous rhythmical ovipositor movements could be reliably elicited by cutting the nerve cord in the abdomen of females (Thompson, 1982). These movements always occurred following nerve cord transections, provided the animals were sexually

mature, and were very similar to those underlying the digging part of oviposition behaviour.

One purpose of this study was to characterize the motor activity produced by the isolated abdomen and to determine its relationship to the oviposition digging motor programme. Another objective was to examine the relationship of these activities to the bursting discharges that occurred spontaneously in the ovipositor nerves of isolated nerve cords of females. This would test for the presence of a central pattern generator driving oviposition digging behaviour. Central pattern generators (CPGs) are groups of neurones which are responsible for the production of many innate rhythmical patterns of behaviour. Although CPG activity usually is strongly integrated with neural information from both sensory and descending systems, this exogenous input, by definition, is not essential to the formation of the basic rhythmicity underlying the behaviour. CPGs are of widespread occurrence, underlying such diverse behaviour as walking in cats, turtle scratching, leech swimming, insect flight and respiration in most animals (see Grillner, 1975; Delcomyn, 1980; Roberts & Roberts, 1983 for reviews). The activity produced by the isolated part of the nervous system that contains the CPG is called the 'fictive' behaviour.

This study and the subsequent one (Thompson, 1986) suggest that the female grasshopper can provide a useful preparation for analysis of the neural basis of behaviour because of the remarkable similarity of the motor programme underlying the real behaviour, in the semi-intact preparation, and in the isolated CNS. This activity is produced by a system of simple neuromuscular anatomy which is easily and reliably activated for study.

MATERIALS AND METHODS

Preparations

Sexually mature female grasshoppers, *Schistocerca americana*, were obtained from a laboratory breeding colony. Intact animals, severed abdomens and isolated nervous systems were used in this investigation. The dissection of severed abdomens for anatomical and physiological study began by decapitation of the animal using a twisting motion followed by a pull which served to remove the gut. The severed abdomen was then pinned open after either a superficial mid-ventral or mid-dorsal incision. The spermatheca and oviducts were removed from ventral preparations to allow access to the terminal abdominal ganglion and ovipositor muscles; in dorsal preparations it was usually necessary to remove a small amount of remaining gut. Ovipositor nerves and muscles were not damaged in either preparation. In some experiments the ventral nerve cord was isolated from the animal by dissecting it free and then severing the nerves with scissors. The preparation was superfused with locust saline (Hoyle, 1953) at $0{\cdot}2\,\mathrm{ml\,min^{-1}}$.

Anatomical methods

Precise locations of muscle insertions and attachments were determined using the account of Snodgrass (1935) as a general guide. The movement that would be

produced by muscle shortening was then deduced. Such determinations were confirmed by observations of ovipositor valve movements during the production of spontaneous digging movements by isolated abdomen preparations and during bilateral electrical stimulation of the muscles in denervated preparations.

The innervation of ovipositor muscles was studied in stained dissections (methylene blue) and corroborated by electrical stimulation of nerve branches to produce contraction of the appropriate muscle. Central motoneurone cell bodies were located by retrograde filling of ovipositor nerves with cobalt (Pitman, Tweedle & Cohen, 1972), and some preparations were silver intensified by the method of Bacon & Altman (1977). Drawings were made using a *camera lucida* attachment on a Leitz compound microscope.

Tissues to be examined by scanning electron microscopy were fixed in 0·1 mol l^{-1} sodium-cacodylate-buffered 6·25 % glutaraldehyde at pH 7·4, and dehydrated to 100 % ethanol followed by critical point drying. Specimens were mounted on stubs with silver conductive paint, sputter coated with gold/palladium, and examined with an AMR Model 1000A scanning electron microscope.

Physiological methods

Extracellular recordings of muscle potentials were obtained from intact animals by anaesthetizing them with gaseous CO_2 and inserting, through pin-holes in the cuticle, pairs of insulated silver wires, 35 μm in diameter, which were fixed in place with small drops of cyanoacrylate glue on the external cuticle. In dissected preparations the wires were implanted directly into the muscles. The amplified electrical signals were displayed on a chart recorder (Gould 220). Extracellular nerve recordings were obtained with silver hook electrodes placed under the final branch of the nerve that directly supplies the muscle of interest, except for the ventral protractor whose final branch cannot be readily dissected. The penultimate branch was used for recordings of the ventral protractor motor nerve. Nerve signals were photographed from the oscilloscope. Intracellular recordings of ovipositor motoneurones were obtained with glass micropipettes filled with 2 mol l^{-1} potassium acetate. Motoneurones were identified initially by the method of Hoyle & Burrows (1973) which correlates intracellular motoneurone action potentials with individual muscle spikes. In the isolated nervous system motoneurones were identified by correlating intracellular spiking discharge with action potentials extracellularly recorded from the final branch of the nerve to the ovipositor muscle. Some motoneurones were filled intracellularly with cobalt injected iontophoretically from the intracellular microelectrode. Current injections were not monitored directly, but as voltage deflections on the trace using a balanced bridge circuit.

RESULTS

Oviposition and morphology of the ovipositor

Depriving female grasshoppers of access to an oviposition site for several days causes them to oviposit readily when a substrate is finally provided. To observe the

natural oviposition behaviour, animals were kept in a wire cage for 5–10 days and then transferred to a specially constructed chamber. The oviposition substrate in this chamber consisted of moist sand in a narrow trough with sides of glass (depth 10 cm). In Fig. 1 the animal has dug to the bottom, and the abdomen has been slightly retracted from the bottom of the hole which now contains a few eggs.

The movable ovipositor valves are shown more clearly in their closed, resting position in Fig. 2A, while in Fig. 2B the valves are fixed in the open position. In mature females the dorsal and ventral pairs of ovipositor valves are heavily sclerotized structures which are pointed distally and flattened on their excavating surfaces. They are hinged together at their bases and function primarily by a forcible separation, alternately opening and closing. In the living preparation protraction, retraction and some degree of tilting of the ovipositor are also observed. A small rudimentary third pair of valves, the inner valves, is also present. These develop from outgrowths of the dorsal valves and in the adult fit into grooves on the ventral valves (Fig. 2B). The inner valves never emerge from their grooves in the ventral valves and are not in a position to excavate soil particles during digging.

The ovipositor valves are connected to large flat internal apodemes that project several segments anterolaterally into the body cavity (Fig. 3). The bases of the apodemes are strongly hinged to the proximal margins of the dorsal ovipositor valves, but are not connected mechanically to the ventral valves. Instead, the articulation point for the ventral valves is a depression on their proximal dorsal surface which fits a ridge on the ventral side of each dorsal valve. The left and right dorsal valves are joined by cuticular bridges, the posterior and anterior intervalvulae (not shown). The bases of the small inner valves are attached to the anterior of these bridges from which a small disc-like medial apodeme (*e*) projects. There are two small sclerotized plates in the soft membranous cuticle between the bases of the ventral valves and the ovipositor apodemes, and three large pairs of cuticular plates (*a*,*b*,*c*) which surround the ventral valves and give them the appearance of being almost as large as the dorsal valves (nomenclature after Thomas, 1965).

Ovipositor muscles and their innervation

Attachment sites for muscles that operate the ovipositor include the internal surfaces of the three pairs of ovipositor valves, the apodemes, and the abdominal body wall of segments eight and nine. The ten pairs of ovipositor muscles comprise three intrinsic openers, two intrinsic and one extrinsic closer; the two protractors and two retractors are all extrinsic. Because the ventral ovipositor valves are appendages of the eighth segment, the four ovipositor muscles of segment eight are considered to operate the ventral valves. They comprise one each of the four functional types: opener, closer, protractor and retractor (Fig. 4A). The dorsal valves are appendages of segment nine and they also have one muscle of each functional type – opener, closer, protractor, retractor and two additional muscles: the accessory dorsal opener, and the muscle of the inner valve which is functionally a closer (Fig. 4B). By far the most prominent ovipositor muscles are the openers of the dorsal and ventral valves. They are attached to the flat surfaces of the ovipositor apodemes and insert

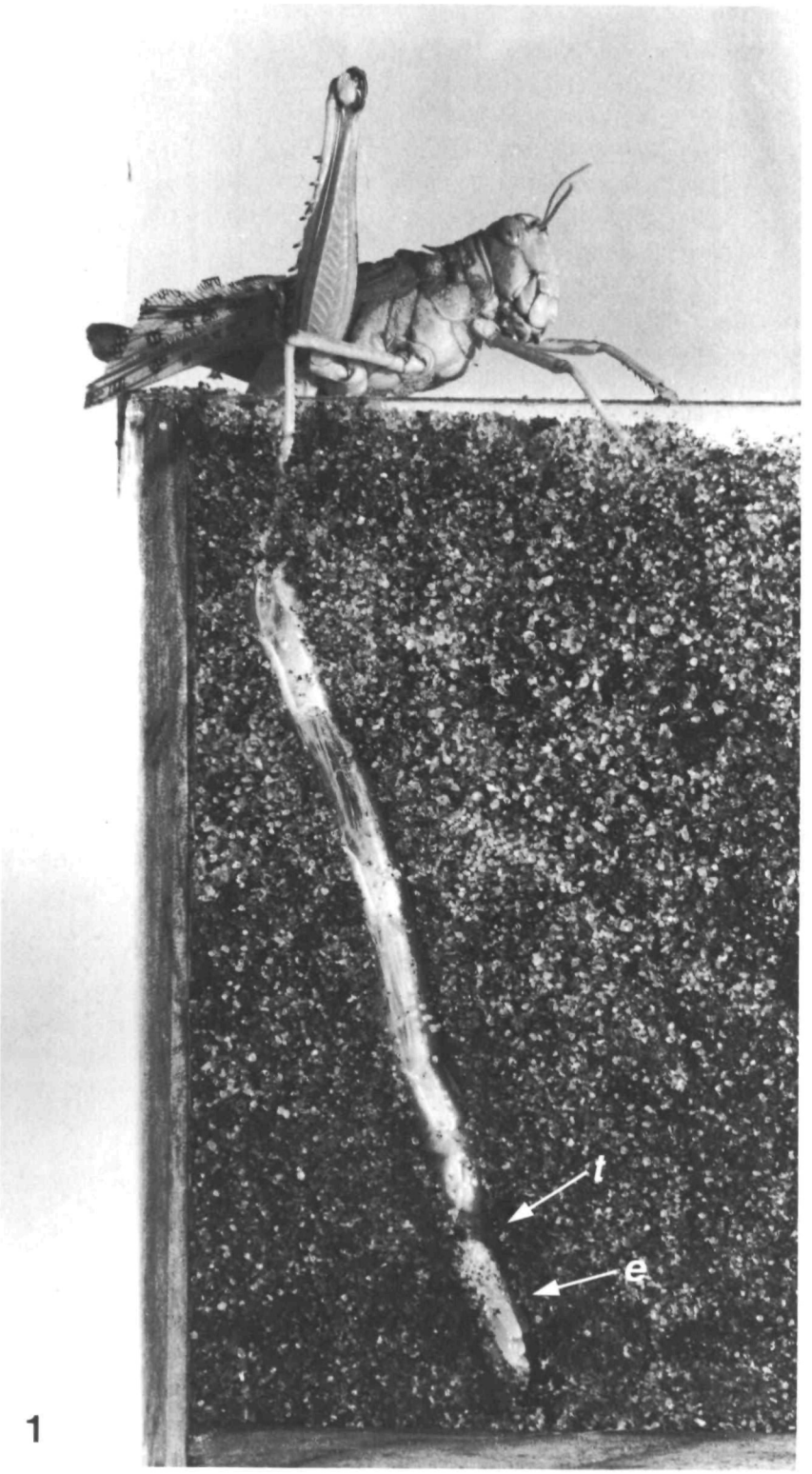

Fig. 1. Ovipositing grasshopper. The abdomen is greatly extended into the sand-filled chamber and a few eggs (*e*) are seen at the bottom of the hole beneath the abdomen tip (*t*). The depth of the chamber is 10 cm.

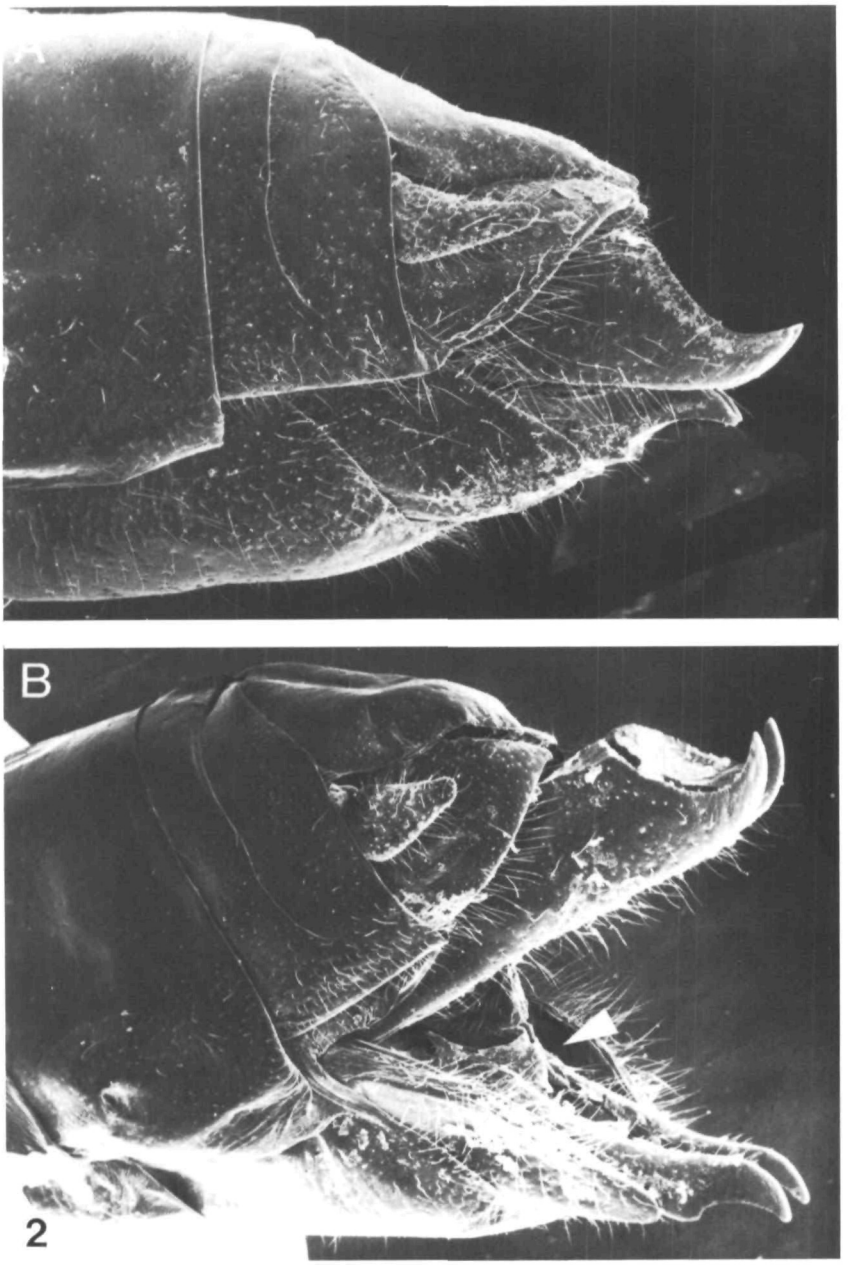

Fig. 2. Scanning electron micrographs of the ovipositor (lateral views). (A) The ovipositor valves are closed and the dorsal valves overlap the ventral valves. (B) The dorsal and ventral pairs of ovipositor valves are widely opened. In the open position the small pair of inner valves are visible with their tips in grooves on the inner surfaces of the ventral valves (arrowhead).

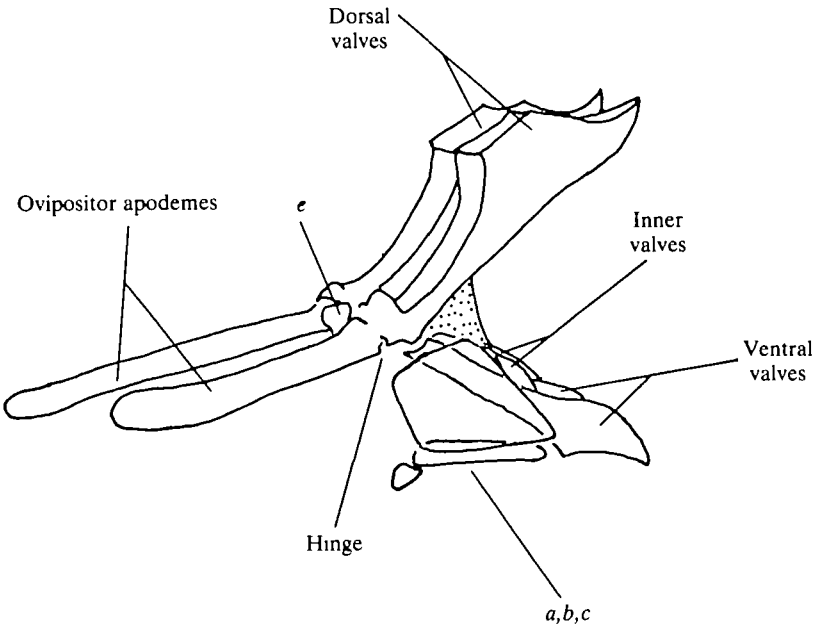

Fig. 3. Skeletal components of the ovipositor (abdominal cuticle removed). The dorsal and ventral pairs of valves are indicated, as is one of the inner valves in position in its groove on the ventral valve. The ventral valves are enclosed by three basivalvular plates (*a*, *b*, *c*) and articulate at their proximal surfaces on ridges on the inner surfaces of the dorsal valves. The bases of the dorsal valves are hinged to the ends of the internal ovipositor apodemes. A small medial apodeme (*e*) is also indicated. A few other cuticular structures (not shown) are described in the text. (Figure labelled after Thomas, 1965.)

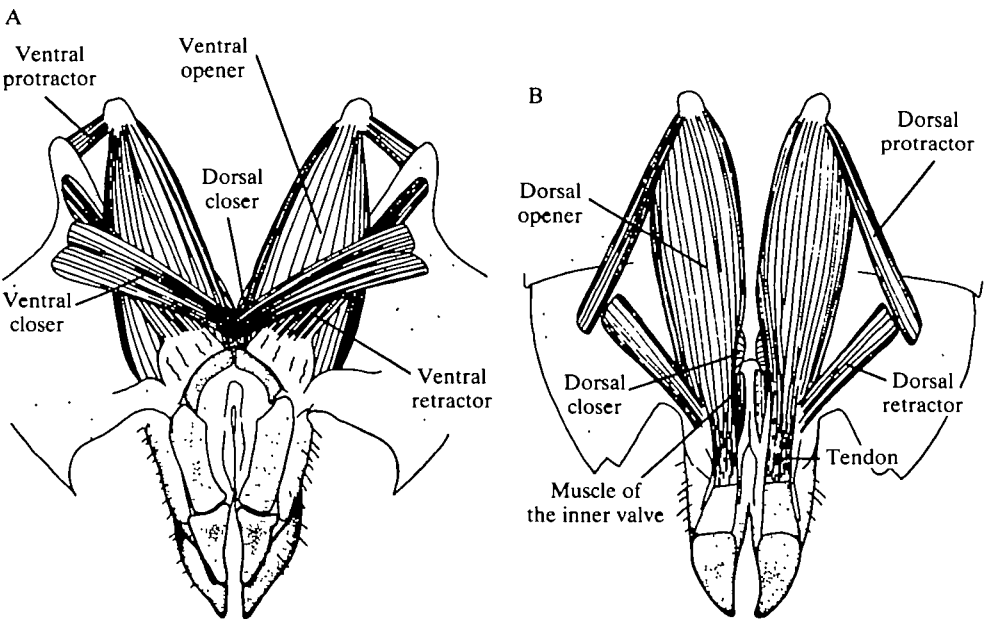

Fig. 4. Ovipositor muscles. (A) The muscles were drawn from a ventral dissection of the ovipositor. (B) The muscles were drawn from a dorsal dissection. Nine of the ten pairs of ovipositor muscles are visible in dissected preparations. The accessory dorsal opener, which is not shown, is a small muscle lying underneath the dorsal opener tendon.

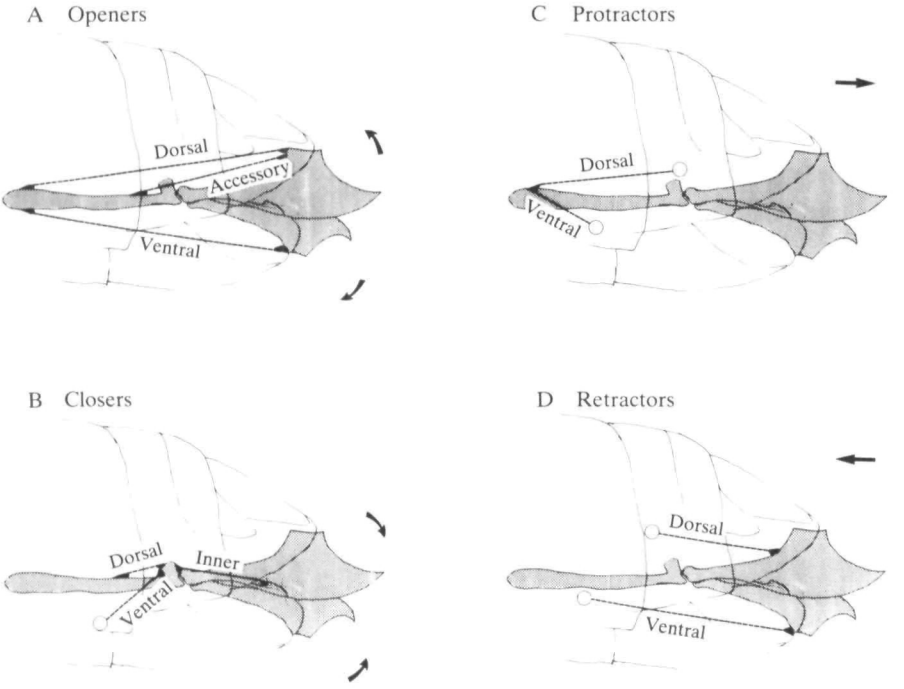

Fig. 5. Functional anatomy of the muscles. In these lateral views, the dorsal and ventral ovipositor muscles are represented in functional groups. Extrinsic muscle attachment to the abdomen is indicated by open circles; intrinsic muscle attachments are shown as filled triangles; ovipositor cuticle is shaded. Movements produced by the contraction of the functional group of muscles are indicated by large arrows. These movements are perhaps best understood by considering that the valve hinges are in the region of their junction with the apodemes, and that the ovipositor (including apodemes) can slide caudally and rostrally. (A) The dorsal opener, ventral opener and accessory opener muscles. Their contractions swing the ovipositor valves open. (B) The dorsal closer, ventral closer and muscles of the inner valves contract to press the valves closed. (C) The dorsal and ventral protractor muscles whose contractions cause the ovipositor to protrude from the end of the abdomen. (D) The dorsal and ventral retractor muscles, which pull the ovipositor back into the abdomen.

posteriorly into the valves. Contraction of these muscles swings the ovipositor valves open (Fig. 5A). Opening of the dorsal valves is assisted by contraction of the accessory dorsal opener muscles. These are not visible in the dissected preparations shown in Fig. 4 because they lie under the dorsal opener muscle and tendon. They lie roughly parallel to the dorsal openers, but attach in a caudal position on the ovipositor apodeme and insert at a more ventromedial site on the dorsal ovipositor valve. Closing of the ovipositor valves is produced mainly by relaxation of the large opener muscles (the rest position of the valves is closed). The valves can be pressed tightly together, however, by the shortening of three small pairs of muscles: the dorsal and ventral closers, and the muscles of the inner valves (Fig. 5B). The muscles of the inner valves directly oppose the openers by pulling on the dorsal side of the ventral valves and crossing to the opposite side of the fulcrum for ventral valve rotation in their attachment to the dorsal valves. The ventral and dorsal protractors

work in concert, by pulling the rostral ends of the apodemes back towards their attachments on the tergites of segments eight and nine. During protraction, the ovipositor protrudes as a unit from the abdomen tip (Fig. 5C). The ventral and dorsal retractors of the ovipositor are attached to the bases of the valves and insert rostrally into the sides of the abdomen. Shortening of these muscles pulls the ovipositor back into the abdomen (Fig. 5D). The ventral closer muscle also contributes to retraction of the ovipositor and causes ventral tilting.

The names used for muscles in this study are based on the movement produced. Some of them differ from those in the work by Snodgrass on *Dissosteria carolina* (1935). Table 1 presents a comparison of the names in this study and the names chosen by Snodgrass, along with muscle numbers and segmental assignments. Some of the names chosen by Snodgrass were selected because they imply possible homologies with muscles of the pregenital segments, but as Snodgrass himself pointed out, the musculature differs so much that few of the ovipositor muscles can be homologized with the muscles of the other abdominal segments.

The ovipositor muscles are innervated by the segmental nerves of the terminal abdominal ganglion which comprises the ganglia of abdominal segments eight to

Table 1. *Segmental assignments and muscle names from the present study and the study by Snodgrass (1935)*

Present study		Snodgrass (1935)		
Abdominal segment	Muscle name	Number	Abdominal segment	Muscle name
8	Ventral opener	272	9	Depressor of the ventral valvula
8	Ventral closer	247	8	Median internal ventral muscle
8	Ventral retractor	248	8	Lateral ventral muscle
8	Ventral protractor	256	8	Short protractor
	Dorsal opener	271	9	Levator of the dorsal valve
	Dorsal closer	273	9	Adductors of the ventral valvulae
	Dorsal retractor	263	9	Retractor
	Dorsal protractor	262	9	Long protractor
	Accessory dorsal opener	274	9	Adductors of the dorsal valvulae
	Muscle of the inner valve	275	9	Muscle of the second valvula

Muscles 273 and 274 produced only barely discernible adductions in *Schistocerca americana*, but the stronger movements observed (closing and opening, respectively) were used in naming them. The first muscle listed in the table was the subject of some uncertainty to Snodgrass, who suggested that an alternative number for this muscle might be 249. Seabrooke (1968) confirmed the segmental assignments for the dorsal muscles.

eleven. The two pairs of eighth segmental nerves supply the four ventral ovipositor muscles (Fig. 6). Nerve 8dn leaves the ganglion dorsolaterally before branching to supply the two parts of the ventral closer muscle. It then passes ventral to the ovipositor muscles, sending a branch dorsally around them to the dorsal surface of the short protractor muscle (see left side of Fig. 6). The larger ventral nerve (8vn) leaves the middle of the ganglion slightly ventrolaterally. Its lateral branch passes under the closer nerve branch of the 8dn and ventral to the closer muscle to supply the ventral retractor muscle on its dorsal surface. The ventral opener muscle is supplied by a branch of the posterior branch of the 8vn. This branch turns rostrally to supply the ventral opener muscle (best seen on the left side of the figure). The distal branch of the 8vn which enters the ventral ovipositor valves seemed to contain no excitatory motoneurones. This is in apparent contrast to the situation in

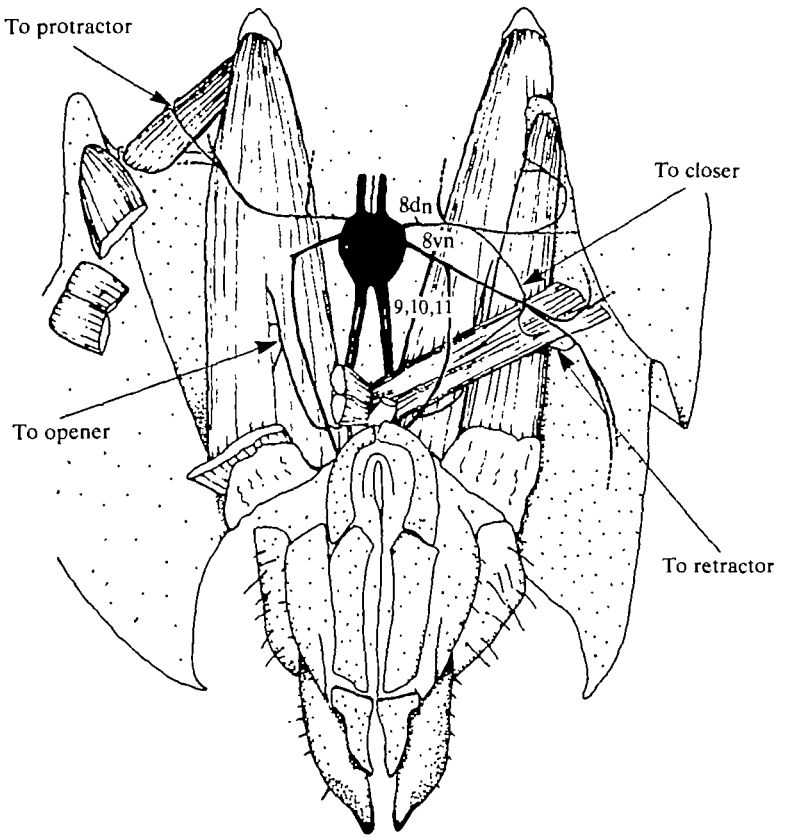

Fig. 6. Innervation of the ovipositor muscles of the eighth segment (ventral view). The ventral opener and ventral retractor muscles are innervated by the eighth ventral nerves (8vn), and the ventral closer and ventral protractor muscles by the eighth dorsal nerves (8dn) of the terminal abdominal ganglion. On the left side of the drawing, the closer and retractor muscles and some nerve branches are shown cut to more clearly reveal the branches supplying the opener and protractor muscles. The segmental nerves of segments nine to eleven leave the ganglion in a large pair of posterior nerves (9, 10, 11).

S. gregaria where this branch of the nerve is mixed sensory and motor (Seabrooke, 1968). The present conclusion was based on the observation that electrical stimulation of this branch did not produce muscle contraction, and based on physiological recordings from this nerve during execution of the oviposition digging motor programme. The possibility of its containing inhibitory or neurosecretory axons, however, cannot be excluded. The muscles of the dorsal valves receive their innervation from the ninth segmental nerves (see Seabrooke, 1968). Sensory innervation of the ovipositor was also described by Seabrooke (1968) for *S. gregaria* which seems similar to the innervation in *S. americana*.

Motoneurones of ovipositor muscles

The central locations of 17 ovipositor motoneurones were determined by retrograde transport of cobalt from the four motor nerve branches to the ventral ovipositor muscles. The opener muscle was found to be supplied by five motoneurones with large ipsilateral somata of 50 μm diameter. These cell bodies are located in a cluster anterolaterally on the ventral surface of the terminal abdominal ganglion near the emergence of the 8vn (Fig. 7A). Backfills of the nerve branch to the retractor muscle revealed seven small cell bodies (diameters of 20 μm). These are ipsilateral and ventral, but are rostral to the opener motoneurones close to the level of the 8dn (Fig. 7B). The two protractor neurone cell bodies are unusual in that they are contralateral and dorsal (diameters of 25 μm, Fig. 7C). The closer motoneurones have axons in the 8dn, but these axons travel through the terminal ganglion without branching, and ascend within the ipsilateral connective. In the seventh abdominal ganglion, which is the ganglion next most rostral to the terminal abdominal ganglion, are found the somata of the three closer motoneurones (25 μm soma diameter) with their bilateral branching patterns (Fig. 7D). This location is curious because the backfill also revealed a dorsal unpaired medial cell (DUM), based upon morphological criteria only, in the terminal ganglion. A putative DUM cell was also found in backfills of the nerve to the ventral opener muscles, but extensive backfilling failed to reveal DUM cells for the other ventral muscles (Thompson, 1982).

Oviposition digging behaviour

During oviposition, females were observed to grip firmly to the surface with the first two pairs of legs. The jumping legs were held in a variety of positions: the one in the photograph (Fig. 1) was common; cocked and ready to deliver a defensive kick. The head often produced auxiliary respiration by pumping. When animals were ovipositing in the glass-sided chamber, it was possible to view the behaviour of the abdomen and to measure the timing of valve movements. Excavation of the hole was accomplished by rhythmical movements of the ovipositor valves which swept the soil from the bottom of the hole and compressed it into the sides. Alternate opening and closing were the most obvious movements seen. In each cycle of movement the valves began tightly closed; they were then pressed into the substrate, opened widely and slightly retracted. This pattern of movements forced the soil particles sideways and somewhat upwards. Protraction and retraction movements appeared to be used

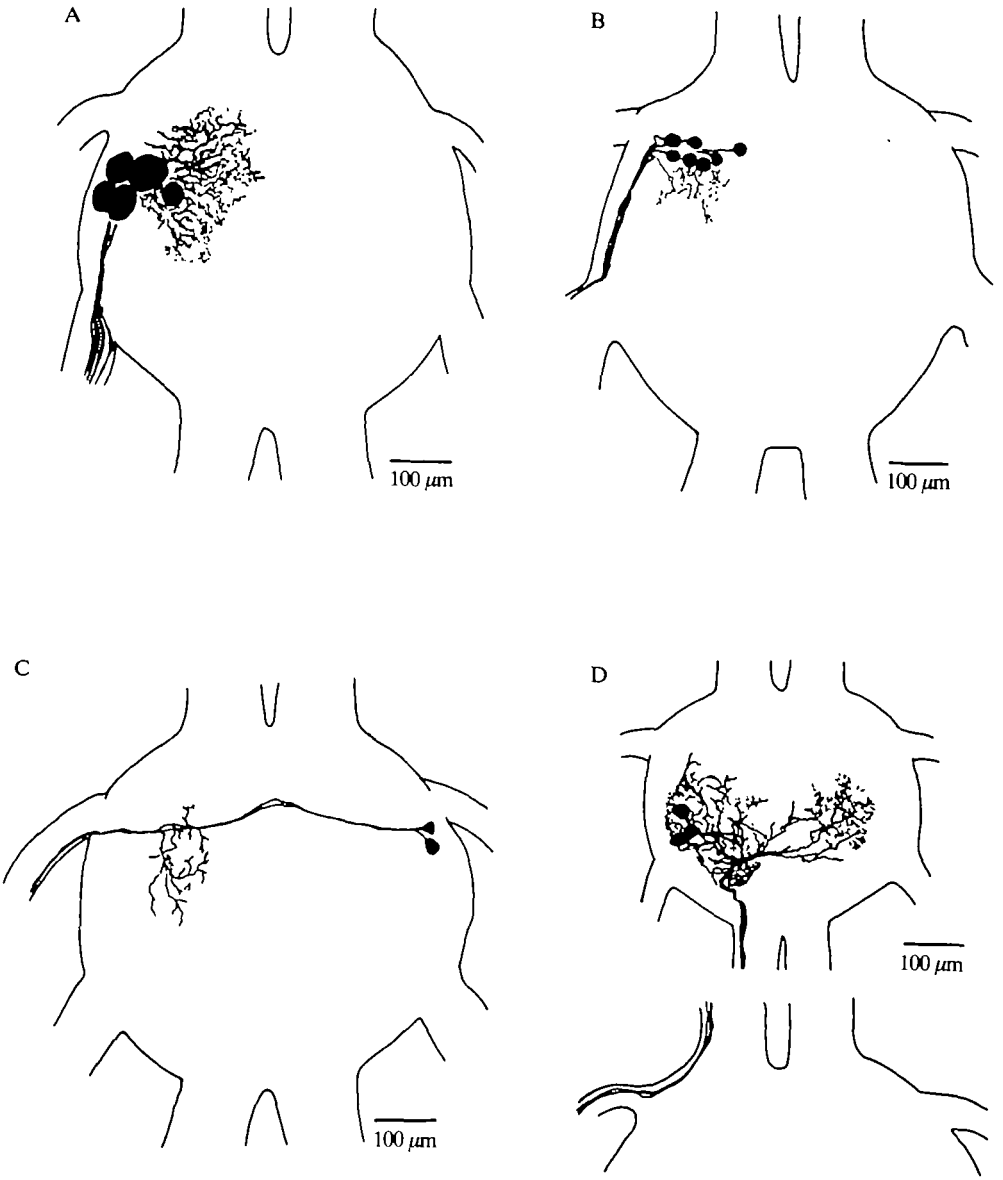

Fig. 7. Ovipositor motoneurones of the eighth abdominal segment. (A) Ventral opener motoneurones; (B) ventral retractor motoneurones; (C) ventral protractor motoneurones. In A, B and C the neurones are located in the terminal abdominal ganglion. (D) The ventral closer motoneurones whose cell bodies and fine branches are in ganglion 7. The path of their axons within the terminal abdominal ganglion is shown at the bottom of the drawing.

primarily for elongation of the abdomen. During the closed phase of each cycle of movement, the ovipositor was tilted ventrally with respect to the abdomen and pressed into the working face of the hole. At this time in each cycle some retraction of the valves took place. The effect of the retraction was to shorten the distance between

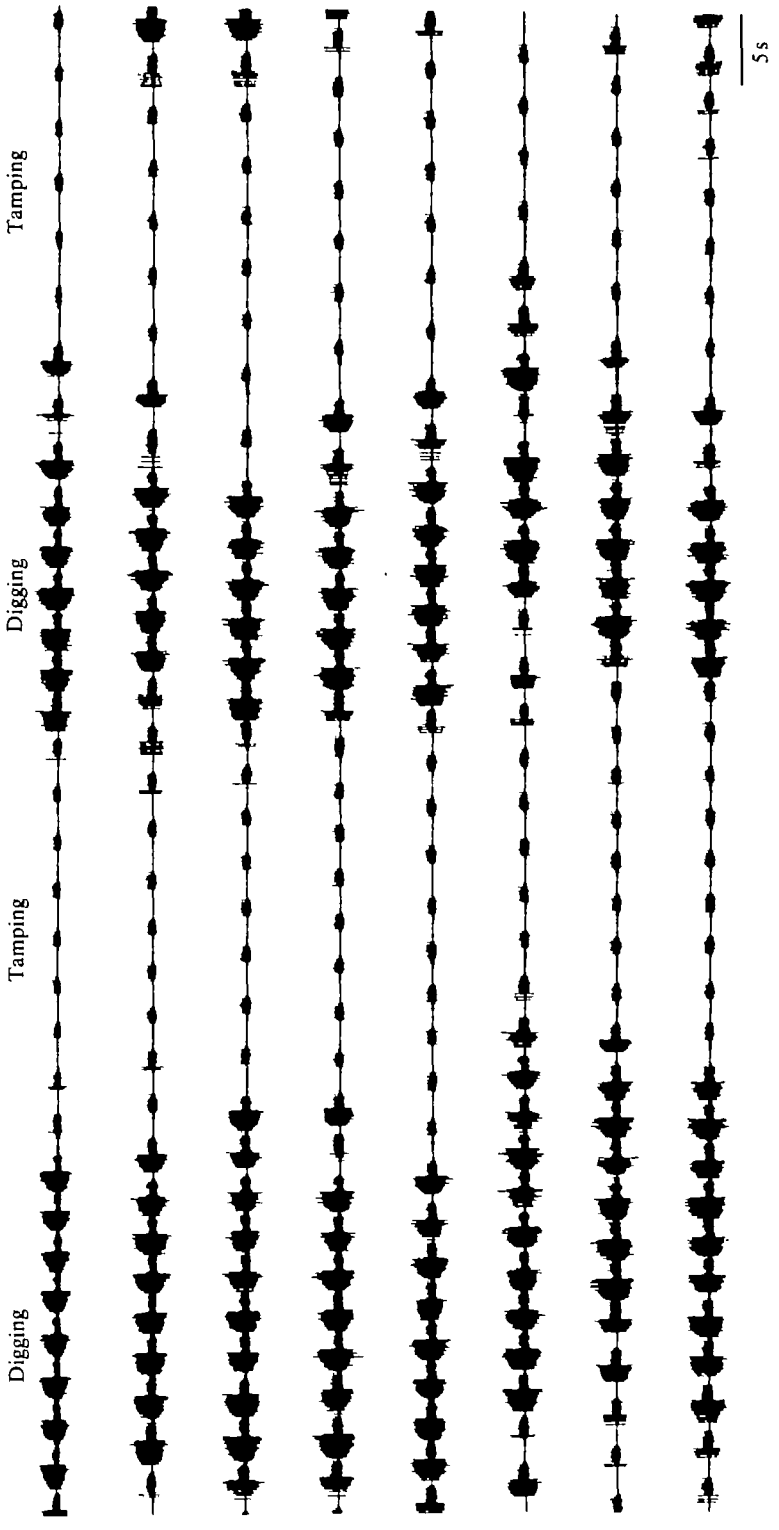

Fig. 8. Electromyographic activity accompanying natural oviposition digging behaviour, continuous record from a ventral opener muscle. The large amplitude bursts correspond to ventral opener activity. The small amplitude bursts are closer muscle activity, which was recorded as cross-talk by the electromyographic wires. Alternating bouts of digging and tamping are indicated at the top of the record.

the valves and the rest of the abdomen. This resulted in a tug on the intersegmental membranes. Protraction during the opening phase allowed the ovipositor to be extended further into the hole, and the protrusion of the ovipositor from the abdomen allowed the valves to open to the maximum extent possible. Furthermore, this enabled the ovipositor to ratchet into the ground while soil particles were being swept to the sides. Periodically, the opening and closing digging movements stopped, and the valves were closed together. The abdomen was then rotated about its long axis, and patted the sides of the chamber, smoothing and tamping. Thus, excavation involved an alternation between digging and tamping.

Electromyographic activity recorded from insects digging in sand included large rhythmical bursts of electrical activity in ventral opener muscles, coinciding with opening movements (Fig. 8). These bursts alternated with bursts of closer activity during digging, but then were absent for several cycles during tamping. The rhythmical bursting activity of the closer, in contrast, continued throughout tamping but caused little movement since the rest position of the valves was already closed.

Motor programme of digging

When the abdomen or ventral nerve cord was severed at the junction with the thorax, continuous rhythmical movements of the ovipositor valves were produced (Fig. 9). These movements were qualitatively very similar to the digging movements observed in intact animals, but tamping-like activity was not present. When placed in sand, severed abdomens were able to displace the substrate, but they were unable to construct complete oviposition chambers. The movements of the isolated abdomen were cyclic and comprised the same basic components as seen in the intact animal during digging. Namely, the ovipositor valves were first retracted and closed together. They were then protracted and opened widely while the ovipositor was tilted slightly ventrally. Next the ovipositor was once again retracted and closed tightly together.

An electromyographic analysis of the activity of all ten ovipositor muscles, recorded in pairs, was undertaken to characterize the motor programme in the isolated abdomen (Fig. 10A,B). As was observed in recordings of intact animals digging in sand, alternating bursts of activity occurred in openers and closers. The ventral and dorsal valves opened and closed in phase with each other and their respective opener muscles were coactive (Fig. 10C). The ventral and dorsal protractor muscles were also coactive, and this activity led the onset of opener activity in each cycle but terminated at the same time as opener activity. The ventral retractor muscle was almost continuously active. It, therefore, co-contracted with the functionally antagonistic protractor muscles. In contrast, the dorsal retractor was activated in antiphase to the protractor muscles. The two additional muscles of the ninth segment, the accessory dorsal opener and the muscle of the inner valve, were also rhythmically active in phase with the other ovipositor muscles. The accessory dorsal opener was active in a short intense burst at the end of each opener phase. Its activity contributed to further opening of the dorsal valves. The muscles of the inner valves were active during the closing phase of the digging cycle. These muscles are in a

position to be exceedingly important in the mechanics of closing (see Fig. 5B) and the recordings are consistent with this function.

Central generation of the digging pattern

Efferent bursts of activity recorded from ovipositor motor nerves in isolated (deafferented) nerve cords retained the essential features of timing and coordination

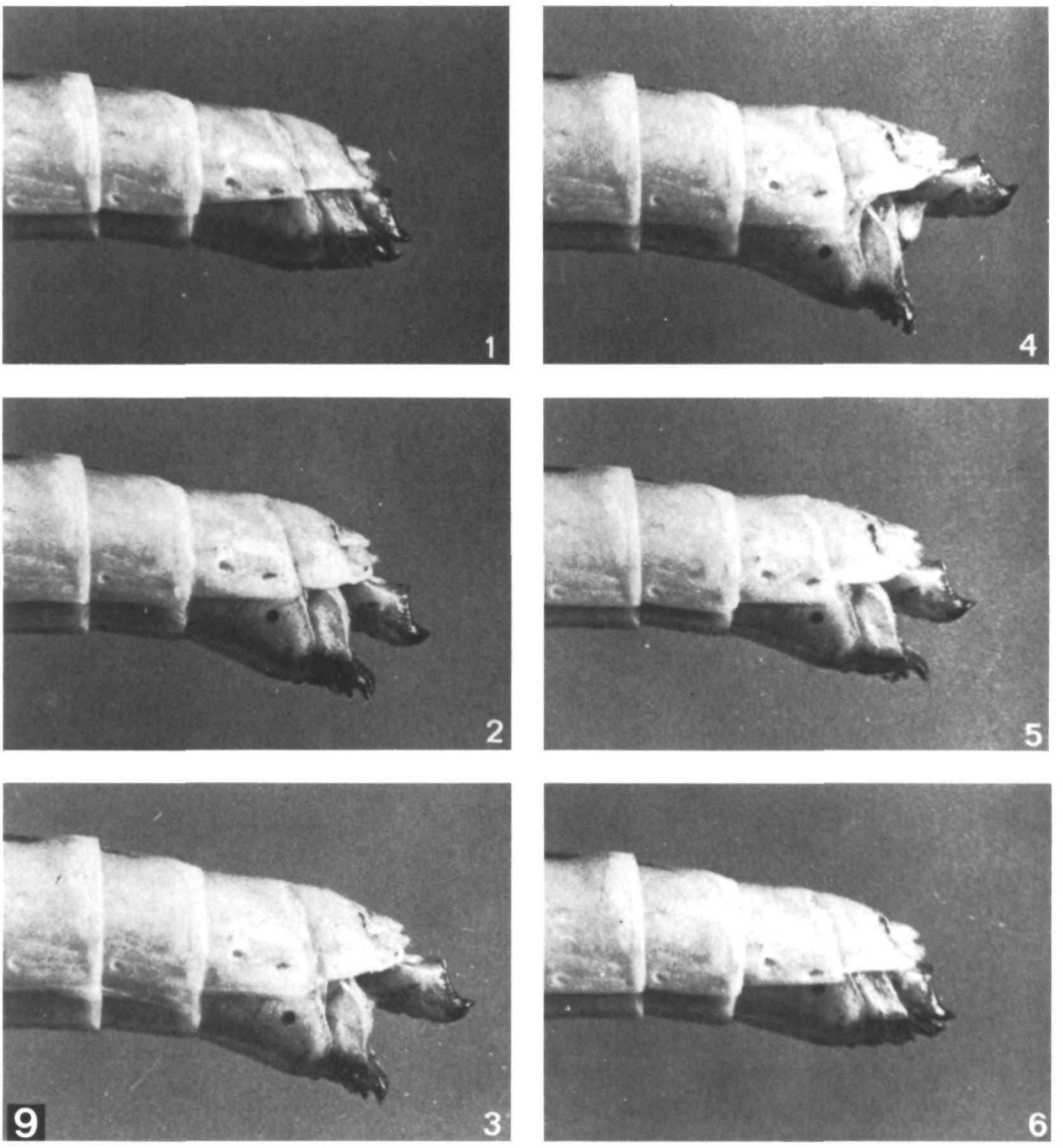

Fig. 9. Oviposition digging movements spontaneously produced by the isolated abdomen. This sequence of photographs shows one complete cycle of ovipositor digging movements. The valves were closed and retracted in frame 1 and then increasingly opened and protracted in frames 2–4. In frames 5 and 6 the ovipositors were once again closed and retracted. Cycle period length in this preparation was approximately 3·1 s. There is an interval of 0·63 s between each frame 1–4, and 0·42 s between frames 4–6.

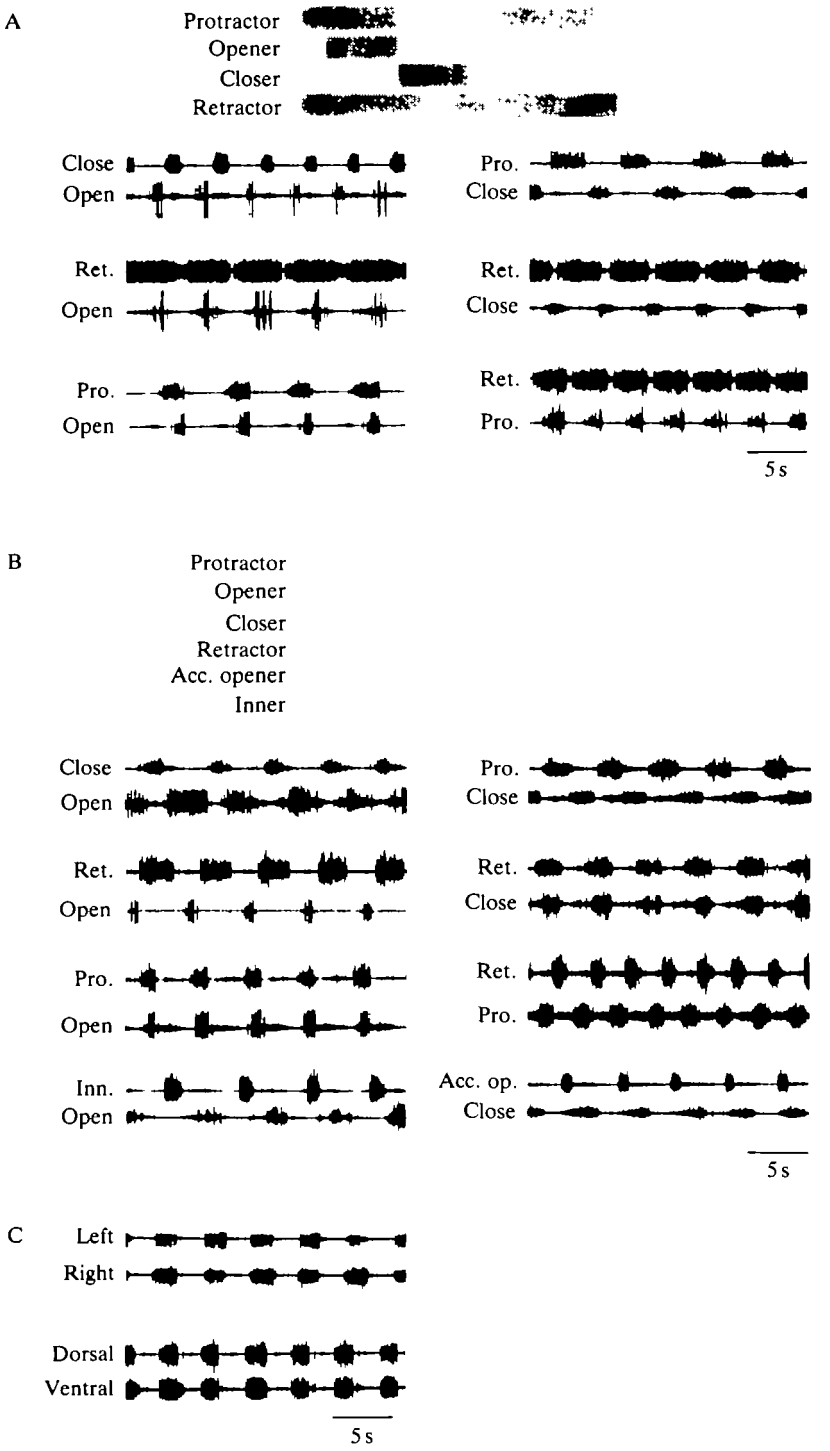

Fig. 10. The motor programme for oviposition digging. These phase diagrams and paired electromyographic recordings of ovipositor muscle activity relationships were obtained from the isolated abdomen undergoing digging movements. In A, the activities of the four muscles of the eighth segment, and in B the activities of four functionally homologous muscles in segment nine and the activities of the two additional ninth segmental muscles are shown. In C, the coactivation of bilateral and dorsal and ventral opener muscles is illustrated. Ret., retractor; Pro., protractor; Acc., accessory; Inn., inner.

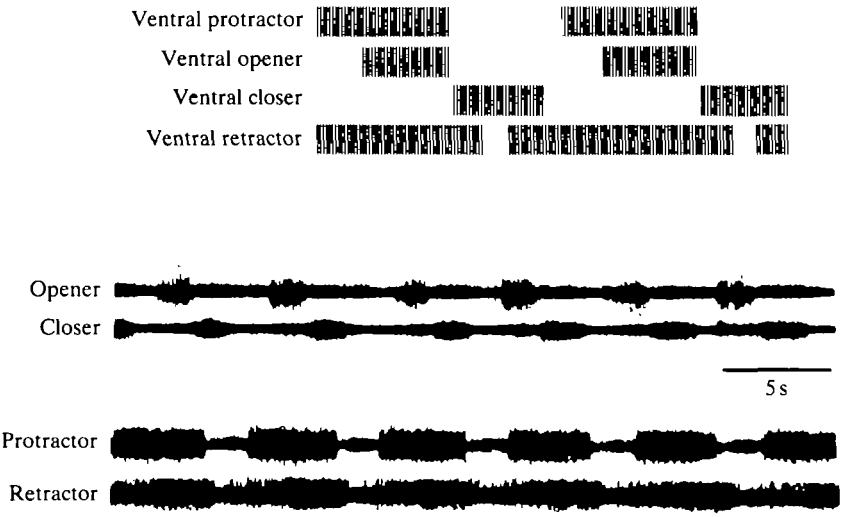

Fig. 11. Extracellular nerve activity during fictive oviposition digging. Pairs of nerve recordings from branches of the eight ventral and dorsal nerves were obtained from the isolated nerve cord. The phase relationships of their spontaneous bursting activity are shown diagrammatically above the records.

exhibited by the appropriate muscles in severed abdomen preparations (Fig. 11). Mean ventral opener burst durations and cycle period were calculated for 50 cycles each in five isolated nerve cord preparations and compared to 10 isolated abdomen preparations. Values calculated for the isolated nerve cord overlapped those of the isolated abdomen, but there was slightly more variability in all measured parameters. In isolated nerve cord preparations cycles occurred with periods ranging from 2·5 to 7·0 s, and opener burst durations occupied 38 % (S.D. = 8) of the cycle duration. In the isolated abdomen preparations used for these calculations the period ranged from 2·9 to 8·0 s, and tended to be relatively constant for an individual preparation (S.D. = 0·36). Opener burst durations were slightly shorter in the isolated abdomen preparation, occupying 34 % (S.D. = 5). In insects laying in sand, the cycles were generally shorter, of the order of 1·0–4·0 s, and could vary if the ovipositor encountered an obstacle.

Recordings from pairs of ventral ovipositor motor nerves were obtained in isolated nerve cord preparations to compare their phase relationships with the activity in the isolated abdomen preparation (Fig. 11). The activity patterns recorded from the nerves coincided closely with the patterns recorded from the ovipositor muscles. Openers and closers were active in alternation, and protractor activity led the onset of activity in the opener but terminated at the same time. Retractor activity showed the nearly-continuous pattern typical of its activity in the severed abdomen, except in approximately 20 % of preparations where it burst in antiphase to protractor activity (not shown). This antiphase pattern was similar to the activity of the dorsal retractor, but was unlike that usually seen in isolated abdomens. Nevertheless, in general the isolated nerve cord was capable of executing the basic rhythmicity and phase

relationships of the digging pattern, and this establishes the presence of a CPG for oviposition digging behaviour in the nerve cord of female grasshoppers.

Oviposition digging activity is strongly influenced by sensory feedback, even in the isolated abdomen preparation. For example, holding the valves closed caused the inhibition of several cycles after which the pattern resumed at a normal rate even though the hold had not been released (Fig. 12A). Immobilizing the valves in other positions, open, protracted or retracted, also disrupted the pattern, but the effect was one of almost continuous activity in the electromyographic records with some hint of maintained rhythmicity (Fig. 12B,C,D). Also, imposed resistance to full opening of an individual valve seemed to cause increased force to be generated against the block.

To carry out further localization of the oviposition digging CPG the abdominal nerve cord was transected at various levels. Transections which preserved the connections between the seventh and terminal abdominal ganglion resulted in a vigorous preparation whose stereotyped activity was indistinguishable from preparations that preserved the entire abdominal nerve cord. However, transection between ganglion 7 and the terminal abdominal ganglion produced a less active preparation. After the terminal abdominal ganglion had been separated from the rest of the nerve cord, the spontaneous oviposition digging pattern was produced in only approximately 40 % of preparations. In many of these preparations, the activity was transient, or the burst intensities were reduced. This difference was observed in both isolated abdomen and isolated ganglion preparations, suggesting that some level of tonic excitability to the CPG in the terminal abdominal ganglion is provided by the normal connections with the seventh ganglion. In addition, the transection eliminated ventral closer nerve bursts, because the ventral closer cell bodies are in the seventh ganglion.

Intracellular motoneurone activity

The intracellular activity of ventral opener motoneurones was examined in severed abdomen preparations which were pinned open with the terminal abdominal ganglion on a platform, and in the completely isolated nervous system while nerve bursts were monitored with extracellular hook electrodes under the final branch of the 8vn cut just proximal to the ventral opener muscle. Of the five ventral opener motoneurones, four are quite large. These were physiologically and anatomically identified in several intracellular experiments, but the small fifth motoneurone was not studied. Intracellular recordings in the isolated abdomen with moving ovipositors were more difficult to obtain than those in the isolated nerve cord preparation because of movement-related instability.

In both types of preparation the characteristic pattern of activity consisted of bursts of spiking discharges riding on rhythmical waves of depolarization. A continuous intracellular recording from one of the large opener motoneurones, showing the spontaneous rhythmical activity typical of recordings from ventral openers in an isolated but active nerve cord, is presented in Fig. 13A (anatomy in Fig. 13C).

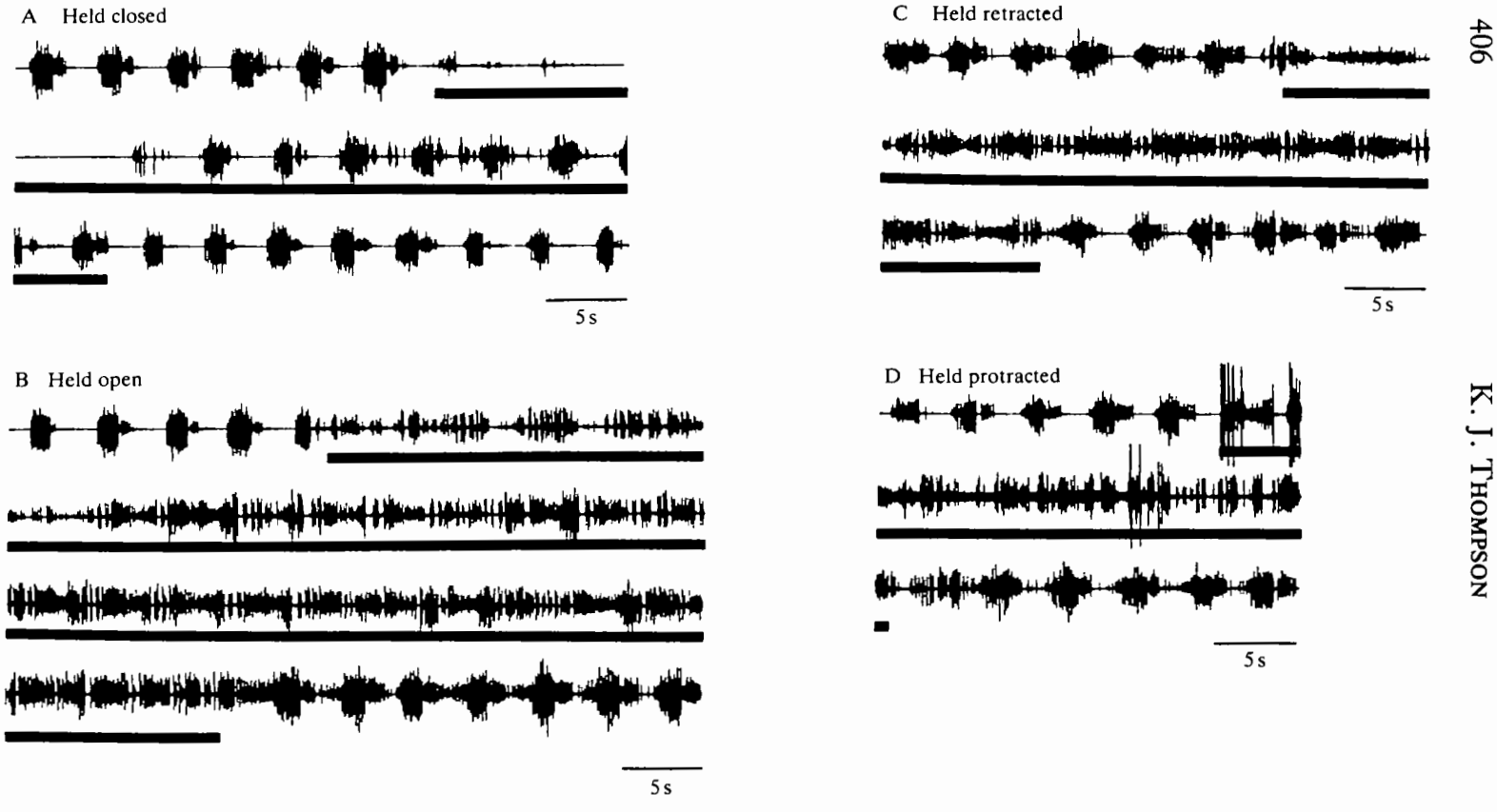

Fig. 12. Effects of immobilizing the ovipositor on the production of the motor programme. The records are all continuous electrical recordings of ventral opener muscle activity in the isolated abdomen. The valves were immobilized by holding them in various positions with forceps (A, closed; B, open; C, retracted; D, protracted) during the times indicated by the thick bars.

Constant current was injected into some ventral opener motoneurones to begin to examine in more detail the synaptic inputs to them and their endogenous membrane properties. In some recordings from these motoneurones it was observed that the characteristic slow depolarizing waves did not always reach threshold for spiking (not shown). Experimentally, spiking discharges could be prevented by injection

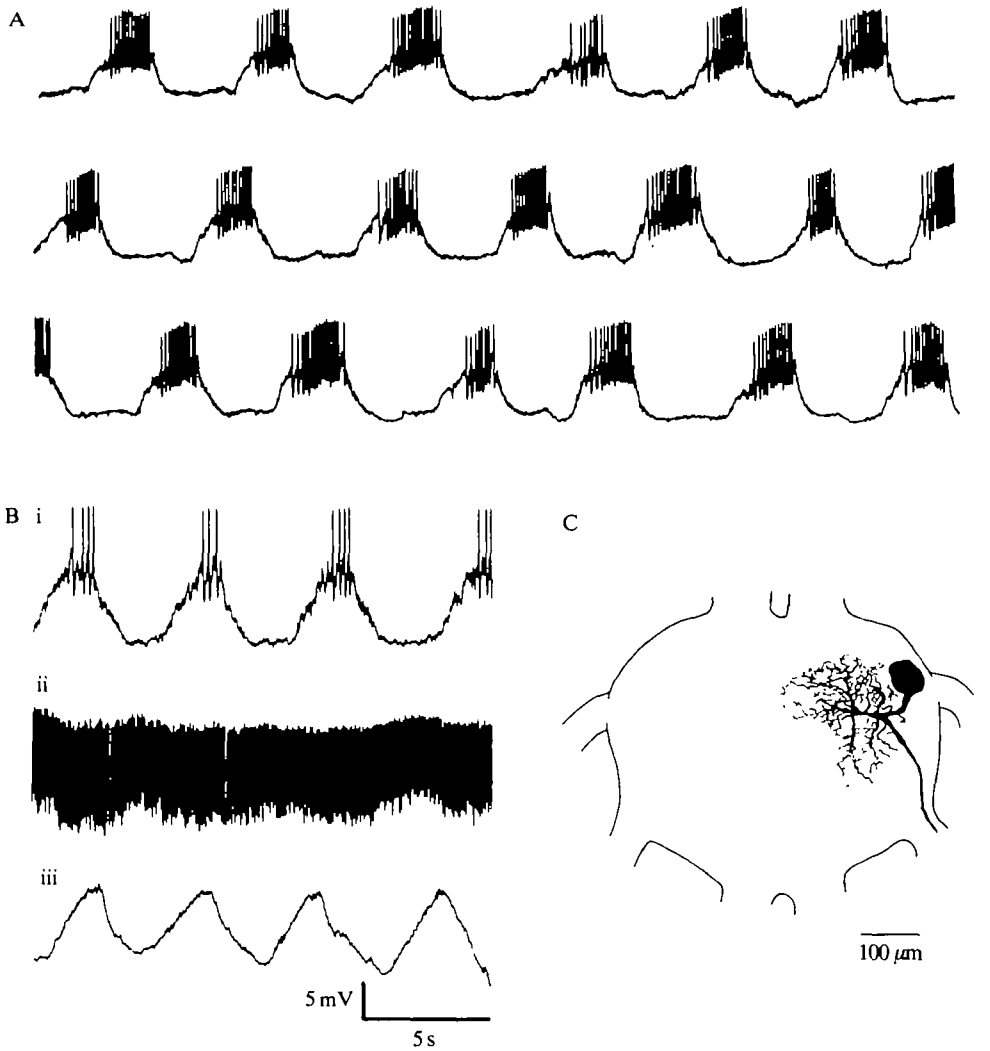

Fig. 13. Intracellular motoneurone activity during fictive oviposition and effects of constant current injections. (A) Continuous recording of slow membrane potential oscillations in a ventral opener motoneurone with bursts of impulses occurring on the depolarizing phases. (Bi) recorded activity of another ventral opener motoneurone in the absence of current injection; (Bii) constant current depolarization caused sustained firing in the motoneurone without eliminating slow wave oscillations; (Biii) constant current hyperpolarization of the motoneurone emphasized the slow wave but did not actually increase its amplitude. (C) Anatomy of the motoneurone recorded in A, drawn from an intensified cobalt direct-fill.

of constant hyperpolarizing current through the intracellular microelectrode (Fig. 13Biii). The slow waves of depolarization continued in the absence of motoneurone action potentials. When constant current depolarization was applied, sustained firing occurred in the motoneurone, but again, the slow wave activity was not eliminated (Fig. 13Bii). Neither form of current injection altered the timing of the oscillations. These results indicate that the motoneurone is not a pacemaker cell itself (see Strumwasser, 1967), but is probably synaptically driven. The reduction in slow wave amplitude observed in response to depolarizing current injection suggests that the motoneurone is driven to produce bursts of spikes by waves of excitatory synaptic input. However, hyperpolarizing current injection did not increase slow wave amplitude. These observations suggest that the form of synaptic input to the opener motoneurones is complex and probably includes synaptic inhibition.

DISCUSSION

The results of this study suggest that oviposition digging behaviour in grasshoppers is driven by a CPG, and that the preparation is a robust and useful one for neural analysis. The present study describes the anatomical and physiological basis of some aspects of the ovipositor. The structural components of the ovipositor were presented from a functional point of view, and the muscles called accordingly by functional names. A discrepancy with the original work by Snodgrass was found in this study and suggested earlier in the innervation study by Seabrooke (1968). The ventral opener muscle was assigned to the ninth segment by Snodgrass (1935), but clearly its innervation, both in terms of supply by the 8vn and position of central motoneurone cell bodies suggests that its proper designation is in the 8th segment. In addition, the conclusions about the function of some of the muscles, in particular the closers, have been modified. Whereas Snodgrass asserts that there are no muscles which directly oppose the openers, the present study points out that the muscles of the inner valve are in just such a position and function as closers. The present study of neuronal anatomy and physiology was concentrated on the structures of the eighth segment. Clearly, to fully characterize the ovipositor, similar nerve backfills, nerve recordings and intracellular recordings need to be done for the dorsal valves and the ninth segment.

One relative advantage of the oviposition system as a preparation for neurophysiology is the ease and reliability with which the pattern can be elicited. The act of isolating the abdomen to make a semi-intact preparation is sufficient to induce spontaneous oviposition activity (Thompson, 1982). This study is not the first to report the occurrence of rhythmical ovipositor movements in the isolated abdomen. Thomas (1965) mentioned the presence of cyclic movements of the ovipositor valves and Vincent (1975*a*) studied these movements in detail, using single-frame analysis to demonstrate that the valve movements provide appropriate forces to accomplish digging of the oviposition hole and the enormous stretching of the intersegmental membranes. Before his study, it was thought that females inflated their abdomen with air to press their ovipositor into the ground.

Intracellular recordings of ganglionic neurones in insects are usually done in young, immature adults because of the difficulty in penetrating the connective tissue in older animals. It may prove difficult to perform further intracellular studies of oviposition, a behaviour that occurs after sexual maturity. However, motoneurones are certainly accessible in mature adults (cf. Fig. 13), and it may even be reasonable to use immature females for some studies. Although young females do not normally express oviposition behaviour, and cutting their nerve cord does not elicit movements of the valves, high-gain electromyographic recordings of developing ovipositor muscles, and intracellular recordings from ventral opener motoneurones in immature females have revealed 'oviposition-like' activity after nerve cord transection (Thompson, 1982; Thompson & Schabtach, 1983).

The rhythmical activity produced by the terminal abdominal ganglion after isolation from more rostral neural centres is characterized by activation of ovipositor muscles in an orderly sequence. This study has confirmed and extended the previous conclusion by Vincent (1975*a*) that the severed abdomen produces *digging* activity. Examination of the pattern of activity in some detail was necessary to rule out involvement in other behaviour. For example, although the ovipositor appendages are not involved in any aspect of copulation or ventilation in these animals, they do participate in motor programmes besides oviposition digging. The ovipositor valves are instrumental in deposition of the eggs which occurs after the hole has been dug. The eggs appear one by one during the maintenance of an extreme open posture of the valves. When the abdomen withdraws and caps the egg-pod, the froth for capping is formed by whipping a milky oviductal secretion into a frothy foam by rapid opening and closing movements of the valves. It is clear from direct observations of oviposition behaviour that distinctly different movements of the valves take place during egg-laying (sustained gaping), during froth formation (3 Hz opening and closing) and during tamping (no discernible movement). These movements contrast with those during digging (slow opening and closing). Furthermore, the activity produced by the deafferented ganglion corresponds closely in phase relationships, burst durations and cycle frequency to the same parameters measured when the ganglion is *in situ*, suggesting that the behaviour, the movements of the severed abdomen, and the activity of the isolated nerve cord have a common neural basis. The major differences found in the reduced preparations were absence of tamping and slower cycle frequencies. Greater variability was found in the isolated nerve cord.

This study has focused primarily on the motor systems involved in grasshopper oviposition, but the importance of sensory systems cannot be minimized. The ovipositor is extremely responsive to its subterranean environment. It is able to burrow around obstacles, such as pieces of gravel, in its path and to detect the level of moisture and salinity of the soil (Woodrow, 1965). The valves also respond to position and load information. Sensory structures on the valves include many tactile sensillae, campaniform sensillae and contact chemoreceptors (Thomas, 1965; Woodrow, 1965; K. J. Thompson & E. Schabtach, unpublished observations).

Nevertheless, the reliable presence of rhythmical neural activity in many preparations of the isolated nerve cord argues against sensory feedback, either phasic or tonic, being necessary for the production of the motor programme. The CPG and ovipositor motoneurones can clearly be spontaneously active in the absence of any feedback at all.

This study has not resolved the neuronal composition of the CPG. However, the failure to alter the timing of the oviposition pattern by constant current injections into motoneurones suggests that the rhythmicity in the opener motoneurones is imposed upon them by other neurones. In other insect systems where more is known about the mechanism of rhythm generation the patterns are typically generated by interneurones. Such systems include locust flight (Burrows, 1973), cockroach walking (Pearson & Iles, 1970), ecdysis in the moth (Truman & Weeks, 1983) and ventilation in locusts (Burrows, 1975). Thus, although the question is not resolved for oviposition, it seems unnecessary to suggest that it would be profoundly different from more well studied behavioural systems in insects.

The results of this study point to additional unanswered questions with potential for future study. Among these are questions concerning the sexually dimorphic nature of the behaviour and its anatomical substrate. A comparison of the terminal ganglia and muscles of males and females has not been undertaken. Also, oviposition behaviour only occurs in sexually mature females, and the mechanism of maturation of this exclusively mature adult behaviour is unknown. How does the nervous system, and possibly the endocrine system, regulate the behavioural transitions from digging to egg emission and froth making? Are these other movements also driven by CPGs? The terminal segments of the female grasshopper should provide a useful preparation for the investigation of such issues and others which bear on the neural organization of animal behaviour. The oviposition digging preparation can be reliably activated for study, its motor programme is continuous after activation, and it has relatively simple neuromuscular anatomy. These advantages commend it for future investigations.

This paper is fondly dedicated to the memory of Professor Graham Hoyle, in whose laboratory the work was done. Dr Graham Hoyle, Dr Edmund Arbas and Eric Schabtach provided helpful comments on the manuscript. Eric Schabtach and Harrison Howard are acknowledged for the microscopy and photography included in this paper. This work was supported by NSF grant BNS 79/23786 to G. Hoyle and NIH training grant GMO 7257 to KT.

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