The Journal of Experimental Biology **198**, 507-520 (1995)
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MOTOR INNERVATION OF THE OVIDUCTS AND CENTRAL GENERATION OF THE OVIDUCTAL CONTRACTIONS IN TWO ORTHOPTERAN SPECIES (*CALLIPTAMUS* SP. AND *DECTICUS ALBIFRONS*)

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Accepted 7 October 1994

Summary

The oviducts of the female *Decticus albifrons* (Orthoptera: Tettigonidae) are innervated by six bilaterally paired neurones, while those of the female *Calliptamus* sp. (Orthoptera: Catantopidae) are innervated by three bilaterally paired neurones, located in the seventh abdominal ganglion. Using intracellular recording and staining, five of the six oviductal neurones of *D. albifrons* and the three oviductal neurones of *Calliptamus* sp. were physiologically and morphologically identified. All three oviductal neurones of *Calliptamus* sp. have a motor function. In *D. albifrons*, however, there is evidence for motor function in only three of the five identified oviductal neurones that appear to participate in the generation of the oviductal contractions. The remaining two identified neurones of *D. albifrons* have a branching pattern similar to that of motor neurones, but their physiological characteristics, large overshooting soma action potentials (30–40 mV) with a long afterhyperpolarising phase, are similar to those of the oviductal unpaired median neurones, which are known to modulate the oviductal contractions. The oviductal muscle exhibits two different modes of contractions: (a) fast and slow myogenic contractions, the fast contractions being produced by spontaneous potentials (30–40 mV) generated by some oviductal muscle fibres; and (b) neurogenic contractions caused by the rhythmic spiking of the oviductal motor neurones. This motor pattern is produced by the oviductal central pattern generator, a neural network residing in the last two abdominal ganglia (seventh and terminal abdominal ganglia) of the species examined here. When isolated both anteriorly and posteriorly, the seventh abdominal ganglion generates rhythmic oviductal contractions of lower frequency and amplitude than those recorded when the connectives between the genital ganglia are intact. The oviductal pattern generator is activated through release from descending inhibition, which originates, in *Calliptamus* sp., from the compound metathoracic ganglion (fused metathoracic and first three abdominal neuromeres) and in, *D. albifrons*, from the first free abdominal ganglion (fused second and third abdominal neuromeres).

Key words: insect oviduct, motor neurones, oviductal contractions, *Calliptamus* sp., *Decticus albifrons*.

Introduction

Several studies have examined the anatomy and motor innervation of the oviductal system in insects (Lange and Orchard, 1984*a*; Stoya *et al.* 1989; Thorn and Truman, 1989; Kalogianni and Pflüger, 1992) and the pharmacology of the oviductal muscle (Lange and Orchard, 1984*b*; Orchard and Lange, 1986; Cook *et al.* 1984). However, with the exception of *Locusta migratoria* (Lange *et al.*1984*b*; Orchard and Lange, 1986), no information is available on the innervation of the oviductal muscle, the firing pattern of the oviductal motor neurones, the origin of the rhythmic drive to the oviductal neurones or its interaction with other neuronal centres.

Many rhythmic motor patterns in both deafferented and intact animals are generated by central neural networks (central pattern generators, CPGs, Bässler, 1986; Delcomyn, 1980). In crickets and locusts, the neural network that is responsible for the generation of the oviposition digging rhythm (a motor pattern elicited at the first stage of egg laying) is distributed in the last two abdominal ganglia (genital ganglia) and is activated by release from descending inhibition, originating from the thoracic and cerebral ganglia (Carrow *et al.* 1982; Thompson, 1986). During oviposition digging in the locust, rhythmic contractions of the oviducts are elicited that prevent the passage of the eggs to the ovipositor (Lange *et al.* 1984*a*,*b*). These contractions are generated by three identified bilaterally paired neurones and are modulated by two groups of dorsal unpaired median (DUM) neurones (Kalogianni and Pflüger,

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1992). In *D. albifrons* and *Calliptamus* sp., the oviductal DUM neurones are also rhythmically activated (in time with the oviductal motor neurones) when the genital ganglia are isolated from the rest of the nerve cord (Kalogianni and Theophilidis, 1993). It thus appears that, in these species, the network producing the oviductal motor pattern is very similar to that generating the oviposition digging rhythm in the locust. Recently, Facciponte and Lange (1993) suggested that the seventh abdominal ganglion (the site of the oviductal motor neurones) is the main site of generation of the oviductal rhythm in locusts.

The aim of the present study is to present an account of the anatomy and physiology of the oviductal neuromuscular system in two orthopteran species. In *Calliptamus* sp., the system is similar to that of the locust, but *D. albifrons* presents some differences, which are outlined here. In addition, selective lesions of the nerve cord were made while monitoring the oviductal rhythmic contractions, in order to locate precisely the oviductal CPG, to examine its relationship to the other parts of the central nervous system (CNS) and to define the site of origin of the descending inhibition that is exercised on it in quiescent preparations. The morphology of the nerve cord in *D. albifrons* has enabled us to locate more precisely the higher inhibitory centres in this species.

Materials and methods

Mature female grasshoppers *Calliptamus* sp. (Catantopidae) and bush crickets *Decticus albifrons* (Tettigonidae), collected from the vicinity of Thessaloniki, Greece, were used in this study.

Physiology

An animal was mounted ventral side uppermost and an incision was made along the ventral midline to expose the oviducts and the seventh abdominal ganglion. Intracellular recordings were obtained from the oviductal muscle fibres with flexibly mounted glass microelectrodes (Woodbury and Brady, 1956), filled with a $2\,mol\,l^{-1}$ potassium acetate solution (resistance 20–50 MΩ) while the preparation was constantly perfused with saline (NaCl, $140\,mmol\,l^{-1}$; KCl, $5\,mmol\,l^{-1}$; $CaCl_2$, $4\,mmol\,l^{-1}$; Hepes, $5\,mmol\,l^{-1}$, pH 6.8). Extracellular recordings were obtained *en passant* from the oviductal nerve using silver hook electrodes. To monitor the neurogenic oviductal contractions, the anterior end of the lateral oviduct was attached by a thread to a Grass isometric force transducer; pins were positioned at the junctional area of the oviduct to prevent detection of the contractions of the other side of the oviducts. To measure the myogenic contractions, the oviducts were removed from the animal, placed in a Petri dish and constantly perfused with saline. The thread of a force transducer was attached to the posterior end of the common oviduct and the lateral oviducts were immobilized with fine pins.

To record intracellularly from the oviductal motor neurones, the seventh abdominal ganglion was stabilized, ventral side up, on a wax platform with minuten pins. The ganglionic sheath was treated with a 1 % solution of Protease (Sigma type XIV) for 30 s to facilitate microelectrode penetration. Intracellular recordings were obtained from the cell bodies of the motor neurones with glass microelectrodes filled with 6 % cobalt hexammine (resistance 70–100 MΩ). All recordings were displayed on a digital oscilloscope and the data were stored on a computer for subsequent analysis and printing. The motor neurones were stained by passing 500 ms depolarising pulses of 10 nA, at 1 Hz, for 20 min through the microelectrode. The dye was left to diffuse for 1 h and the cobalt was precipitated with ammonium sulphide. The ganglia were then silver intensified (Bacon and Altman, 1977), dehydrated and cleared in methyl salicylate, and selected preparations were drawn using a *camera lucida* attached to a microscope.

Anatomy

Backfills of the oviductal motor neurones were obtained by immersing a part of the oviductal muscle, with its innervation intact, in a Vaseline well filled with 6 % cobalt hexammine. This method was used because it was difficult to isolate the numerous fine branches that supply the oviducts in the Vaseline well. Furthermore, it ensured that only neurones supplying the oviducts would be stained. The preparation was left for 12–24 h at 4 °C and the cobalt was precipitated with ammonium sulphide and intensified as outlined above. At least 20 backfills were made in each of the two species.

To reveal the fine structure of the oviductal muscle, it was embedded in Spurr's resin, prior to cutting transverse semi-thick sections (5–10 μm) from the lateral oviducts of both species, using standard light microscopy techniques. The muscle profiles were then drawn using a *camera lucida*.

Results

Anatomy of the oviductal muscle

In both *Calliptamus* sp. and *D. albifrons*, a pair of ovaries is located in the anterior part of the abdominal cavity. They open posteriorly into a pair of muscular lateral oviducts (LO, Fig. 1A,C) which, at their posterior end, join to form the common oviduct (CO). Transverse sections of the lateral oviduct in both species reveal a network of muscle fibres surrounding an inner layer of epithelial cells (EL, Fig. 1B,D, see also insets). In *D. albifrons*, there are two layers of muscle fibres: an inner discontinuous layer of longitudinal fibres (LL, Fig. 1B) and an outer layer of circular fibres (CL). The longitudinal layer consists of muscle bundles located at the folds of the epithelial layer, whereas the circular layer is superficial (Fig. 1B). Some longitudinal fibres are scattered at the surface of the oviduct (LF, Fig. 1B). In *Calliptamus* sp., the circular layer (Fig. 1D) is directly adjacent to the epithelium, whereas the longitudinal fibres are superficial and form a continuous layer.

In both species, the oviduct is innervated by a nerve branch (oviductal nerve) originating from the seventh abdominal

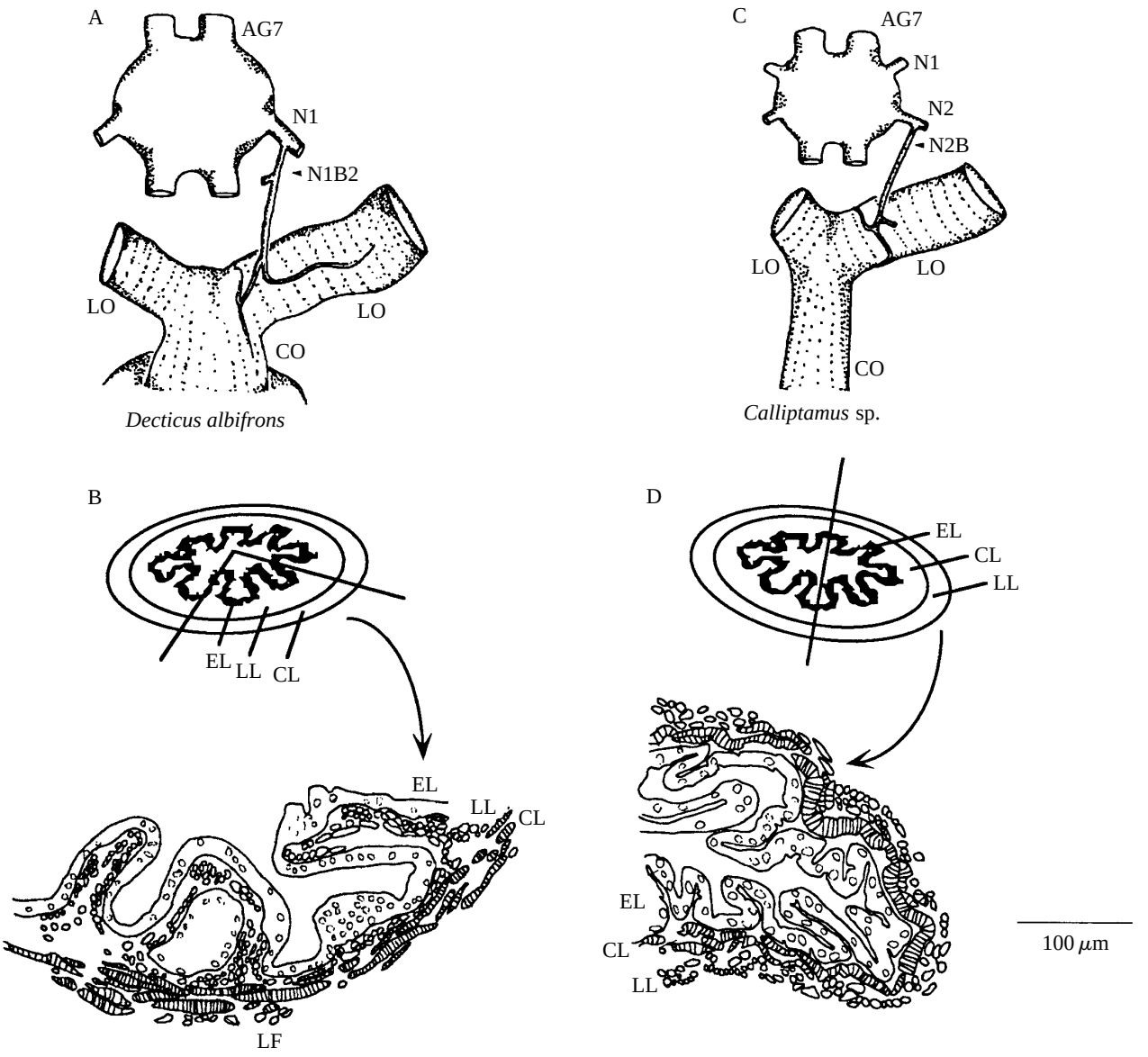

Fig. 1. Semi-diagramatic drawings of the oviductal neuromuscular system (A,C) and fine structure of the oviduct (B,D) of the bush cricket *Decticus albifrons* (A,B) and the grasshopper *Calliptamus* sp. (C,D). (A) In *Decticus albifrons*, nerve branch N1B2 originating from the main nerve N1 of the seventh abdominal ganglion (AG7) supplies the lateral oviduct (LO) and the common oviduct (CO). (B) Part of a transverse section of the lateral oviduct of *Decticus albifrons* showing the epithelial layer (EL), the inner longitudinal muscle layer (LL), the outer circular muscle layer (CL) and the superficial longitudinal muscle fibres (LF). (C) In *Calliptamus* sp., nerve branch N2B originating from nerve 2 (N2) of the seventh abdominal ganglion innervates the lateral oviduct and the common oviduct. (D) A hemisection of the lateral oviduct of *Calliptamus* sp. showing the epithelial layer, the inner circular layer and the outer longitudinal layer.

ganglion (AG7). In *D. albifrons*, the seventh abdominal ganglion has one pair of segmental nerves, termed nerve N1 (see Fig. 1A). In this species, the oviduct is innervated by branch N1B2 of nerve N1 of AG7 (Fig. 1A), whereas in *Calliptamus* sp. it is innervated by branch N2B of the sternal nerve N2 of AG7 (Fig. 1C). In both species, the oviductal nerve gives rise to two secondary branches innervating the posterior part of the lateral oviduct, the anterior part of the common oviduct and their junctional area (Fig. 1A,C). A more detailed account of the peripheral innervation of the oviducts is given in Kalogianni and Theophilidis (1993). The nomenclature used in this study was based on that used for the locust by Lange and Orchard (1984*a*) and Kalogianni and Pflüger (1992).

Motor innervation of the oviductal muscle

Cobalt backfills of the terminals on the oviductal muscle of the efferent oviductal neurones revealed their cell bodies and central projections. In both species, three clusters of cell bodies were stained in the seventh abdominal ganglion: a lateral cluster of bilaterally paired neurones, located at the ventrolateral region of the ganglion (AG7 in Fig. 2A,B) and

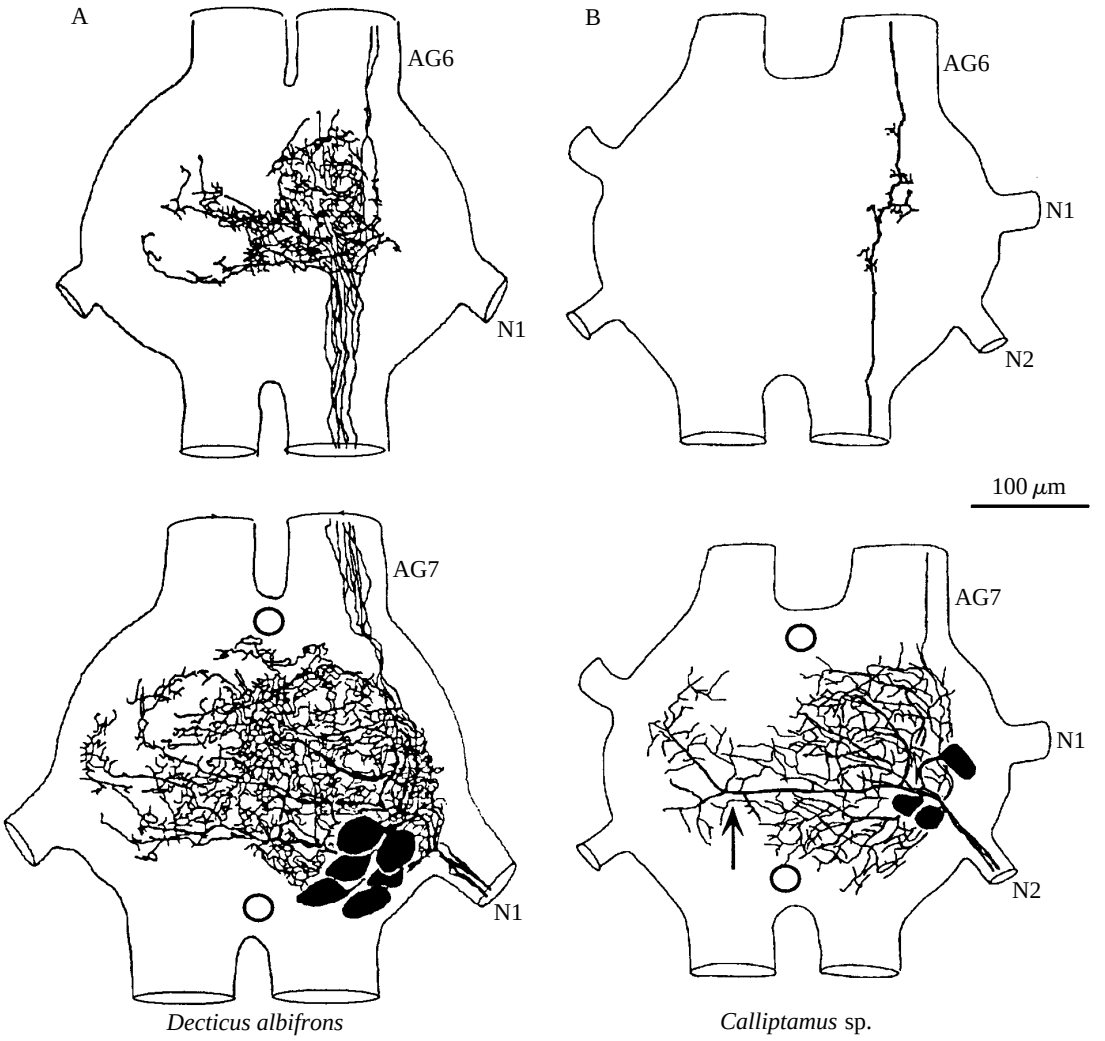

Fig. 2. Backfills of the oviductal nerve (A) in *Decticus albifrons* and (B) in *Calliptamus* sp. revealed the cell bodies and the central projections of the oviductal bilaterally paired neurones in the seventh abdominal ganglion (AG7). Six bilaterally paired neurones (oviductal neurones 1–6, OVN1–OVN6) innervated the oviducts of *Decticus albifrons* (A), whereas three bilaterally paired neurones (oviductal neurones 1–3, OVN1–OVN3) innervated the oviducts of *Calliptamus* sp. (B). Open circles in AG7 indicate the cell bodies of the bilaterally projecting oviductal neurones. Note the large sensory arborizations in the sixth abdominal ganglion (AG6) of *Decticus albifrons* (A). Arrow in B indicates the large neurite invading the contralateral neuropile of AG7 in *Calliptamus* sp.

two clusters of bilaterally projecting neurones (open circles in Fig. 2), located at a median position in AG7 (the latter were described by Kalogianni and Theophilidis, 1993). The number and the morphology of the oviductal bilaterally paired neurones differed, however, in the two species. In *D. albifrons*, six large cell bodies (diameter 30–35 μm) formed the cluster of oviductal bilaterally paired neurones. They possessed dense arborizations that extended dorsally in the ipsilateral neuropile, but also had a number of branches in the contralateral neuropile (Fig. 2A, AG7). In contrast, in *Calliptamus* sp., the cluster of the oviductal bilaterally paired neurones consisted of only three cell bodies (diameter 25 μm) (Fig. 2B, AG7). The overall branching pattern of these cells in the seventh abdominal ganglion differed from that of the homologous neurones of *D. albifrons* mainly in the presence of a thick (approximately 10 μm) contralateral neurite (arrow in Fig. 2B) which sent its arborizations to the contralateral neuropile (see also Fig. 5C).

Backfills of the oviductal nerve also revealed a dense dendritic field in the sixth abdominal ganglion of *D. albifrons* (AG6, Fig. 2A). This is probably of sensory origin and is formed by axons that, without entering the neuropile of AG7, ascended to the next anterior ganglion (AG6) and ramified extensively in its medioventral neuropile. Some arborizations crossed the ganglionic midline. In *Calliptamus* sp., only a single axon ascending through the ipsilateral connective to the AG6 could be detected (AG6, Fig. 2B).

Identification of the oviductal motor neurones

D. albifrons

Four large action potentials with different amplitudes

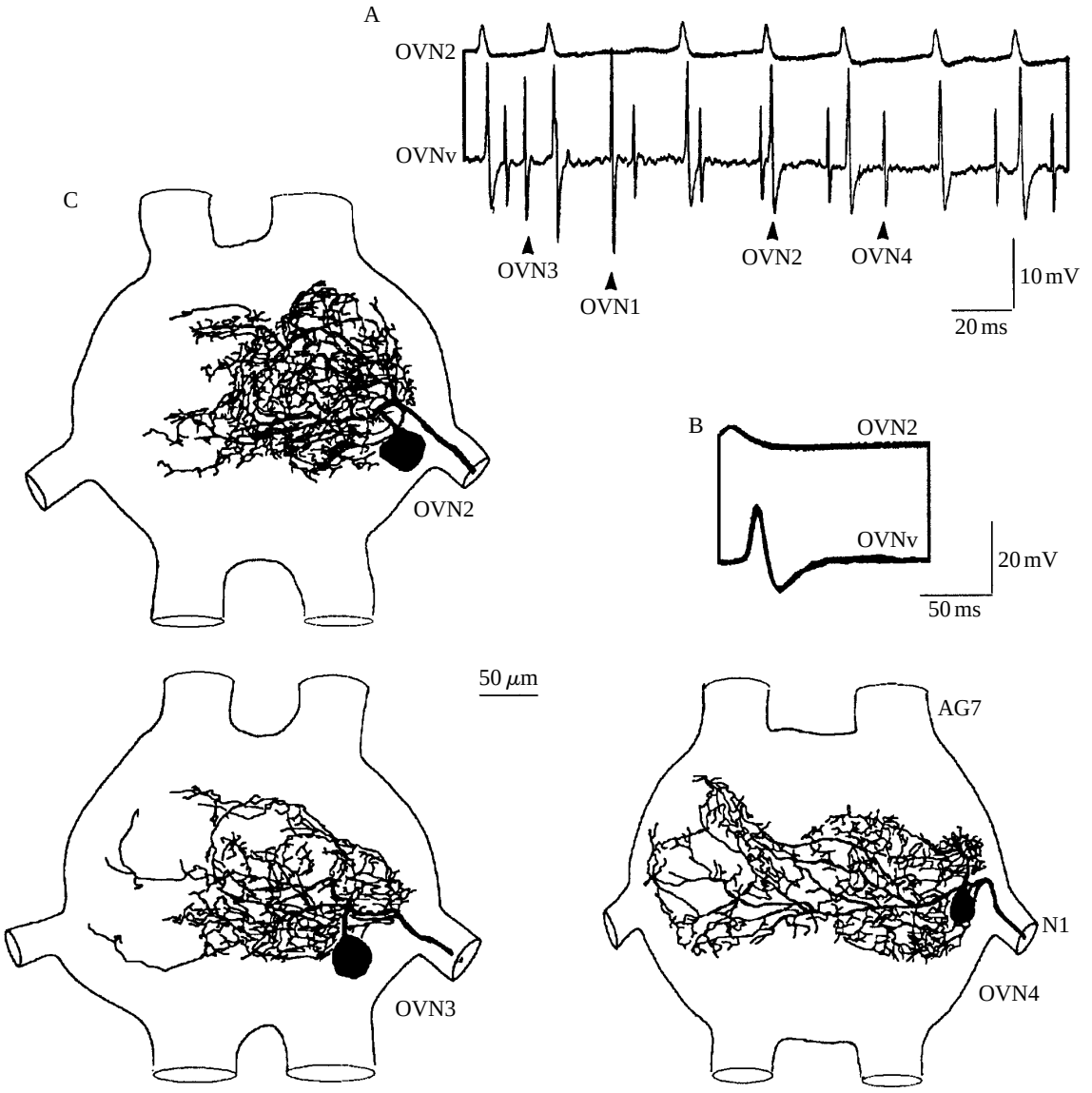

Fig. 3. Physiological and morphological identification of oviductal neurone 2 (OVN2) of *Decticus albifrons*. (A) In the extracellular recordings from the oviductal nerve (OVNv, lower trace), four large potentials of different amplitude could be distinguished (OVN1, OVN2, OVN3, OVN4). Paired intracellular recordings from the cell body of OVN2 and extracellular recordings from the oviductal nerve (OVNv) show that OVN2 produces the second largest action potential. (B) Multiple sweeps are superimposed to show that each soma potential of OVN2 was followed by an action potential in the oviductal nerve. (C) The intraganglionic morphology of OVN2, OVN3 and OVN4 as revealed by intracellular cobalt injection, following physiological characterisation of the motor neurones. Whereas OVN2 and OVN3 have arborizations mainly in the ipsilateral half of the ganglion, OVN4 projected to both the ipsilateral and contralateral neuropile.

(oviductal neurones 1–4, OVN1–OVN4, Fig. 3A, second trace) could be distinguished in extracellular recordings monitoring spontaneous activity in the oviductal nerve (OVNv). Simultaneous extracellular recordings from the oviductal nerve and intracellular recordings from the cell bodies of the oviductal bilaterally paired neurones enabled the physiological and morphological identification of OVN2, OVN3 and OVN4. The neurone producing the largest action potential, designated as OVN1, was active in a few preparations and only at the start of the experiments, so it could not be reliably characterised. The soma spike of OVN2 was 5–7 mV in amplitude and was followed at a short and constant latency (Fig. 3B) by the second largest extracellular action potential in the oviductal nerve (Fig. 3A). After physiological identification, the neurone was stained intracellularly to reveal its morphology. Its cell body is approximately 30 μm in diameter and it has dense arborizations in the ipsilateral dorsal neuropile (OVN2, Fig. 3C). It also sends some projections into the contralateral neuropile. The neurones producing the third and the fourth largest action potentials (OVN3 and OVN4, Fig. 3A) were also physiologically and morphologically identified. OVN3, which had the third largest action potential, arborizes mainly

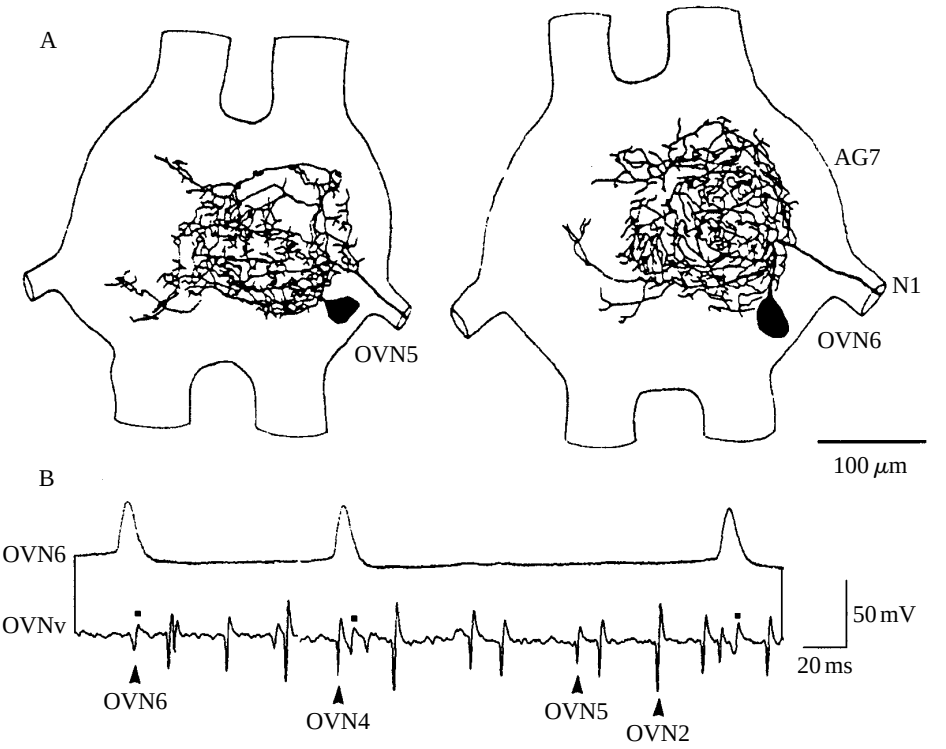

Fig. 4. The physiological characteristics of oviductal neurones 5 and 6 (OVN5 and OVN6) of *Decticus albifrons* are quite different from those of OVN2, OVN3 and OVN4. The former possessed large soma potentials (30–40 mV) recorded intracellularly from their cell bodies (OVN6, upper trace in B) and very small axon potentials, recorded extracellularly from the oviductal nerve (OVNv, marked in lower trace of B). Their morphology, however, as revealed by intracellular staining (A), was very similar to that of OVN2 and OVN3, shown in Fig. 3.

ipsilaterally, but it also has a few contralateral branches (OVN3, Fig. 3C). OVN4, which generated the smallest action potential, has arborizations in both the ipsilateral and contralateral neuropile (OVN4, Fig. 3C). In contrast to the small soma spike of OVN2, OVN3 and OVN4, the remaining two bilaterally paired oviductal neurones, designated as OVN5 and OVN6, had much larger soma spikes (30–40 mV) with a long afterhyperpolarising phase (see OVN6 in Figs 4B, 6D). They were identified with paired intracellular soma recordings and extracellular nerve recordings (Fig. 4B) and their morphology, as revealed by intracellular cobalt injection (Fig. 4A), was very similar to that of OVN2 and OVN3. The arborizations of all five oviductal neurones, which were intracellularly stained, were confined to the seventh abdominal ganglion. Each neurone was fully stained in at least two and up to four preparations.

Calliptamus *sp.*

Three action potentials of different amplitude can be distinguished in the extracellular recordings from the oviductal nerve, which correlate to the three bilaterally paired oviductal neurones revealed by backfills. Simultaneous intracellular recordings from the cell body of one of the three oviductal neurones of *Calliptamus* sp. (OVN2, Fig. 5A) and extracellular recordings from the oviductal nerve (OVNv) showed that each intracellularly recorded spike was followed by an extracellular action potential (Fig. 5A). Intracellular cobalt staining of OVN2 showed that it projects both ipsilaterally and contralaterally into the seventh abdominal ganglion (Fig. 5B), a pattern very similar to that of OVN4 of *D. albifrons*. In contrast to OVN2, OVN3 (which produced the largest extracellular action potential) has a thick secondary neurite (see arrow in Fig. 2B), ramifying exclusively in the contralateral half of the ganglion (OVN3, Fig. 5C). OVN1, which generates the smallest extracellular potential, possesses mainly ipsilateral central projections in AG7 (OVN1, Fig. 5D).

Rhythmic motor activity and neurogenic oviductal contractions

In *D. albifrons*, intracellular recordings from an oviductal muscle fibre reveal rhythmic bursts of excitatory junction potentials (EJPs, Fig. 6A). When these recordings were combined with extracellular records from the oviductal nerve, it was evident that they were produced by the phasic excitation of OVN2 (arrowheads in Fig. 6B). In contrast, intracellular recordings from the cell body of OVN4 (Fig. 6C) showed largely tonic activity, with an increase in its firing frequency during the bursts of OVN2 activity (Fig. 6C). OVN3 fired at a low frequency during the bursts of OVN2 and OVN4 (not shown). In contrast, the firing frequency of the remaining two oviductal neurones (OVN5 and OVN6) was reduced during the bursting phase of OVN2 and OVN4 (for OVN6, see Fig. 6D).

In *Calliptamus* sp., intracellular recordings from a muscle fibre of the common oviduct (OVM, Fig. 7A) also revealed bursts of EJPs, in which two different amplitudes could be distinguished. These were generated by OVN1 and OVN2, as revealed by paired intracellular recordings from their cell bodies and from an oviductal muscle fibre (for OVN1, Fig. 7A). The bursts of EJPs occurred synchronously on the left and right sides of the oviductal muscle, as revealed by simultaneous intracellular recordings from left and right muscle fibres (Fig. 7B). These bursts of EJPs generated

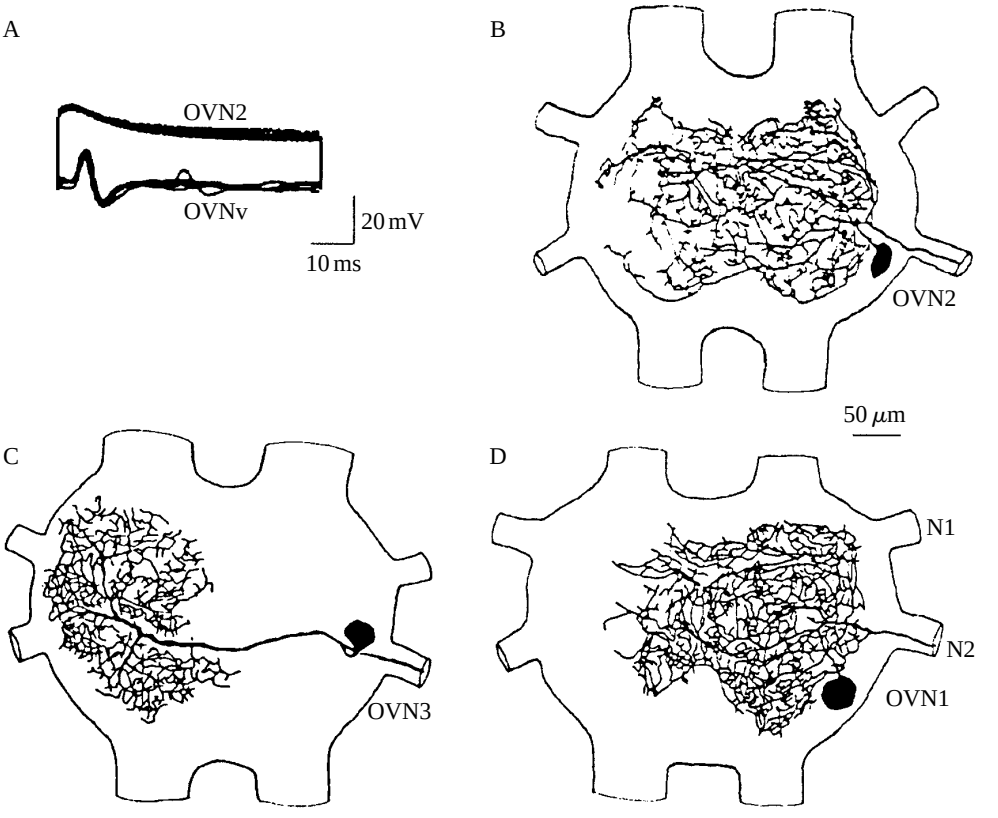

Fig. 5. The oviductal neurones of *Calliptamus* sp. were identified with intracellular recordings from their cell bodies and extracellular recordings from the oviductal nerve (OVNv). Oviductal neurone 2 (OVN2) of *Calliptamus* sp. produced the second largest action potential (A). OVN2 possesses both ipsilateral and contralateral arborizations (B) and OVN1 possesses mainly ipsilateral projections (D). OVN3, however, has a unique morphology, as it projects exclusively to the contralateral half of the ganglion (C).

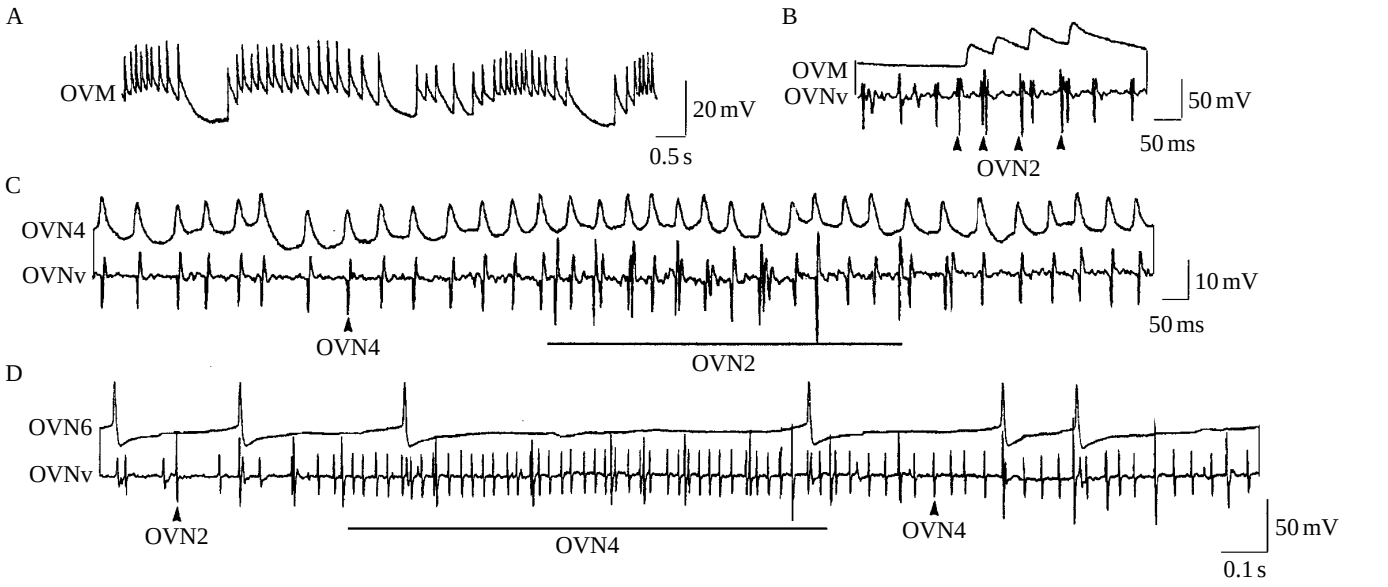

Fig. 6. The firing pattern of the oviductal neurones (OVN2, OVN4, OVN6) of *Decticus albifrons*. Bursts of EJPs recorded intracellularly from an oviductal muscle fibre (OVM, A) were produced by OVN2, which had a phasic firing pattern (B). OVN4, in contrast, fired tonically (C). OVN6 fired during the interbursts of OVN2 (D). Note the large amplitude and the prolonged undershoot of the soma potentials of OVN6 (D).

rhythmic synchronous contractions of the left and right halves of the oviductal muscle (OVC, lower trace in Fig. 7C), which ceased after the isolation of the oviduct from the seventh abdominal ganglion (not shown). A similar synchronous contraction of the left and right sides of the oviductal muscle and the neurogenic origin of the oviductal contractions were also established in *D. albifrons* (see Fig. 10). Other aspects of the oviductal rhythm are described in Kalogianni and Theophilidis (1993).

Stimulation of the oviductal nerve in *Calliptamus* sp. with

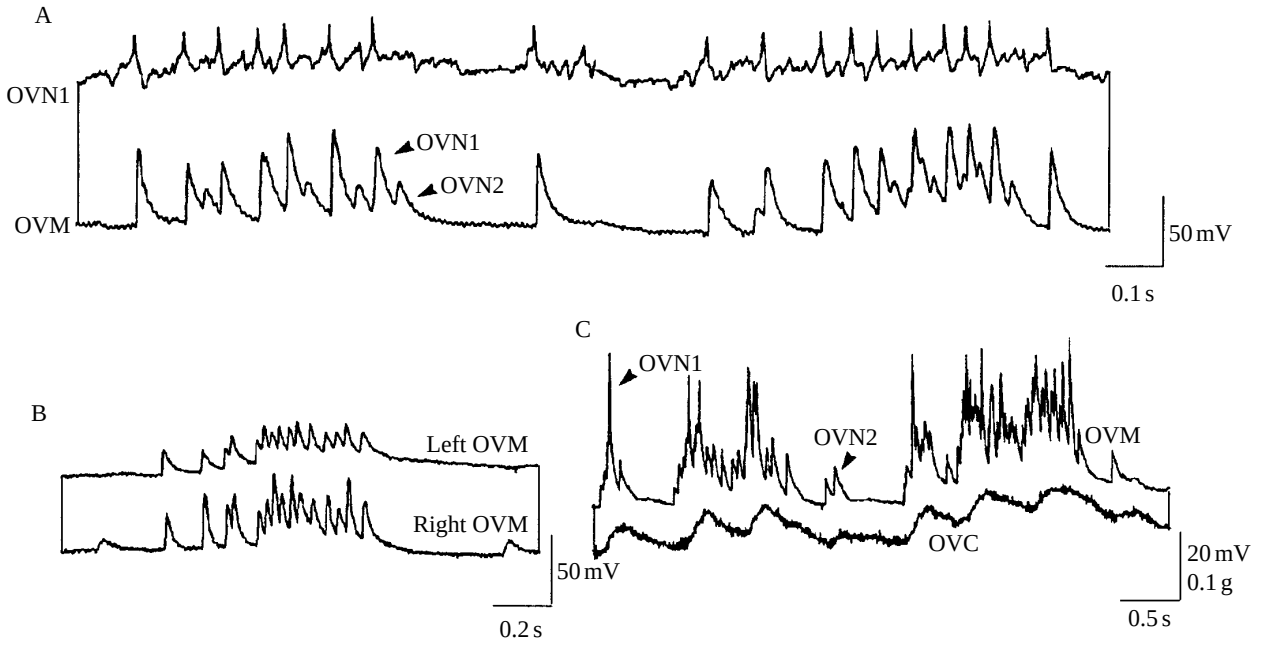

Fig. 7. (A) OVN1 in *Calliptamus* sp. produced the large phasic EJPs recorded from the oviductal muscle fibres and OVN2 produced the smaller EJPs. (B) Recordings from muscle fibres from the left and right sides of the common oviduct (OVM) reveal time-locked bursts of EJPs. (C) These bursts generate contractions of the oviducts (OVC), which are produced by the summation of the EJPs generated by OVN1 and OVN2.

1 ms pulses evoked EJPs in an oviductal muscle fibre which had rise times of 10–12 ms and decay times of 45–50 ms (Fig. 8A). During repetitive stimulation, the EJPs summated (Fig. 8B). Electrical stimulation of the oviductal nerve with single pulses of increasing strength evoked, in both species, EJPs of three different amplitudes (for *D. albifrons*, see Fig. 8C).

Electrical nerve stimulation with bursts of pulses at a range of frequencies (1–40 Hz) resulted, in both species, in oviductal contractions of increasing amplitude (Fig. 8D,E).

In *Calliptamus* sp., nerve stimulation with pulses at 1 Hz resulted in individual contractions of the oviduct (Fig. 8D, 1 Hz). Stimulation with higher frequencies caused a graded contraction of the oviduct (Fig. 8D, 10–40 Hz), which reached its maximal value at 40 Hz with a considerable delay (approximately 1 s after the onset of stimulation) and maintained this value until the end of stimulation (Fig. 8D).

In *D. albifrons*, repetitive stimulation at 1 Hz also evoked individual contractions, but of decreasing amplitude (Fig. 8E, 1 Hz). At higher frequencies (Fig. 8E), the contraction reached its maximal value rapidly after the onset of stimulation but this was followed by a decrease in the force generated by the oviductal muscle. For example, at 30 Hz stimulation, the contraction amplitude declined to 50 % of its maximal value 2 s after the onset of stimulation. The contraction maintained this value until the end of stimulation. Maximal responses of the oviductal muscle, i.e. maximal peak contraction strength, occurred at 30 Hz stimulation (Fig. 8E).

Oviductal motor programme and its central generation

In quiescent preparations, isolation of the seventh and terminal abdominal ganglia (genital ganglia) from the anterior parts of the nerve cord evoked a rhythmic activation of the oviductal motor neurones. This activity was also present after depriving the CNS of afferent information, thus establishing the presence of an oviductal central pattern generator (oviductal CPG, see Kalogianni and Theophilidis, 1993). In the present study, an effort was made to locate the oviductal CPG precisely and to examine its interaction with other parts of the nervous system. For this purpose, the oviductal contractions were measured with a force transducer during stepwise isolation of the seventh abdominal ganglion. All experiments were carried out on minimally dissected animals (i.e. a midventral incision was made to expose the oviducts and the abdominal cavity was constantly perfused with saline). All records were obtained 1 h after dissection and at 10 min intervals after each connective lesion. Each series of lesions shown in Figs 9 and 10 is derived from one individual, and is regarded as representative of the total of 10 successful experiments performed for each species.

Calliptamus *sp.*

When the nerve cord of *Calliptamus* sp. was intact, the oviduct exhibited contractions that had no rhythmicity (Fig. 9A). In a few individuals, however (one in five animals), the oviducts showed large rhythmic contractions (Fig. 9B). In some quiscent preparations, decapitation or transection of the connectives between the mesothoracic and metathoracic ganglia evoked a rhythmic activation of the oviducts. However, lesion of the connectives between the metathoracic (MTG) and the first free abdominal ganglion (fourth abdominal ganglion, AG4, see Fig. 9 inset) consistently induced rhythmic oviductal

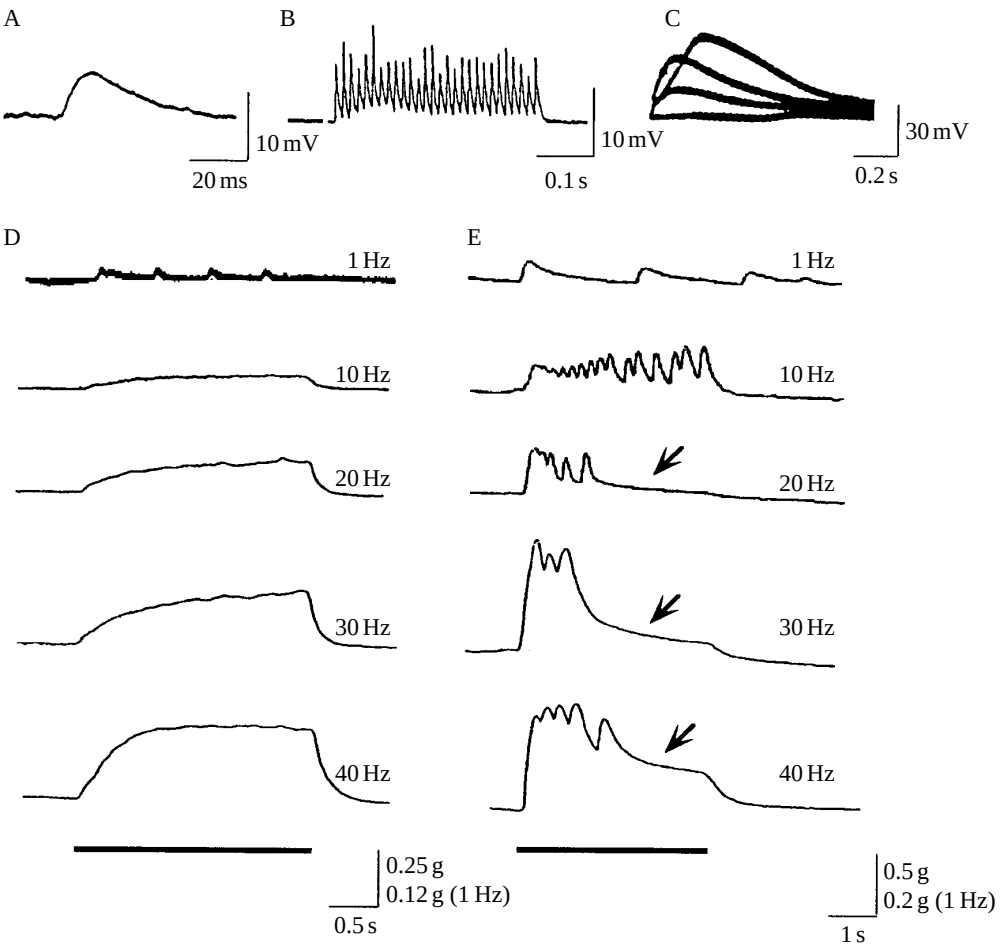

Fig. 8. (A,B) Intracellular recordings from an oviductal muscle fibre in *Calliptamus* sp. during electrical stimulation of the oviductal nerve at 1 Hz (A) and 10 Hz (summation of the EJPs in B). (C) When the oviductal nerve was stimulated with 1 Hz pulses of increasing strength, EJPs with three different amplitudes were recorded from the oviductal muscle of both species. (D,E) Oviductal contractions recorded during repetitive electrical stimulation (bar) of the oviductal nerve, with pulse trains of increasing frequency, in *Calliptamus* sp. (D) and *Decticus albifrons* (E). Whereas the response of the oviduct of *Calliptamus* sp. was a contraction of increasing amplitude, the response of the oviduct of *Decticus albifrons* was a contraction which, after reaching its peak, decreased and then maintained this lower value (marked with arrows in E). Note the individual contractions of decreasing amplitude, at 1 Hz stimulation of the oviductal nerve in *D. albifrons* (E).

contractions, of neurogenic origin, in previously quiescent oviducts (Fig. 9C). In preparations already exhibiting rhythmic oviductal contractions (as in Fig. 9B), isolation of the abdominal from the thoracic ganglia had no detectable effect. Total isolation of AG7 from the anterior nerve cord, by transection of the connectives between AG4–AG5, AG5–AG6 or AG6–AG7, had no apparent effect on the induced contractions (Fig. 9D). Under these conditions, isolation of the seventh abdominal ganglion from the terminal abdominal ganglion (TAG) caused a reduction in both the amplitude and frequency of the oviductal contractions (to approximately 25 % of the original amplitude and 75 % of the original frequency, Fig. 9E). These contractions were of neurogenic origin, because they ceased when the oviducts were isolated from the CNS by severing the oviductal nerve. The above data indicate (a) that, in quiescent preparations, the oviductal CPG is under descending inhibition, which derives from the compound metathoracic ganglion – the metathoracic (MTN) and the first three abdominal neuromeres (AN1–AN3) fused – and (b) that connections between the seventh and terminal abdominal ganglia are necessary for the expression of the oviductal rhythm, as elicited during oviposition behaviour.

D. albifrons

Arrhythmic contractions were recorded from the oviduct when the nerve cord was intact (Fig. 10A), but, as for the oviduct of *Calliptamus* sp., rhythmic oviductal contractions of high amplitude and frequency were evoked after transection of the connectives between the first (abdominal neuromeres 2 and 3 fused, AN2, AN3) and the second (abdominal ganglion 4, AG4) free abdominal ganglia (Fig. 10C, see inset for sites of lesion). Transection of the connectives at a more anterior position also evoked rhythmic contractions, but these were of lower amplitude and frequency (Fig. 10B). As in *Calliptamus* sp., total anterior isolation of the seventh abdominal ganglion had no detectable effect on the oviductal contractions (for lesion, see unmarked arrow in inset). Similarly, isolation of the seventh abdominal ganglion from the terminal abdominal ganglion led to a decrease in the frequency and amplitude of oviductal contractions (to 30 % of the original amplitude and 60 % of the original frequency, Fig. 10D), which ceased after transection of the oviductal nerve (Fig. 10E). It is thus evident that the oviductal CPG in *D. albifrons* is also under descending inhibition. The different structure of its nerve cord enables us to locate its site of origin more precisely; i.e. in the first free abdominal ganglion (second and third abdominal neuromeres fused). As in *Calliptamus* sp., elements of the oviductal CPG seem to be distributed in both genital ganglia.

Myogenic oviductal activity

A common feature of visceral muscles is that, when isolated from the CNS, they undergo spontaneous myogenic

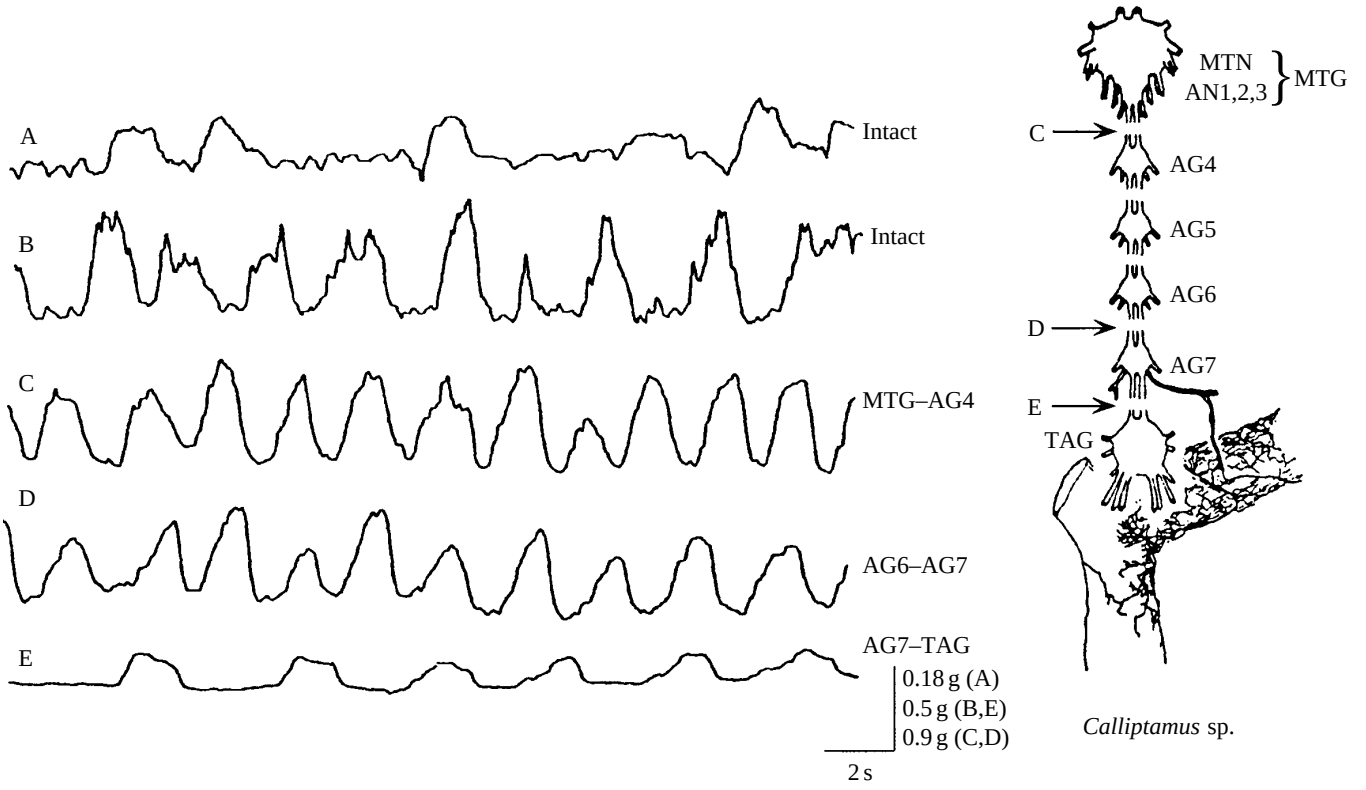

Fig. 9. Recordings of the oviductal contractions in *Calliptamus* sp. during stepwise isolation of the seventh abdominal ganglion from the rest of the nerve cord (see inset). (A,B) Two different degrees of activation of the oviduct in insects with an intact nerve cord. (C) Contractions induced after isolation of the abdominal ganglia (AG) from the thoracic ganglia (oviductal rhythm). (D) Complete isolation of the seventh abdominal ganglion from the anterior nervous system had no effect on the induced contractions. (E) Isolation of the seventh abdominal ganglion from the terminal abdominal ganglion (TAG) resulted in a decrease in both the amplitude and frequency of the oviductal contractions. For sites of connective transections, see inset. AN, abdominal neuromere; MTN, metanthoracic neuromere; MTG, metathoracic ganglion.

contractions (Fig. 11A), which do not occur when the oviductal motor rhythm is elicited. The oviducts of both the species studied here exhibited two different modes of myogenic contractions: (a) slow peristaltic contractions of very low amplitude occurring mainly in the lateral oviducts (SC, Fig. 11A) and (b) strong fast contractions occurring mainly in the anterior part of the common oviduct (FC, Fig. 11A,B). Intracellular recordings from the muscle fibres of the lateral oviducts did not reveal any action potentials. Recordings from muscle fibres in the anterior part of the common oviduct, however, did show large potentials (30–40 mV) with a prolonged depolarising phase (upper trace, Fig. 11B), which were found to be temporally correlated with fast myogenic contractions (Fig. 11B). These potentials appeared to be responsible for the generation of the phasic contractions, as can be seen from the paired intracellular recordings from an oviductal muscle fibre and tension measurements from the common oviduct (Fig. 11B). They were present in only a limited number of muscle fibres located on each side of the anterior part of the common oviduct. As the intracellular electrode was moved to a more central position on the common oviduct, the potentials decreased in amplitude, and they could not be recorded from muscle fibres located near the midline of the common oviduct.

Discussion

Oviductal structure and myogenicity

The muscle fibre arrangement of the oviduct of *Calliptamus* sp., as revealed by histological sections, follows a pattern very similar to that described in other insect species (in moths, Cook *et al.* 1984; in locusts, Lange *et al.* 1984*b*). This pattern consists of an inner layer of circular muscle fibres, directly adjacent to the epithelium, surrounded by a layer of longitudinal fibres. In *D. albifrons*, however, there exists an additional discontinuous inner longitudinal layer similar to that in the lateral oviduct of the stick insect *Carausius morosus* (Thomas, 1979) and in the junctional area of the locust oviduct (Lange *et al.* 1984*b*). The different muscle fibre layers are related to the multiple modes of contraction expressed by the insect oviduct. When isolated from the CNS, the oviductal muscle, although under direct neural control, also displays slow peristaltic and fast phasic myogenic contractions (Cook *et al.* 1984; Lange *et al.* 1984*b*). The peristaltic contractions appear to result from contractions of the circular muscle fibres, but both the neurogenic and the fast myogenic contractions could result from the excitation of longitudinal muscle fibres. The present study shows that the fast myogenic contractions are produced by spontaneous action potentials with a long

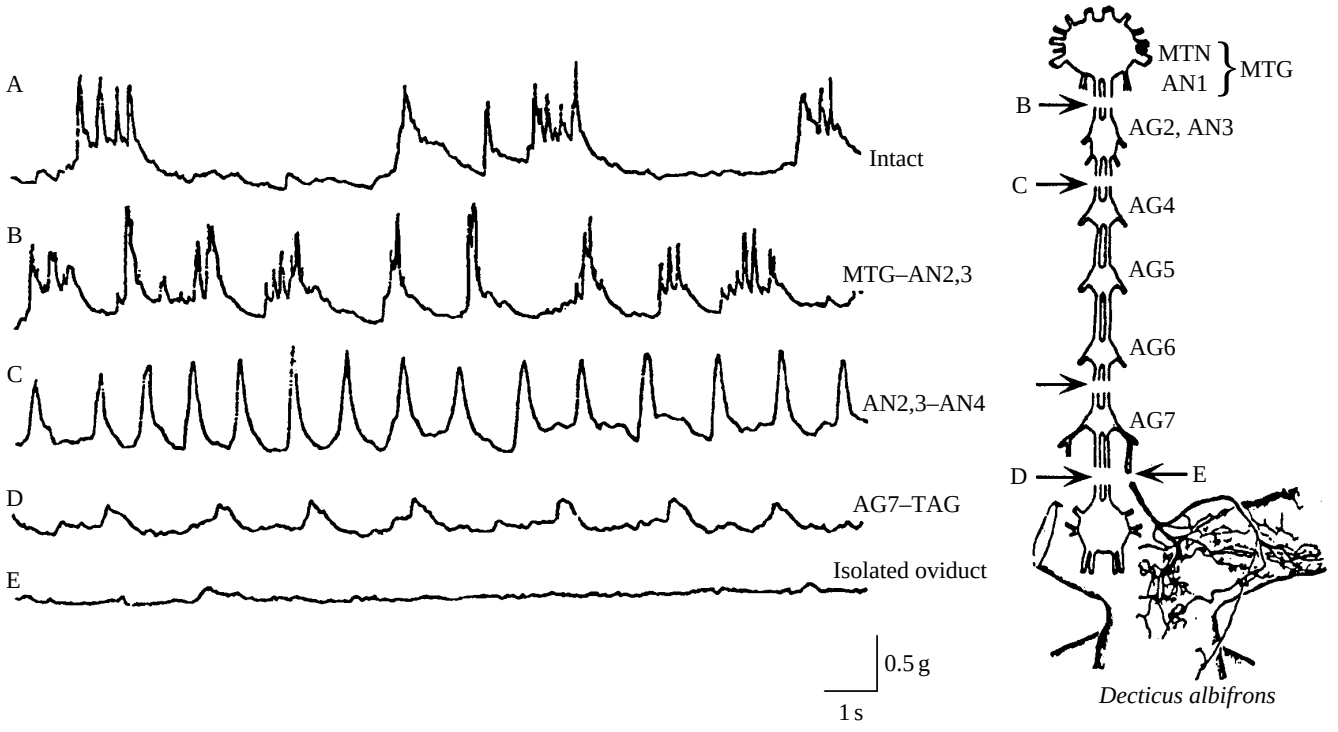

Fig. 10. Recordings of the oviductal contractions in *Decticus albifrons* during stepwise isolation of the seventh abdominal ganglion from the rest of the nerve cord (see inset). (A) Oviductal contractions in a female with an intact nerve cord. (B) Transection of the connective between the metathoracic ganglion and the first free abdominal ganglion increased the frequency, but not the amplitude, of the oviductal contractions. (C) Only when the fused first free abdominal ganglion (abdominal neuromeres 2 and 3) was removed, were rhythmic oviductal contractions of high amplitude and frequency induced (oviductal rhythm). (D) Isolation of the seventh abdominal ganglion from the terminal abdominal ganglion resulted in a decrease of the amplitude and frequency of the oviductal contractions. These contractions ceased upon cutting the oviductal nerve (E). Abbreviations are explained in Fig. 9.

depolarising phase and prolonged afterhyperpolarisation. Similar potentials have been recorded from the locust oviduct (Orchard and Lange, 1986) and beetle cardiac muscle (Ebara *et al.* 1990). In the oviduct of the species examined here, only a few longitudinal fibres, located on each side of the common oviduct, are capable of generating these spontaneous potentials. The low amplitude of these potentials, when recorded from neighbouring muscle fibres, and their absence from fibres at the midline of the common oviduct, could indicate that some fibres act as pacemaker cells and the potentials then spread electrotonically to the neighbouring fibres. Orchard and Lange (1986) have shown that locust oviductal muscle fibres are both electrically and dye coupled and suggested a similar pattern of propagation of these potentials. It is possible that the phasic myogenic contractions are induced during the second stage of oviposition, i.e. during the actual egg laying when the eggs are expelled from the oviductal system, after they have been propelled to the common oviduct by the slow peristaltic contractions of the lateral oviducts. During this phase, there is no neurogenic oviductal rhythm. The oviductal myogenic contractions, believed to be induced by neurohormonal factors (Okelo, 1971; Sugawara, 1986), can be modulated by a wide variety of substances, such as glutamate, octopamine and proctolin (Cook and Meola, 1978; Orchard and Lange, 1986). Kalogianni and Theophilidis (1993) have suggested that these contractions are inhibited, when the oviductal rhythm is elicited, by the oviductal dorsal unpaired median (DUM) neurones. The oviduct is thus under hormonal, neuromodulatory and direct neural control, shaping its contractions during the different stages of egg laying.

Motor innervation and neurogenic contractions

The location of the bilaterally paired neurones that innervate the internal genital tract of female insects within the CNS is variable (Cook *et al.* 1980; Stoya *et al.* 1989; Sugawara, 1986). There is also diversity in the number of oviductal bilaterally paired neurones found in different species (Stoya *et al.* 1989, Thorn and Truman, 1989; Lange and Orchard, 1984*a*; Kalogianni and Pflüger, 1992). Morphological characterisation, by intracellular staining of the three oviductal bilaterally paired neurones in *Calliptamus* sp., shows that they possess an almost identical morphology to that of the previously identified oviductal motor neurones in *Locusta migratoria* (Kalogianni and Pflüger, 1992). Physiological experiments have also shown that they generate rhythmic neurogenic contractions of the oviductal muscle. Intracellular recordings from the muscle fibres of the common oviduct revealed two EJPs of different amplitude, which is in accord with a previous report on the spatial distribution of the

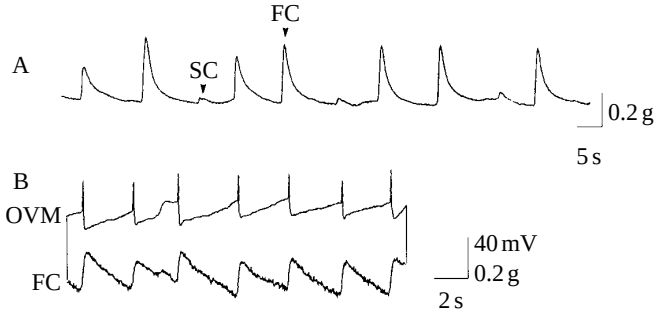

Fig. 11. (A) *In vitro* contractions, measured from the oviduct after its isolation from the CNS. Two modes of contraction can be distinguished: slow peristaltic contractions, of low amplitude (SC) and larger fast phasic contractions (FC). (B) The latter were produced by high-amplitude action potentials (upper trace) generated spontaneously by the oviductal muscle fibres (OVM).

oviductal motor neurones on the locust oviduct, showing that the common oviduct is innervated by two out of the three oviductal motor neurones (Kalogianni and Pflüger, 1992). These neurones, termed OVN1 and OVN2, are homologues of OVN1 and OVN2 in *Calliptamus* sp. The third motor neurone in *L. migratoria* (OVN3), which appears to be homologous to OVN3 of *Calliptamus* sp., was found to innervate only the junctional oviductal area (Kalogianni and Pflüger, 1992). Polyneuronal innervation of the oviductal muscle by a maximum of three motor neurones, reported for *L. migratoria* (Orchard and Lange, 1986), has also been established in both *Calliptamus* sp. and *D. albifrons* (recorded from muscle fibres of the junctional area). In the latter, however, backfilling and intracellular recording experiments have shown that six pairs of bilaterally paired neurones supply the oviductal muscle. The three different EJPs could account for OVN2, OVN3 and OVN4 which, according to this study, have physiological properties typical of motor neurones. In *D. albifrons*, OVN2 and, to a lesser degree, OVN3 have a phasic firing pattern, similar to that of OVN1 of *Calliptamus* sp. and *L. migratoria* (Kalogianni and Pflüger, 1992). OVN4 of *D. albifrons* has a tonic firing pattern and is probably homologous to OVN2 of *Calliptamus* sp. and *L. migratoria* (Kalogianni and Pflüger, 1992). However, only three EJPs of different amplitude were recorded from the muscle fibres of *D. albifrons.* The possibility that there are muscle fibres receiving innervation from more than three neurones cannot be excluded but, if this is not the case, this discrepancy might be explained in a different manner, involving the functional properties of oviductal neurones 5 and 6 (OVN5, OVN6). These neurones support large soma spikes, with a characteristic afterhyperpolarisation. These features are similar to those of the putatively octopaminergic dorsal unpaired median (DUM) neurones innervating the locust oviduct (Lange and Orchard, 1984*a*; Kalogianni and Pflüger, 1992), the oviduct of *D. albifrons* and *Calliptamus* sp. (Kalogianni and Theophilidis, 1993) and many insect skeletal muscles (Heitler and Goodman, 1978; Brookes and Weevers, 1988). These cells (OVN5 and OVN6) fire in interphase with the other oviductal motor neurones, following the same pattern as the oviductal unpaired median neurones of *D. albifrons*, described elsewhere (Kalogianni and Theophilidis, 1993).

The possibility that these neurones have a modulatory rather than a motor function could also help to interpret the response of the oviductal muscle of *D. albifrons* to electrical stimulation of the oviductal nerve. The oviduct of *Calliptamus* sp. responds to high-frequency nerve stimulation with a slowly rising contraction which maintains its peak value throughout stimulation, and is thus similar to that of the locust oviduct (Lange *et al.* 1984*b*). In *D. albifrons*, in contrast, electrical stimulation at 1 Hz induces individual contractions of decreasing amplitude, and high-frequency stimulation initiates a fast-rising contraction which, after reaching a peak value, stabilizes at a decreased plateau value. The delayed decline of the contraction amplitude, if not due to failure of stimulation, could be attributed to the effect of some neuromodulatory substance released during high-frequency stimulation of the oviductal nerve. Neurosecretory cells, such as the DUM neurones, that innervate the oviduct, have been reported in both species (Kalogianni and Theophilidis, 1993) and their counterparts in the locust inhibit neurogenic contractions of the oviduct (Kalogianni and Pflüger, 1992). This, however, suggests that the oviductal neurosecretory cells of *D. albifrons* release different transmitters onto the oviduct from those released in *Calliptamus* sp. and/or that the excitation of OVN5 and OVN6 is responsible for the different response of the oviductal muscle in *D. albifrons*. Although it has been established that unpaired median neurones act as modulators of both neurogenicity and myogenicity, we know of no other bilaterally paired neurones with a modulatory function like that suggested for OVN5 and OVN6. However, additional experiments are required to exclude the possibility of a failure in the electrical stimulation of the oviductal nerve. In addition, immunocytochemical studies would be required to identify the transmitters released by OVN5 and OVN6, so that their putative modulatory role could be tested.

Central generation of the oviductal rhythm

The oviductal motor pattern is generated by a neural circuit located in the genital ganglia. The capacity of this network to generate the pattern in the absence of sensory information has been shown previously (Kalogianni and Theophilidis, 1993), establishing the concept of a CPG initiating the oviductal rhythm. Many motor patterns related to egg laying reside in the abdominal nerve cord; the isolated abdomen of locusts, crickets and moths (Thompson, 1986; Carrow *et al.* 1982; McCracken, 1907) is able to perform ovipositional movements, upon isolation of the abdominal cord from thoracic and cerebral centres. Activation of a motor pattern by release from descending inhibition has also been implied for motor programmes as diverse as copulation in the male mantis (Roeder *et al.* 1960) and the calling song in crickets (Bentley and Hoy, 1970). This also appears to be the case for the initiation of the oviductal rhythm. In the present study, the use of species with anatomically different nerve cords allowed a

more precise localisation of the anterior inhibitory centres. In both *D. albifrons* and *Calliptamus* sp., removal of the cerebral, prothoracic and mesothoracic neuromeres failed to initiate the oviductal pattern, as it also fails to induce oviposition digging in *Locusta migratoria* (Thompson, 1986). In *Calliptamus* sp., removal of the compound metathoracic ganglion (i.e. deactivation of inhibition originating from this ganglion) is necessary for the expression of the rhythm, as it is for the initiation of locust oviposition (Thompson, 1986). In *D. albifrons*, however, it was only removal of the second and third neuromeres, which in *Calliptamus* sp. are fused with the metathoracic and first abdominal neuromere, that always initiated the oviductal pattern. These results suggest that the descending inhibition of the oviductal circuit derives in *D. albifrons*, and possibly also in *Calliptamus* sp., from the second and/or the third abdominal neuromeres. A similar inhibition of the oviposition digging rhythm may involve a neuromodulatory substance (Thompson, 1986), because suppression of the rhythm by electrical stimulation of the nerve cord bears strong similarities to the effect of ionophoretic application of octopamine into the terminal ganglion (Sombati and Hoyle, 1984).

Recently, evidence from frequency measurements of the overall neural activity in the oviductal nerve during step-wise isolation of the seventh abdominal ganglion has indicated that the oviductal motor circuit in the locust is also under descending inhibition, and it has been suggested that the locust oviductal CPG resides almost entirely in the seventh abdominal ganglion (Facciponte and Lange, 1993). This activity was not reduced after isolation of the seventh abdominal ganglion from the terminal ganglion. The effect of this isolation on the bursting frequencies of the three oviductal motor neurones varied, however, with spike 2 (homologous to OVN2 in *Calliptamus* sp.) showing a decrease in its frequency. OVN2 is the only one of the three oviductal neurones that projects to all regions of the oviduct (Kalogianni and Pflüger, 1992), so the decline in its bursting frequency could account for the decrease in both amplitude and frequency of the oviductal contractions in the isolated seventh abdominal ganglion described in the present study. Thus, in *Calliptamus* sp. and *D. albifrons*, the seventh abdominal ganglion can generate a rhythmic pattern, but inputs from the terminal abdominal ganglion are essential for the production of the oviductal rhythm. This input could be related to the neural network producing the locust oviposition digging rhythm, which is also distributed in the two genital ganglia (Thompson, 1986). Another rhythmic pattern, the ventilatory rhythm, recently studied in *D. albifrons*, is produced by a CPG located in the first free abdominal ganglion, which shapes the output of local pattern generators residing in the abdominal ganglia (Consoulas, 1990).

The species studied here show extensive differences in the anatomy and innervation of their oviductal system but the neurogenic oviductal contractions, the origin of the central rhythmic drive to the oviductal motor neurones and the site of origin of the descending inhibition to the oviductal CPG appear to be similar. Thus, the results of this study, by providing insight in the anatomy and physiology of the oviductal system of two insect species, could be further extended to identify the interneurones that generate the oviductal rhythm and to study the role of neuromodulatory elements and sensory information in the shaping of oviductal contractions during egg-laying behaviour.

This research was supported by a grant from the Greek Ministry of Industry, Energy and Technology (Research and Technology Branch). Special thanks to Philip Newland, Tom Matheson and Alex Norman in Cambridge for their comments on the manuscript.

References

BACON, J. P. AND ALTMAN, J. S. (1977). A silver intensification method for cobalt-filled neurones in whole mount preparations. *Brain Res.* **138**, 359–363.

BÄSSLER, U. (1986). On the definition of a Central Pattern Generator and its sensory control. *Biol. Cybernetics* **54**, 65–69.

BENTLEY, D. R. AND HOY, R. (1970). Post-embryonic development of adult motor patterns in crickets: a neural analysis. *Science* **170**, 1409–1411.

BROOKES, S. J. H. AND WEEVERS, R. DE G. (1988). Unpaired median neurones in a lepidopteran larva *Antheraea pernyi*. I. Anatomy and physiology. *J. exp. Biol.* **136**, 311–332.

CARROW, G. M., CABEZA, R. DE J. AND FLORES, G. (1982). Isolation of the abdomen releases oviposition behaviour in females of the cricket *Acheta domesticus*. *J. Insect Physiol.* **28**, 401–404.

CONSOULAS, C. (1990). A study of the neuromuscular system that controls the abdominal movement in *D. albifrons*. PhD thesis, University of Thessaloniki, Greece.

COOK, B. J., HOLMAN, G. M. AND MEOLA, S. M. (1984). The oviduct musculature of the cockroach *Leucophaea maderae* and its response to various neurotransmitters and hormones. *Archs Insect Biochem. Physiol.* **1**, 167–178.

COOK, B. J. AND MEOLA, S. M. (1978). The oviduct musculature of the horsefly, *Tabanus sulcifrons* and its response to 5-hydroxytryptamine and proctolin. *Physiol. Ent.* **3**, 273–280.

COOK, B. J., THOMPSON, J. M. AND SHELTON, W. D. (1980). Some structural and functional properties of nerves and muscles in the oviduct of the pink bollworm moth, *Pectinophora gossypiella* (Saund.) *Int. J. Invert. Reprod.* **2**, 351–362.

DELCOMYN, F. (1980). Neural basis of rhythmic behaviour in animals. *Science* **210**, 492–498.

EBARA, A., UESAKA, H. AND YAMAGISHI, H. (1990). Pacemaker activity in the heart of the beetle, *Allomyrina dichotomus*. *Comp. Biochem. Physiol.* **97**A, 223–228.

FACCIPONTE, G. AND LANGE, A. B. (1993). Characterisation of a novel central pattern generator located in the VII abdominal ganglion of *Locusta*. *J. Insect Physiol.* **38**, 1011–1022.

HEITLER, W. J. AND GOODMAN, C. S. (1978). Multiple sites of spike initiation in a bifurcating locust neurone. *J. exp. Biol.* **76**, 63–84.

KALOGIANNI, E. AND PFLÜGER, H. J. (1992). The identification of motor and unpaired median neurones innervating the locust oviduct. *J. exp. Biol.* **168**, 177–198.

KALOGIANNI, E. AND THEOPHILIDIS, G. (1993). Centrally generated

rhythmic activity and modulatory function of the oviductal dorsal unpaired median (DUM) neurones in two orthopteran species (*Calliptamus* sp. and *Decticus albifrons*). *J. exp. Biol.* **174**, 123–138.

LANGE, A. B. AND ORCHARD, I. (1984*a*). Dorsal unpaired median neurones and ventral bilaterally paired neurones project to a visceral muscle in an insect. *J. Neurobiol.* **15**, 441–453.

LANGE, A. B. AND ORCHARD, I. (1984*b*). Some pharmacological properties of neuromuscular transmission in the oviduct of the locust *Locusta migratoria*. *Archs Insect Biochem. Physiol.* **1**, 231–241.

LANGE, A. B., ORCHARD, I. AND LOUGHTON, B. G. (1984*a*). Neural inhibition of egg laying in the locust *Locusta migratoria*. *J. Insect Physiol.* **30**, 271–278.

LANGE, A. B., ORCHARD, I. AND LOUGHTON, B. G. (1984*b*). Spontaneous and neurally evoked contractions of visceral muscles in the oviduct of *Locusta migratoria*. *Archs Insect Biochem. Physiol.* **1**, 179–190.

MCCRACKEN, I. (1907). The egg-laying apparatus in the silkworm (*Bombyx mori*) as a reflex apparatus. *J. comp. Neurol* **. 17**, 262–285.

OKELO, O. (1971). Physiological control of oviposition in the female desert locust *Schistocerca gregaria* Forsk. *Can. J. Zool.* **49**, 969–974.

ORCHARD, I. AND LANGE, A. B. (1986). Neuromuscular transmission in an insect visceral muscle. *J. Neurobiol.* **17**, 359–372.

ROEDER, K. D., TOZIAN, L. AND WIEANT, T. E. (1960). Endogenous nerve activity and behaviour in the mantis and the cockroach. *J. Insect Physiol.* **4**, 45–62.

SOMBATI, S. AND HOYLE, G. (1984). Generation of specific behaviours in a locust by local release into neuropile of the natural neuromodulator octopamine. *J. Neurobiol.* **15**, 481–506.

STOYA, G., AGRICOLA, H., ECKERT, M. AND PENZLIN, H. (1989). Investigations on the innervation of the oviduct muscle of the cockroach *Periplaneta americana*. *Zool. Jb. Physiol.* **93**, 75–86.

SUGAWARA, T. (1986). Oviposition behaviour of the cricket *Teleogryllus commodus*: the site of action of an oviposition stimulating factor and the role of the nervous system. *J. Insect Physiol.* **32**, 179–188.

THOMAS, A. (1979). Nervous control of egg progression into the common oviduct and genital chamber of the stick insect *Carausius morosus*. *J. Insect Physiol.* **25**, 811–823.

THOMPSON, K. (1986). Oviposition digging in the grasshopper. II. Descending neural control. *J. exp. Biol.* **122**, 413–425.

THORN, R. S. AND TRUMAN, J. W. (1989). Sex-specific neuronal respecification during the metamorphosis of the genital segments of the tobacco hornworm moth *Manduca sexta*. *J. comp. Neurol.* **284**, 489–503.

WOODBURY, J. W. AND BRADY, A. J. (1956). Intracellular recordings from moving tissues with a flexibly mounted microelectrode. *Science* **123**, 100–101.