J. exp. Biol. **148**, 313–324 (1990)
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THE MANDIBULAR GANGLION – A NEW PERIPHERAL GANGLION OF THE LOCUST

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Accepted 22 August 1989

Summary

Paired peripheral ganglia within the locust mandibular segment are described. Each mandibular ganglion contains the cell bodies of 22–25 neurones. Four of these are sensory neurones which innervate the receptor strand of one of the mandibular proprioceptors. The other neurones connect the suboesophageal ganglion with the tritocerebral lobes of the brain, and with the first ganglion of the stomatogastric nervous system, the frontal ganglion.

Introduction

In addition to the chain of segmental ganglia of the central nervous system (CNS), insects possess a stomatogastric nervous system which innervates the foregut. It consists of the unpaired frontal and hypocerebral ganglia and the paired paraventricular ganglia. It is connected with the central nervous system (CNS) *via* the frontal connectives, which link the frontal ganglion to both tritocerebral lobes of the supraoesophageal ganglion, or brain (see Fig. 1).

Nerve cells making connections with the frontal ganglion have been found chiefly in the brain, but there are also a few in the suboesophageal ganglion (Aubele and Klemm, 1977; Gundel and Penzlin, 1978; Kirby *et al.* 1984). Thus, both head ganglia appear to participate in the control of the stomatogastric nervous system.

In the course of a study of the peripheral nervous system of the locust suboesophageal ganglion (Bräunig, 1987), peculiar structures were found in association with one of the major branches of the mandibular nerve. As will be shown here, these structures are hitherto undescribed peripheral ganglia. They contain neurones which project into the suboesophageal ganglion, the tritocerebrum and the stomatogastric nervous system. They may therefore participate in the control of mouthpart and foregut function.

Materials and methods

Adult male and female locusts, *Locusta migratoria migratorioides* (R. & F.)

Key words: insects, stomatogastric ganglion, strand receptor, visceral nervous system.

were used for the present study. They were taken from the culture 2–4 days after the final moult, since at that stage fatty tissue around nerves and sense organs is not yet fully developed, greatly facilitating the preparation of delicate components of the peripheral nervous system.

Nerves were stained either by ionophoretically injecting cobalt chloride into the suboesophageal ganglion *via* a blunt microelectrode, as described previously (Bräunig, 1987), or by the conventional method of exposing single nerve stumps to the staining solution for anterograde or retrograde diffusion. In one set of experiments, the circumoesophageal connectives were stained differentially with cobalt and nickel salts (Quicke and Brace, 1979; Sakai and Yamaguchi, 1983). When necessary, stains within ganglia or small pieces of other tissues were intensified with silver, using the method for wholemounts (Bacon and Altman, 1977).

Since the backfilling method frequently fails to stain all cells of a given population, mandibular ganglia were also stained with Toluidine Blue (Altman and Bell, 1973) to obtain comparative data for cell body numbers. Two mandibular ganglia were embedded in plastic and serially sectioned at 2 μm.

Intracellular staining was carried out using microelectrodes filled with 0.1 mol l^{-1} hexamminecobaltic chloride (Brogan and Pitman, 1981) and having resistances between 80 and 120 MΩ. The dye was injected for 5–10 min, applying 2–3 nA depolarizing current pulses of 200 ms duration at a frequency of 2.5 Hz.

Nomenclature follows Snodgrass (1928) for muscles and skeletal structures, Altman and Kien (1979) for peripheral nerves of the suboesophageal ganglion and Honomichl (1978*a*,*b*) for mandibular mechanoreceptors.

Results

Location of the mandibular ganglion (Fig. 1)

The mandibular ganglion is located about 1 mm from the suboesophageal ganglion. It is intricately associated with the mandibular sensory complex, which consists of two muscle receptor organs (DMR, VMR; Honomichl, 1978*a*,*b*) and a strand receptor (SR; Honomichl, 1978*a*). Proximally, the ganglion is attached to the dorsolateral edge of the ventral branch of the mandibular nerve (nerve 1B), just where this nerve enters the mandibular hollow. The ganglion tapers towards its dorsal (distal) end, and from it emerge two flattened nerves (PRN, DRN) which connect the ganglion with the tendon of the strand receptor (SR). This tendon is suspended between the posterior margin of the anterior arm of the tentorium and the anterior face of the apodeme of the mandibular closer muscle (M9).

Cell classes of the mandibular ganglion

In cobalt-stained material, the mandibular ganglion appears as a group of 22–25 neuronal somata. They either form a cluster close to the lateral edge of nerve 1B, or are loosely dispersed along the length of the ganglion between nerve 1B and the origin of proximal (PRN) and distal (DRN) strand receptor nerves (Figs 2A and

3). The same number and distribution of cell bodies are observed after Toluidine Blue staining (Fig. 2B).

All cells within the mandibular ganglion are unipolar neurones with elongated somata. Their length ranges from 30 to 50 μm, and their width from 10 to 15 μm. They can be divided into two classes. (i) Four larger cells, located within the distal half of the ganglion, have distally directed neurites (Figs 2A and 3), which proceed into the strand receptor tendon *via* the distal receptor nerve (DRN). Within this tendon they establish a dense meshwork of dendritic ramifications, from which

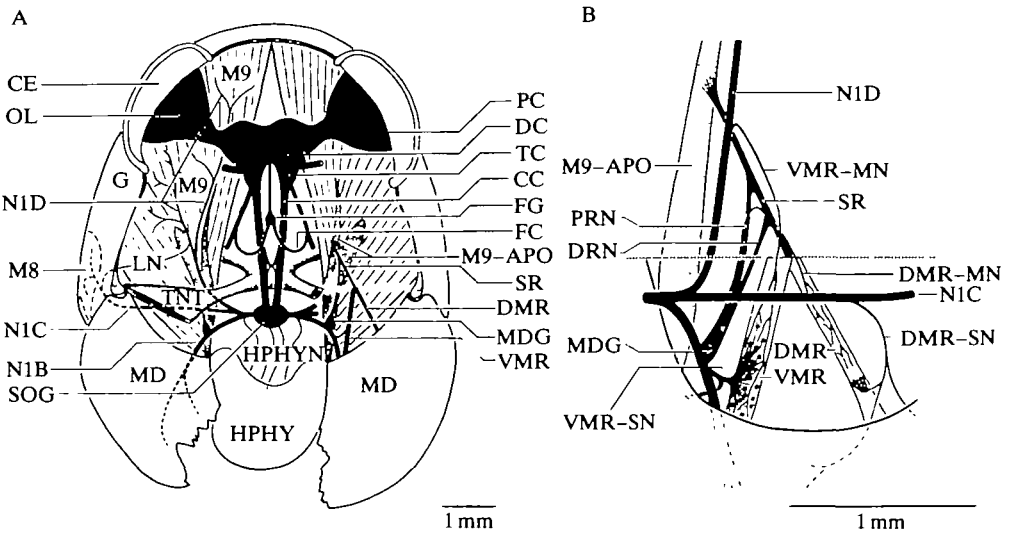

Fig. 1. Location and innervation of the mandibular ganglion. (A) Schematic frontal view of the locust head after removal of frons, labrum and pharynx. On the left are shown the major branches of the mandibular nerve (N1B, N1C, N1D), which innervate the mandible (MD), the mandibular opener (M8) and the closer muscle (M9). On the right, the anterior arm of the tentorium (TNT) as well as nerves 1C and 1D have been removed for an unobstructed view of the mandibular sensory complex, which consists of two muscle receptors (DMR, VMR), a strand receptor (SR) and the mandibular ganglion (MDG). The dotted line indicates the posterior rim of the removed tentorium, to which the sense organs are attached. The size of the mandibular ganglia is exaggerated to show their location in relation to suboesophageal (SOG), supraoesophageal (PC, DC, TC) and frontal (FG) ganglia. (B) The mandibular sensory complex. The mandibular ganglion is attached proximally to nerve 1B, the sensory nerve of the mandible. Distally, two flattened receptor nerves (PRN, DRN) connect the ganglion with the strand receptor (SR) between tentorium (dotted line, compare A) and closer apodeme (M9–APO). For reasons of clarity, the motor nerve (DMR–MN) of the dorsal muscle receptor (DMR) is shown as a separate nerve. In actual preparations, only the short section where it crosses the gap between the two receptor nerves can occasionally be seen. APO, apodeme; CC, circumoesophageal connectives; CE, compound eye; DC, deutocerebrum; FC, frontal connective; G, gena; HPHY, hypopharynx; HPHYN, hypopharyngeal nerves (nerves 1A and 2 of SOG); LN, link nerve between opener and closer motor nerves; MN, motor nerve; OL, optic lobe; PC, protocerebrum; SN, sensory nerve; TC, tritocerebrum; VMR, ventral muscle receptor.

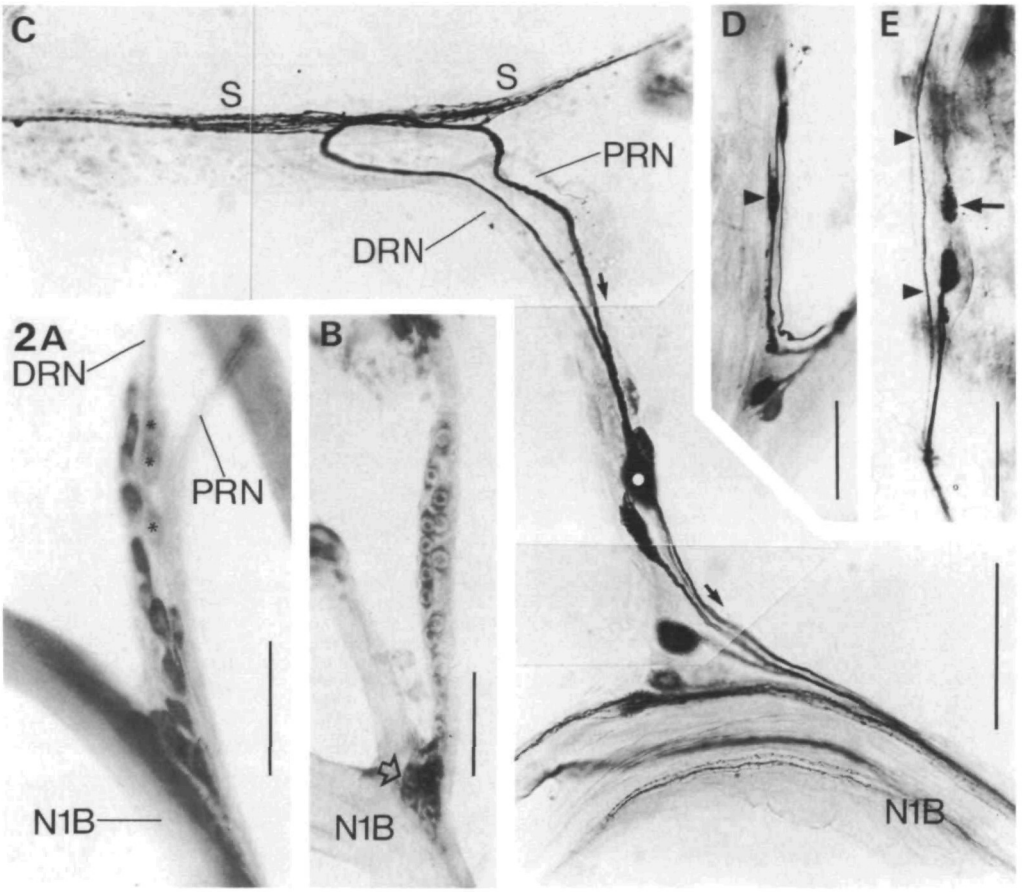

Fig. 2. Photomicrographs of mandibular ganglia (anterior views; A–D show left ganglia, E shows a right ganglion; D and E are from the same preparation; scale bars, 100 μm). (A) Wholemount of a mandibular ganglion showing the location of cells between nerve 1B (N1B) and the two strand receptor nerves (DRN, PRN). Three weakly stained SR cells are marked by asterisks (cobalt-stained, unintensified preparation; dorsal to the top, distal to the left; for more detail see Fig. 3). (B) The cell bodies of a mandibular ganglion stained with Toluidine Blue. Note proximal cluster of somata (arrow) close to the edge of mandibular nerve 1B (N1B). (C) Cells of the mandibular ganglion backfilled from the ipsilateral circumoesophageal connective (cobalt, silver-intensified). A single SR cell (the cell body, which is slightly out of focus, is marked with a white dot) and five MDG cells are labelled. The SR cell sends its neurite towards the receptor strand (S) within the distal receptor nerve (DRN). Its axon (arrows) returns in the proximal receptor nerve (PRN), runs across the cell body and proceeds towards the suboesophageal ganglion in nerve 1B (N1B). Note numerous dendritic branches in the receptor strand (S). (D, E) Backfills from the frontal connective (cobalt, silver-intensified). In the ganglion ipsilateral to the filled frontal connective (E) two MDG cells and one SR cells are stained (arrow marks SR cell body, arrowheads mark its axon). In the contralateral ganglion (D) five MDG cells are stained. One of them has a short, distally directed process (arrowhead, compare Fig. 3).

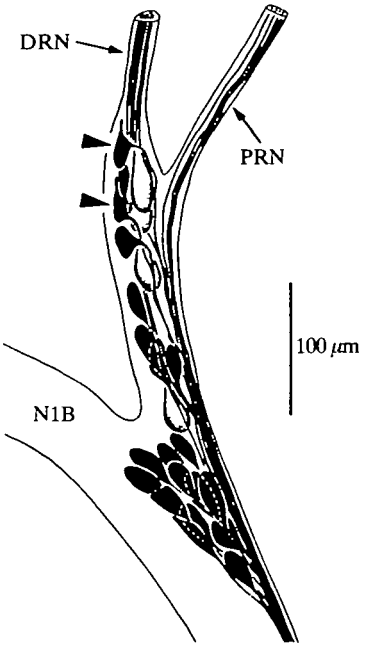

Fig. 3. *Camera lucida* drawing of the preparation shown in Fig. 2A. The MDG cells are shown filled, the SR cells in outline. One MDG cell obscuring the most proximal SR cell has been omitted. Note that MDG cells are clustered at the edge of nerve 1B (N1B) or dispersed along the length of the ganglion between this nerve and the two strand receptor nerves (DRN, PRN). Two MDG cells show short, distally directed processes (arrowheads).

their axons originate (Fig. 2C). These return towards the mandibular ganglion in the proximal receptor nerve (PRN), bypass other ganglion cells (Figs 2A,C,E and 3), and proceed towards the suboesophageal ganglion in nerve 1B. In the following, these neurones will be called strand receptor cells (SR cells). (ii) The second group of cells, named the mandibular ganglion cells (MDG cells), consists of about 20 neurones which are slightly smaller than the SR neurones (Fig. 2A). They usually stain more intensely because their neurites run directly to the suboesophageal ganglion (Fig. 3). Thus, their cell bodies are much closer to the site of dye application (injection of $CoCl_2$ into the mandibular neuromere of the suboesophageal ganglion) than the SR cell bodies. Two to three MDG cells, always located close to the distal end of the ganglion, have short, distally directed processes (Figs 2E and 3).

Central projections of the strand receptor cells

The central projections of the SR cells were stained selectively by introducing cobalt chloride into their dendritic tips in the distal end of the receptor tendon (Fig. 4, inset). A maximum of four axons enters the suboesophageal ganglion through the mandibular nerve. Their diameter appears to be rather large, but this

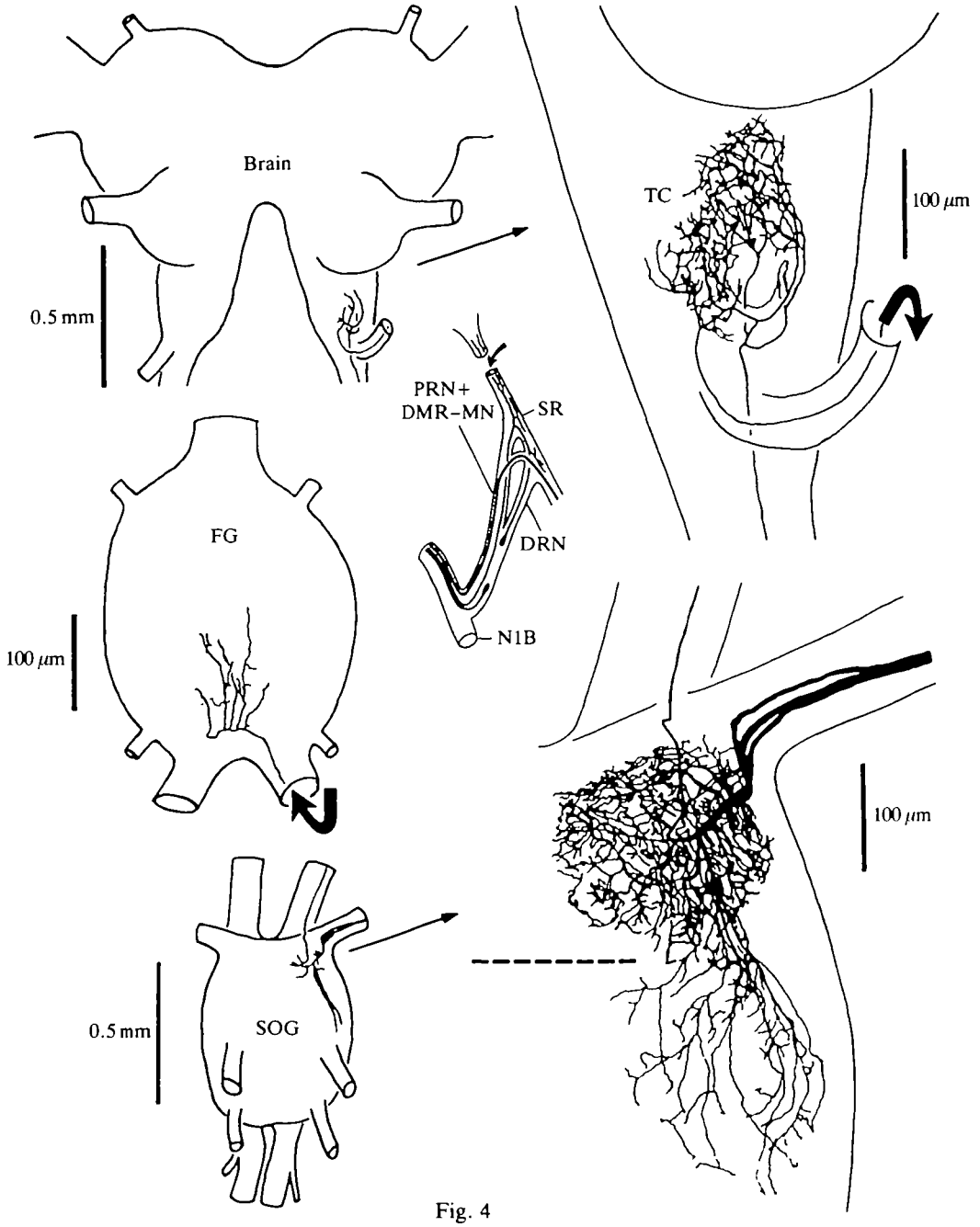

Fig. 4

is probably only the result of the prolonged silver intensification necessary to show the ramifications within the ganglion (Fig. 4, SOG). Profuse ramifications originate from the axons: they fill the entire ventral mandibular neuropile on the ipsilateral side, so that in wholemounts this neuropile appears to be cupped within a close-meshed basket. Two to three longer collaterals proceed lateroventrally

Fig. 4. Central projections of strand receptor cells within the suboesophageal ganglion (SOG), tritocerebral lobe (TC) of the brain and frontal ganglion (FG) (*camera lucida* drawings, ventral views, anterior to the top). The inset illustrates the technique employed to stain selectively the SR cells through their cut dendrites (curved arrow, compare Fig. 1B). The broken line indicates the border between mandibular and maxillary neuromeres of the SOG. The curved arrows indicate the continuity of the fibre which leaves the tritocerebrum and enters the frontal ganglion through the frontal connective.

into the maxillary neuromere. Their secondary ramifications are found along the lateral and ventral margin of the maxillary neuropile (Fig. 4, SOG).

One SR fibre makes a U-turn into the circumoesophageal connective. Upon reaching the tritocerebral lobe of the brain, it makes another U-turn into the labrofrontal nerve (Fig. 4, TC) and eventually terminates within the frontal ganglion (Fig. 4, FG). Within the tritocerebrum, there are lateral and ventral areas of ramifications, separated by a major trachea entering the brain.

Of 37 preparations, the tritocerebral ramifications were completely stained in only six cases; those in the frontal ganglion in only one case. This low success rate is due both to the difficult dissection and to the great distances between the ganglia (see Fig. 1).

Retrograde labelling of mandibular ganglion cells

Attempts were made to stain the single, intersegmentally projecting SR cell by introducing cobalt into its ascending axon either *via* the frontal or *via* the circumoesophageal connective. Surprisingly, in all preparations treated in this way, a variable number of MDG cells were stained together with the SR cell (Fig. 2C–E). These MDG cells appear not only within the mandibular ganglion ipsilateral to the filled connective but also in the contralateral ganglion (Fig. 2D, E). This indicates that MDG cells also possess axons which ascend towards the brain and/or the frontal ganglion within the ipsilateral or the contralateral connectives.

Intracellular staining of MDG cells

For morphological reasons, it is not possible to stain the central projections of MDG cells selectively with the backfilling method. Cutting the MDG and exposing the stump to cobalt stains the MDG cells together with the SR cells and the DMR motor neurone, the axon of which also runs within the proximal receptor nerve (Fig. 1B, and Fig. 4, inset). Such preparations, however, confirmed the above-mentioned results, in that they showed additional ascending fibres in both circumoesophageal connectives.

To investigate the central projections of MDG cells, attempts were made to penetrate individual cell bodies with microelectrodes. The success rate in these experiments was very low. The cells could not be held for more than a few minutes, a time sufficient only to stain their ramifications within the suboeso-

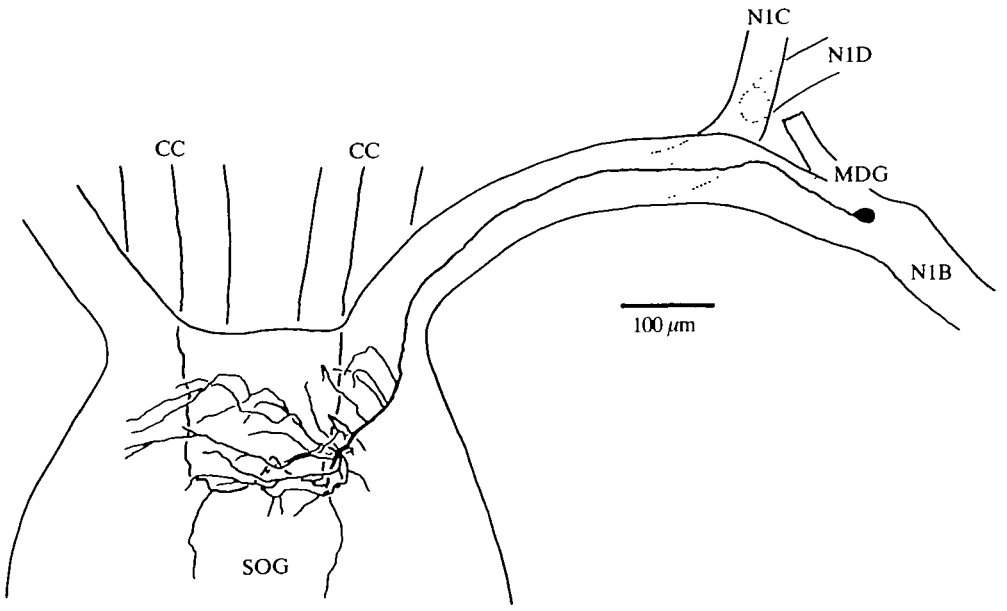

Fig. 5. The morphology of an MDG neurone revealed by intracellular dye injection. Note the axons in both circumoesophageal connectives (CC) and the extremely long neurite which connects the cell body in the mandibular ganglion (MDG) with the ramifications within the suboesophageal ganglion (SOG). Other labels as in Fig. 1.

phageal ganglion (Fig. 5), but not those within the tritocerebrum and frontal ganglion. Fig. 5 shows that individual MDG cells project into both ipsi- and contralateral mandibular neuromeres, crossing the sagittal plane of the central nervous system in many places. They also send ascending axons into both circumoesophageal connectives. These features were observed in three successful fills.

Attempts to stain the projections of individual SR cells with intracellular methods failed. Only their neurites within the distal receptor nerve (DRN, inset in Fig. 4) and a few dendritic branches within the receptor strand could be labelled.

Differential staining of circumoesophageal connectives

To estimate how many MDG cells send axons into both connectives, in one set of experiments one connective was stained with cobalt and the other with nickel. After incubation, mandibular ganglia were exposed to dithiooxamide (rubeanic acid). Precipitation of cobalt and nickel ions with this agent results in complexes of different colours (Quicke and Brace, 1979; Sakai and Yamaguchi, 1983): nickel stains blue, cobalt stains orange, and a mixture of both stains red or purplish. Accordingly, MDG cells with axons in only one connective ought to stain blue or orange, those with axons in both connectives ought to stain red.

Because of the inherent variability of the backfilling method, the number of stained cells in individual mandibular ganglia varied considerably. The total

number of stained cells ranged between five and 19 MDG cells (plus one SR cell). However, up to 10 MDG cells appeared red or purplish, indicating that at least half the MDG cells of one ganglion project into both connectives. Cells appearing orange or blue might indicate either that individual neurones failed to take up both dyes or the existence of MDG cells with only one ascending axon.

Discussion

A new visceral ganglion in the locust?

The mandibular ganglion fits a general definition of a ganglion such as that given in *Henderson's Dictionary of Biological Terms*: 'a mass of nerve cell bodies giving rise to nerve fibres'. In contrast to the segmental ganglia of the CNS and the ganglia of the stomatogastric nervous system, however, it contains only cell bodies; there is no neuropile. None of the SR or MDG cells stained by backfilling or by intracellular methods exhibited any side branches within the mandibular ganglion itself (Figs 2C–E, 5). Also, serial semithin sections of the ganglia revealed only somata and fibres of passage (neurites and axons of MDG and SR cells, and the motor axon of the DMR).

The neurones of the mandibular ganglia are also clearly different from the peripheral neurosecretory cells found in various insect species (reviewed by Myers and Evans, 1988). The latter are also located on peripheral nerves, their somata in some cases also form small clusters (Fifield and Finlayson, 1978; Garcia-Scheible and Honegger, 1989), but they show ramifications in the immediate vicinity of the cell bodies and appear to lack projections into the central nervous system.

Some previous studies of the peripheral nervous system in other insects make fleeting reference to a 'swelling of the mandibular nerve', a 'mandibular ganglion' or 'mandibular association center' (Haub, 1971; Marquardt, 1940; Rähle, 1970; Swaine, 1920; Zacharuk, 1962), but do not provide any details. Masuko (1986) uses the term 'ganglionic mass' for the sensory cells of mandibular mechanoreceptors of the honeybee. It is possible, therefore, that previous authors have seen the MDG cells together with the numerous multipolar sensory cells of the mandibular muscle receptors. In other insects, particularly small species, these are located even closer together than in locusts (beetle: Honomichl, 1978*a*,*b*; honeybee: Masuko, 1986). However, as discussed by Moulins (1971), nearby groups of hypopharyngeal sensory neurones have also been mistaken for ganglia. One has to be careful, therefore, with previous interpretations.

In embryos of various orthopteroid insect species, paired peripheral ganglia have been observed in the basal regions of the gnathal appendages (Neumann-Visscher, 1972). Their neurones appear to originate from neuroblasts of the CNS. Stem cells, which do not belong to the segmental sets of neuroblasts that establish the three gnathal ganglionic primordia, have also been observed in locust embryos (Doe and Goodman, 1985). These observations suggest that the neurones of the mandibular ganglia are of central rather than peripheral origin.

The MDG cells and one of the SR cells (Fig. 4) connect the suboesophageal

ganglion with the brain. At least seven MDG cells and the SR cell proceed further into the frontal ganglion (Fig. 2D, E). Thus the neurones connect those neural centres that control the function of the mouthparts and foregut. This suggests that the neurones of the mandibular ganglia are elements of the network involved in the control of feeding. They might participate in the coordination of biting and salivation (suboesophageal ganglion) and swallowing (tritocerebrum and stomatogastric ganglia). In this sense the mandibular ganglion may be regarded as an additional stomatogastric ganglion.

Strand receptor cells

The mandibular strand receptor was first described in a coleopteran insect (Honomichl, 1978*a*). Honomichl noted the close proximity of the SR cells to the numerous multipolar sensory neurones of the ventral muscle receptor (VMR, Fig. 1B). However, the peculiar morphology of the SR cells was not completely demonstrated, nor were any cells mentioned that might correspond to the locust MDG cells.

It is difficult to compare the mandibular strand receptor with those described for locust legs and antennae (Bräunig and Hustert, 1980; Bräunig, 1985*a*,*b*; Pflüger and Burrows, 1987). These have their cell bodies within thoracic ganglia or the brain and do not project to other ganglia, as at least one mandibular SR cell does. Like strand receptors in the thorax (Bräunig, 1985*b*; Bräunig and Hustert, 1980, 1985) and in the antennae (Bräunig, 1985*a*), the mandibular strand receptor is a mechanoreceptor which responds to elongation of its receptor strand. Extracellular recordings from the proximal receptor nerve (P. Bräunig, unpublished results) demonstrate units responding to either direct elongation of the strand or elongation caused by upward movement of the closer muscle apodeme. This supports Honomichl's (1978*a*) proposed function of the receptor: as a sensor for adduction of the mandible.

Mandibular ganglion cells

The function of the MDG cells remains totally obscure at present in contrast to that of the SR cells. Their cell bodies, for some unknown reason, have been shifted away from the suboesophageal ganglion to form a separate peripheral ganglion together with the SR cells. This displacement of the somata gives rise to the most striking feature of the neurones contained within the mandibular ganglia: the extraordinary length of their neurites (the processes connecting the soma with the nearest dendritic or integrative segment). The MDG cell bodies are 0.7–0.9 mm from the CNS, the SR cell bodies are 0.3–0.4 mm from the SR tendon. Accordingly, the neurites of both cell types are probably the longest so far encountered within an insect nervous system.

One consequence of this peculiar morphology is that the cell bodies are electrically completely silent. During the intracellular studies, an initial potential drop occurred upon penetration of a cell, but no synaptic potentials or spikes passively invading the cell bodies were observed. To investigate the function of

MDG cells, future studies will have to rely on intracellular recordings from their integrative segments within the suboesophageal ganglion. In view of the small diameters of these processes, this will not be an easy task.

I wish to thank Christine Böhme and Ulrike Paulus for technical assistance. I also wish to thank G. A. Manley for helpful comments on the manuscript and for correcting the English. Finally many thanks go to two unknown referees for their criticism and their extraordinary speed! Supported by Deutsche Forschungsgemeinschaft (Br 882).

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