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CENTRALLY GENERATED RHYTHMIC ACTIVITY AND MODULATORY FUNCTION OF THE OVIDUCTAL DORSAL UNPAIRED MEDIAN (DUM) NEURONES IN TWO ORTHOPTERAN SPECIES (*CALLIPTAMUS* SP. AND *DECTICUS ALBIFRONS)*

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Summary

The rhythmic firing pattern of the putatively octopaminergic dorsal unpaired median (DUM) neurones supplying the oviductal system of female orthopterans, *Calliptamus* sp. and *Decticus albifrons*, was examined. Our data provide evidence that the oviductal DUM neurones in the seventh abdominal ganglion modulate the oviductal motor pattern, both peripherally and centrally, during the inhibition of egg-laying behaviour.

In a minimally dissected animal, rhythmic activation of the oviductal DUM and motor neurones can be readily elicited by isolation of the seventh abdominal ganglion from the anterior part of the nerve cord. The bursting activity of the DUM neurones is temporally correlated with the oviductal motor rhythm. Both populations of oviductal neurones retain their rhythmic firing pattern after total isolation of the genital ganglia, indicating the presence of an oviductal central pattern generator.

The effects of stimulation of oviductal DUM neurones on the oviductal motor activity were monitored by recording intracellularly from oviductal muscle fibres and extracellularly from motor axons. These effects consist of a reduction in the amplitude and frequency of excitatory postsynaptic potentials (EPSPs) in the muscle fibre and in the firing rate in oviductal motor neurones. We suggest that the change in EPSP amplitude results from peripheral release of octopamine by DUM neurones. The decreased firing rate of motor neurones, however, appears to be a central effect, possibly caused by central release of octopamine by DUM neurones.

Introduction

The morphology and physiology of the dorsal unpaired median (DUM) neurones of insects have been studied extensively during the past two decades (Hoyle *et al.* 1974;

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Heitler and Goodman, 1978; Hoyle and Dagan, 1978; Lange and Orchard, 1984; Watson, 1984; Pflüger and Watson, 1988). DUM neurones innervate both skeletal and visceral muscles, but the effects of DUM neurone activity on the physiological properties of muscle fibres and the activation of DUM neurones during various forms of behaviour have not yet been fully clarified. It has been shown that DUM neurones enhance skeletal muscle contractions (Hoyle *et al.* 1974; Evans and O'Shea, 1977; O'Shea and Evans, 1979; Brookes, 1988), whereas their effects on visceral muscles are inhibitory. Orthodromic stimulation of oviductal DUM neurones and direct application of octopamine to oviductal muscle inhibit neurogenic and myogenic oviductal contractions (Kalogianni and Pflüger, 1992; Orchard and Lange, 1985, 1986). Octopamine has also been found to have central effects on motor patterns, since local application of octopamine in the thoracic ganglia of the locust can generate specific behavioural patterns (Sombati and Hoyle, 1984; Stevenson and Kutsch, 1987). Also, there is evidence that octopamine can induce plateau potentials in flight interneurones (Ramirez and Pearson, 1991). A few recent studies have presented evidence for the activation of DUM neurones during centrally generated motor activities, such as the glow response of larval fireflies (Christensen and Carlson, 1982), body movements of lepidopteran larvae (Brookes, 1988) and locust flight (Ramirez and Orchard, 1990). In these studies, it has been suggested that the DUM neurones release octopamine and either trigger or modulate peripherally the output of the central nervous system (CNS).

The purpose of this study is to investigate the firing patterns of oviductal DUM neurones in relation to the oviductal motor rhythm in two species of Orthoptera (*Decticus albifrons*, *Calliptamus* sp.). Their effects on the activity of the oviductal motor neurones are inhibitory and occur both peripherally and centrally. The possible role of the oviductal DUM neurones during oviposition is discussed.

Materials and methods

Adult female *Decticus albifrons* and *Calliptamus* sp. collected near Thessaloniki, Greece, were used in this study. For anatomical and physiological experiments, the animal was immobilized with the ventral side up and a midventral incision was made in the posterior abdominal segments to reveal the oviductal system and the seventh abdominal ganglion (minimally dissected animal). The two sides of the body wall were pinned down laterally so that the abdominal cavity formed a small pool. During physiological experiments, the preparation was superfused 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).

For intracellular recordings from the cell bodies of the oviductal dorsal unpaired median (DUM) neurones the seventh abdominal ganglion was stabilized with minuten pins on a silver platform covered with wax and the ganglionic sheath was softened with a 1% solution of Protease (Sigma, type XIV). Records were obtained using glass microelectrodes (resistance 50–70 MΩ), filled with 6% hexammine cobaltic chloride (Brogan and Pitman, 1981). The records were displayed on a digital oscilloscope (Hameg 205) and stored on computer for subsequent analysis and printing. Intracellular staining

of an impaled cell was made with 500ms depolarising pulses of 10nA at 1Hz for 15min. The preparation was left for another 60min to allow for passive diffusion of the dye into the impaled cell. Cobalt was precipitated with ammonium sulphide and the preparation fixed and subsequently silver-intensified (Bacon and Altman, 1977). The intensified preparations were cleared in methyl salicylate and drawings of the stained neurones were made using a *camera lucida.*

Intracellular recordings from oviductal muscle fibres were obtained using low-resistance (10–20 MΩ) floating glass microelectrodes (Woodbury and Brady, 1956) filled with $2 mol\,l^{-1}$ potassium acetate. Extracellular records from the oviductal nerve were obtained using monopolar hook electrodes. Oviductal contractions were monitored by attaching the thread of a Grass isometric tension transducer to the lateral or the common oviduct. For antidromic stimulation of the oviductal DUM neurones, 2–4V square-wave pulses of 1ms duration at various frequencies were delivered through a stimulus isolation unit to a silver hook electrode upon which the cut end of the contralateral oviductal nerve was mounted.

To locate the cell bodies of the oviductal DUM neurones a differential staining technique (Quicke and Brace, 1979; Sakai and Yamagushi, 1983) was applied. For this purpose, the seventh abdominal ganglion and the oviductal system were removed and placed in a Petri dish. Pieces were cut from the left and right parts of the oviduct and placed in Vaseline pools containing a $1 mol\,l^{-1}$ solution of $NiCl_2$ and $CoCl_2$ respectively. The preparation was left for 24h at 4˚C and then developed in 3ml of sodium cacodylate buffer containing three drops of saturated alcoholic rubeanic acid solution. Neurones possessing axons in both oviductal nerves appear red as a result of the mixture of the two dyes.

To stain exclusively the peripheral projections of DUM neurones the proximal cut end of the oviductal nerve of a minimally dissected animal was placed in a Vaseline pool filled with distilled water, which was later replaced with 6% hexammine cobaltic chloride. The preparation was left at 4˚C for 24h to allow passive diffusion of the dye through the contralateral neurites of the DUM neurones to their projections on the contralateral part of the oviduct. The stained preparations were then treated according to the procedure described above for cobalt chloride.

Results

Oviductal DUM neurones

The oviductal system of female *Calliptamus* sp. and *D. albifrons* consists of a pair of lateral oviducts (Fig. 1A,B) which, at their posterior end, fuse to form a common oviduct (Fig. 1A,B). In *Calliptamus* sp. (Fig. 1A) the common oviduct opens posteriorly into the ovipositor, whereas in *D. albifrons* the common oviduct is very short and opens posteriorly into the genital chamber (Fig. 1B). In both species the oviducts are innervated by the seventh abdominal ganglion through a pair of nerve branches, designated as the oviductal nerves. In *Calliptamus* sp. the oviductal nerve arises from nerve N2 (branch N2B, Fig. 1A), while in *D. albifrons* it arises from nerve N1 (branch N1B2, Fig. 1B).

To establish the exact number and location of the DUM neurones which innervate the oviductal system, the left and right oviductal nerves were backfilled using a differential staining technique (Quicke and Brace, 1979). In *Calliptamus* sp. 8–9 bilaterally projecting neurones (10 preparations) were located near the midline of the seventh abdominal ganglion (Fig. 1A, see AG7 in inset). Thirteen oviductal bilaterally projecting neurones (eight preparations) were found in *D. albifrons* (Fig. 1B, see AG7 in inset). In

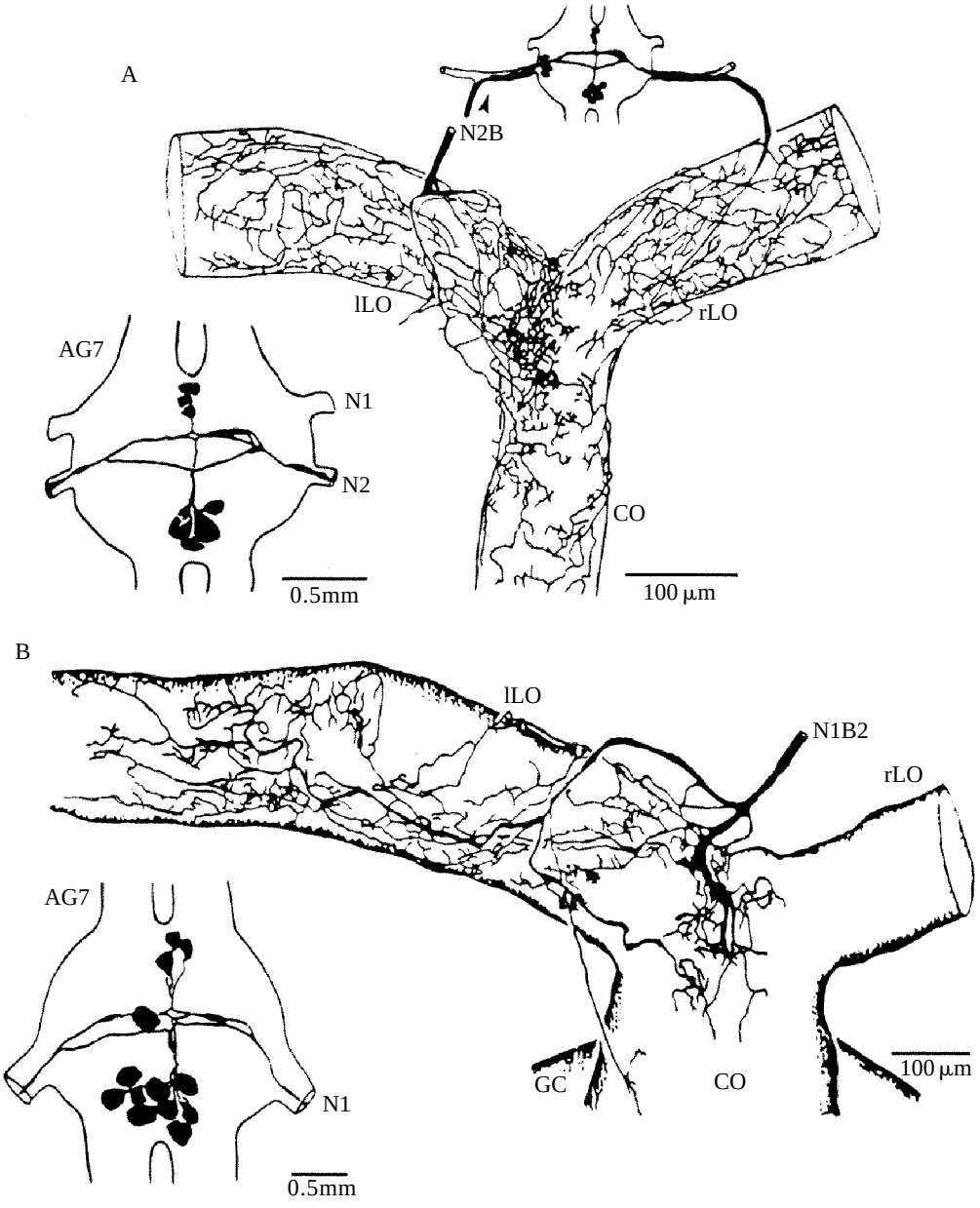

Fig.1

both species, the median neurones form two distinct groups within the seventh abdominal ganglion. The anterior group consists of 3–4 neurones located near the origin of the anterior connectives (AG7, Fig. 1A,B). The posterior group consists of 5–6 neurones in *Calliptamus* sp. and nine neurones in *D. albifrons* and is located near the origin of the posterior connectives (AG7, Fig. 1A,B). In *D. albifrons*, there is also a neurone located at the centre of the ganglion. In both species, the stained neurones have the characteristic morphology of DUM neurones, i.e. they possess a large cell body located dorsomedially in the ganglionic cortex and their primary neurite projects towards the centre of the ganglion, where it bifurcates into a pair of secondary neurites which project to the oviducts through the oviductal nerves.

Peripheral stainings of the left oviductal nerve (N2B in Fig. 1A and N1B2 in Fig. 1B) revealed the distribution pattern of both DUM and motor axons on the left half of the oviduct (Fig. 1A,B, lLO). The innervation pattern of the oviductal DUM axons was established in the same preparation by simultaneous backfilling of the left oviductal nerve (Fig. 1A, see arrowhead), since dye first passes to the cell bodies of the oviductal DUM neurones and then through their right oviductal neurites to the right half of the oviducts (Fig. 1A, rLO). A comparison of the two oviductal halves shows that the DUM neurones mainly innervate the lateral oviduct and a small part of the common oviduct, but they send only a few branches to the junctional area, i.e. the point where the lateral and the common oviduct are joined (Fig. 1A, right half of the oviducts). In contrast, the junctional area is mainly innervated by the oviductal motor neurones (dense network of motor fibres on the left half of the oviducts in Fig. 1A).

Central activation of oviductal DUM neurones

Interruption of a female locust during egg laying evokes rhythmic contractions of the oviducts, brought about by rhythmic activation of the oviductal motor neurones. Their probable function is to propel the eggs back to the ovaries and thus inhibit egg laying (Lange *et al.* 1984). In the course of our experiments it was found that, in non-egg-laying females which were immobilized and minimally dissected (see Materials and methods), such contractions could be elicited after isolation of the genital ganglia (seventh and terminal abdominal ganglia) from the rest of the nerve cord (Fig. 2A). In both *Calliptamus* sp. and *D. albifrons* these contractions are of neurogenic origin because they are produced by bursts of summating EPSPs (Fig. 2B) generated by bursts of action potentials in the oviductal motor neurones (Fig. 2C) of the seventh abdominal ganglion.

Fig. 1. (A) Innervation pattern of the oviduct of *Calliptamus* sp. revealed by both peripheral and central cobalt filling of the left oviductal nerve (arrowhead indicates site of central filling). Arborizations of both the motor and DUM neurones are visible on the left half of the oviduct, whereas the right half of the oviduct shows only arborizations of the bilaterally projecting median neurones. Central filling reveals three motor neurones, 3–4 anterior and 5–6 posterior median neurones with bilaterally projecting axons (see inset). (B) Peripheral filling of the oviductal nerve in *D. albifrons* reveals the innervation pattern of the oviduct by both motor and DUM neurones. Central filling reveals six motor neurones (not shown) and three anterior, one medial and nine posterior median neurones with bilaterally projecting axons. N1, N2, main nerves; N2B, N1B2, oviductal nerves; AG7, seventh abdominal ganglion; lLO, left lateral oviduct; rLO, right lateral oviduct; CO, common oviduct; GC, genital chamber.

A 1:1 correlation between the action potentials and the muscle fibre EPSPs was evident (see action potentials marked with dots in Fig. 2D).

Under these conditions, the oviductal DUM neurones were also rhythmically active (Fig. 2E,F, upper traces). Intracellular records from their cell bodies (Fig. 2E,F, upper traces) during the rhythmic oviductal contractions (Fig. 2E,F, lower traces) show that the posterior DUM neurones support bursts of overshooting action potentials (70–80mV) in

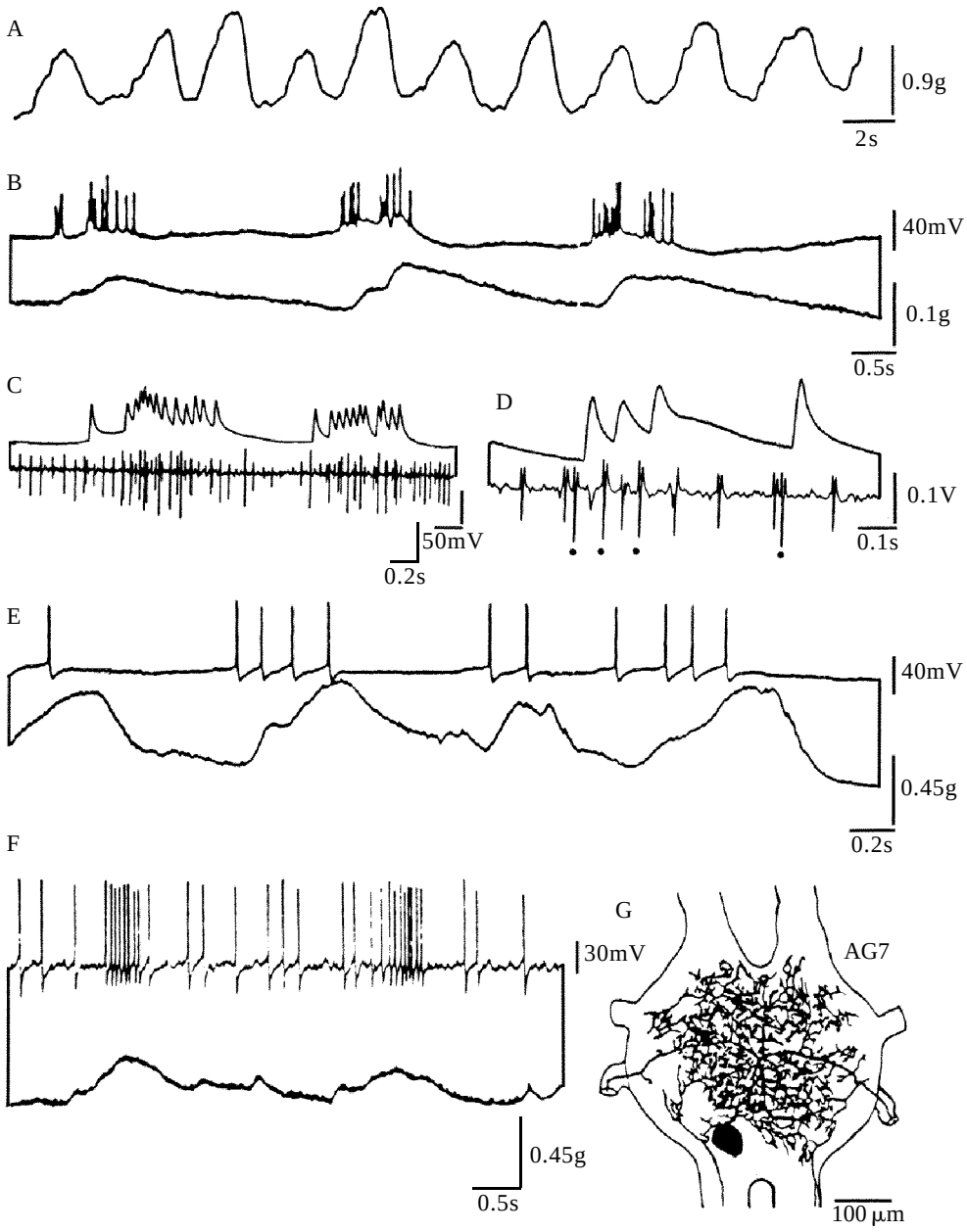

Fig. 2

phase with the oviductal neurogenic contractions in both D. *albifrons* (Fig. 2E) and *Calliptamus* sp. (Fig. 2F). Intracellular staining of these neurones has shown that they possess the typical intraganglionic morphology of abdominal DUM neurones described elsewhere (Pflüger and Watson, 1988).

The firing pattern of the oviductal DUM neurones was most extensively studied in *Calliptamus* sp. (Fig. 3). In non-egg-laying females with the CNS intact, both oviductal DUM and motor neurones fired tonically (Fig. 3A), as can be seen from paired intracellular records from the cell bodies of the posterior oviductal DUM neurones (Fig. 3A, upper trace, see R1 in inset) and the oviductal muscle fibres (Fig. 3A, lower trace, see R2 in inset). Severing the nerve cord anterior to AG7 (see inset of Fig. 3) evoked rhythmic activation of both oviductal DUM and motor neurones. This rhythmic pattern consisted of bursts of motor neurone discharges (revealed by EPSPs in the muscle) followed by bursts of DUM neurone discharges. The bursts in the DUM neurones appeared mainly at the end of the barrage of EPSPs and continued through the EPSP burst interval (Fig. 3B,C,D). Their intraburst frequency varied in different DUM neurones in the same preparation. The most common pattern was that shown in Fig. 3C,D, where the DUM neurones fired 5–7 impulses per burst at 0.4–0.5Hz. However, there were DUM neurones that had a very low rate of activation (Fig. 3B) and others that fired tonically but still exhibited a decrease in their firing rate at the peak of the bursts of EPSPs (Fig. 3E).

The persistence of the oviductal motor pattern, recorded extracellularly from the oviductal nerve, in isolated and deafferented seventh and terminal abdominal ganglia (Fig. 4) indicates the presence of an oviductal central pattern generator (oviductal CPG). Nevertheless, in an intact animal, tactile stimulation of the oviduct or the ovipositor (see bars in Fig. 3F) resulted in a bursting response of both oviductal DUM and motor neurones, indicating that sensory feedback originating from these structures can influence the activity of the oviductal neurones.

Modulatory function of oviductal DUM neurones

The effects of the oviductal DUM neurones on the oviductal system were also examined. For this purpose, EPSPs generated by the rhythmic activation of the oviductal motor neurones were recorded before and immediately after antidromic stimulation of DUM neurones through the contralateral oviductal nerve (Fig. 5). In a female with an intact CNS, tonically occurring EPSPs were recorded from the oviduct (Fig. 5A). In this case, electrical stimulation of the contralateral oviductal nerve at 3Hz (S in Fig. 5A)

Fig. 2. (A) *Calliptamus* sp.: oviductal contractions recorded after isolation of the genital (seventh and terminal abdominal) ganglia from the remaining nerve cord. (B) *D. albifrons*: simultaneous intracellular records from an oviductal muscle fibre (upper trace) and tension measurements from the oviduct (lower trace). (C,D) *D. albifrons*: intracellular records from an oviductal muscle fibre (upper traces) and extracellular records from the oviductal nerve (lower traces). (E) *D. albifrons*, (F) *Calliptamus* sp.: simultaneous intracellular records from the cell body of a posterior DUM neurone (upper trace) and tension measurements from the oviduct (lower trace). (G) Intracellular staining of the DUM neurone in F reveals the central branching pattern of this neurone.

caused a decrease in EPSP amplitude (EPSPs marked with dots in Fig. 5A are superimposed in Fig. 5D) and a delayed decrease in the frequency of the EPSPs (arrow in Fig. 5A). In females where the rhythmic activation of oviductal motor neurones was evoked by severing the nerve cord, antidromic stimulation of DUM neurones (S in

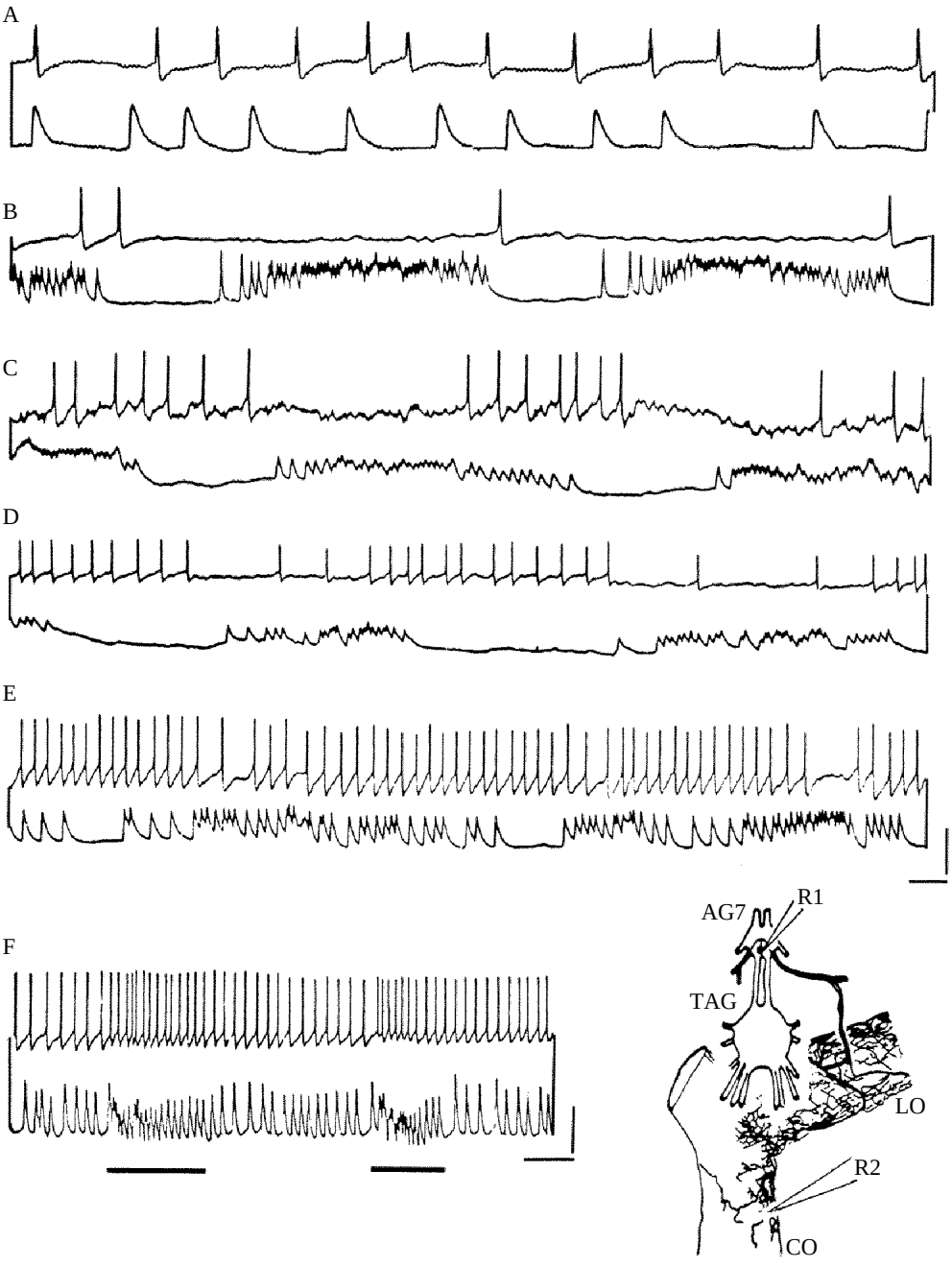

Fig. 3

Fig. 5B) caused a slight decrease in EPSP amplitude and a considerable decrease in the firing rate of the motor neurones, which were identified by the EPSPs they generated (Fig. 5B). The activity of the tonic motor neurone (small EPSPs) was completely suppressed, whereas the activity of the phasic motor neurone (large EPSPs) was reduced to a few EPSPs per burst. The frequency of the EPSPs was gradually re-established after several seconds. These inhibitory effects were observed in more than 20 preparations but their strength varied, as can be seen in Fig. 5C where antidromic stimulation of the DUM neurones mainly affected the frequency of the tonic neurone. The recovery of the EPSPs' frequency was observed after approximately 7s (Fig. 5C).

In these experiments, however, the activity of the oviductal motor neurones was monitored by recording the EPSPs they generated in the oviductal muscle and therefore it is not clear whether the decrease in the frequency of the EPSPs is a peripheral effect of the DUM neurones on the muscle fibres or an effect occurring in the central nervous system. To clarify this, simultaneous extracellular records from the oviductal nerve (Fig. 6A,B upper traces) and intracellular records from the oviductal muscle fibres (Fig. 6A,B, lower traces) were made during electrical nerve stimulation. Antidromic stimulation of the DUM neurones resulted in a decrease in the frequency of the EPSPs approximately 4 s after the onset of stimulation by decreasing the frequency of the motor neurone action potentials that generated them (Fig. 6A,B, arrows). This is strong evidence that the frequency change is a result of central interactions.

The drawback of this kind of experiment is that antidromic stimulation may activate contralateral reflexes which would affect the excitability of the oviductal motor neurones. Therefore, an attempt was made to examine whether orthodromic stimulation of individual DUM neurones produces long-term inhibitory effects on the muscle fibre EPSPs similar to those caused by antidromic stimulation of the whole population of oviductal DUM neurones. For this purpose, in females where oviductal motor neurones exhibited tonic firing (CNS intact, see inset of Fig. 7), individual DUM neurones were stimulated intracellularly to fire at high frequency (Fig. 7, upper traces, see R1/S1 in inset). Continuous intracellular records from oviductal muscle fibres before, during and after the end of DUM neurone stimulation (Fig. 7A,B, lower traces, see R2 in inset) revealed an inhibitory effect of single DUM neurones on the firing rate of oviductal motor neurones (arrows in Fig. 7A,B). This effect appeared several seconds after the onset of stimulation (17s for A and 13s for B) and full recovery was observed several seconds later. The effects of orthodromic DUM neurone stimulation were also examined in females exhibiting rhythmic oviductal motor activity induced by severing the nerve cord (see inset of Fig. 8). Stimulating a DUM neurone to fire at 7Hz (Fig. 8A, upper traces) caused a decrease in the intraburst frequency of EPSPs from 40–50Hz to 15Hz (lower

Fig. 3. Intracellular records from several posterior DUM neurones of *Calliptamus* sp. (upper trace) and intracellular records from an oviductal muscle fibre (lower trace), before (A and F) and after severence of the nerve cord (B,C,D,E). See inset for recording configuration. (F) Response of oviductal DUM and motor neurones to tactile stimulation of the oviduct (indicated by bars). TAG, terminal abdominal ganglion; R1, R2, recording sites 1 and 2. Scale bars: A, 40mV, 20mV, 0.12s; B,C,E, 40mV, 20mV, 0.2s; D, 80mV, 20mV, 0.2s; F, 40mV, 50mV, 0.5s.

traces in Fig. 8A), approximately 2.5–3s after stimulus onset (arrow in Fig. 8A). This effect was more evident when a second DUM neurone (Fig. 8B, upper trace) was depolarised so that it fired at a much higher frequency. This caused a decrease in intraburst frequency of EPSPs from 16 to 6Hz. These experiments verified that the central inhibition of the oviductal EPSPs observed during contralateral stimulation of the

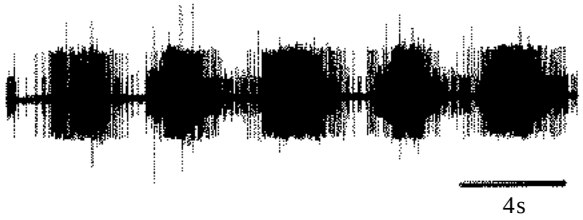

Fig. 4. Extracellular recording from the oviductal nerve after isolation and deafferentation of the seventh and terminal abdominal ganglia.

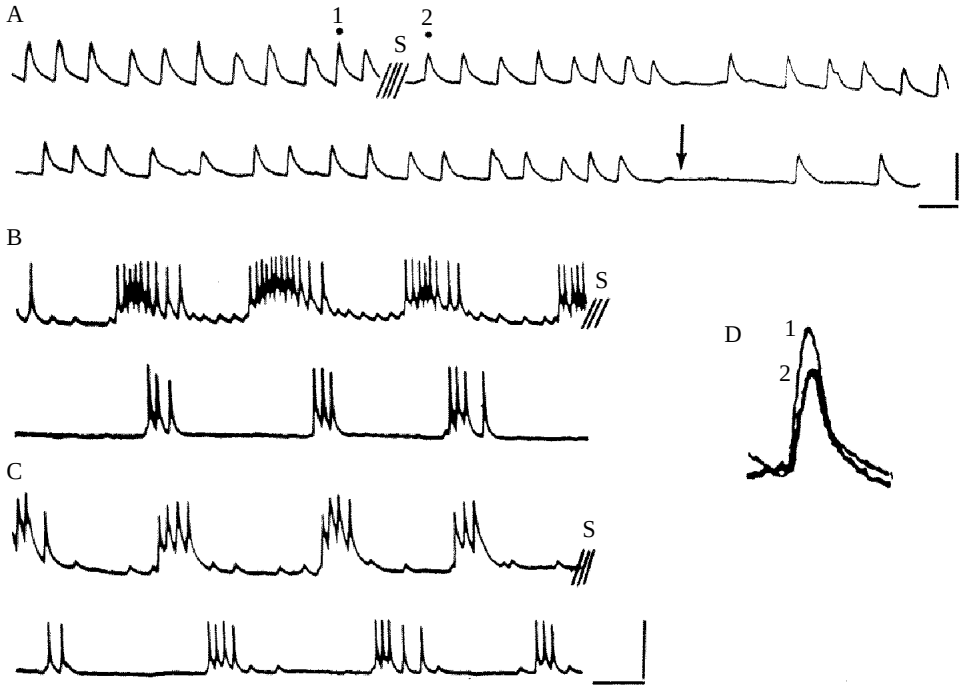

Fig. 5. (A) Continuous intracellular records from a right-side oviductal muscle fibre before and after antidromic stimulation (S) of oviductal DUM neurones through the distally severed contralateral oviductal nerve, in a female with the CNS intact. The arrow marks the delayed decrease in the frequency of EPSPs. (B,C) Intracellular records from an oviductal muscle fibre before (first trace) and after (second trace) antidromic stimulation of the oviductal DUM neurones in a female with severed nerve cord. (D) The two EPSPs marked with dots (1 and 2) in A are superimposed. Scale bars: A, 50mV, 0.2s; B,C, 50mV, 0.5s; D, 15mV, 70ms.

oviductal nerve (Figs 5 and 6) can be attributed to a modulatory action of the DUM neurones.

Discussion

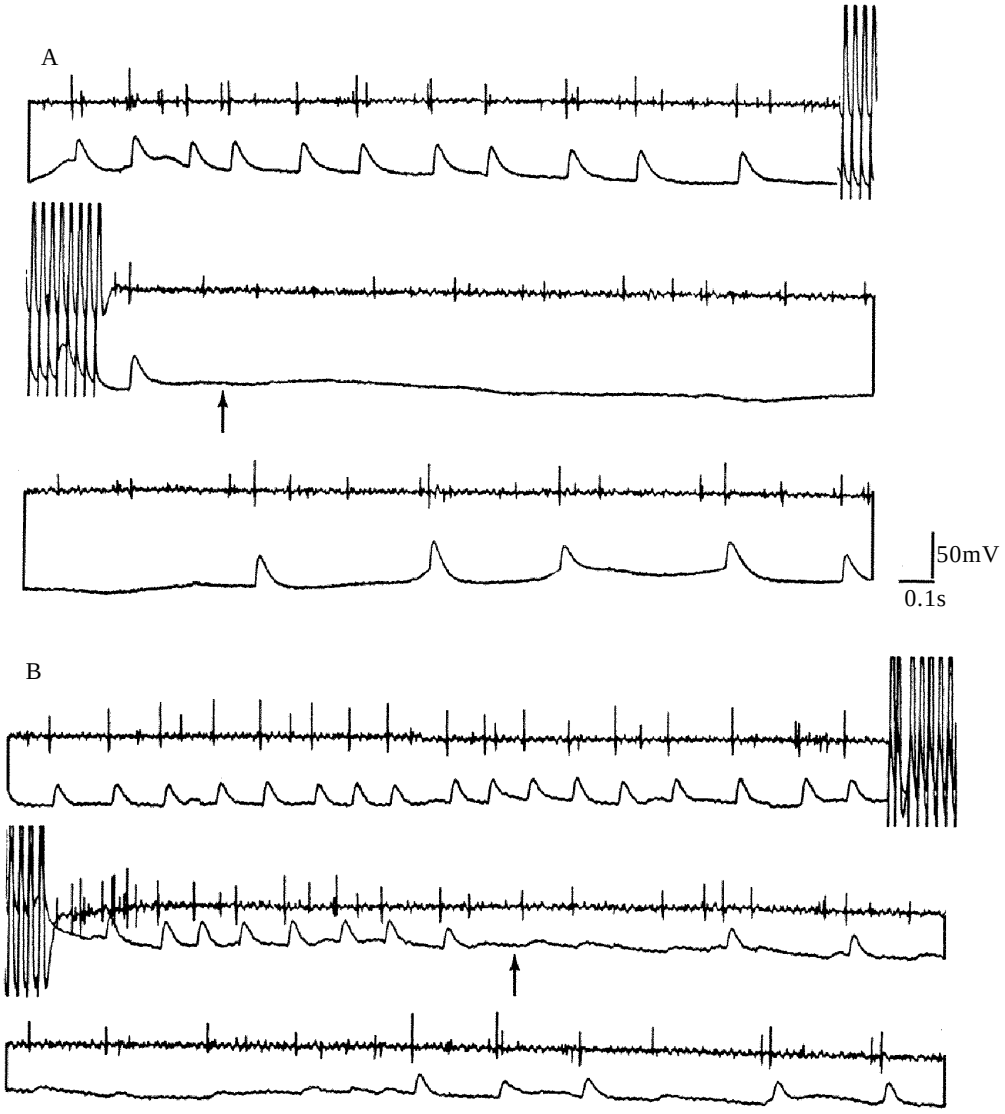

Fig. 6. (A,B) Paired extracellular records from the oviductal nerve (upper traces) and intracellular records from an oviductal muscle fibre (lower traces) during antidromic stimulation of the oviductal DUM neurones through the distally severed contralateral oviductal nerve. Arrows indicate the reduction in EPSP frequency.

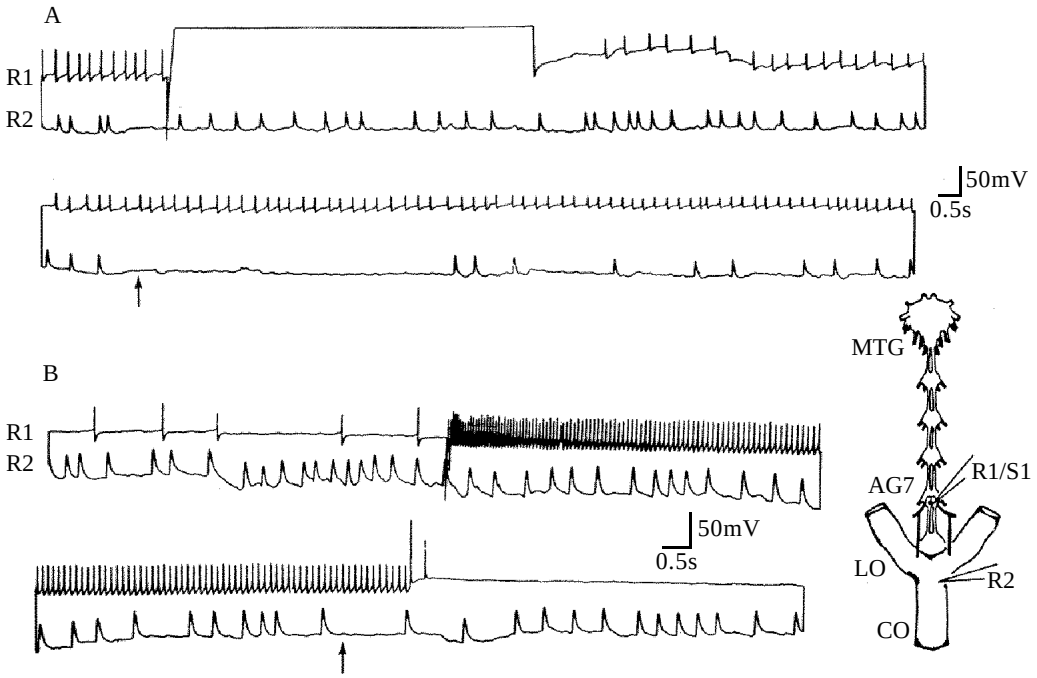

Fig. 7. (A,B) Intracellular suprathreshold stimulation of a single oviductal DUM neurone (upper trace, R1/S1 in inset) showing resultant slowing of EPSP frequency (arrows) recorded from an oviductal muscle fibre (lower trace, R2 in inset) in a female with an intact nerve cord (see inset for recording and stimulation configuration). A and B show the two extremes in the spectrum of responses observed. In A the microelectrode bridge was unbalanced so the high-frequency burst of spikes during stimulation was not recorded. MTG, metathoracic ganglion; R1/S1, recording/stimulating site 1; R2, recording site 2.

Central activation of oviductal DUM neurones

In this study, the activation pattern of oviductal DUM neurones in relation to the oviductal motor programme and the effects of the DUM neurones on the oviductal system were examined. In females with an intact nerve cord, both oviductal DUM and motor neurones fire tonically. However, isolation of the seventh and terminal abdominal ganglia from the rest of the nerve cord evokes a rhythmic activation of both DUM and motor neurones, which is temporally correlated with the oviductal neurogenic contractions. The bursts in the motor neurones, which cause the rhythmic contractions of the oviductal muscle, are followed by the bursts in the DUM neurones. This pattern, with little change in its temporal sequence and frequency, persists after deafferentation, indicating that both oviductal neurone populations are driven by a central pattern generator (oviductal CPG, for a definition of CPG see Bässler, 1986). This rhythmic pattern can only be produced when the connectives between the seventh and terminal abdominal ganglia are intact, suggesting that the oviductal CPG network is spread in both genital ganglia (Kalogianni, 1991).

Rhythmic activation of DUM neurones correlated with behavioural motor patterns has

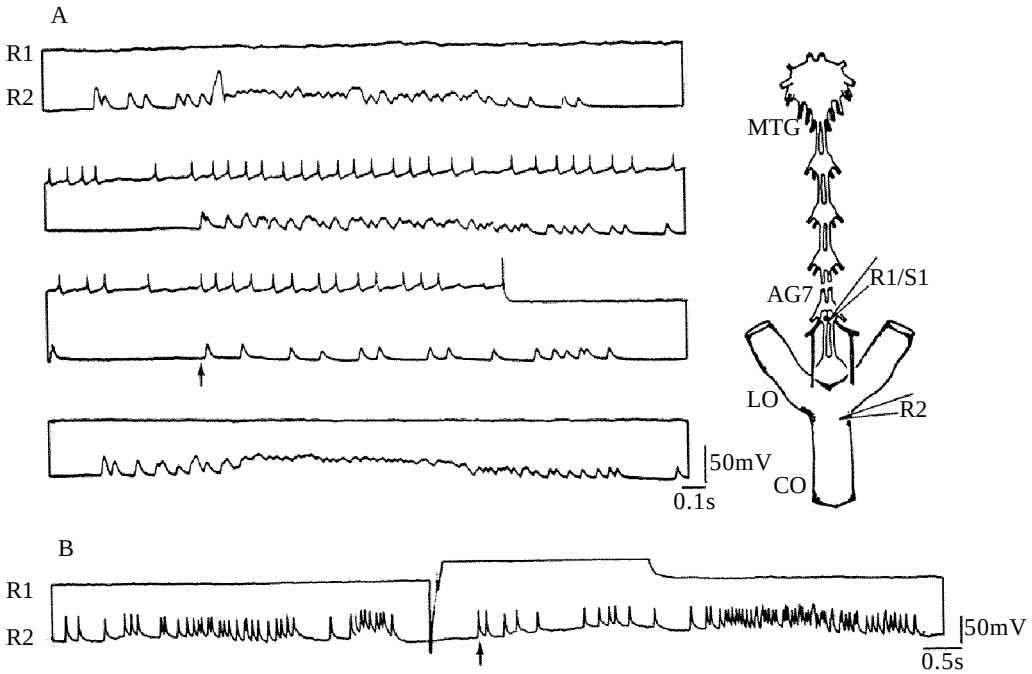

Fig. 8. (A,B) Intracellular suprathreshold stimulation of a single oviductal DUM neurone (upper trace, R1/S1) showing resultant slowing of EPSP intraburst frequency (arrows) recorded from an oviductal muscle fibre in a female with anteriorly isolated genital ganglia (see inset). A and B show the two extremes in the spectrum of the responses observed.

been reported in only a few studies. In the locust, some mesothoracic DUM neurones are active during flight and it has been suggested that they may modulate the forewing stretch receptor through the release of endogenous octopamine (Ramirez and Orchard, 1990). In the larva of the lepidopteran *Antheraea pernyi*, two identified abdominal DUM neurones (MC1, MC2) are active during phasic muscular activity and enhance skeletal muscle contractions (Brookes and Weevers, 1988; Brookes, 1988). This enhancing effect of DUM neurones consists mainly of an increase in the amplitude of the muscle fibres' excitatory junctional potentials and is probably mediated through the release of octopamine (Brookes, 1988).

Effects of oviductal DUM neurones

The effects of the DUM neurones on the oviductal system were examined using both antidromic and orthodromic stimulation of these neurones. In both cases, it was found that the DUM neurones cause a reduction in the amplitude and frequency of the EPSPs generated by the oviductal motor neurones. In the locust, a similar inhibitory effect on the amplitude of EPSPs was shown after application of octopamine (3.2×10^{-5}–1.7×10^{-4} mol l^{-1}) to the oviduct (Orchard and Lange, 1986). Thus, DUM neurones probably mediate this response by the peripheral release of octopamine from their terminals. Oviductal neurosecretory terminals have been detected by electron microscopy

(Kiss *et al.* 1984) and a dense network of octopamine-like immunoreactive fibres can be demonstrated immunocytochemically (E. Kalogianni and P. A. Stevenson, unpublished data). In the present study, we showed DUM neurones inhibiting the firing rate of the motor neurones. This effect cannot be attributed to a peripheral action of DUM neurones, so we suggest that the release of octopamine by oviductal DUM neurones in the CNS has a direct effect on the rhythm-generating circuit. Central effects of octopamine have been reported previously. Octopamine injected into specific regions of the thoracic ganglia initiates flight in locusts (Sombati and Hoyle, 1984; Stevenson and Kutsch, 1987) and moths (Claasen and Kammer, 1986). However, when octopamine is injected into the terminal abdominal ganglion, it suppresses oviposition digging behaviour (Sombati and Hoyle, 1984). This inhibition occurs approximately 10s after the onset of octopamine release, is dose-dependent and affects primarily the fast motor units (Sombati and Hoyle, 1984). The effects upon the EPSPs recorded from the oviducts after stimulation of the oviductal DUM neurones in this study are similar, supporting the hypothesis that DUM neurones could act at a central level through the release of octopamine.

The role of oviductal DUM neurones during oviposition behaviour

The oviductal and ovipositional digging patterns are functionally related during oviposition behaviour. It has been shown that the oviducts exhibit different modes of contractions during different stages of oviposition. During egg deposition the oviducts contract myogenically and the eggs are transferred to the ovipositor. At this stage only tonic motor activity can be recorded from the oviductal nerve (Lange *et al.* 1984). Our results also show that, at this stage, both oviductal DUM and motor neurones are tonically active. The rhythmic activation of the oviductal motor neurones is elicited during oviposition digging (the first stage of egg laying) and following interruption of egg laying, leading to neurogenic contractions of the junctional area of the oviducts, which propel the eggs back to the ovaries (Lange *et al.* 1984). At this stage, activation of the oviductal DUM neurones could serve to inhibit the myogenic oviductal contractions. Such an inhibition of myogenicity during electrical stimulation of the oviductal nerve has already been reported (Lange *et al*. 1984) and could readily be attributed to the peripheral release of octopamine from the oviductal DUM neurones, since they mainly project to the myogenically active parts of the oviducts, i.e. the lateral and the common oviduct. Thus, the DUM neurones seem to have a dual function: inhibition of the myogenic rhythm in the periphery and slowing of the oviductal motor pattern in the CNS. Both these effects, in cooperation with the oviductal motor neurones, could serve to inhibit egg laying.

A possible additional central function of the oviductal DUM neurones is inhibition of the oviductal motor rhythm. Our results suggest that in non-egg-laying females (in our experiments, females with the CNS intact) the oviductal CPG is under descending inhibition. At this stage the oviductal DUM neurones are tonically active at high frequency, suggesting that they may participate in the complete inhibition of the oviductal rhythm in non-egg-laying females. Sombati and Hoyle (1984) proposed that the inhibition of the ovipositional CPG in non-egg-laying females that derives from the thoracic and head ganglia (Thompson, 1986) may also be mediated by the local release of

octopamine. The present study demonstrates that the putatively octopaminergic DUM neurones have effects in the central nervous system.

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