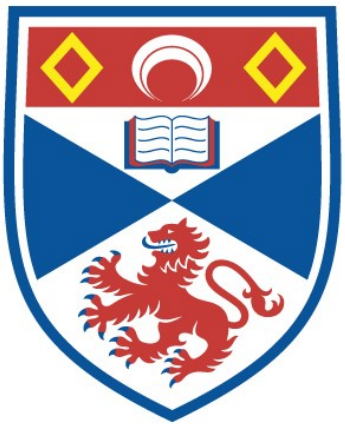
THE COMMISSURAL GANGLIA OF THE LOBSTER, HOMARUS GAMMARUS, (L)

S. M. Martin

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Abstract

The bilaterally paired commissural ganglia of the lobster, Homarus gammarus, form part of the stomatogastric nervous system. The functional organization of these ganglia has been investigated using a variety of anatomical and physiological techniques.

There are no consistent differences between right and left commissural ganglia. Each contains the somata of several hundred interneurons and about sixty motorneurons which supply the labrum and oesophagus. There are many input and output pathways to and from the commissural ganglia, including a large number of sensory fibres. There appears to be much interaction between these ganglia and the central and stomatogastric nervous systems.

A comparison of the in vitro preparation and previous studies of the semi-intact animal have shown that the isolated commissural ganglion contains at least the minimal pattern generator for oesophageal peristalsis. The isolated ganglion can also produce other rhythms, including a putative labral rhythm.

The small size of most commissural ganglion neurons precludes intracellular recording and dye injection. Most "recordable" somata are silent. The activity of several neurons, including labral motorneurons, is closely related to the oesophageal rhythm. At least one neuron exhibits rhythmic activity unrelated to the oesophageal cycle. Other neurons show a tonic firing pattern; their possible functions are discussed.

A large dopamine-containing neuron sends a corollary discharge of foregut activity to at least the brain. The structure and function of this neuron is discussed in detail.

This study suggests that the commissural ganglia have the integratory potential to act as coordination centres for the foregut. The findings are considered in the context of the operation of the stomatogastric nervous system and suggestions are made for further research.

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by S.M. Martin

A thesis presented for the degree of Doctor of Philosophy

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SUPERVISOR'S CERTIFICATE

I certify that S.M. Martin has fulfilled the conditions laid down under Ordinance General No. 12 and Resolution of the University Court 1967, No. 1, of the University of St. Andrews and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

DECLARATION

I declare that the work reported in this thesis is my own and has not been submitted for any other degree.

CURRICULUM VITAE

I graduated from Bedford College, University of London in 1976 with a first class honours degree in zoology. The work described in this thesis was carried out between October, 1977 and September, 1980.

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Chapter 1 : General Introduction

a. Why we study lobsters - the concept of "simple" nervous systems

KROGH's principle states (sic) "For many problems there is an animal on which it can be most conveniently studied" (KREBS, 1975). If an animal is to be the subject of protracted research it must fulfil certain requirements : it must be fairly abundant in the appropriate locality, survive under laboratory conditions, be easily handled and be resilient to experimental procedures. Many representatives of invertebrate groups, such as molluscs and arthropods, meet these requirements. However, their widespread use as objects of neurobiological research reflects a less pragmatic motive. Understanding the function of nervous systems involves analysis of the cellular properties and activities of individual elements, the neurons, and of the way they interact. Most nervous systems, for example in "higher" vertebrates, contain very great numbers of neurons with widely varying properties and such an approach is difficult. Numerically simpler systems provide models for systems of greater organizational complexity. Many invertebrate nervous systems and their component ganglia furnish examples of such "simple" nervous systems and are amenable to analysis with current technical and conceptual skills. So, in studying invertebrate nervous systems we are not simply asking "How does this animal work ?" but are addressing the broader question "How do nervous systems work ?". In other words by studying "simple" nervous systems we hope not only to understand important differences in nervous organization between different animal groups, for example between invertebrates and vertebrates, but to elucidate principles applicable to nervous systems in general.

Amongst the invertebrates arthropods have featured markedly in investigations of the functional organization of nervous systems (KANDEL and KUPFERMANN, 1970). In particular, insects such as locusts and cockroaches and "higher" crustaceans such as crabs, lobsters and crayfish

have provided favourable preparations. Their merits include the accessibility of the nervous system, which can be investigated in situ or in vitro, the paucity and often large size of the neurons and the distribution of neuron somata on the outside of the ganglionic neuropile. These last features enable maps of neuron somata to be constructed (COHEN and JACKLET, 1967; MAYNARD, 1972; KENNEDY and DAVIS, 1977) and facilitate intracellular recording and dye injection in single identified neurons. Such techniques can be applied to the nervous systems of most animal groups, for example intracellular recordings can be made from single neurons in the the vertebrate CNS. However, only in invertebrates such as molluscs and arthropods can individual neurons be consistently and uniquely characterised in terms of their membrane properties, synaptic physiology and neuronal geometry. There is often a marked uniformity in ganglionic architecture and neuron distribution amongst individuals of the same species (OTSUKA, KRAVITZ and POTTER, 1967). Furthermore, arthropod preparations exhibit a range of complexity. This may not only in itself enhance our understanding but may allow the selection of a preparation appropriate to a particular investigation. Studying a few specific systems in depth is germane to a comparative understanding of nervous systems.

b. Rhythmic behaviour and pattern generation

Rhythmic behaviour features in many aspects of an animal's life, for example in respiration, feeding, locomotory behaviours such as walking, swimming and flying, courtship and mating. The neural basis of such behaviours is studied not simply because of their biological importance and widespread occurrence but for the practical reason that they generate large samples of data for analysis.

Central to modern invertebrate neurobiology is the concept that part

of the nervous system contains the "programme" for a particular behavioral act, which is dependent on the properties of the component neurons and their connectivity pattern. The programme may be started, stopped or modified but not structured by peripheral input or "higher-level" control. This neuronal ensemble constitutes the "pattern generator" or "motor tape" (HOYLE, 1964).

The classic work of WILSON (1961) demonstrated that flight in the locust is centrally programmed in the thoracic ganglia. Similarly, the work of WILLOWS and his co-workers on the swimming escape response of the nudibranch mollusc Tritonia (WILLOWS, 1967; DORSETT, WILLOWS and HOYLE, 1973) showed that the patterned activity responsible for this behaviour is generated by a group of cells in the brain which could produce this programme even when the brain was completely isolated. Since then many examples of pattern generation by the central nervous system have been demonstrated in both invertebrates and vertebrates. For example, central patterning is involved in vertebrate and invertebrate respiration (WYMAN, 1977; YOUNG, 1975; BURROWS, 1974) and locomotion (DELONG, 1971; GRILLNER, 1975; STEIN, 1978).

An important question in considering a coordinated behaviour is how peripheral feedback and central patterning are integrated in its production. Such sensory input may have a tonic excitatory or inhibitory effect or a phasic (timing) influence. The importance of phasic input is becoming increasingly recognised. For example, WILSON (1961; 1964) considered that in the locust phasic input from the wing hinge proprioceptors was averaged out to exert a tonic excitatory effect on the central pattern generator for flight. However, WENDLER (1974) later showed by artificially driving a wing that this receptor input can phasically entrain the flight rhythm, increasing the precision of wing coordination. Recent research has elucidated the complex effects and

interactions of input from other wing sense organs (KIEN and ALTMAN, 1979). In extreme cases continuous sensory feedback may be necessary to generate behaviour, for example in scallop swimming which may be considered as a sequence of individual reflex acts (MELLON, 1969). However, it is generally accepted that most, if not all, rhythmic behaviours involve some degree of central patterning and current research concerns the mechanisms of pattern generation, its modulation by peripheral feedback and higher-level centres and how it affects and is affected by the operation of other parts of the nervous system.

c. Oscillators

The basis of centrally-generated rhythmic behaviour resides in neural oscillators. These are represented by two conceptual extremes : single cell oscillators and connectivity oscillators.

1. Single cell oscillators show endogenous rhythmicity, their membrane potential oscillating regularly. Membrane potential oscillations can be produced by regular fluctuations in the activity of an electrogenic pump or in ionic permeability. A current model for neuronal oscillatory activity proposes cyclic variations between an inward (depolarising) and outward (hyperpolarising) current dependent on several ionic channels (BERRIDGE and RAPP, 1979). Endogenous oscillators may be of two types :-

(i) Spiking oscillator cells produce bursts of action potentials on their depolarising phase. There are many examples of endogenous bursting neurons in invertebrates, for instance the parabolic burster of Aplysia (STRUMWASSER, 1967).

(ii) The membrane potential of non-spiking oscillator cells fluctuates sinusoidally without the production of action potentials. MENDELSON

(1971) claimed that two oscillatory interneurons in the suboesophageal ganglion of the lobster and hermit crab caused spiking in antagonist scaphognathite motorneurons in their depolarising or hyperpolarising phases without spiking themselves. PEARSON and FOURTNER (1975) implicate such a non-spiking oscillatory interneuron in levator activity during cockroach walking.

In few cases have the single oscillator neurons been isolated and shown to exhibit endogenous bursting activity. For example, the parabolic burster (R15) of Aplysia continues to exhibit cyclic bursting on ligaturing the soma, functionally isolating it from synaptic input (ALVING, 1968). This implies that the oscillation in the membrane potential of R15 is basically endogenous. However, the membrane potential of a bursting neuron may oscillate not because of its intrinsic properties but because it receives cyclic synaptic input. Furthermore, non-oscillating neurons can be converted to oscillators and vice versa, for example by the application of catecholamines or the manipulation of the calcium level (CHALAZONITIS, 1977). For instance, IFSHIN, GAINER and BARKER (1975) found that a peptide factor extracted from the circumoesophageal ganglia of the molluscs Otala lactea and Aplysia californica induced or enhanced bursting pacemaker potentials in an identified neurosecretory cell in O. lactea. The importance of single cell oscillators may have been overemphasised (DAVIS, 1976).

2. Connectivity oscillators comprise two or more neurons. Patterned output arises from their individual properties and from their synaptic relationships. Connectivity oscillators may be of two types :-

(i) In electrotonically coupled oscillators a general depolarisation of a group of neurons connected by non-rectifying electrotonic synapses leads to near-synchronous firing through positive feedback. When exact synchrony

occurs the burst is terminated by accentuation and prolongation of the spike after-hyperpolarisation. Hence self-limiting bursts are produced Such a neuronal network has been described for the trigger-group cells in Tritonia (GETTING and WILLOWS, 1974) and for the cyberchron network controlling the feeding behaviour of the pulmonate mollusc Helisoma trivolvis (KATER, 1974).

(ii) In resonating circuit oscillators or network connectivity oscillators when a certain level of excitation is reached the interaction of the neurons induces the network to "resonate" at a particular burst frequency. A simple model for such a network proposes two neurons, or groups of synergistic neurons, with reciprocal inhibitory connections. PERKEL and MULLONEY (1974) showed by computer modelling studies that in such a system if the neurons exhibit non-linear properties such as post-inhibitory rebound (or presumably habituation of inhibitory input) then a stable, alternating burst pattern can be produced. WILSON (1966) proposed a model of the locust flight system involving two pairs of pacemakers coupled by reciprocal inhibition and crossed excitation. The swimming escape response in Tritonia involves alternating bursts of impulses in functional antagonists associated with reciprocal inhibition between the two networks. Regenerative feedback in part maintains the system in an excited state after initiation of the behaviour. Swimming is terminated by active inhibition of certain neurons (WILLOWS, DORSETT and HOYLE, 1973; DORSETT, WILLOWS and HOYLE, 1973). In contrast, DAVIS, SIEGLER and MPITSOS (1973) proposed that the coupling of antagonistic bursts in the feeding system of Pleurobranchaea is mediated by long-delay unilateral excitatory connections. Post-inhibitory rebound is also implicated (SIEGLER, MPITSOS and DAVIS, 1974). In this system the feeding rhythm is generated by at least three neuronal oscillators which are functionally coupled by coordinating "efference copy" interneurons. Segmentally distributed

oscillators linked by coordinating fibres are involved in many locomotory systems (STEIN, 1978) (see part d below). Additionally, a single oscillator may be distributed into more than one ganglion as in the proposed leech swimming oscillatory network (KRISTAN, STENT and ORT, 1974; FRIESEN, POON and STENT, 1978). The central patterning of leech swimming also demonstrates another principle of network connectivity oscillators : recurrent cyclic inhibition (FRIESEN et al., 1978). A ring comprising an odd number of neurons, each neuron inhibiting the next in the chain, can produce oscillatory activity even in the absence of non-linear phenomena such as post-inhibitory rebound. Reciprocal inhibition is also involved in the leech swimming pattern generator (FRIESEN et al., 1978) emphasising the critical role of synaptic inhibition in rhythm generation and phasing.

d. The control of pattern generators

An important advance in invertebrate neurobiology was the concept and subsequent study of command fibres. Our understanding stems chiefly from work on crustacean systems initiated by WIERSMA and his colleagues (for example WIERSMA and IKEDA, 1964; ATWOOD and WIERSMA, 1967). The action of command fibres has been demonstrated or inferred in other animal groups such as insects (MILLER, 1971), molluscs (GILLETTE, KOVAC and DAVIS, 1978) and vertebrates (GRILLNER, 1975). This topic has been reviewed extensively (BOWERMAN and LARIMER, 1976; LARIMER, 1976; KUPFERMANN and WEISS, 1978).

BOWERMAN and LARIMER (1976) defined command neurons as identified cells releasing organized segments of behaviour. This operational definition includes intersegmental sensory neurons but it is generally assumed that command fibres are descending interneurons controlling local,

autonomous motor networks (DAVIS, 1976). Command fibres may receive direct sensory input or peripheral information, often multimodal, via an hierachical arrangement of interneurons (LARIMER, 1976) putting them in a "decision-making" role with regard to the evocation of a behaviour by sensory input. Command fibres generally synapse onto central pattern generators to turn them on or off but in some cases they may form bypass connections directly with motorneurons (KENNEDY and DAVIS, 1977). Single command fibres can evoke behaviours such as the defence posture and the tail-flip escape response in the crayfish Procambarus clarkii (BOWERMAN and LARIMER, 1974a and b) but generally motor systems are controlled by more than one command neuron acting in concert (DAVIS and KENNEDY, 1972; BOWERMAN and LARIMER, 1974a). For example, about fifteen command fibres act on the scaphognathite and heart rhythms of the crab, Cancer magister (WILKENS, WILKENS and McMAHON, 1974). Most of these fibres are bivalent, controlling both heart and scaphognathite systems. Studies on swimmeret beating in the lobster, Homarus americanus, (DAVIS and KENNEDY, 1972) demonstrated that each command neuron in this system controls a part of the range of output period (range fractionation). Command neurons may exhibit adaptation, a threshold frequency and a frequency-dependent output (BOWERMAN and LARIMER, 1976). In extreme cases the evoked behaviour may change radically with different frequencies of stimulation (ATWOOD and WIERSMA, 1967). In addition, a distinction may be drawn between trigger command neurons which elicit motor sequences that last longer than the stimulus, for example those elicited by a single impulse in the command fibre and gate command neurons which must be continually activated for the continued expression of the evoked behaviour (STEIN, 1978).

An important qualification of the command neuron concept is that in most cases it remains to be demonstrated that command fibres represent the normal pathway by which a behaviour is evoked. Moreover, the

organizational complexity of systems involving command neurons has usually precluded detailed analysis; for example little is known of possible feedback mechanisms from the motor level to the command neurons (DAVIS, 1976). However, systems in which such feedback occurs have recently been demonstrated (GILLETTE and DAVIS, 1977 ; GILLETTE, KOVAC and DAVIS, 1978). KUPFERMANNand WEISS (1978) critically assessed these and other limitations of the concept of command neurons. They proposed stringent criteria for defining command fibres as both necessary and sufficient in evoking a behaviour. They distinguish between "command elements" which can act individually, a "command system" comprising a group of neurons which are effective only in concert and "modulatory elements" which modify but do not elicit a behaviour. KUPFERMANNand WEISS point out that few known candidates may satisfy these criteria for "command neurons".

Coordinating neurons may provide coupling between parts of a distributed oscillator system (see part c above), for example in the segmental coordination of crayfish swimmeret movements (STEIN, 1974). Interneurons driven by one local centre may carry an "efference copy" of its activity to another to effect coordination (STEIN, 1978). DAVIS (1976) suggests that coordinating neurons may also play a significant role in the control of non-rhythmical behaviour, for example in the suppression of one motor act during the execution of another.

"Oscillator", "command neuron" and "coordinating neuron" are terms of convenience and do not reflect an absolute functional division. This is illustrated by the work of GILLETTE and DAVIS (1977) on the role of the 5HT-containing metacerebral giant neuron (MCG) in the feeding behaviour of the gastropod mollusc Pleurobranchaea. MCG acts as a command neuron on the feeding rhythm. It receives synaptic input from other neurons in the feeding network. These inputs are accentuated by the intrinsic biophysical

properties of the cell membrane (anomalous rectification and post-spike conductance increase) causing its membrane potential to oscillate in phase with the feeding rhythm, which may contribute to network oscillation. It also receives input from a population of corollary discharge interneurons. It can thus sample the behaviour that it drives. Furthermore, it may itself act as a coordinating neuron by corollary discharge and its many peripheral branches may fulfill a sensory or motor function. GILLETTE, KOVAC and DAVIS (1978) described a similar feedback system for the paracerebral "command neurons" in Pleurobranchaea which are connected to other neurons in the motor network by a positive feedback loop. The functional redundancy implied by the reciprocity in such systems suggests that the command role of a neuron may derive from its special access to the sensory input which drives the behaviour. The complexity of these systems underlines the inadequacy of the traditional concepts of command-oscillator-motor neuronal hierachy (DAVIS, 1976).

e. The stomatogastric nervous system

The passage of food through the gut of decapod crustaceans involves a series of rhythmic activities : the action of the mandibles (WALES, MACMILLAN and LAVERACK, 1976), the labrum (ROBERTSON, 1978; ROBERTSON and LAVERACK, 1979), the oesophagus (SPIRITO, 1975; this thesis), the cardiac sac (VEDEL and MOULINS, 1977; MOULINS and VEDEL, 1977), the gastric mill (MULLONEY and SELVERSTON, 1974a and b; SELVERSTON and MULLONEY, 1974), the pyloric filter (MAYNARD and SELVERSTON, 1975) and the hindgut (WINLOW and LAVERACK, 1972a, b and c; MURAMOTO, 1977). Studies of the neuronal mechanisms underlying these rhythmic behaviours have provided much information concerning pattern generation and its control.

MAYNARD and SELVERSTON and their co-workers pioneered work on the stomatogastric system (MAYNARD, 1966 and 1972; SELVERSTON, 1974; SELVERSTON, RUSSELL, MILLER and KING, 1976). In these early studies the stomach was conceptualised as an "internalised appendage" under the control of the stomatogastric ganglion, which was considered to comprise restricted neural networks controlling the well-defined behaviours of the gastric mill and the pyloric filter. The neuronal bases of the gastric and pyloric rhythms are now largely elucidated and the mechanisms underlying the other gut rhythms are beginning to be understood. As knowledge increases it is clear that the early concepts of the stomatogastric nervous system are inadequate. Networks for pattern generation are not restricted to one part of the nervous system but are distributed into more than one centre. The modulation and interaction of the various networks exhibit a complexity and subtlety that could not be envisaged in early studies (see Chapter 3 Introduction parts d and e for a detailed review).

One fact that has emerged clearly is the intimate involvement of the commissural ganglia in the production, modulation and integration of foregut rhythms. These ganglia are involved in the production of the oesophageal rhythm, in the control of the gastric and pyloric rhythms and in integrating sensory information from the foregut. However, despite their importance the commissural ganglia have been little studied.

f. Objects of research

The general object of this thesis was to make an initial study of the functional organization of the commissural ganglia. When this work was started there was very little anatomical information available apart from the classic work of ORLOV (1929). Since then a few anatomical and histochemical studies have been done (KUSHNER, 1979; KUSHNER and MAYNARD,

1977) but these do not provide a comprehensive picture. The first aim of the work was to investigate the number and nature of commissural ganglion neurons, to relate them to the input and output pathways of the ganglion and to examine evidence for the interaction of the different parts of the nervous system. For example, little was known of possible coordinating pathways between the labrum and the oesophagus, of pathways between the commissural and stomatogastric ganglia and of the interaction of the commissural ganglia with higher nervous centres such as the brain and the suboesophageal ganglion. Such pathways may be of fundamental importance in the expression and modulation of gut rhythms in the context of the animal's behaviour.

Similarly, when this work was started very few physiological studies had been carried out on the commissural ganglia. Intracellular studies had been briefly executed for a few commissural ganglion cells : the L cell, the E neuron and the P cells (RUSSELL, unpublished, in SELVERSTON et al., 1976 : see Chapter 3 Introduction part e). The interaction of commissural ganglion neurons with the stomatogastric pattern generators had been inferred by recording intracellularly from stomatogastric neurons and extracellularly from nerves linking the ganglia (RUSSELL, 1976 and 1977). Since then further inference has been made concerning the effects of commissural ganglion input on the action of stomatogastric neurons (RUSSELL and HARTLINE, 1978; RUSSELL, 1979) but only very recently have two other commissural ganglion cells been physiologically characterised (ROBERTSON and MOULINS, unpublished a,b,d and e). The aim of this part of the study was an initial physiological investigation of commissural ganglion cells and a determination of their structure using intracellular dye injection techniques, which had not previously been done for any commissural ganglion neuron. In particular, the production of the oesophageal rhythm was investigated to assess the output of the <u>in vitro</u>

isolated commissural ganglion compared with the *in vivo* situation previously described (ROBERTSON, 1978), to search for intracellular correlates of this rhythm in the commissural ganglion and to see if any deductions could be made concerning the nature of the oesophageal pattern generator.

As the second part of the work progressed it became clear that a particular commissural ganglion neuron, the L cell, was suitable for a more protracted study. Physiological, anatomical and histochemical investigations of this cell were carried out in an attempt to understand its coordinating role in the stomatogastric nervous system.

Chapter 2 : Anatomy

Introduction

a. The foregut nervous system

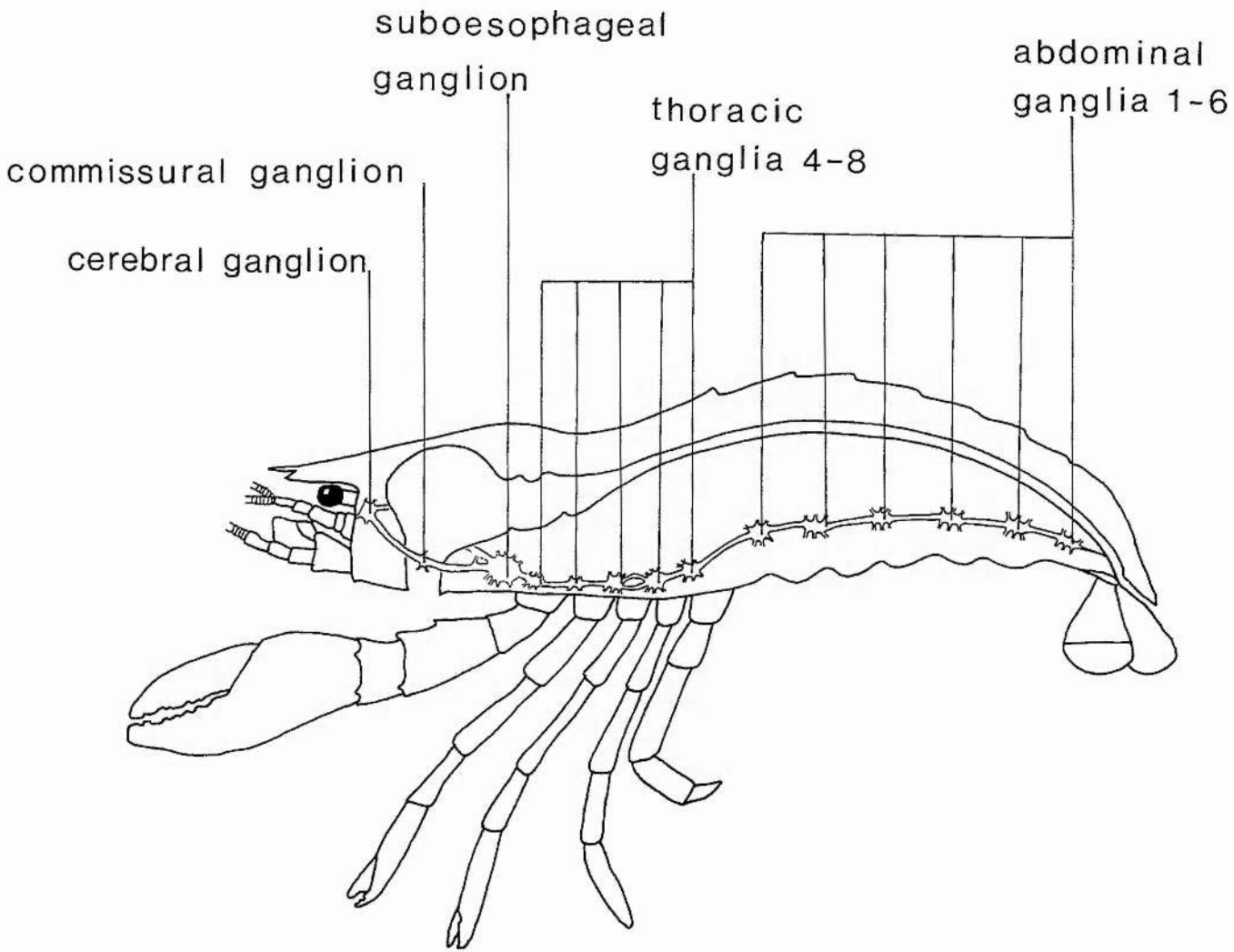
The layout of the commissural ganglion nerves and their contribution to the innervation of the oesophagus and the stomach has been described in varying detail for many species of decapod Crustacea including Homarus gammarus (H. vulgaris) (ALLEN, 1894b; MOCQUARD, 1883; POLICE, 1908 and ROBERTSON, 1978), Homarus americanus, Callinectes sapidus and Panulirus argus (MAYNARD and DANDO, 1974), Cancer pagurus (PEARSON, 1908), Jasus lalandii (PATERSON, 1968), Astacus fluviatilis (Potamobius astacus) (LEMOINE, 1868; KEIM, 1915 and ORLOV, 1929), Galathea squamifera (PIKE, 1947), Eupagurus bernhardus (JACKSON, 1913) and Pugettia producta (HEATH, 1941). Both MOCQUARD (1883) and POLICE (1908) describe and compare the stomatogastric nervous system in many species. In addition brief descriptions appear in most relevant papers, for example in MOULINS and VEDEL (1977) and in KUSHNER (1979). The most comprehensive and accurate description of the commissural ganglion, oesophageal and labral innervation of Homarus gammarus is given by ROBERTSON (1978) and was used for reference throughout this work.

b. Review of the commissural ganglion nerves

The commissural ganglia separate from the brain during embryological development and move posteriorly (ALLEN, 1894a). In the adult they are situated ventrally on the circumoesophageal connectives which run each side of the oesophagus to connect the cerebral and suboesophageal ganglia (Figure 2.1). A short tract, the post-oesophageal commissure, situated

Figure 2.1

Diagram of the lobster showing the position of the commissural ganglion in relation to the rest of the nervous system and to the gut. For clarity the oesophageal and stomatogastric ganglia and their nerves are not shown.


suboesophageal
ganglion
abdominal
ganglia 1-6
thoracic
ganglia 4-8
commissural ganglion
cerebral ganglion

posterior to the oesophagus and anterior to the suboesophageal ganglion connects the two commissural ganglia (see part c below). The ganglia each give rise to several nerve trunks (Figure 2.2) :-

1. Superior oesophageal nerve

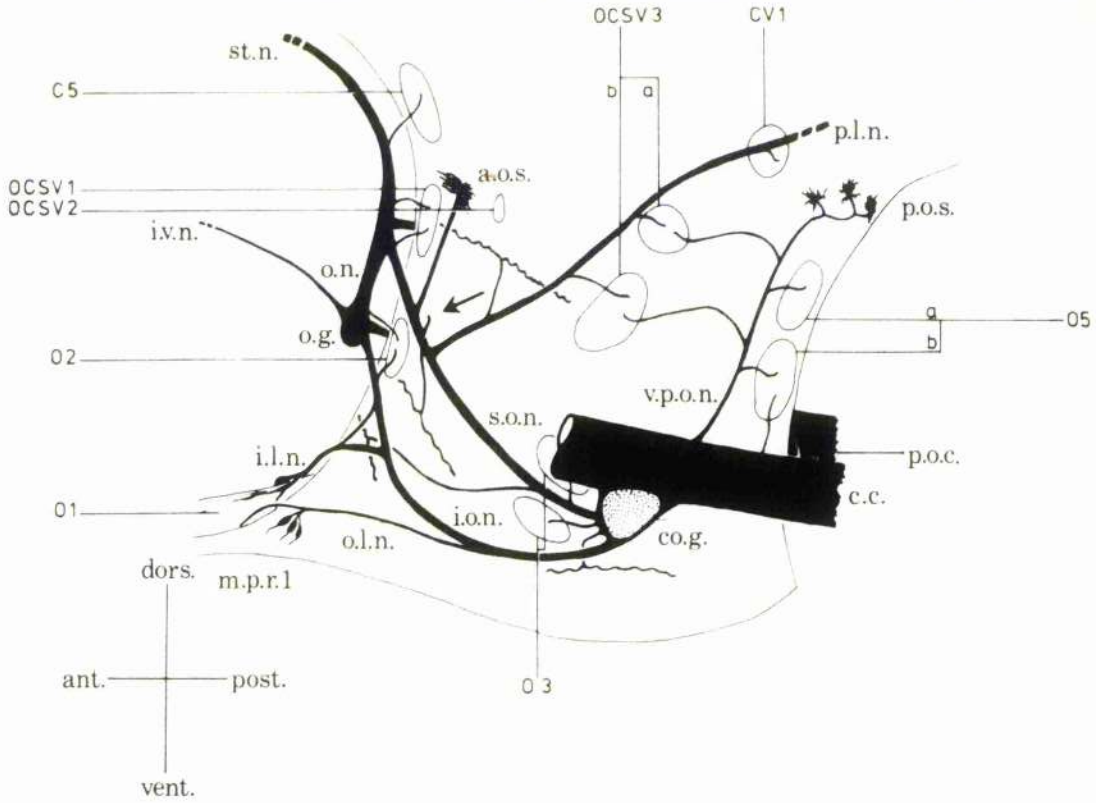
Each superior oesophageal nerve exits anteriorly from the commissural ganglion, passing through the lateral oesophageal dilator muscle (O3). The superior oesophageal nerves join in the midline at the junction of the oesophageal nerve and the stomatogastric nerve. The superior oesophageal nerve gives rise to the postero-lateral nerve which innervates the oesophageal constrictor muscle (O4), the upper lateral oesophageal dilator (OSCV3 a and b) and the ventral cardiac sac muscle (CV1) before continuing to become part of the stomatogastric system. The superior oesophageal nerve also supplies branches innervating the lateral oesophageal dilator muscle (O3), the oesophageal constrictor muscle (O4) and the oesophageal cardiac sac valve dilator muscles OSCV 1 and 2. A sensory nerve innervates the anterior oesophageal sensors (A.O.S.) (see Chapter 3 Introduction part b).

2. Inferior oesophageal nerve

The inferior oesophageal nerves arise posteriorly from each commissural ganglion and run on the anterior surface of the oesophagus. The inferior oesophageal nerves meet in the anterior midline at the oesophageal ganglion after each branching to form the outer labral nerve and the inner labral nerve. The outer labral nerve runs antero-medially from the commissural ganglion. It supplies a group of receptor cells (M.P.R.1) which appear to act as a tonic proprioceptor (see Chapter 3 Introduction part b). Anteriorly it continues to innervate part of the labrum. ROBERTSON (1978) describes the outer labral nerve as "purely a sensory nerve". The inner labral nerve originates from the inferior

Figure 2.2

The innervation of the oesophagus (from ROBERTSON and LAVERACK, 1979).



. Oesophageal innervation, left lateral aspect. The extrinsic muscles are indicated by the positions of their insertions. The arrow indicates the branch from the s.o.n. that innervates OCSV2 as it passes in front of this nerve. Abbreviations: a.o.s., anterior oesophageal sensor; C, cardiac muscle; c.c., circumoesophageal connective; co.g., commissural ganglion; CV, ventral cardiac muscle; i.l.n., inner labral nerve; i.o.n., inferior oesophageal nerve; i.v.n., inferior ventricular nerve; MPR1, mouth part receptor 1; O, oesophageal muscle; OCSV, oesophageal–cardiac sac valve muscle; o.g., oesophageal ganglion; o.l.n., outer labral nerve; o.n., oesophageal nerve; p.l.n., postero-lateral nerve; p.o.c., posterior oesophageal commissure; p.o.s., posterior oesophageal sensor; s.o.n., superior oesophageal nerve; st.n., stomatogastric nerve; v.p.o.n., ventral–posterior oesophageal nerve.

oesophageal nerve further from the commissural ganglion. Its branches supply the lower anterior oesophageal dilator muscle (O1), the intrinsic labral muscles (L1, L2, L3, L7 and L8) and the extrinsic labral muscles (L4, L5 and L6). According to ROBERTSON (1978) the inner labral nerve also contains sensory fibres.

3. Ventral-posterior oesophageal nerve

The ventral-posterior oesophageal nerve exits dorsally from the circumoesophageal connective, supplying the upper lateral oesophageal dilator muscle (OSCV3 a and b) and the posterior oesophageal dilator muscle (O5 a and b) before terminating in the posterior oesophageal sensors (P.O.S.) (see Chapter 3 Introduction part b).

4. Minor nerves

Two small commissural ganglion nerves exit from the ganglion near the superior and inferior oesophageal nerves and supply the lateral oesophageal dilator (O3) and the oesophageal constrictor (O4).

A dorsal-posterior oesophageal nerve is not described in Homarus gammarus for the reasons given by ROBERTSON and LAVERACK (1979).

c. The post-oesophageal commissure

The post-oesophageal commissure merits consideration since it is in a position to coordinate information between the two commissural ganglia. ALLEN (1894b) states that "...all the fibres observed to pass through this transverse cord [the post-oesophageal commissure] come down the commissures [circumoesophageal connectives], a few possibly from the commissural ganglia, the majority from the brain". BULLOCK and HORRIDGE

(1965) say of the post-oesophageal commissure "...most of the fibres in it originate in the brain". ALLEN's studies on embryonic lobsters (1894a) depict the axon of a cell in the brain crossing the post-oesophageal commissure before bifurcating, sending a branch to the contralateral half of the brain and a branch down the contralateral circumoesophageal connective (see ALLEN, 1894a, cell A(a) Ant II in Figure 2). The large neurosecretory X cells described by KNOWLES (1953) in the commissural ganglion of Leander serratus (see part d below) send axon branches to the contralateral commissural ganglion via the post-oesophageal commissure. Both KNOWLES (1953) and BROWN (1946) comment on groups of cells situated on the tritocerebral commissure (the post-oesophageal commissure). KNOWLES gives a detailed description of the post-oesophageal commissure nerves. In Leander serratus, Penaeus braziliensis (KNOWLES, 1953) and Crago septemspinosus (BROWN, 1946) the post-oesophageal commissure and its nerves appear to be involved in the hormonal control of pigment migration. Apart from these general statements and a few specific examples little is known of the role of the commissure (see also Chapter 3).

d. Structural studies of the commissural ganglion

There are few structural studies on the commissural ganglion and there is no comprehensive description for any one species. Consequently the relevant literature is often vague or confusing. For example, KNOWLES (1953) says that in Leander serratus "Each connective [commissural] ganglion contains many cell bodies...". SPIRITO (1975) states that there are "well over fifty" cell bodies in each commissural ganglion of Procambarus clarkii. MOULINS, DANDO and LAVERACK (1970), in a general description of the decapod stomatogastric nervous system say "This system...contains several [i.e. stomatogastric, oesophageal and commissural] small ganglia with a restricted number of cells...". Such statements are inaccurate since each commissural ganglion contains several hundred neuron somata (see below and results part b). An informed description of the commissural ganglion is given by SELVERSTON et al. (1976) but unfortunately it is extremely brief.

ALLEN (1894b) used methylene blue techniques in his study of the stomatogastric nervous system of Astacus (unspecified species) and Homarus (presumably H. gammarus) but gives little structural detail of individual commissural ganglion cells. KNOWLES (1953) comments that most commissural ganglion cells in Leander serratus range from 15-20 μm in diameter but two large neurosecretory cells (X cells) have a diameter of 70-85 μm. He describes the course of the X cell axons (see part c above) but gives no other structural details.

ORLOV (1929) described neurons in the commissural and oesophageal ganglia of Astacus fluviatilis (Potamobius astacus) and A. leptodactylus (P. leptodactylus) using methylene blue staining. His work is summarised in BULLOCK and HORRIDGE (1965). Despite the capricious nature of methylene blue staining ORLOV's descriptions are detailed and provide a

useful comparison for some aspects of structural studies on Homarus.

WIERSMA (1957) counted the number of cell bodies in the commissural ganglion. Using Bouins-fixed, toluidene blue-stained serial sections of a commissural ganglion from the crayfish, Cambarus clarkii, he assessed the number of neuron somata as 390 by counting nucleoli. RUSSELL (1977) used serial sections of a Bouins-fixed, silver-stained commissural ganglion from the spiny lobster, Panulirus interruptus, to count the number of cell bodies, using similar criteria to WIERSMA. RUSSELL found the nature of the large number of small cells in the commissural ganglion equivocal, especially since silver staining methods are capricious and are not entirely specific for neurons. He concluded that there are at least 64-240 neurons in the commissural ganglion of Panulirus interruptus and possibly as many as 685. RUSSELL measured the diameter of cell bodies but the statistical accuracy with which he treats these results is inappropriate since "No allowance was made for possible tissue shrinkage [during fixation]". However, his results give a useful guide to the size ranges of commissural ganglion cells, emphasising the small number of large cells (more than about 40 μm in diameter) and the large number of small cells (less than about 20 μm in diameter). Both WIERSMA and RUSSELL draw attention to the limitations of their methods. RUSSELL states "The present count is still at best an estimate, since only one ganglion was studied, since silver staining is capricious and might have been incomplete and since there is probably some biological variation in the number of neurons per commissural ganglion". In view of these limitations their estimates should be used cautiously especially when considering other species.

KUSHNER (1979) used cobalt chloride backfilling techniques to identify interganglionic neurons in the stomatogastric nervous system of Panulirus interruptus. She described groups of cells filling from the superior and inferior oesophageal nerves and emphasised the "...apparent

constancy of cell position...". Her evidence implies that "...there may be as few as three CG [commissural ganglion] cells sending an axon to the SGG [stomatogastric ganglion] via the SON [superior oesophageal nerve]...". KUSHNER suggested that these small commissural ganglion somata may be dopamine-containing modulators of stomatogastric ganglion activity (see Chapter 4). There is also some evidence for axons going to, or through, the commissural ganglion from Ex cells in the stomatogastric ganglion (see Chapter 3 Introduction part e). She concluded : "Cobalt preparations...permit insight into CG [commissural ganglion] topography and hopefully provide a guide to electrophysiological investigations". However, her study concentrates on a search for interneurons and is far from comprehensive.

Materials and methods

General

a. Animals

Homarus gammarus were caught locally and maintained in large tanks of circulating, aerated sea-water at a temperature ranging from 1-15 °C. They were fed about once a week on chopped whiting. Healthy adult males and females of all sizes were used for experiments.

b. Dissection

The lobster was restrained in a rigid perspex holder with strong elastic bands after autotomising the chelipeds. A dental drill was used to cut through the cephalothoracic dorsal carapace from behind the cervical groove, along the sides of the body and just posterior to the rostrum. The carapace and underlying epidermis were removed by scraping away the muscle insertions. The mandibular adductor muscles were excised and their stout apodemes cut near the base and removed to facilitate removal of the digestive glands.

1. Single commissural ganglion preparation

After cutting the extrinsic stomach muscles the oesophagus was clamped with artery forceps and cut near the oesophageal-cardiac sac valve. The midgut was cut posterior to the stomach. The stomach, digestive glands and gonads were then carefully removed and discarded. If a lengthy experiment or dissection was to be performed the green glands were also removed. However, it was difficult to excise each gland as a whole in one

attempt and removal thus risked contamination of the preparation. When a commissural ganglion was to be removed quickly the glands were left intact. During this preliminary dissection the animal was constantly flushed with chilled, oxygenated sea-water or saline (see part c below). The lobster was then transferred to a tank holding the perspex frame and immersed under oxygenated sea-water or saline. A Nikon binocular microscope was used during the subsequent dissection.

To remove each commissural ganglion and its nerves the appropriate oesophageal muscles were cut, a process described fully by ROBERTSON (1978). The superior oesophageal nerve, inferior oesophageal nerve, ventral-posterior oesophageal nerve, minor commissural ganglion nerves and outer mandibular nerve were cut. The circumoesophageal connective was cut anterior to the commissural ganglion and posterior to the junction of the outer mandibular nerve. The ganglion was then removed.

2. Combined preparation

This preparation comprised the stomatogastric nerve, oesophageal ganglion, the two commissural ganglia and their connecting nerves (see Results part d).

After removal of the carapace the lateral and dorsal ventricular nerves were traced anteriorly along the midline of the dorsal surface of the stomach to locate the stomatogastric ganglion. The lateral ventricular nerves, the medial ventricular nerves and the smaller motor nerves were cut. The stomatogastric ganglion was carefully removed from the anterior aorta and deflected anteriorly. The stomach was removed as in part 1 above. The inferior ventricular nerve was cut and the two commissural ganglia were detached as in part 1 above, without cutting the superior and inferior oesophageal nerves. The preparation was then removed and the stomatogastric nerve was cut, removing the stomatogastric ganglion.

In procedures 1 and 2 it was sometimes necessary to remove other parts of the nervous system and their connections to the commissural ganglion. The brain could be removed by cutting all the nerve trunks except the circumoesophageal connectives. By dissecting away the appropriate parts of the endophragmal skeleton the post-oesophageal commissure and the suboesophageal ganglion were revealed. Removal of the post-oesophageal commissure with the rest of the preparation involved cutting the small commissure nerves to the oesophagus. The suboesophageal ganglion could be included by cutting all its nerves except the anterior circumoesophageal connectives.

During some experiments it was necessary to remove the connective tissue sheath from the commissural ganglion. The ganglion was pinned lateral (cell body) side up in a Sylgard-lined Petri dish with fine pins. The sheath was carefully removed from the circumoesophageal connective and the ganglion using fine spring scissors and electrolytically-sharpened tungsten needles. Most of the perineural tissue could be removed with a fine jet of saline.

c. Saline

Sea-water was found to be suitable during quick dissections, for example in removal of a commissural ganglion for histological processing. Tissue so treated showed no obvious deleterious effects. However, a saline more consistent with the internal medium of Homarus gammarus in osmolarity, ionic composition and pH was used for lengthier experiments and for those in which the ionic composition of the medium may be very important, for example in physiological experiments. The saline used was a modification of PANTIN's (1948), buffered to pH 7.8 with borate/ boric acid. Its composition and full details of its derivation are given in Appendix A.

Both sea-water and saline were constantly perfused with a 95% oxygen/ 5%carbon dioxide mixture from a gas cylinder and renewed periodically to ensure that the temperature did not rise above 13°C.

Anatomical

a. Nomarski interference-contrast microscopy

A single commissural ganglion was gently squashed on a slide under a coverslip. In most preparations the sheath was first removed. The ganglion was viewed with a Zeiss STANDARD RA microscope equipped for Nomarski interference-contrast microscopy. Preparations were photographed with a Praktica VLC2 camera using Ilford Pan F film.

b. Vital staining

Methylene blue was occasionally used to trace nerve branches during dissection. A stock solution of 1% methylene blue in distilled water was applied directly to the tissue. Alternatively sufficient stain was added to the sea-water or saline surrounding the preparation to colour it light blue. Further dissection and staining were done until optimal staining occurred.

Methylene blue and neutral red were used to stain whole desheathed ganglia, pinned out in a Sylgard-lined Petri dish. A few drops of 1% methylene blue (as above) or 1% neutral red in distilled water were added to the saline, colouring it light blue or light red accordingly. When satisfactory staining had occurred, usually after about twenty minutes, the medium was replaced with clear saline. The preparation was immediately examined and photographed using a Zeiss Tessovar photomacrographic zoom

system with an EXA 1 camera and Ilford Pan F or FP4 film.

Methylene blue preparations were fixed overnight in 10% ammonium molybdate in distilled water, washed for one hour under running tap-water, dehydrated through an ascending ethanol series with three changes of absolute alcohol (ten minutes each), cleared and mounted in methyl salicylate. Permanent preparations were examined and photographed as in part c below.

c. Histology

Block silver-staining methods stained neurons capriciously. Simple histological methods were found to be preferable. Single commissural ganglia were fixed in Bouins (HUMASON, 1972) made up in sea-water and processed as described in Appendix B. For comparison other fixatives were also used : Bouins made up in isotonic sucrose and formol-alcohol (HUMASON, 1972). Wax-embedded tissue was serially sectioned at 10 µm. Slides were brought to water through a descending ethanol series, stained with Heidenhain's iron haematoxylin and alcoholic eosin (HUMASON, 1972), cleared in xylene and mounted in DPX. They were examined and photographed with a Zeiss UNIVERSAL transmitted light microscope equipped with a MC 63 photographic camera using Ilford Pan F film.

Counts of neuron somata involved the examination of serial sections. Neurons generally had a dark cytoplasm, a lightly-stained nucleus and a prominent nucleolus. Where the large number or small size of the cells made counting difficult camera lucida drawings of a section were made and superimposed on the next section.

d. Semi-thin epoxy resin sections

Single commissural ganglia and the appropriate nerves were processed for electron microscopy. They were fixed in glutaraldehyde, post-fixed in osmium, dehydrated and embedded in Durcupan or Spurr resin according to the procedure in Appendix C. Tissue blocks were trimmed and were sectioned on a Huxley ultramicrotome. Semi-thin (about 1 μm) sections were placed on a slide and stained with toluidene blue in the conventional way. They were mounted in immersion oil, examined and photographed as in part c above.

e. Cobalt chloride backfilling

The position of the commissural ganglia and their nerves prohibited in vivo backfilling. In vitro preparations usually involved single commissural ganglia but occasionally combined preparations (see General Materials and methods part b2 above) and those involving the brain or the suboesophageal ganglion and the post-oesophageal commissure were used. The backfilled nerve was kept as long as the technique permitted since pronounced difficulties arose from trying to backfill through short nerves. Great care was taken not to stretch or damage the nerve.

The preparation was placed in a perspex chamber (Figure 2.3) with a small amount of saline covering the ganglion. The nerve was draped through the vaseline seals which formed a gap containing slightly hypotonic (750 mM) sucrose (MULLONEY, 1973). The seals were built up with vaseline from a hypodermic syringe and checked to ensure that leakage would not occur. The end of the nerve was cut cleanly under distilled water to remove any damaged parts and left for a few minutes to swell osmotically the ends of the axons. The distilled water was removed and replaced by a small drop of 60, 150 or 300 mM cobalt chloride made up in distilled water,

Figure 2.3

Perspex chamber for cobalt chloride backfilling

a. Top view

b. Side view

Key :-

CoCl2.....cobalt chloride

g.....commissural ganglion

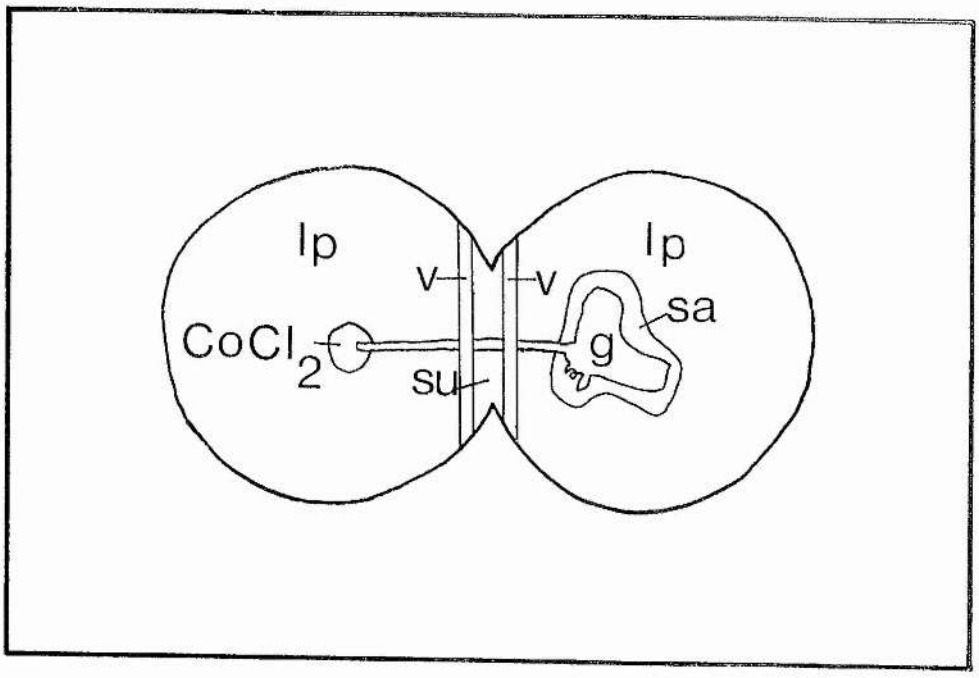
lp.....liquid paraffin

sa.....saline

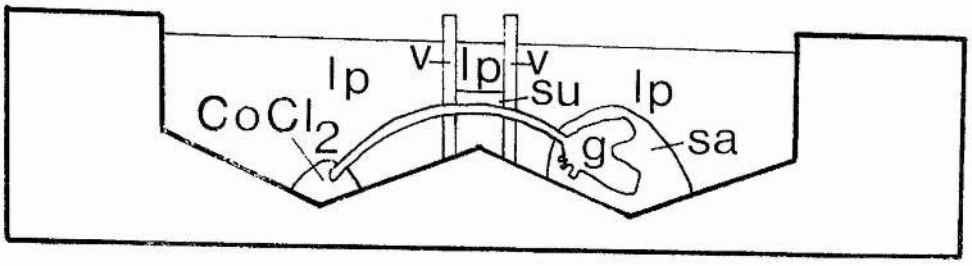
su.....hypotonic sucrose

v.....vaseline wall

a



b



just sufficient to cover the end of the nerve. With larger amounts of cobalt chloride and saline there was a disproportionate tendency for leakage and mixing of the solutions to occur. The preparation was covered with liquid paraffin and incubated at 4 °C for 6-48 hours. Usually it was left overnight. Results showed that application of current was not necessary for adequate staining and since its use may enhance transynaptic cobalt migration it was avoided. The preparation was washed for ten minutes in several changes of saline to remove superfluous cobalt chloride and liquid paraffin. It was treated with a freshly-made dilute solution of ammonium sulphide (a few drops in 20 ml saline) for 15-30 minutes in a fume cupboard to precipitate cobalt sulphide. After washing in several changes of saline for fifteen minutes it was fixed for five minutes in Carnoy's fixative (KUSHNER, 1979), dehydrated through an ethanol series (90% and 3 x 100% for five minutes each), cleared and mounted in a cavity slide in methyl salicylate. Wholemount preparations were examined and photographed as in part c above. They were drawn using a Zeiss camera lucida and a light source of variable intensity. Preparations were stored in methyl salicylate in a deep freeze to minimise fading.

Silver intensification was used to enhance cobalt staining. Several trials revealed that the method of BACON and ALTMAN (1977) with slight modifications suggested by J.S. ALTMAN (pers. comm.) was superior to that of PITMAN (1979). It gave better results and was quicker and easier. PITMAN's method involves fixation in formaldehyde : aldehydes cause reduction of silver nitrate and silver precipitation on the outside of the preparation (BACON and ALTMAN, 1977).

Intensification involved treating the preparation as described above until the fixation stage. It was fixed in sea-water Bouins for 24 hours, rinsed thoroughly in 50% ethanol (ten minutes) and treated with 30% ethanol (five minutes). It was transferred to warm distilled water in a

dark oven at 60 ℃ for five minutes and then "pre-soaked" in developer base (Appendix D) at 60 ℃ for 1-4 hours. The preparation was intensified for 30-90 minutes in a freshly-made mixture of one part 1% silver nitrate solution and ten parts developer base, changing the solution every twenty minutes because of silver precipitation. It was swiftly examined under a binocular microscope every ten minutes and when sufficient intensification had occurred the preparation was immediately transferred to distilled water at 60 ℃ for ten minutes. It was passed to cold distilled water and dehydrated through 30%, 50%, 70%, 90% and 3 x 100% ethanol (five minutes each), cleared and mounted in methyl salicylate and examined, drawn and photographed as described above. Some preparations were fixed, dehydrated and examined and then rehydrated, intensified and re-examined.

Results

a. General structure

The commissural ganglion with its sheath intact is about 1 mm in diameter (see Figure 2.4 a). It appears to be a typical arthropod ganglion, comprising an area of neuropile with an outer layer of cell bodies, most on the lateral surface (see Figure 2.4 b and c). The connective tissue sheath and perineural tissue form a thick layer which contains blood sinuses and glia. No consistent differences were found between right (side) and left (side) commissural ganglia which are roughly mirror images in a single animal.

b. Cell number, size and position

Rough counts of cell bodies from montages of photographs taken with Nomarski interference-contrast microscopy and more accurate counts in histological serial sections showed that each commissural ganglion contains about 400 $\pm$ 100 neuron somata. As WIERSMA (1957) and RUSSELL (1977) found it was very difficult to determine exactly the number of cells in each preparation. Small cells (less than about 15 μm in diameter) were often confused with elements such as glial nuclei. As many as sixty cells appeared in a single section through the anterior part of the ganglion which also made counting difficult. Although there is a striking constancy from animal to animal in the position of certain cells and groups of cells (see below) as RUSSELL surmises there is some individual variation. The figure obtained in this study agrees roughly with WIERSMA's (1957) estimate of 390 neuron somata for the commissural ganglion of Cambarus

Figure 2.4

a. A lateral view of a desheathed left commissural ganglion stained with the vital dye neutral red. Note the numerous cell bodies and the very large soma, the L cell, near the exit point of the superior oesophageal nerve.

Scale : 500 µm

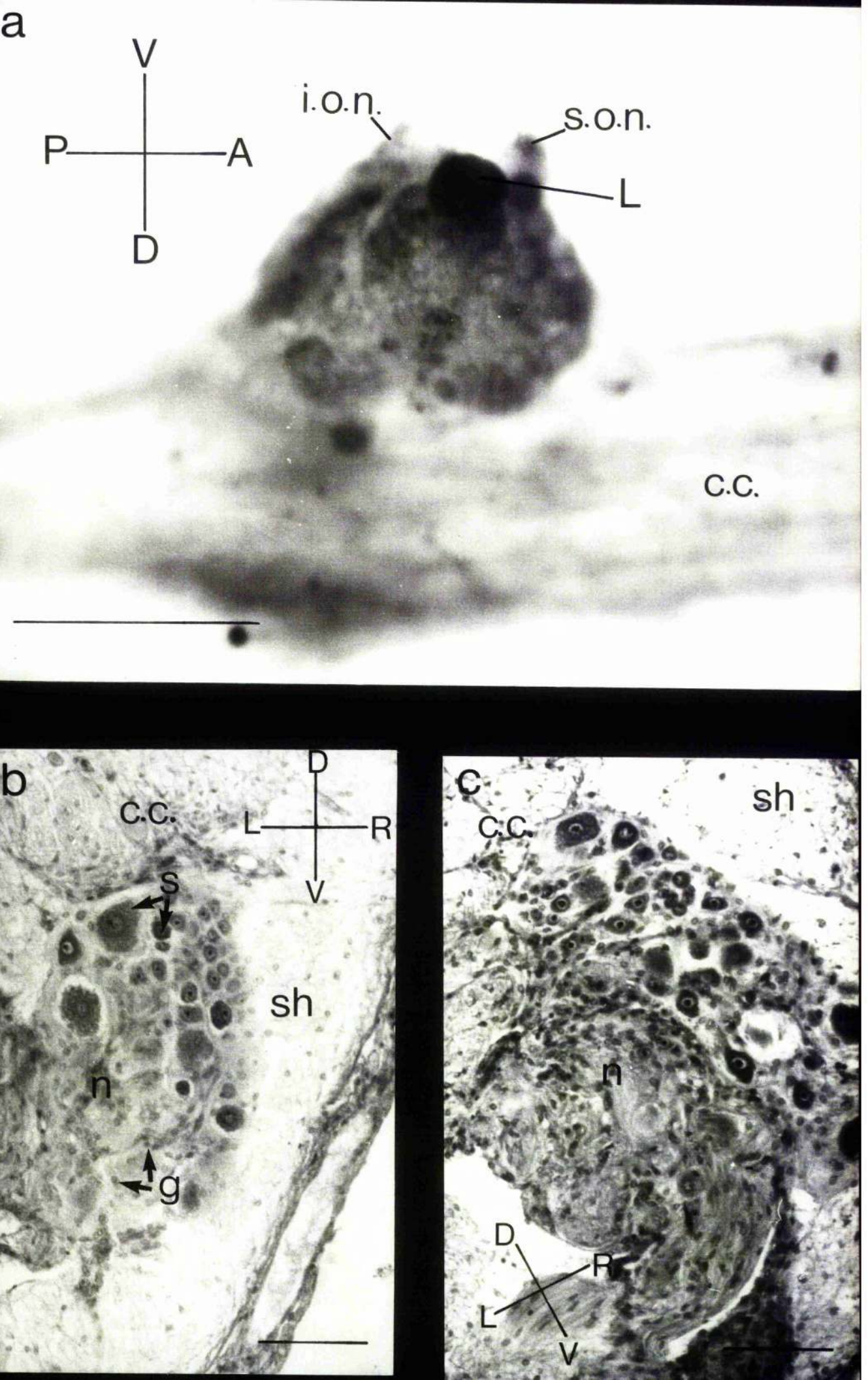
b and c. 10 µm wax sections cut dorsoventrally

b. anteriorly and

c. posteriorly

through a right commissural ganglion fixed in sea-water Bouins and stained with haematoxylin and eosin. Neuron somata (arrowed s) are distributed around a neuropile area (n) surrounded by a connective tissue sheath (sh) supplied by blood vessels (bv). Some of the glia (g) are also arrowed. Neuron somata are distributed all round the anterior part of the ganglion (see b) but more posteriorly are located only on the lateral surface (see c). Shrinkage has occurred during fixation resulting in gaps in the tissue.

Scale in b and c : 20 µm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clarkii and is within RUSSELL's (1977) wide limits of 64-685 neuron somata for the commissural ganglion of Panulirus interruptus. However, in view of the limitations outlined it can be stated only that each commissural ganglion in Homarus gammarus contains several hundred neuron somata.

Most somata are located anteriorly on the lateral surface of the ganglion but many cells are distributed all round the anterior aspect of the ganglion (see Figure 2.4 b). A group of about fifteen cells occurs medially near the exit point of the ventral-posterior oesophageal nerve from the commissural ganglion (see part d below). RUSSELL (1977) found 13-20 such cells in Panulirus interruptus. The most anteriorly-located cell is the large soma denoted the L cell after SELVERSTON et. al. (1976) which ranges from about 90-150 μm in diameter and serves as a landmark in nearly all preparations (see Figure 2.4 a). A group of about fifteen large cells (about 50-80 μm in diameter) occurs ventrally to the L cell, near the exit point of the superior oesophageal nerve from the ganglion. A striking feature of the commissural ganglion is the large number of small cells (less than about 20 μm in diameter), possibly more than 300 (see above). A very large group of small cells is located dorsally next to the circumoesophageal connective (see part d below). It was difficult to determine exactly the diameter of cells. Severe shrinkage of the commissural ganglion (up to 50%) often occurred during histological processing. The size ranges given are based on observations of vitally-stained living tissue compared with histological sections and are accurate to about ± 30%. The important point to note is that the constancy of size and position of certain individual cells and groups of cells permitted their identification from animal to animal.

c. The commissural ganglion nerves

The review of the commissural ganglion nerves given above (see Introduction part b) can only provide a general guide. The disposition of the nerves is variable, for example the outer labral nerve may separate from the inferior oesophageal nerve before leaving the commissural ganglion. The minor nerves usually branch profusely on leaving the ganglion, restricting identification of constrictor and dilator nerves. In an extreme case all commissural ganglion nerves except the ventral-posterior oesophageal nerve formed one large nerve trunk, separating a few millimetres from the ganglion. However, familiarity with the preparation ensured that all nerves and their branches, except the minor nerves, could be easily identified. No details can be added to ROBERTSON's (1978) description of the commissural ganglion nerves except to note that several small nerve trunks arise from the junction of the post-oesophageal commissure and the circumoesophageal connective and from the commissure itself. Some of these appear to innervate the oesophageal constrictor muscle but they were not investigated in detail.

Semi-thin epoxy resin sections revealed the structure of the commissural ganglion nerves seen in cross-section (see Figure 2.5). All three commissural ganglion nerves and the post-oesophageal commissure are each surrounded by a thick connective tissue sheath and are heavily invested with connective tissue and glia. In the superior oesophageal nerves over 200 "medium" axons (about 3-10 µm in diameter : SUTHERLAND and NUNNEMACHER, 1968) were counted. A discrete bundle of axons (see Figure 2.5 a) contains hundreds of very fine fibres. Over 100 "medium" axons were counted in the inferior oesophageal nerve and over 50 in the ventral-posterior oesophageal nerve. Both these nerves also contain tracts of several hundred fine fibres (see Figure 2.5 b and c). Note that the

Figure 2.5

Semi-thin epoxy resin sections through commissural ganglion nerves stained with toluidene blue

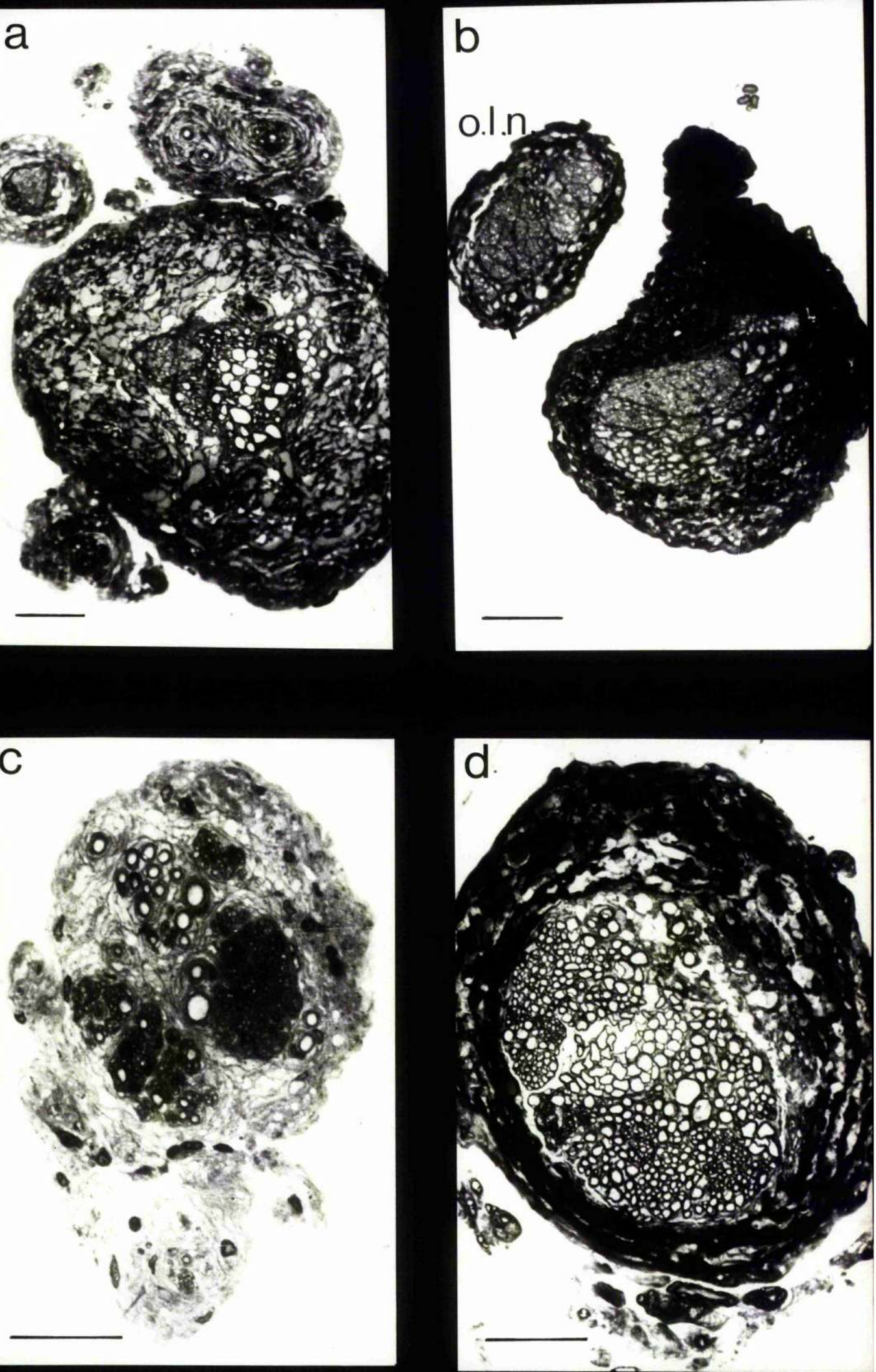
a. The superior oesophageal nerve and minor branches supplying the oesophageal constrictor muscle (04). Over 200 "medium" axons (about 3-10 μm in diameter) and bundles of several hundred fine fibres are surrounded by a thick sheath.

b. The inferior oesophageal nerve and the outer labral nerve. In the inferior oesophageal nerve over 100 "medium" axons and bundles of numerous fine fibres are surrounded by a thick sheath. Most of the axons in the outer labral nerve a group of which is arrowed.

c. The ventral-posterior oesophageal nerve. It contains over 50 "medium" axons and bundles of fine fibres scattered through the connective tissue matrix. Many "medium" axons are heavily sheathed in glial processes.

d. The post-oesophageal commissure. It contains over 1,000 axons ranging up to 12 μm in diameter, surrounded by a thick sheath. There are no conspicuous bundles of fine fibres as seen in a, b and c.

Scale in a, b, c and d : 50 μm


a
b
o.l.n.
c
d

outer labral nerve contains chiefly bundles of fine fibres but also some "medium" axons. The post-oesophageal commissure contains over 1,000 axons, ranging up to 12 μm in diameter.

d. Mapping the commissural ganglion

Attempts were made to backfill the commissural ganglion through the superior oesophageal nerve (including the postero-lateral nerve tract), the postero-lateral nerve, the inferior oesophageal nerve (including the outer and inner labral nerve tracts), the outer labral nerve, the inner labral nerve, the ventral-posterior oesophageal nerve, the outer mandibular nerve (all three branches), the anterior circumoesophageal connective (cut at the level of the brain), the posterior circumoesophageal connective (cut at the level of the suboesophageal ganglion), the stomatogastric nerve and the post-oesophageal commissure. Each nerve was filled as close to the commissural ganglion as the technique permitted.

A minimum of five successful preparations was considered necessary to get a representative description since there was some variability amongst preparations. In a successful preparation features in the commissural ganglion relating to axons in that particular nerve could be clearly observed. This minimum was achieved for all nerves except the postero-lateral nerve (only three preparations) and the post-oesophageal commissure, which was not successfully backfilled in fifteen attempts, probably due to technical difficulties imposed by its shortness. Often more than half the preparations failed due to lack of cobalt migration, or cobalt travelling in the extracellular space or on the outside of the nerve, despite attempts to minimise these problems (see Materials and Methods part e). Over 200 preparations were attempted to obtain the results given below.

Unintensified preparations were usually adequate and although silver intensification generally revealed more detail, especially in the neuropile, this occasionally obscured other features. Intensified preparations are indicated in the text and figure legends.

In each preparation the number of filled neuron somata in the ganglion and the number of axons filled in other nerves were noted. These results are presented in Table 2.1, giving the minimum, maximum and average number of somata and axons observed for each nerve preparation. Camera lucida drawings of these preparations are given as they convey far more information than photographs (compare Figures 2.6 and 2.7 a). Views are taken from the lateral surface, bearing nearly all of the cell bodies, unless otherwise stated. Shrinkage during tissue processing did not seem to be pronounced and so the size ranges given for cells are probably only slight underestimates. Some distortion of the preparation usually occurred when mounting it but with careful examination it was possible to define the location of filled cells in the ganglion.

Superior oesophageal nerve

See Table 2.1 and Figures 2.6 and 2.7. The most striking feature of superior oesophageal nerve preparations is the prominent cluster of cells located anteriorly at the exit point of this nerve from the commissural ganglion. This group of cells is very similar to that described by KUSHNER (1979) in Panulirus interruptus (compare Figure 2.6 b and Figure 5 in KUSHNER, 1979). The similarity in size (about 50-80 µm in diameter), number (about ten) and position of these cells is pronounced. A cell of similar size occasionally filled in the posterior part of the ganglion (see Figure 2.7). Up to seventeen smaller cells (about 15-30 µm in diameter) were seen near the prominent group of large cells and in the middle of the ganglion (see Figure 2.7). KUSHNER describes only 2-3 such

Table 2.1

Cobalt chloride backfilling the commissural ganglion through various nerves

Figures denote minimum and maximum numbers of commissural ganglion somata and axons observed with means (expressed to nearest whole number) in brackets. See Glossary for abbreviations.

Key:-

?.....uncertain

-.....it was generally not possible to count axons in the nerve which was backfilled due to intense staining

Nerve	Number of fills	Number filled C.G. somata	Axons in other nerves s.o.n.	i.o.n.	v.p.o.n	m.n.	c.c.ant	c.c.post
s.o.n.	10	14-26 (20)	-	1-4 (3)	0-5 (2)	0-8 (3)	0-2 (0)	2-6 (4)
p.-l.n.	3	2 (2)	2 (2)?	0	0	0	0	2 (2)
i.o.n.*1	10	20-42 (29)	0-3 (1)	-	0-4 (1)	0-4 (1)	0-2 (1)	4-12 (7)
o.l.n.	10	0	0	-	0	0	0	4-10 (7)
i.l.n.	8	7-15 (10)	0	-	0	0	0-1 (0)	2-9 (4)
v.-p.o.n.	5	2-4 (3)	0-2 (0)	1-2 (1)	-	0	0	1-4 (3)
o.m.n.	10	0	0	0-1 (1)	0	0-1 (0)	0	>10
c.c.ant	15	>50	0-1 (1)	0-4 (2)	0	0-3 (2)	-	-
c.c.post	10	>50	2-10? (3)?	3-10? (4)?	0-3? (2)?	2-4? (3)?	-	-
st.n.*2	7	3-15?	6-10 (8)	2-5 (3)	0-2 (1)	2-6 (3)	0	0

*1.....0-1 (0) axons in p.-o.c.

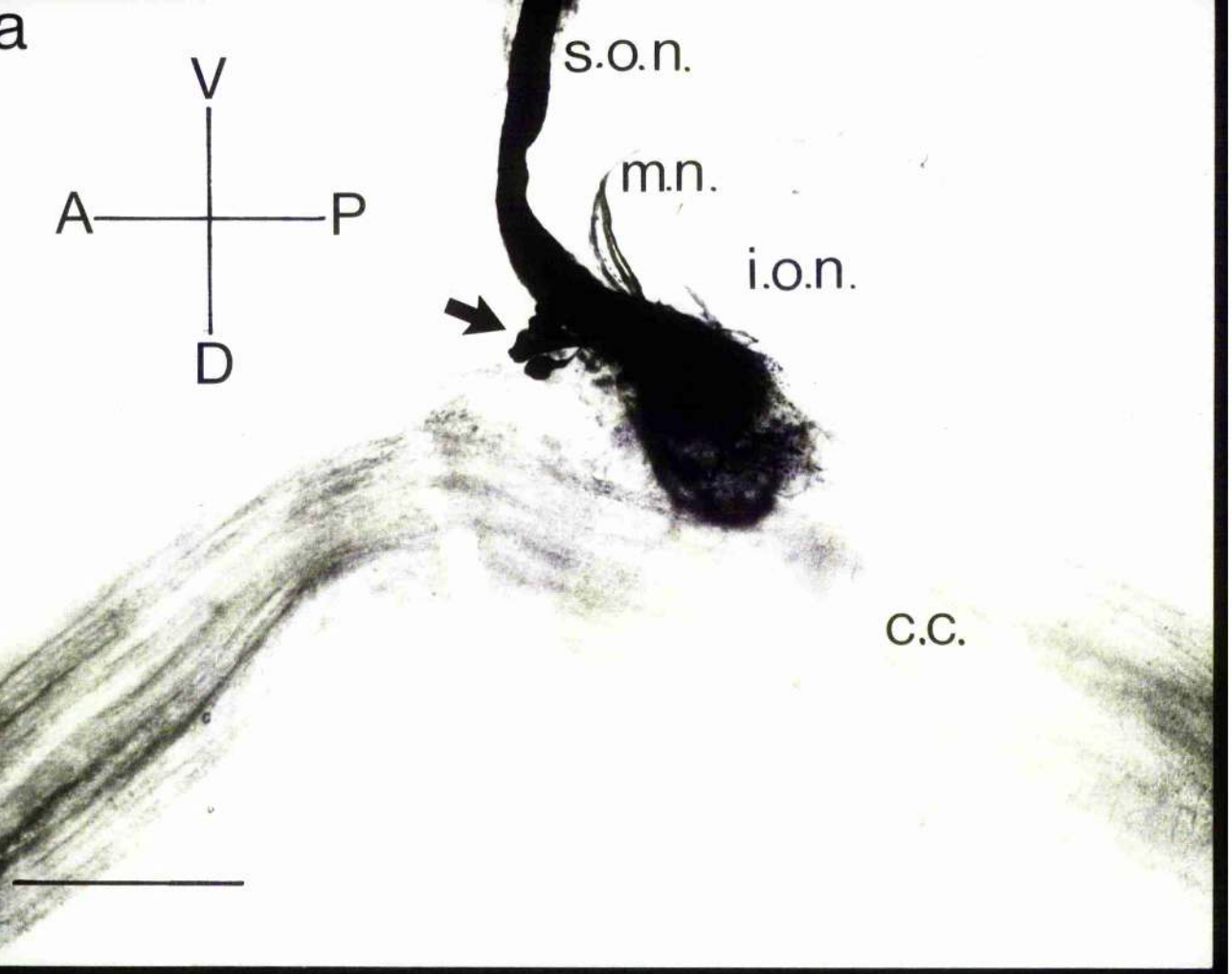
*2.....6-12 (8) axons in o.n.

Figure 2.6

Cobalt backfilling the commissural ganglion through the superior oesophageal nerve

Photographs of a right commissural ganglion backfilled through the superior oesophageal nerve. Note the prominent group of cell bodies (arrowed in a, shown at higher magnification in b) at the exit point of the superior oesophageal nerve from the ganglion. There is a dense area of neuropile in the posterior part of the ganglion and several filled axons in the minor nerves and the inferior oesophageal nerve.

Scale in a : 500 µm, in b : 100 µm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b

Figure 2.7

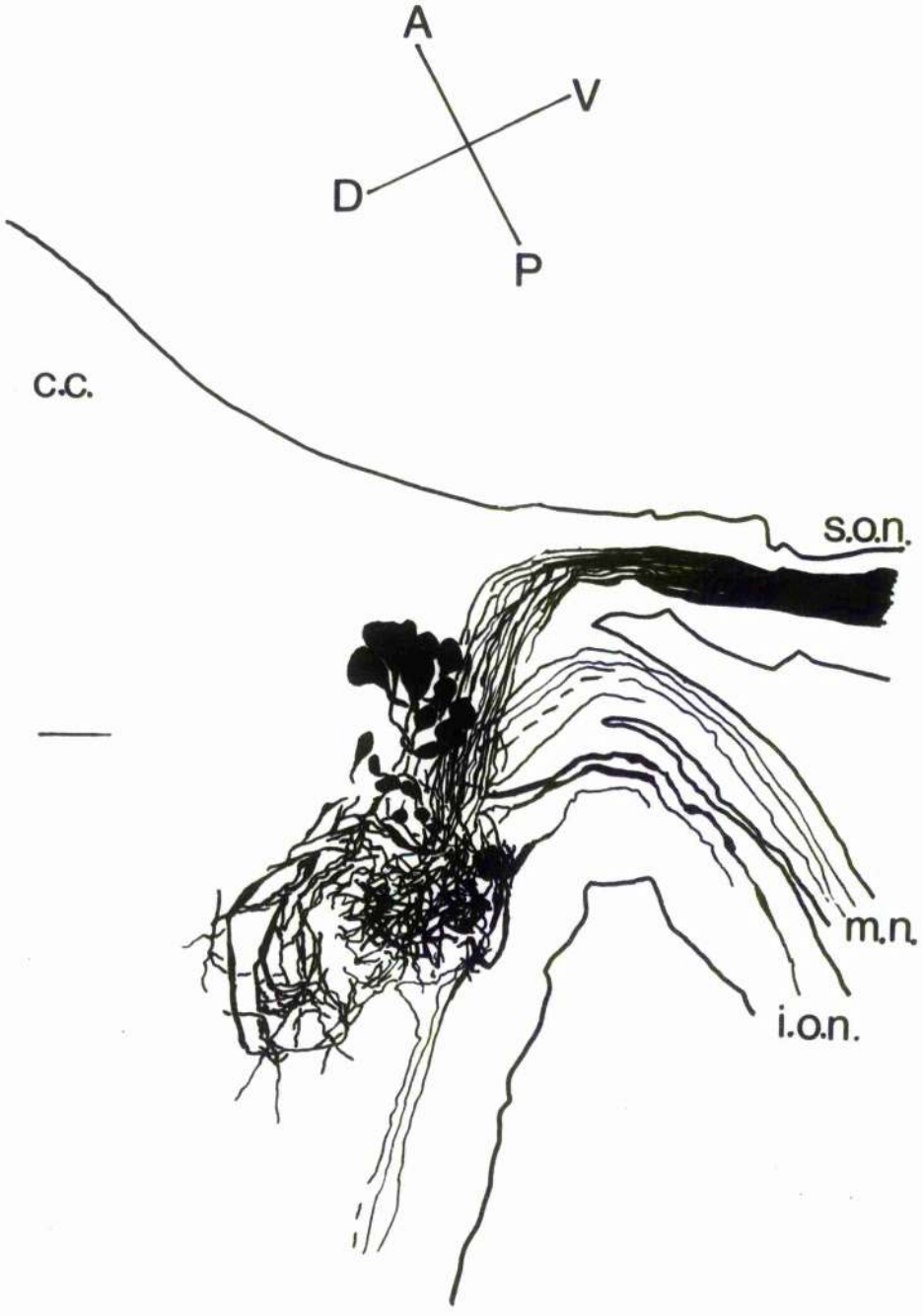
Cobalt chloride backfilling the commissural ganglion through the superior oesophageal nerve

a. A right commissural ganglion. Note the prominent group of cells near the exit point of the superior oesophageal nerve from the ganglion and the smaller cells scattered more posteriorly in the dense area of neuropile. There are several filled axons in the inferior oesophageal nerve, minor nerves and posterior circumoesophageal connective. Compare with Figure 2.6 which shows photographs of this preparation.

b. A right commissural ganglion showing features similar to those in a. There are also several filled axons in the ventral-posterior oesophageal nerve.

Scale in a and b : 100 µm

a



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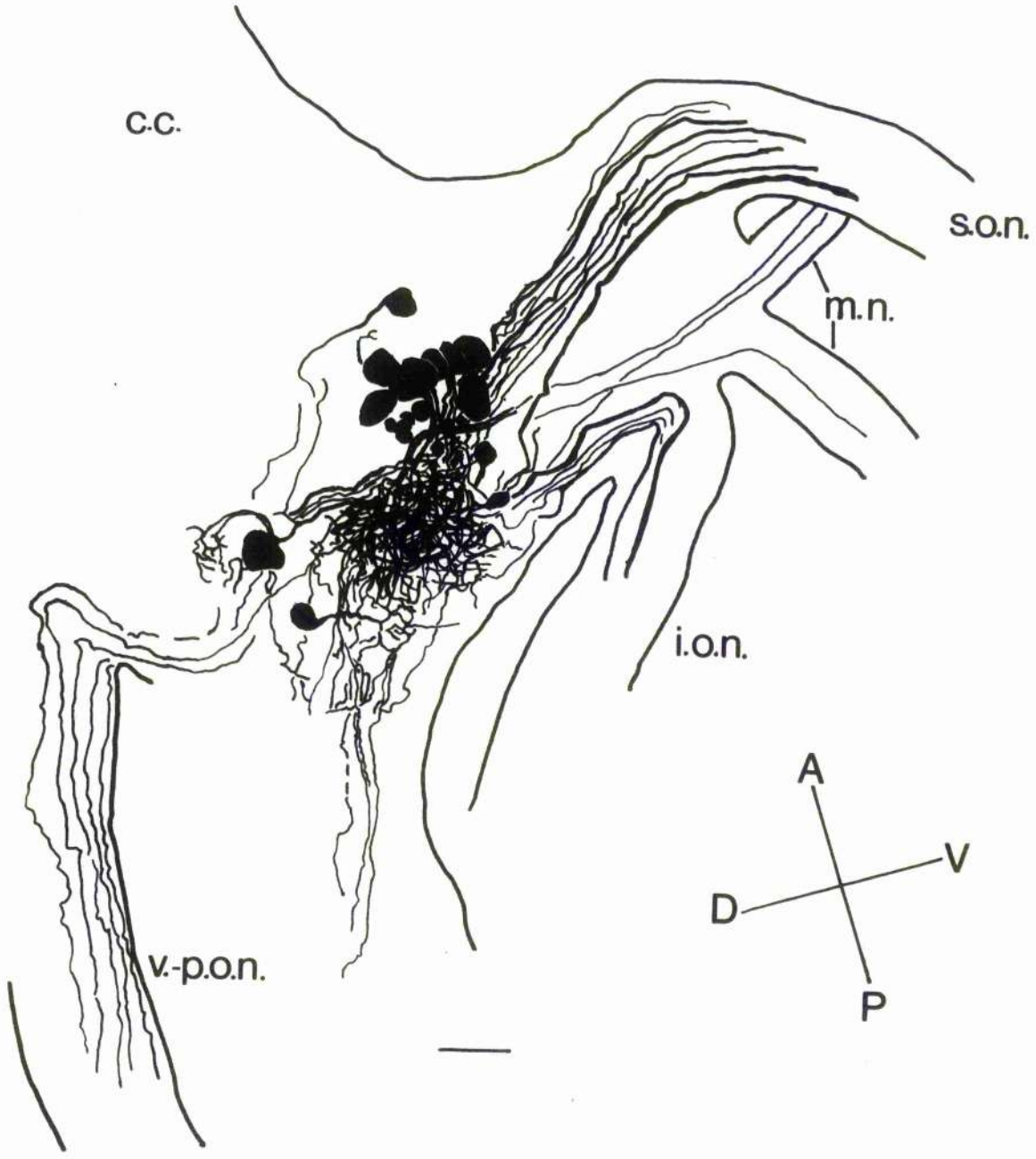


cells in P. interruptus.

The dense area of neuropile in the posterior part of the ganglion is characteristic of all superior oesophageal nerve fills. Again, an homologous feature is seen in P. interruptus (see Figure 5 in KUSHNER, 1979). The size and complexity of this neuropile area generally precluded detailed investigation of individual cell processes. The large somata in the superior oesophageal nerve group appear to have thick axons which do not branch near the cell. However, it was not possible to see if the smaller cells have "extensively branching neurites" as KUSHNER claims; presumably they are contributing to the neuropile area.

Axon tracts in the inferior oesophageal nerve, ventral-posterior oesophageal nerve and minor commissural ganglion nerves were usually clearly seen (Table 2.1). Note the large axons which often filled in the inferior oesophageal nerve (Figure 2.7). Axons travelling anteriorly towards the brain in the circumoesophageal connective were possibly seen in two backfills but the interpretation of these two preparations was difficult and open to question. Up to six axons filled in the posterior circumoesophageal connective, travelling towards the suboesophageal ganglion. These axons could not be traced more than about 5 mm, even in intensified preparations. KUSHNER remarks on the apparent absence of cobalt-filled fibres in the circumoesophageal connective on backfilling the superior oesophageal nerve of P. interruptus.

Postero-lateral nerve

See Table 2.1. Only three postero-lateral nerve preparations were moderately successful out of nineteen attempts and silver intensification did little to clarify the results. Two somata filled from the postero-lateral nerve, each about 50 µm in diameter, situated antero-dorsally and in the middle of the commissural ganglion. These

somata seemed to give rise to the two axons which filled from the postero-lateral nerve in the superior oesophageal nerve. Two axons also filled in the posterior circumoesophageal connective. In one preparation one of the two filled cells appeared to have a bifurcating axon, one branch travelling in the postero-lateral nerve and one in the posterior connective. Even in intensified preparations the area of neuropile in the middle of the commissural ganglion was sparse and appeared to make little contribution to the dense area of neuropile seen on backfilling the entire superior oesophageal nerve. There was no clear evidence for axons filling in other commissural ganglion nerves. In the postero-lateral nerve axons filled distally in its branches to muscle OSCV3 (refer to Figure 2.2). There was an indication that some postero-lateral nerve axons were travelling in the superior oesophageal nerve away from the commissural ganglion, i.e. towards the junction of the oesophageal and the stomatogastric nerves.

Inferior oesophageal nerve

See Table 2.1 and Figure 2.8. About thirty cells ranging in diameter from 60 μm to less than 10 μm filled from the inferior oesophageal nerve. These were widely distributed in the posterior region of the commissural ganglion and as KUSHNER (1979) found in Panulirus interruptus showed "no simple pattern of soma location". Usually some cells were located near the exit point of the inferior oesophageal nerve from the ganglion but did not form a "prominent group" as KUSHNER describes.

A dense area of neuropile in the posterior part of the ganglion similar to that seen in superior oesophageal nerve fills was observed. In general individual cell processes could not be resolved but some small cells (less than 20 μm in diameter) appeared to have complex, thin, branching arborisations.

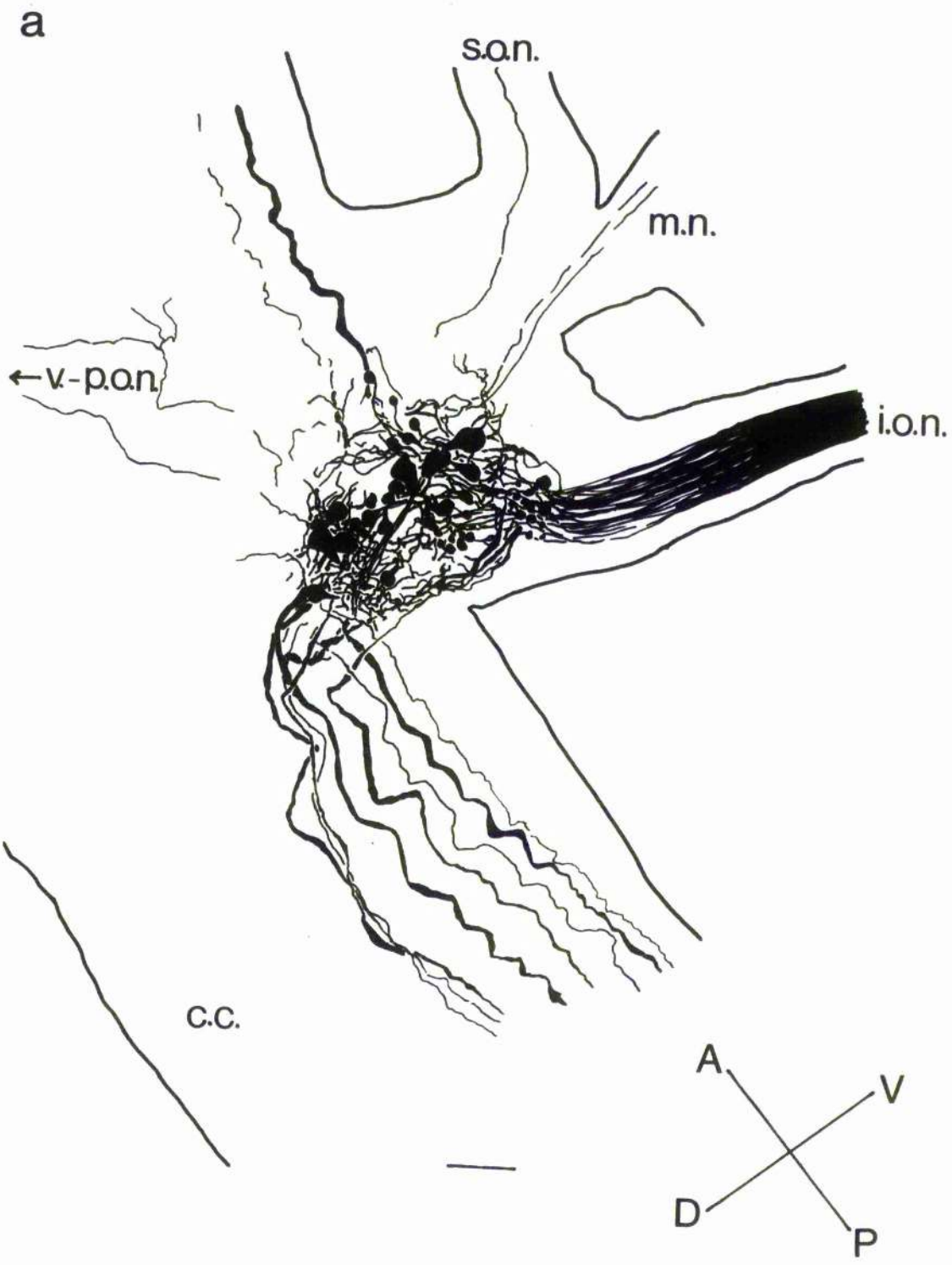
Figure 2.8

Cobalt chloride backfilling the commissural ganglion through the inferior oesophageal nerve

a. A right commissural ganglion. Note the wide distribution of cells in the posterior part of the ganglion and the dense neuropile area. There are filled axons in all other commissural ganglion nerves and in the anterior and posterior circumoesophageal connective.

b. A right commissural ganglion showing features similar to those in a. In this preparation axons have not filled in the minor commissural ganglion nerves.

Scale in a and b : 100 μm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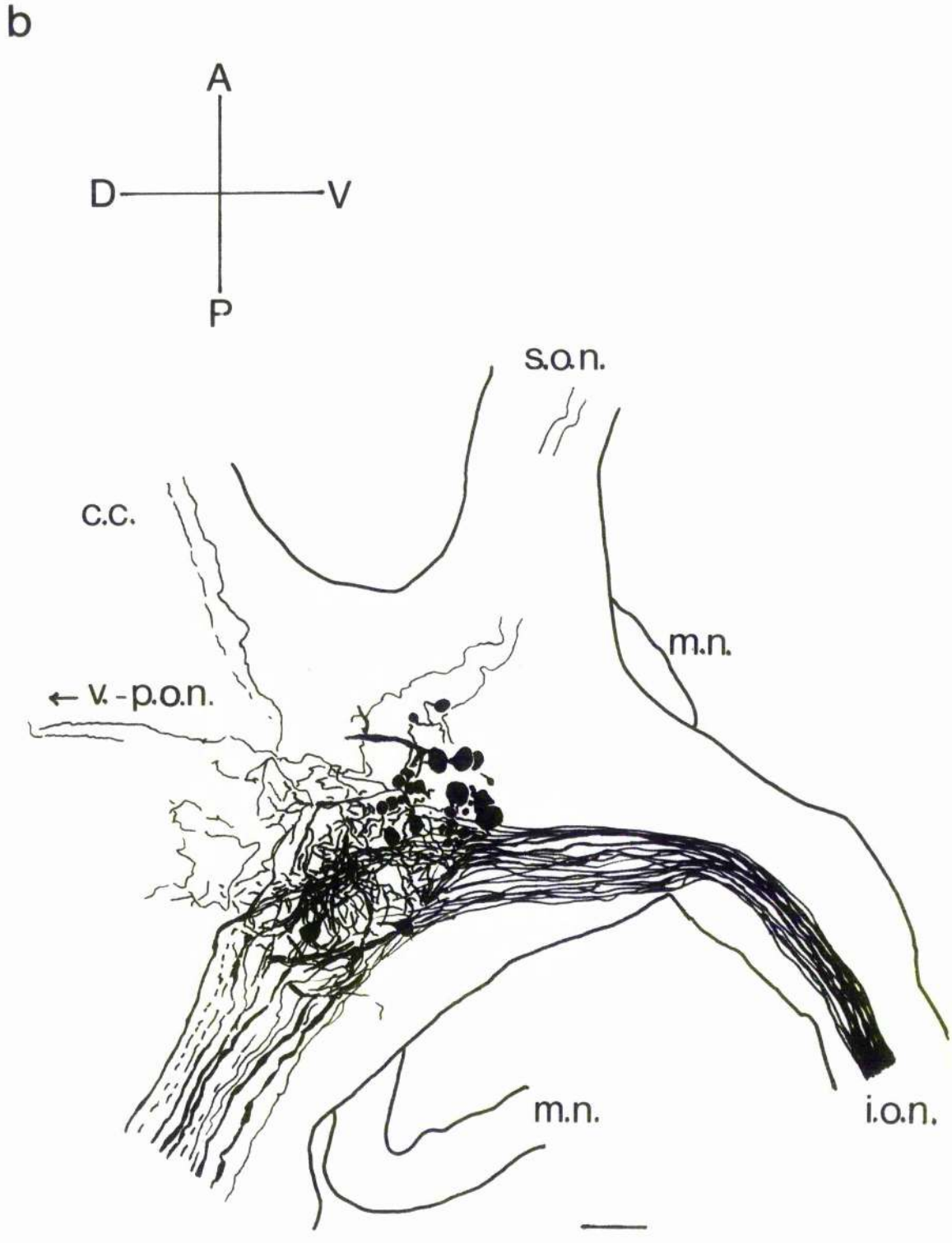
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Filled axons were observed in the superior oesophageal nerve, ventral-posterior oesophageal nerve and minor nerves (see Table 2.1). One or two axons were seen travelling towards the brain in the anterior circumoesophageal connective. In the posterior connective up to twelve axons travelled towards the suboesophageal ganglion. At least one of these axons traversed the post-oesophageal commissure and in one preparation one axon appeared to cross the connective at the level of the nerve supplying muscle O5b. A prominent group of through fibres passed posteriorly through the commissural ganglion and often formed a discrete axon bundle in the posterior connective (see Figure 2.8). A similar tract was observed in P. interruptus (KUSHNER, 1979).

Outer labral nerve

See Table 2.1 and Figure 2.9. No cell bodies in the commissural ganglion filled from the outer labral nerve. An area of neuropile was seen in the posterior part of the ganglion and some of the axons appeared to make complex arborisations. No axon branches were observed travelling in other commissural ganglion nerves or towards the brain in the anterior circumoesophageal connective. Up to ten axons were seen in the posterior connective; their course usually became indistinct after a few millimetres but in one preparation they were seen to reach the suboesophageal ganglion. Some of these axons were large (over 5 μm in diameter). Nerve fibres from the outer labral nerve may represent the through fibre tract seen on filling the entire inferior oesophageal nerve.

Inner labral nerve

See Table 2.1 and Figure 2.10. No more than fifteen cells filled from the inner labral nerve although up to twenty have been observed in Homarus gammarus (R.M. ROBERTSON, pers. comm.). These cells were

Figure 2.9

Cobalt chloride backfilling the commissural ganglion through the outer labral nerve

A right commissural ganglion. No cell bodies filled in the ganglion. There is a filled neuropile area in the posterior part of the ganglion. Several axons in the posterior circumoesophageal connective are filled but none in other nerves.

Scale : 100 μm

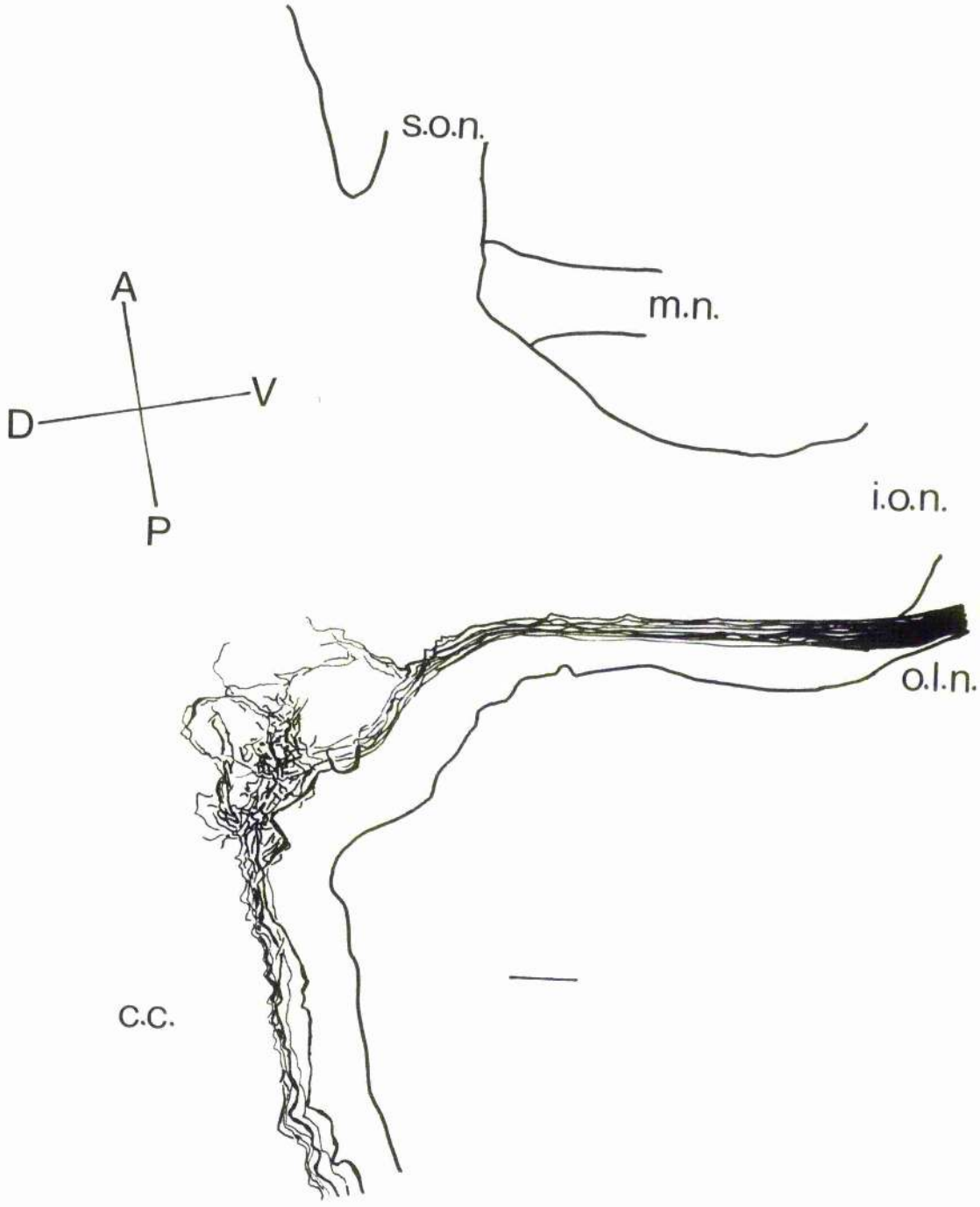
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Figure 2.10

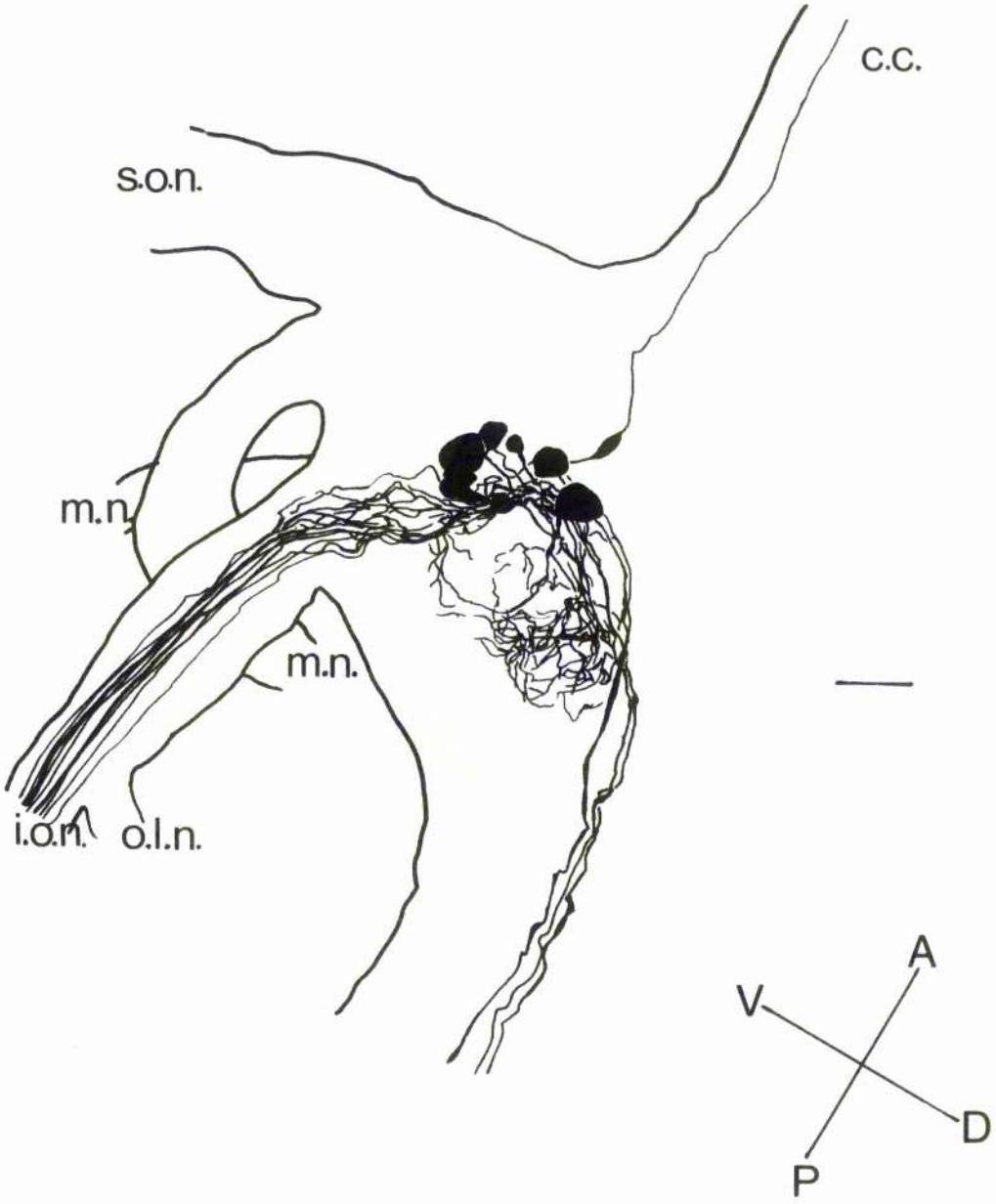
Cobalt chloride backfilling the commissural ganglion through the inner labral nerve

a. A left commissural ganglion. There is a small group of cells in the centre of the ganglion and a sparse area of neuropile located posteriorly. There are some filled axons in the anterior and posterior circumoesophageal connective but none in other commissural ganglion nerves.

b. A right commissural ganglion. Note features similar to those seen in a and the large number of axons in the posterior circumoesophageal connective.

Scale in a and b : 100 μm

a



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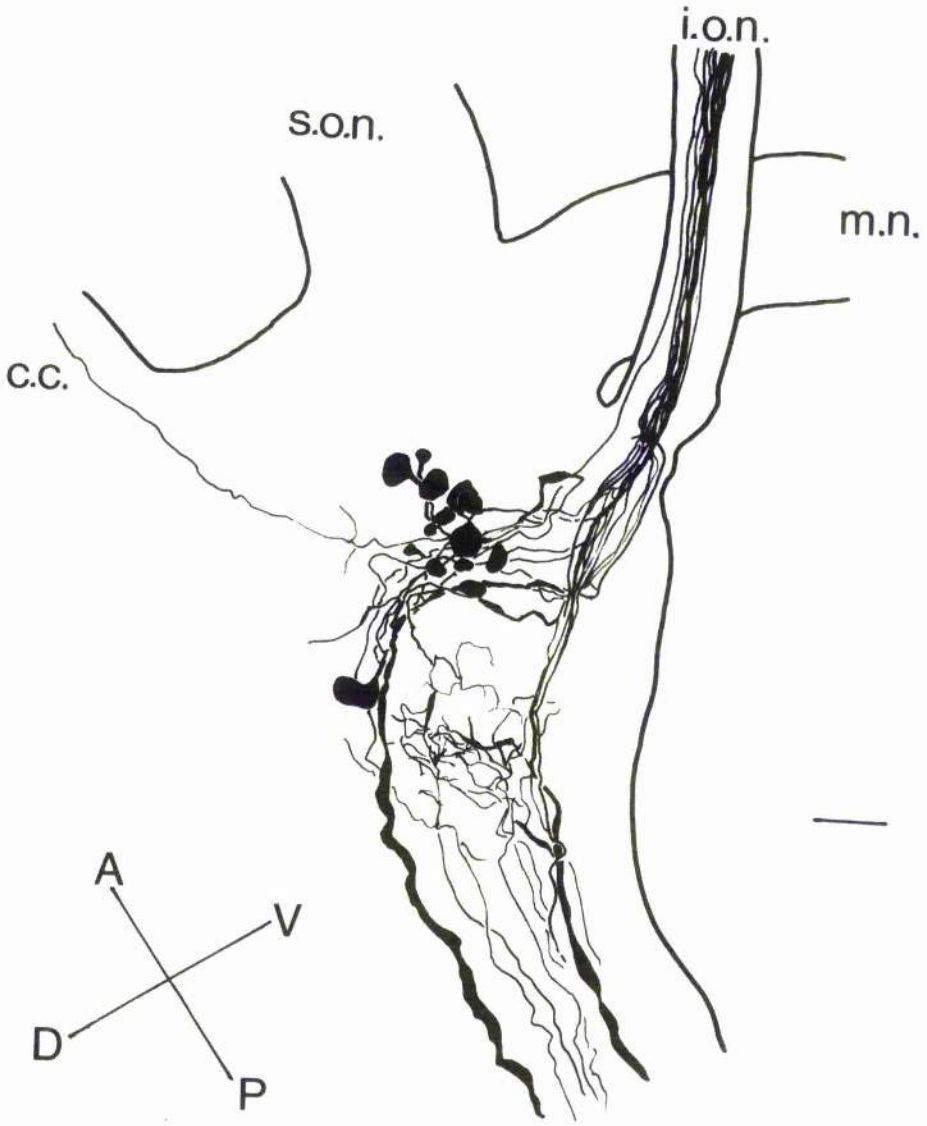

located roughly in the centre of the commissural ganglion and ranged in diameter from about 10-60 μm. An area of neuropile containing thin, branching processes occurred posteriorly in the ganglion. No filled axons were seen in other commissural ganglion nerves. One axon was often observed travelling anteriorly in the circumoesophageal connective towards the brain. Up to nine axons travelled posteriorly in the connective, some of them appearing to traverse the connective at the level of the post-oesophageal commissure and some continuing towards the suboesophageal ganglion.

Ventral-posterior oesophageal nerve

See Table 2.1 and Figures 2.11 and 2.12. About three somata located on the medial surface of the commissural ganglion at the exit point of the ventral-posterior oesophageal nerve were usually seen. These cells appeared to be very variable in distribution over the medial surface and it is possible that due to the difficulties encountered in backfilling this nerve (see below) only a few neurons were "sampled" in each preparation. Methylene blue studies showed that there are about fifteen somata on the medial surface at the exit point of the ventral-posterior oesophageal nerve from the commissural ganglion (see Figure 2.12 a). These may be closely clustered as in Figure 2.12 a or more scattered. Some of these neurons appear to be bipolar (see Figure 2.12 a and b). The neuropile area in the posterior and centre of the ganglion was often more extensive than in the preparation shown in Figure 2.11 which is otherwise representative. One axon in the inferior oesophageal nerve was consistently filled and up to four axons were seen travelling posteriorly in the circumoesophageal connective towards the suboesophageal ganglion. It was not clear if any axons in the superior oesophageal nerve were filled.

These results from backfilling the commissural ganglion through the

Figure 2.11

Cobalt chloride backfilling the commissural ganglion through the ventral-posterior oesophageal nerve

Medial view of a right commissural ganglion. Note the three filled somata at the exit point of the ventral-posterior oesophageal nerve from the ganglion. The filled neuropile area is atypically sparse in this preparation. There are filled axons in the posterior circumoesophageal connective and one in the inferior oesophageal nerve.

Scale : 100 μm

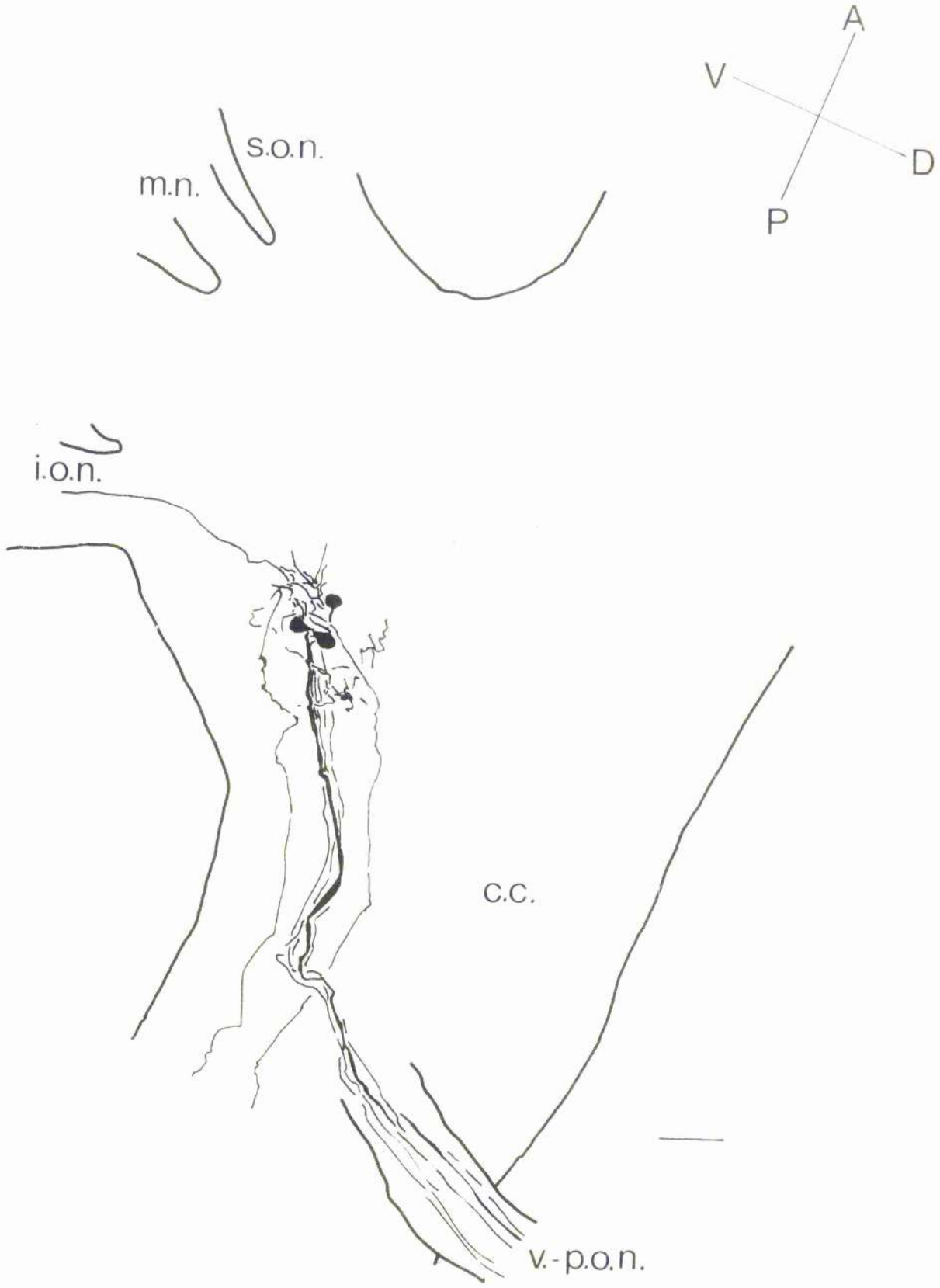
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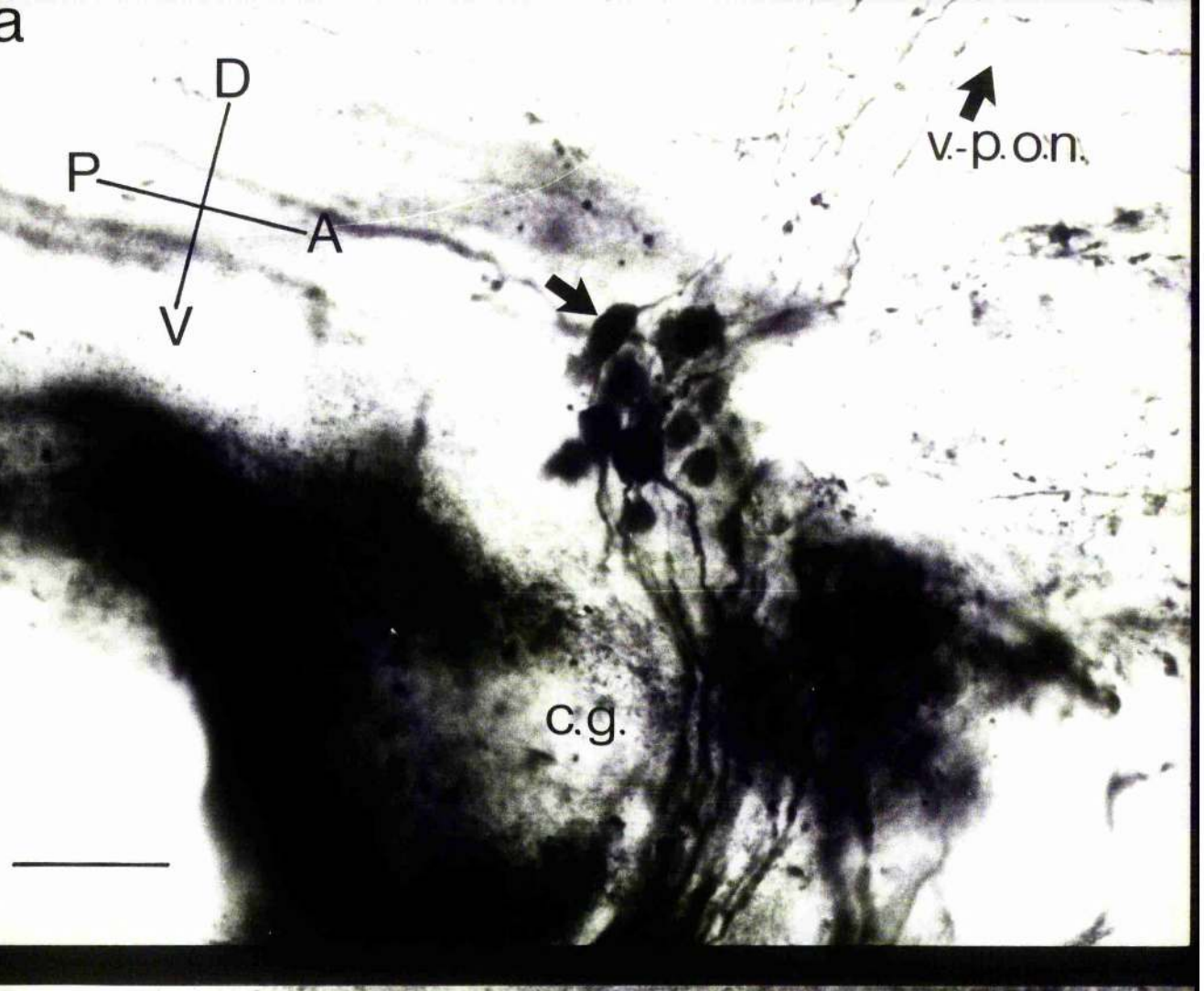
Figure 2.12

Ventral-posterior oesophageal nerve somata

a. Medial view of a left commissural ganglion stained with methylene blue (permanent preparation). Over ten neuron somata are clustered at the exit point of the ventral-posterior oesophageal nerve from the commissural ganglion, sending axons into this nerve and into the ganglion. At least one of these cells is bipolar (arrow).

b. Medial view of a left commissural ganglion backfilled with cobalt chloride through the ventral-posterior oesophageal nerve. Three bipolar cells, presumably from the group shown in a above, are filled.

Scale in a and b : 100 µm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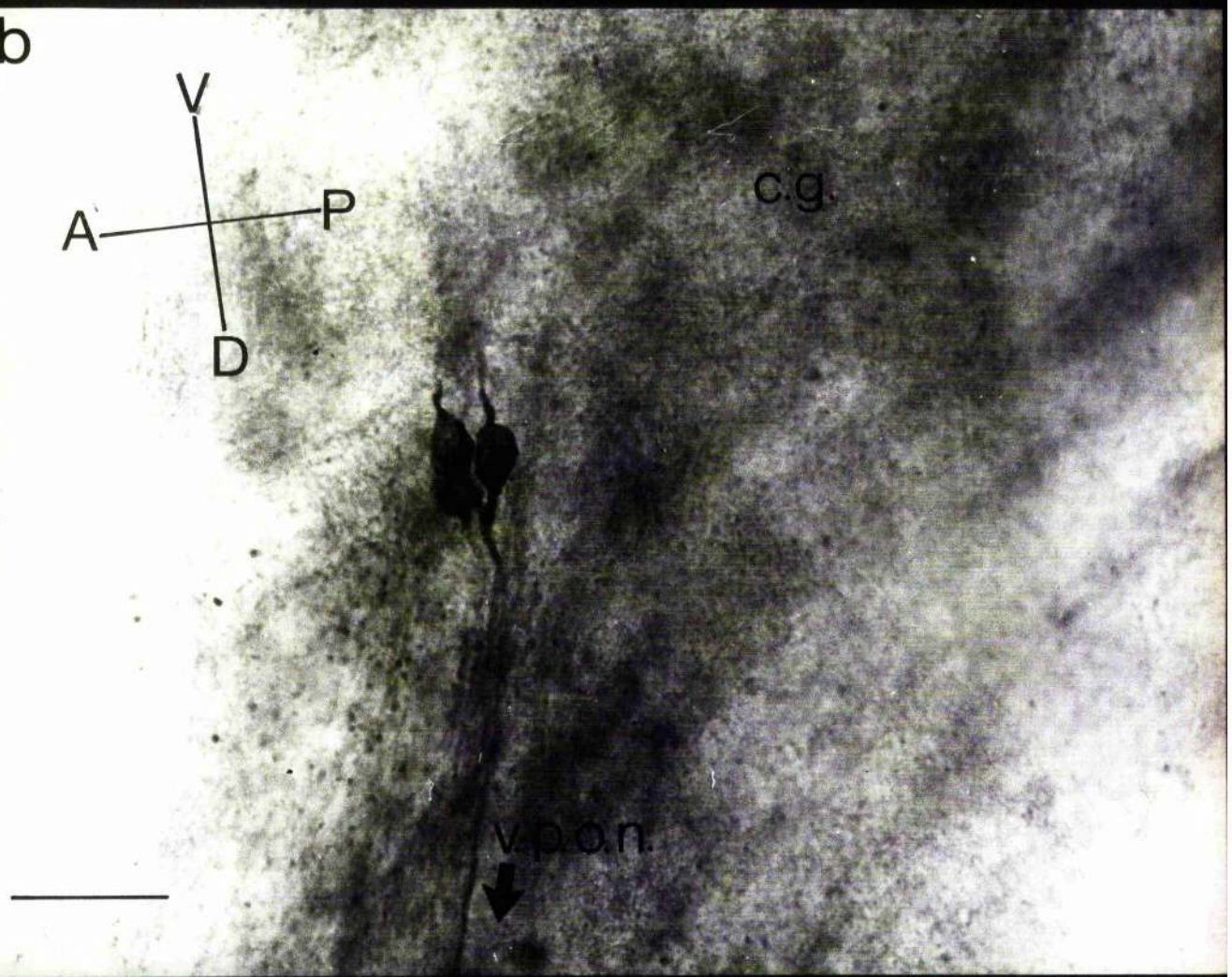
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ventral-posterior oesophageal nerve must be treated cautiously in view of the difficulties experienced with the preparation. Backfills were often unsuccessful because of the shortness of the nerve. Often several other neurons were seen as faint profiles, for example on the lateral surface, which is characteristic of transynaptic cobalt migration. Why this problem occurred in the ventral-posterior oesophageal nerve preparations and hardly at all in others is unclear.

Outer mandibular nerve

See Table 2.1. No filled commissural ganglion somata were observed. In all ten preparations a tract of axons travelled posteriorly down the circumoesophageal connective, past the post-oesophageal commissure towards the sudoesophageal ganglion. A smaller tract travelled anteriorly in the connective, subdividing into two groups of axons. Both groups appeared to arborise in the commissural ganglion, one postero-ventrally and one mid-dorsally.

One axon in the former group appeared to run in the inferior oesophageal nerve although its whole course was not very clear in any preparation. In one preparation an axon was observed in one of the commissural ganglion minor nerves but it was very faint and may have filled from cobalt leaking in the extracellular space. No outer mandibular nerve axons were observed to travel more anteriorly than the commissural ganglion, i.e. none ran to the brain.

Anterior circumoesophageal connective

See Table 2.1 and Figure 2.13. Over fifty somata were filled through the anterior connective, cut at the level of the brain, including a large group of small cells (less than about 15 μm in diameter) located mid-dorsally in the ganglion. It was difficult to count these cells as they tended to overlap each other and the connective in which most axons

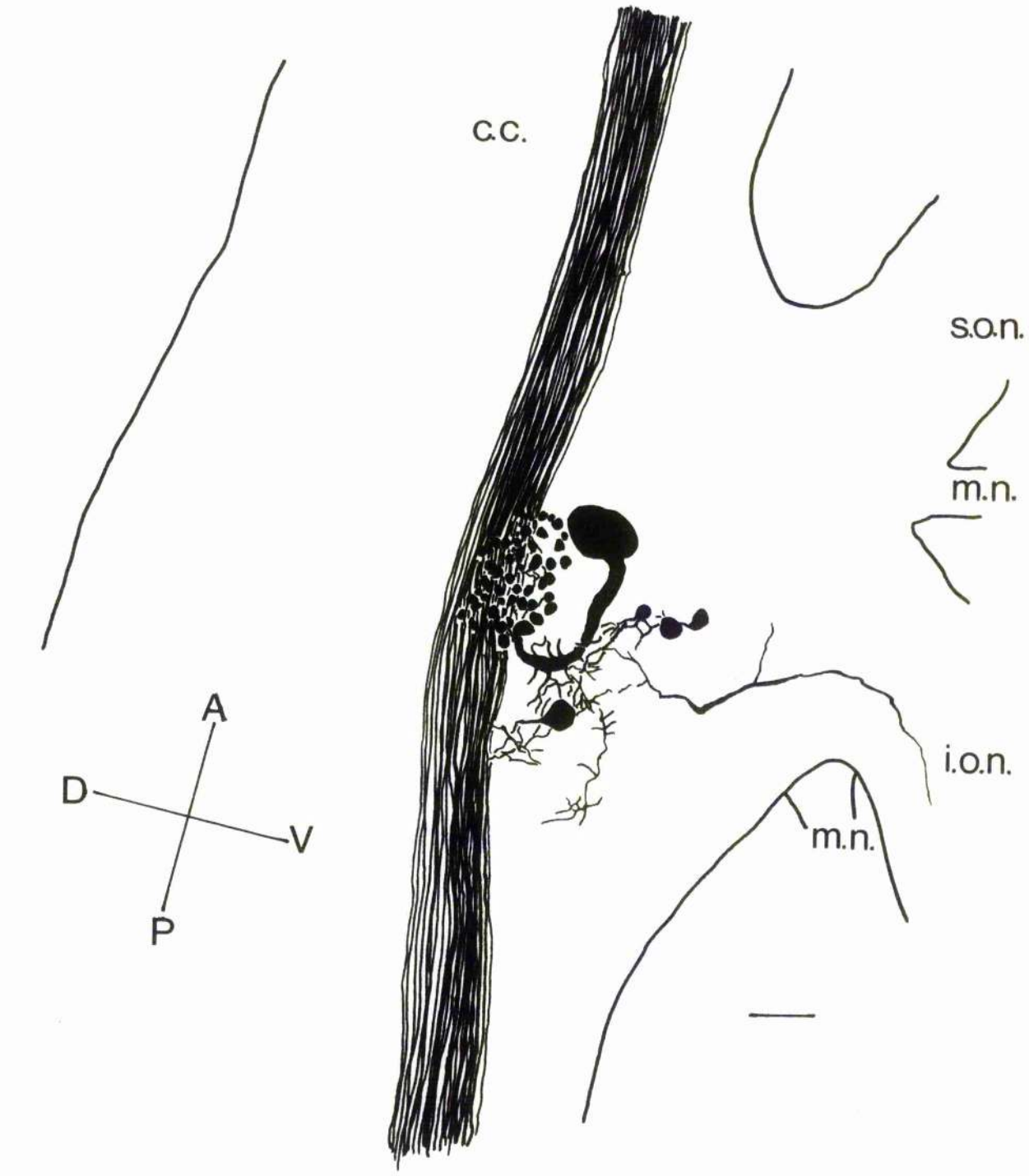
Figure 2.13

Cobalt chloride backfilling the commissural ganglion through the anterior circumoesophageal connective

a. A right commissural ganglion. Filled axons in the circumoesophageal connective are depicted only at its margin with the ganglion. A large group of small cells located mid-dorsally, the L cell and a few other somata are filled, totalling over fifty. The neuropile area appears to be sparse. No axons are filled in the superior oesophageal nerve or minor nerves which is not typical of these preparations.

b. A left commissural ganglion. Note features similar to those seen in a.

Scale in a and b : 100 μm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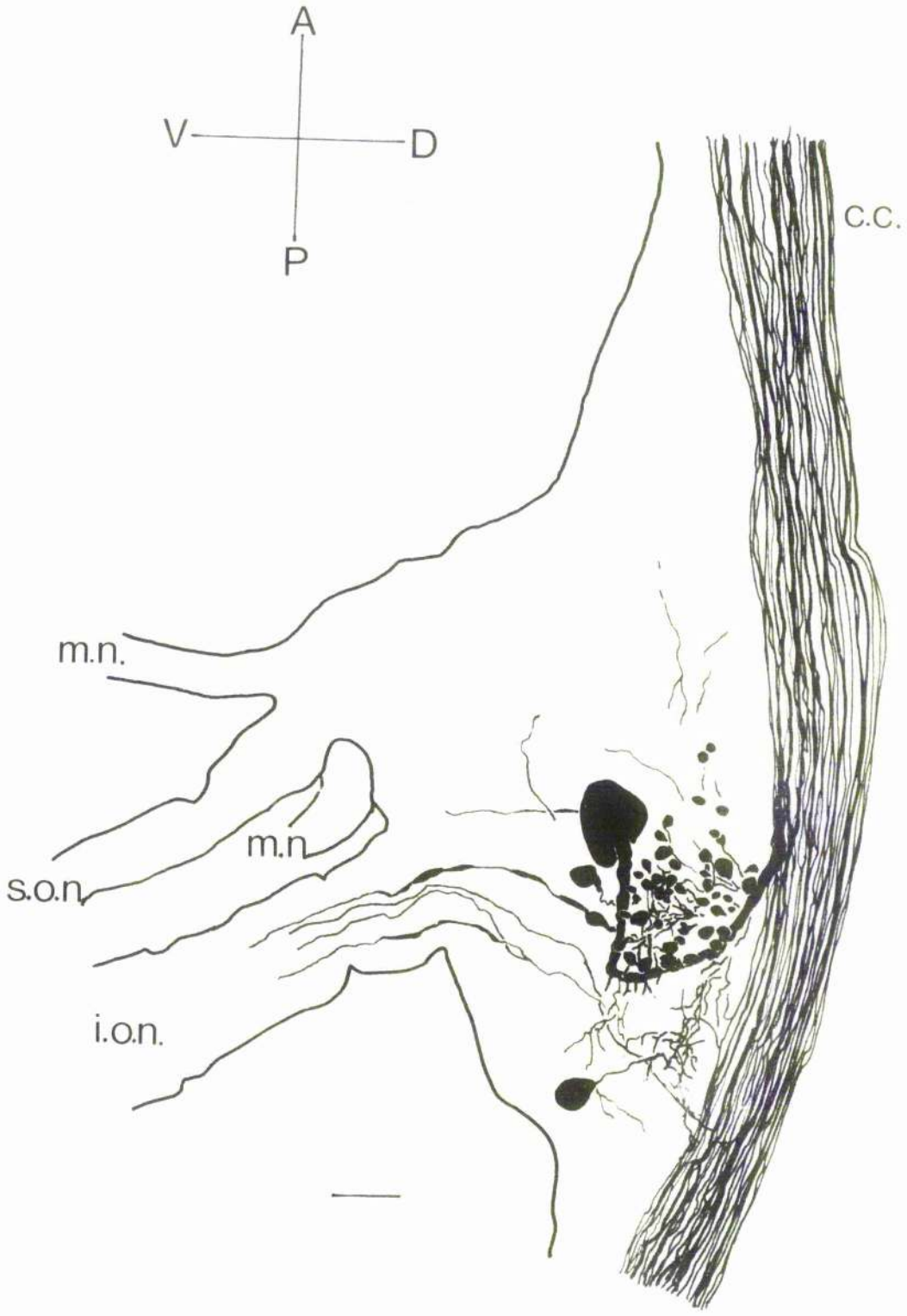


had filled. Some preparations indicated that there may be as many as 200 small cells in this group. The L cell was always filled in these preparations indicating that its axon travels to the brain after looping in the commissural ganglion as shown in Figure 2.13 (see Chapter 4). Two or three cells, about 20-30 μm in diameter, ventral to the L cell and a cell about 50 μm in diameter located posteriorly were also consistently filled. The neuropile area in these preparations was sparse but in intensified preparations it was revealed to be more extensive. The larger cells, especially the L cell, appeared to arborise profusely in the ganglion, but it was not possible to observe the processes of the large group of small cells. Axons filled in all commissural ganglion nerves except the ventral-posterior oesophageal nerve. Usually only one axon was seen in the superior oesophageal nerve but up to four in the inferior oeosphageal nerve. Up to three axons filled in the minor commissural ganglion nerves. It was not generally possible to observe the processes of individual commissural ganglion cells running in the connective since it filled almost entirely. However, in one intensified preparation one small neuron in the mid-dorsal group was seen to have a T-shaped branching axon, one branch travelling anteriorly and one posteriorly in the connective (compare with ORLOV, 1929, neuron D in Figure 19; neuron 43 in Figure 16.52 in BULLOCK and HORRIDGE, 1965).

Posterior circumoesophageal connective

See Table 2.1 and Figure 2.14. In these preparations extracellular cobalt migration caused problems in deriving and interpreting results. Hence these observations can only be used as a general guide. In all preparations over fifty neuron somata filled. These had an extensive size range (about 10-100 μm in diameter) and were widely distributed throughout the commissural ganglion. Some of them appeared to correspond

Figure 2.14

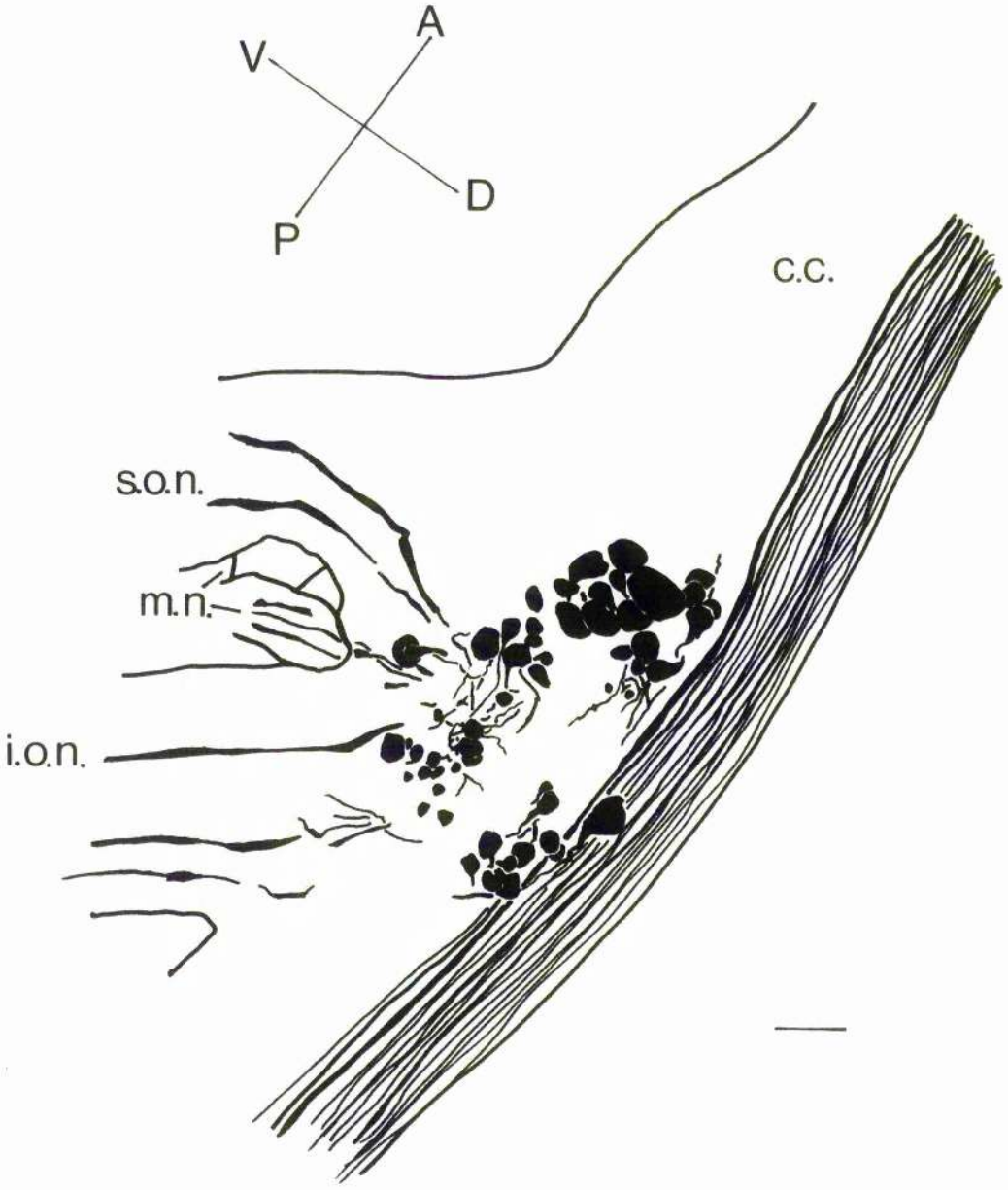
Cobalt chloride backfilling the commissural ganglion through the posterior circumoesophageal connective

a. A left commissural ganglion. Filled axons in the connective are depicted only at its margin with the ganglion. Over fifty cell bodies exhibiting a wide size range and distribution are filled. Several axons appear to have filled in the superior and inferior oesophageal nerves and the minor nerves but this may be due to extracellular cobalt leakage in these preparations, which also obscured neuropilar detail.

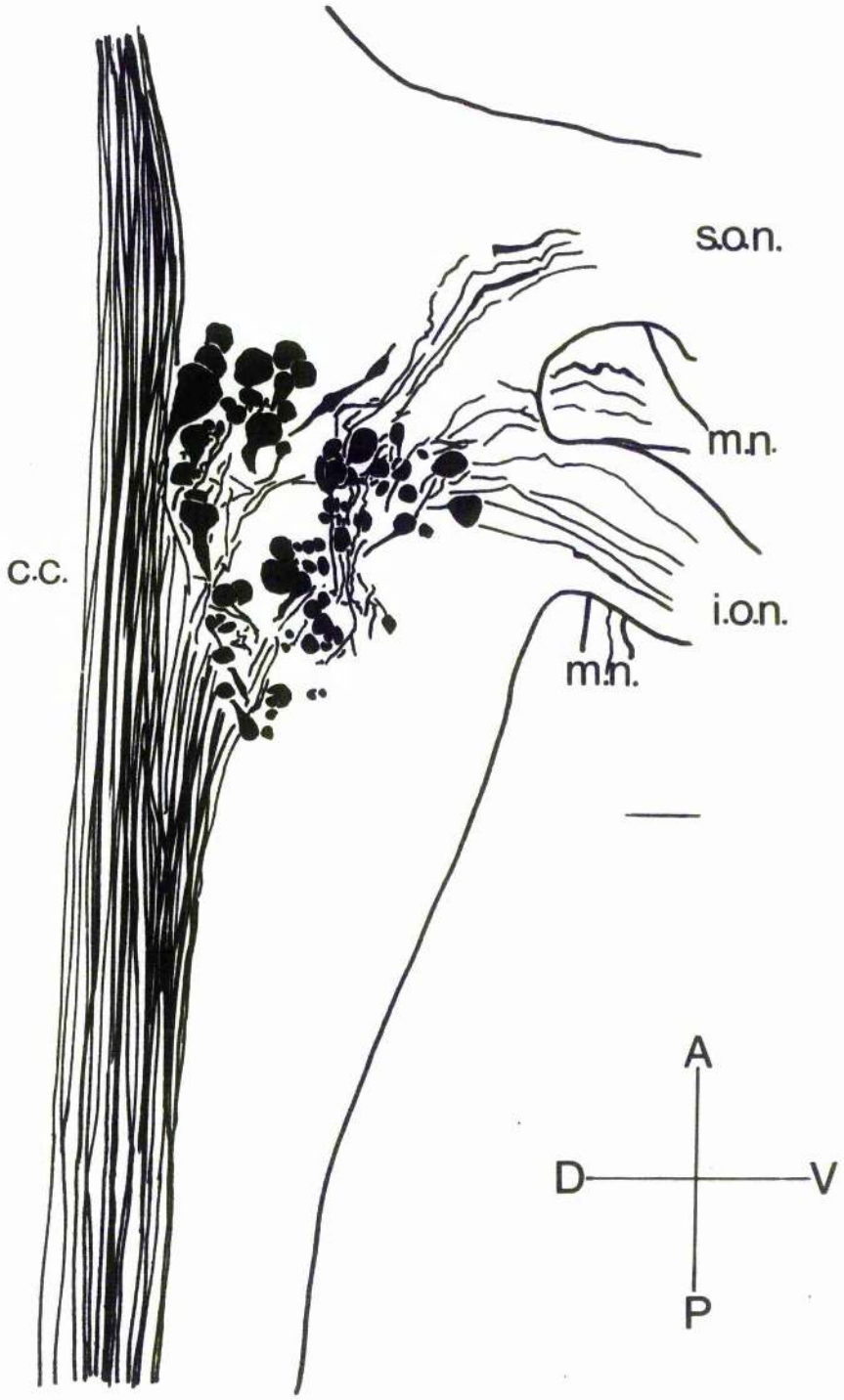
b. A right commissural ganglion. Note features similar to those seen in a.

Scale : 100 μm

a



b



to cells which also filled from the superior or inferior oesophageal nerve. One cell, located anteriorly, was very large (up to 100 µm in diameter) and sent an unbranching axon posteriorly down the connective. Most neuropilar detail could not be seen as cobalt leakage produced a brown background stain throughout the ganglion against which individual cell processes usually could not be discerned. Most cells seemed to send their axons postero-dorsally to the connective (see Figure 2.14 b). There was some indication that several nerves were filling in all the commissural ganglion nerves (see Table 2.1) but these data must be interpreted with caution because of the problems of cobalt leakage.

Stomatogastric nerve

See Table 2.1 and Figures 2.15 and 2.16. Only seven out of eighteen preparations were successful in that stomatogastric nerve axons could be traced into the commissural ganglia. This may be partly due to the distance between the point of filling the stomatogastric nerve (about five millimetres from the junction of the oesophageal, stomatogastric and two superior oesophageal nerves) and the commissural ganglion, which is over one centimetre.

The same number of axons (about eight) tended to fill in the right and left superior oesophageal nerves in a single preparation. This symmetry is partly due to bifurcating stomatogastric nerve axons which send a branch in each superior oesophageal nerve (axon a in Figure 2.15 b : compare with ORLOV, 1929, neuron N in Figure 5; neuron 14 in Figure 16.51 in BULLOCK and HORRIDGE, 1965). Another class of bifurcating axons sends a branch in one superior oesophageal nerve and a branch in the oesophageal nerve towards the oesophageal ganglion (axon b in Figure 2.15 b). Several axons did not branch but travelled from the stomatogastric nerve in the right or left superior oesophageal nerve (axon c in Figure 2.15 a :

Figure 2.15

Cobalt chloride backfilling through the stomatogastric nerve

a. The junction of the oesophageal, inferior ventricular and inferior oesophageal nerves. Note the three filled oesophageal ganglion somata. One axon bifurcates, sending a branch down each inferior oesophageal nerve and others travel unbranched from the oesophageal nerve down the right or left inferior oesophageal nerve.

b. The junction of the stomatogastric, oesophageal and superior oesophageal nerves. Only a selection of axons are depicted :-
a : bifurcating axons sending a branch in each superior oesophageal nerve
b : bifurcating axons sending a branch in the right or left superior oesophageal nerve and a branch in the oesophageal nerve
c : unbranching axons travelling from the stomatogastric nerve to the right or left superior oesophageal nerve

Scale (applies to a and b) : 100 μm

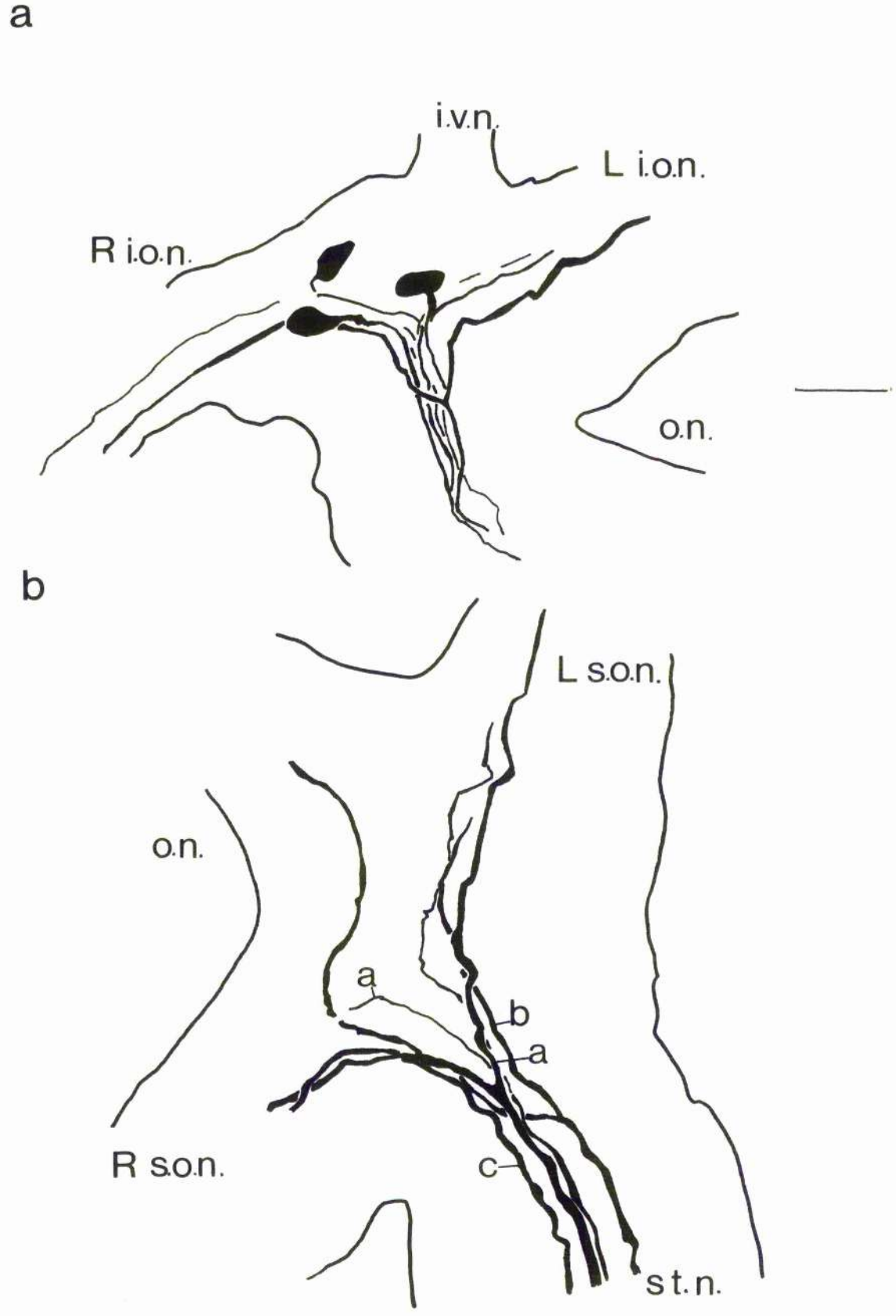

a
i.v.n.
L i.o.n.
R i.o.n.
o.n.
b
L s.o.n.
o.n.
a
b
a
R s.o.n.
c
s t. n.

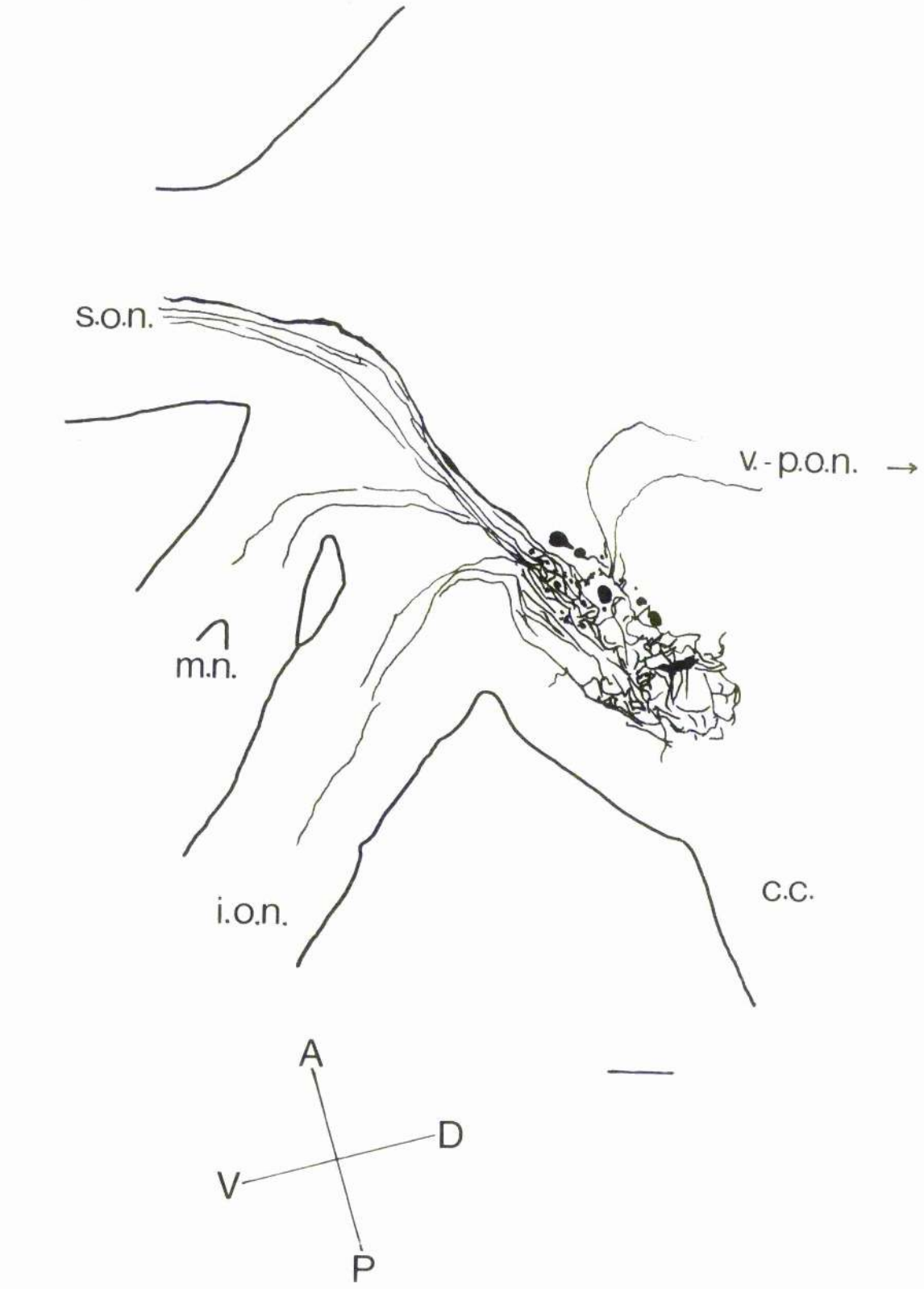
Figure 2.16

Cobalt chloride backfilling the commissural ganglion through the stomatogastric nerve via the superior and inferior oesophageal nerves

a. A left commissural ganglion. Note several small filled cell bodies and other very small features which may represent neuron somata. There is a pronounced area of filled neuropile and filled axons in the ventral-posterior oesophageal nerve and the minor nerves.

b. A right commissural ganglion. Note features similar to those seen in a.

Scale in a and b : 100 μm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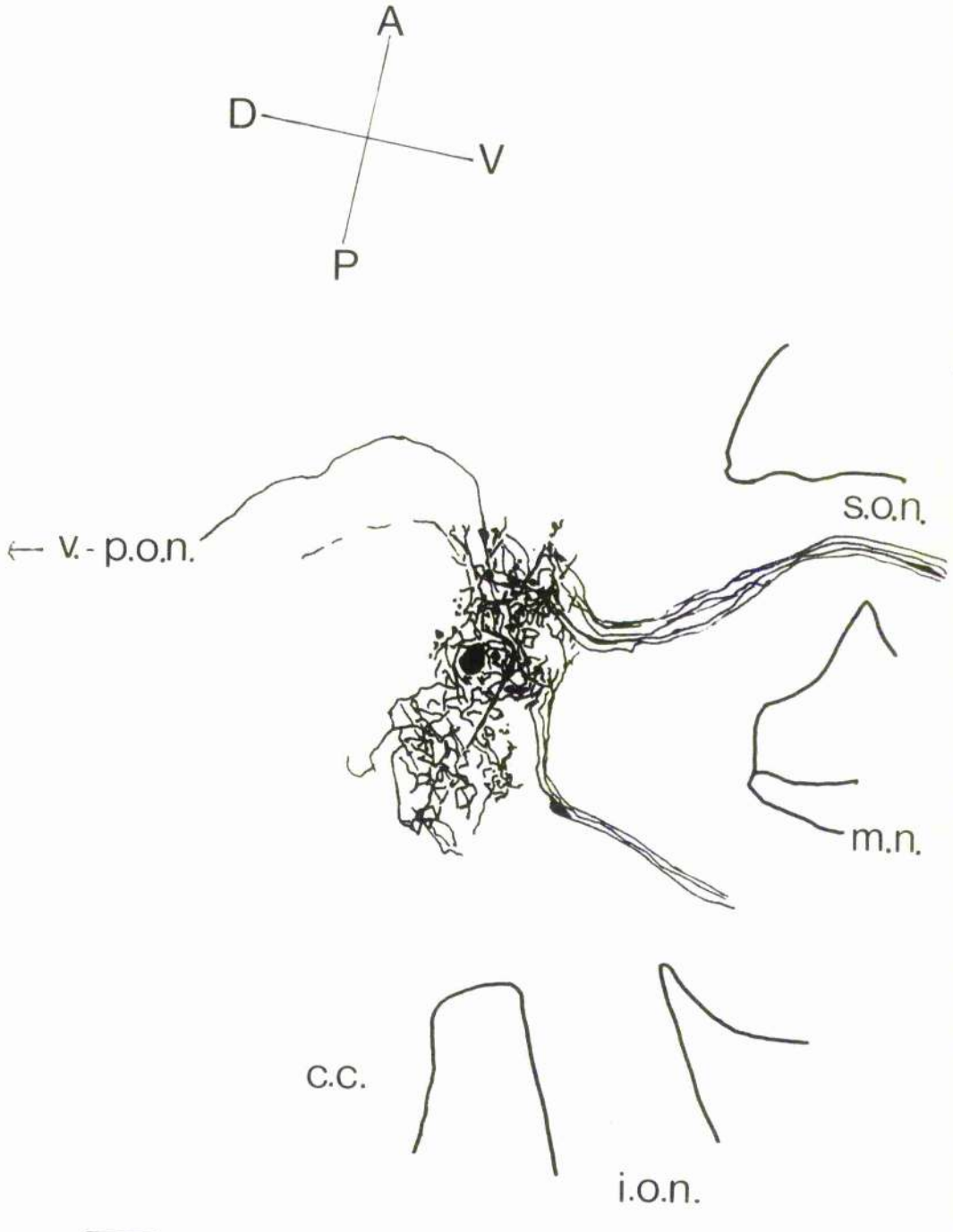


compare with ORLOV, 1929, neurons a and a1 in Figure 3; neuron 1 in Figure 16.51 in BULLOCK and HORRIDGE, 1965). The large number of axons at this junction presented a complex picture and it was not possible to observe any other classes of axons. For example, ORLOV (1929) decribes axons travelling from one superior oesophageal nerve to the other without branching.

About eight axons stained in the oesophageal nerve. Three oesophageal ganglion somata always filled from the stomatogastric nerve (see Figure 2.15 a) sending axons down the oesophageal nerve. MOULINS and VEDEL (1977) found that four oesophageal ganglion somata took up cobalt from the stomatogastric nerve in Palinurus vulgaris. There was no clear evidence for fibres filling in the inferior ventricular nerve from the stomatogastric nerve (in contrast see ORLOV, 1929, e.g. cells h2 and g4 in Figure 5; MOULINS and VEDEL, 1977, Figure 2A). At this junction bifurcating axons were seen sending a branch down each inferior oesophageal nerve. Other axons travelled from the oesophageal nerve to the right or left inferior oesophageal nerve without branching (compare with ORLOV, 1929, neuron E in Figure 3 and neurons B and C in Figure 5; neuron 4 in Figure 16.51 in BULLOCK and HORRIDGE, 1965). In a single preparation there was usually symmetry in the number of axons filling in the right and left inferior oesophageal nerves. This number, about three, was markedly less than the number of axons filling from the stomatogastric nerve in the superior oesophageal nerves.

The inferior oesophageal nerves are relatively longer than the superior oesophageal nerves and cobalt travelling in them did not usually reach the commissural ganglia, even with an incubation time of 48 hours. Thus most cobalt-filled features seen in the commissural ganglia presumably correspond to stomatogastric nerve axons filled in the superior oesophageal nerves.

It was difficult to assess the number of commissural ganglion cells filled from the stomatogastric nerve; for example due to long-distance cobalt migration some features were often faint. Up to five small cells (about 15 μm in diameter) usually filled. Several very small features (less than about 8 μm in diameter) were usually seen (see Figure 2.16). Their small size and distribution in the neuropile area precluded identification. Some probably represent neuron somata but others may be varicosities or thickenings in cobalt-filled axons. Somata of this size were not seen in backfills of the entire superior oesophageal nerve but they could have been obscured by the dense neuropile area (see Figures 2.6 and 2.7). These results contrast with those of KUSHNER (1979) who states that only two or three commissural ganglion cells send axons to the stomatogastric ganglion via the superior oesophageal and stomatogastric nerves. If cobalt has reached the commissural ganglion some of these cells may correspond to cells seen on filling the entire inferior oesophageal nerve. If inferior oesophageal nerve, outer labral nerve and inner labral nerve preparations are compared (Figures 2.8, 2.9 and 2.10) it can be demonstrated that there are a number of small cells (less than about fifteen μm in diameter) seen in backfills of the entire inferior oesophageal nerve which apparently do not send axons in the outer or inner labral nerves. At least some of these cells may be sending axons to the stomatogastric ganglion via the inferior oesophageal nerves. KUSHNER (1979) was unsuccessful in characterising cells sending axons in the stomatogastric nerve via the inferior oesophageal nerves.

About two axons filled in the commissural ganglion minor nerves and up to two in the ventral-posterior oesophageal nerve. No axons appeared to fill in the circumoesophageal connective.

Discussion

Each commissural ganglion contains several hundred neurons. Some of them appear to be motorneurons, for example large neurons (more than about 40 μm in diameter) sending axons in the superior or inferior oesophageal nerves, presumably to innervate the oesophagus and the labrum respectively. Considering the mean number of neurons filled from the superior and inferior oesophageal nerves (Table 2.1) and taking the number of motorneurons sending an axon in the ventral-posterior oesophageal nerve as ten suggests that possibly only about sixty commissural ganglion neurons are motorneurons. Most commissural ganglion neurons are small (less than about 20 μm in diameter) and at least some of these are interneurons, for example the large dorsally-located group of small neurons sending an axon to the brain in the anterior circumoesophageal connective. It is possible that some commissural ganglion somata represent centrally-located sensory cells. The peripheral location of some sensory neurons sending input to the commissural ganglion is known, for example the anterior and posterior oesophageal sensors and mouthpart receptor 1 (see Chapter 3 Introduction part b) but this does not preclude the possibility of centrally-situated somata of other sense organs. In particular, little is known about oesophageal proprioceptors (see Chapter 3 Introduction part b) and there are several examples of non-spiking mechanosensory neurons in the central nervous system of "higher" Crustacea (PAUL, 1976; RIPLEY, BUSH and ROBERTS, 1968). However, it seems that several hundred commissural ganglion neurons may be interneurons. Such a large population of interneurons is not uncommon in crustacean central nervous ganglia. For example, WILSON and SHERMAN (1975) deduced from cobalt chloride backfilling studies of the third thoracic ganglion of the lobster, Homarus americanus, that about 90% of the neuron somata are interneurons.

There is a large number of input and output pathways in the commissural ganglion. Bundles of fine fibres seen in cross-sections of all commissural ganglion nerves may represent sensory fibres as SUTHERLAND and NUNNEMACHER (1968) suggest for the thoracic roots of the crayfish Orconectes virilis. For example, bundles of fine fibres in the ventral-posterior oesophageal nerve may derive at least partly from the posterior oesophageal sensors, fine fibres in the superior oesophageal nerve from the anterior oesophageal sensors and those in the inferior oesophageal nerve from mouthpart receptor 1 (see Chapter 3 Introduction part b). However, as SUTHERLAND and NUNNEMACHER (1968) point out, axon size is not an absolute criterion of function. Physiological study is needed to define the role of these fine fibres. The relatively small proportion of motorneurons in the commissural ganglion and the paucity of neuromuscular innervation in arthropods suggests that the large number of medium-sized fibres (about 3-10 μm in diameter) in the commissural ganglion nerves, totalling over 350, do not all represent motor axons. Presumably some of them are sensory axons and in the superior and inferior oesophageal nerves the axons of interneurons travelling at least to the oesophageal ganglion.

Cobalt chloride backfilling studies emphasise the multiplicity of interactions between the commissural ganglia and other parts of the nervous system. However, there are severe limitations to the interpretation of cobalt backfilling (see below) and conclusions can only be tentative and qualitative. In most cases it was difficult to differentiate axons passing through the commissural ganglion and those derived from commissural ganglion somata. However, there is strong evidence for common pathways between all the commissural ganglion nerves, the superior, inferior and ventral-posterior oesophageal nerves and the minor nerves. For example, on backfilling the superior oesophageal nerve at least one inferior

oesophageal nerve axon was consistently filled. Some of the neurons filled from the inferior oesophageal nerve may represent some of those filled from the superior oesophageal nerve, i.e. neurons with an axon branch in each nerve. No such cells were observed with intracellular dye injections (see Chapter 3) but relatively few cells were sampled. ORLOV (1929) described neurons sending large fibres in both superior and inferior oesophageal nerves in Astacus (see ORLOV, 1929, neuron s in Figure 18; neuron 50 in Figure 16.52 in BULLOCK and HORIDGE, 1965); it is interesting to note that one or two large fibres in the inferior oesophageal nerve often filled on backfilling the superior oesophageal nerve. Similarly, on backfilling the superior oesophageal nerve axons filled in the ventral-posterior oesophageal nerve. There is some physiological evidence for common units in these nerves (see Chapter 3 Results part a). The activity of all the commissural ganglion nerves is closely related (see Chapter 3 Results part a); presumably this is underlain partly by these common anatomical pathways as well as by interactions within the ganglion, for example common driving of oesophageal and labral motorneurons by the oesophageal pattern generator (see Chapter 3).

There is some evidence for control of the labrum by the suboesophageal ganglion. The outer labral nerve has been described as purely sensory (ROBERTSON, 1978) and there is no evidence for motorneuron somata backfilling from this nerve. A few of the outer labral nerve axons seen in cross-section are medium-sized, which may imply that they are mechanosensory axons of mouthpart receptor 1 (M.S. LAVERACK, pers. comm.) or alternatively motor axons. Motor activity in this nerve has been observed in at least one species, Jasus lalandii (R.M. ROBERTSON, pers. comm.). Hence it is possible that some axons in the posteriorly-going tract seen on backfilling the outer labral nerve

represent motor axons with somata in the suboesophageal ganglion. It is not possible to tell what contribution, if any, these axons make to the outer labral nerve neuropile area in the posterior region of the ganglion.

Axons from the outer mandibular nerve tracts arborise in the commissural ganglion which suggests that information from the mandibles may provide some input to the ganglion. There is only slight evidence from these studies that outer mandibular nerve axons pass into any commissural ganglion nerves but ORLOV (1929) suggests that these axons pass into the superior and inferior oesophageal nerves in Astacus (see BULLOCK and HORRIDGE, 1965). Outer mandibular nerve tracts travel up the circumoesophageal connective only as far as the commissural ganglion; presumably integration of information from the mandibles takes place in the ganglion and is relayed to the brain. The large number of commissural ganglion neurons sending axons to the suboesophageal ganglion suggests interaction with the mandibular control system, in particular to effect coordination of the mandibles, labrum and oesophagus.

There appears to be great potential for the interaction of the commissural ganglia with higher nervous centres : the brain and the suboesophageal ganglion. Two interneurons sending an unbranched axon to the brain have been studied (see Chapter 3 Results part e(1), Figures 3.12 e and 3.13 e and Chapter 4). On backfilling the connective axons appear to fill in other commissural ganglion nerves which suggests that such coordination may be mediated not only by interneurons but by motorneurons sending a collateral in a commissural ganglion nerve and one in the connective. Intracellular dye injection confirmed that this is so in at least one case : Figure 3.6 shows a neuron with a bifurcating axon, one branch travelling in the inferior oesophageal nerve and one travelling in the posterior connective towards the suboesophageal ganglion (compare with ORLOV, 1929, neurons L,M and N in Figure 13; neurons 44 in Figure 16.52 in

BULLOCK and HORRIDGE, 1965). ORLOV (1929) also describes a neuron sending axon branches in the anterior and posterior connective and in the superior oesophageal nerve (see ORLOV, 1929, neuron A in Figure 18; unlabelled neuron in Figure 16.52c in BULLOCK and HORRIDGE, 1965).

Cobalt backfilling the stomatogastric nerve suggests that over ten commissural ganglion somata may send an axon to the stomatogastric ganglion via the superior and inferior oesophageal nerves. It seems unlikely that there are "as few as three" commissural ganglion neurons projecting to the stomatogastric ganglion via the superior oesophageal nerve as KUSHNER (1979) suggests for Panulirus interruptus. At least two commissural ganglion neurons send axons to the stomatogastric ganglion via this nerve : the E neurons, one in each commissural ganglion (RUSSELL, 1976) and the commissural gastric drivers, also one in each ganglion (ROBERTSON and MOULINS, unpublished a and e) (see Chapter 3 Introduction part e). Lesion experiments emphasise the importance of the superior and inferior oesophageal nerves in commissural ganglion inputs to the gastric and pyloric pattern generators in the stomatogastric ganglion (RUSSELL, 1977 : see Chapter 3 Introduction part e). These nerves must carry input concerning the oesophageal rhythm to the stomatogastric pattern generators, for example inputs to the AB interneuron and to the Ex cells (SELVERSTON et al., 1976), input from the commissural ganglion P neurons (RUSSELL, unpublished, in SELVERSTON et al., 1976) and from the commissural pyloric oscillators (ROBERTSON and MOULINS, unpublished a and d) (see Chapter 3 Introduction part e). There are about 240 axons in the stomatogastric nerve of Homarus americanus (MAYNARD, 1971a) and about 120 in Panulirus interruptus (KING, 1975, quoted in SELVERSTON et al., 1976). According to SELVERSTON et al. (1976) at least eight axons represent centrally projecting axons from stomatogastric neurons (AB, Int 1 and Ex cells) and a few from sensory neurons. The rest are thought to be input fibres to the

stomatogastric ganglion. Some of these derive from the brain via the inferior ventricular nerve, for example two "through fibres" (DANDO and SELVERSTON, 1972 ; see Chapter 3 Introduction parts d and e), some from the oesophageal ganglion (ORLOV, 1929; SPIRITO, 1975) but most presumably from the commissural ganglia. Hence it is suggested that well over ten neurons in each commissural ganglion send axons to the stomatogastric ganglion via the superior and inferior oesophageal nerves.

Little is known of stomatogastric ganglion inputs to the commissural ganglia but at least two stomatogastric ganglion neurons send an axon to each commissural ganglion : Int 1, synapsing onto the E neurons (RUSSELL, 1976) and interneuron AB, possibly responsible for the patterning of P neuron firing (RUSSELL, unpublished, in SELVERSTON et al., 1976) (see Chapter 3 Introduction part e). Backfilling the stomatogastric nerve revealed several bifurcating axons at the junction of the superior oesophageal nerves with the stomatogastric and oesophageal nerves, as SPIRITO (1975) found in Procambarus clarkii. Such bifurcating axons were also seen at the junction of the inferior oesophageal nerves with the oesophageal and inferior ventricular nerves. The axon branches of the filled oesophageal ganglion somata were unclear; SPIRITO (1975) found that at least six oesophageal ganglion neurons send axon branches symmetrically in the inferior oesophageal nerves or in the inferior and superior oesophageal nerves (see SPIRITO, 1975, Figure 5). SPIRITO apparently found no oesophageal ganglion cell homologous to neuron OD1 in Palinurus vulgaris (MOULINS and VEDEL, 1977). At least some of these pathways may underlie common input to both commissural ganglia from the stomatogastric and oesophageal ganglia. Such input may have an important coordinating role (see Chapter 3 Discussion).

Some difficulties and limitations of the methodology of this study have already been discussed, for example difficulties in counting neuron

somata (see Results part b) and in cobalt chloride backfilling techniques (see Materials and methods part e). The results underline not only the value of cobalt as a mapping technique but some of its limitations. Differences between retrograde (towards the soma) and anterograde (away from the soma) neuronal transport mechanisms may partly explain lack of reciprocity in the numbers of axons filled in one of a pair of nerves, for instance in the superior and inferior oesophageal nerves (see Table 2.1). It is not clear how cobalt travels in neurons, but it is commonly believed to be by a combination of active transport and passive diffusion (M. TYRER, pers. comm.). Furthermore, crustacean sensory and motor axons have different potentialities for "normal" physiological functioning after removal of the soma (BITTNER and JOHNSON, 1974). Hence there may be differences in cobalt transport in sensory axons (for example from the anterior and posterior oesophageal sensors and from mouthpart receptor 1 : see Chapter 3 Introduction part b), motor axons (from putative motorneurons in the suboesophageal ganglion projecting to the labrum via the outer labral nerve : see above) and interneuronal axons (from the brain, suboesophageal, oesophageal and stomatogastric ganglia) whose cell bodies have been removed on isolation of the commissural ganglion. It is also interesting that in at least one case known pathways were not revealed by this technique, further emphasising its limitations. Backfilling the stomatogastric nerve did not disclose the two through fibres from the brain in the inferior ventricular nerve (Figure 2.15 b). Physiological evidence has shown that these fibres are present in Homarus gammarus (R.M. ROBERTSON, pers. comm.; ROBERTSON and MOULINS, unpublished a, c and e). Perhaps the most severe limitation of cobalt chloride backfilling and intracellular injection techniques is the ability of cobalt to fill neurons other than those under investigation. STRAUSFELD and OBERMAYER (1976) showed that in the visual neuropile of dipterous insects cobalt could

migrate through at least two synapses between functionally contiguous neurons. FREDMAN and JAHAN-PARWAR (1980) have recently demonstrated that under at least some circumstances cobalt can migrate across neuronal membranes. As STRAUSFELD and OBERMAYER (1976) say : "Cobalt marking may be a far more powerful technique than hitherto realised" but its results must be treated with caution. Clearly, cobalt mapping must be supplemented by physiological studies, in particular by intracellular physiological and dye injection techniques to determine the structure and function of single, identified neurons.

The limitations of this anatomical study result in some features of commissural ganglion organisation remaining enigmatic. Cobalt backfilling studies have accounted for about 200-300 neuron somata. Considering the total number of commissural ganglion somata as about 400 implies that 100 or more neuron somata may represent local interneurons whose processes are confined to the ganglion itself and which cannot be revealed by backfilling techniques (see Figure 3.12 d and Figure 3.13 d). In particular, little can be deduced about the role of the post-oesophageal commissure. The results suggest that some axons from the inferior oesophageal nerve, in particular from the inner labral nerve, may cross the commissure. R.M. ROBERTSON (pers. comm.) has shown that in Homarus gammarus at least two oesophageal ganglion neurons send branches in both inner labral nerves and confirms that some inner labral nerve axons cross the commissure and travel to the contralateral commissural ganglion, one or two of them passing into the contralateral inner labral nerve. This suggests that the post-oesophageal commissure may be involved in labral control but at present the thousand or more axons that it contains remain largely unaccounted for (see also Chapter 3 Discussion).

Chapter 3 : The oesophageal rhythm

Introduction

a. The oesophagus

The oesophagus is a short muscular tube lined with thin, flexible cuticle which connects the mouth to the cardiac sac region of the stomach. ROBERTSON (1978), summarised in ROBERTSON and LAVERACK (1979), gives detailed descriptions of oesophageal morphology, musculature and innervation in Homarus gammarus.

b. Oesophageal sense organs

1. Chemoreceptors

ROBERTSON and LAVERACK (1978 and 1979) describe two bilaterally-located groups of chemoreceptors at the junction of the oesophagus and the cardiac sac. The posterior oesophageal sensors (refer to Figure 2.2) consist of groups of many small bipolar neurons whose dendritic endings are associated with structures on the cuticle of the oesophageal lumen. Their axons travel in the ventral-posterior oesophageal nerve to the commissural ganglion. Chemical or electrical stimulation of the posterior oesophageal sensors appears to initiate or augment oesophageal peristalsis (see part c below). The anterior oesophageal sensors also consist of populations of small bipolar neurons with associated epicuticular features. Their axons travel in the superior oesophageal nerve, presumably to the ipsilateral commissural ganglion. Electrical or chemical stimulation of these neurons results in the slowing or termination of oesophageal peristalsis.

2. Mechanoreceptors

There are no clear findings concerning proprioceptors located on the oesophagus itself. Early workers, for example ALLEN (1894b) described receptor cells on the oesophagus, which may represent mechanoreceptors. DANDO (1969) tentatively mentions "...scattered (probable) mechanoreceptor neurones...on the oesophagus". ROBERTSON and LAVERACK (1979) describe a small group of presumptive stretch receptors near the anterior oesophageal sensors whose axons travel in the same nerve. A detailed review of foregut sense organs is given by DANDO and MAYNARD (1974) who describe multiterminal and small uniterminal cells on the oesophagus which are probably mechanoreceptors. These are also briefly discussed by WALES (1976). However, there is no detailed information on their structure or function.

LAVERACK and DANDO (1968) and MOULINS, DANDO and LAVERACK (1970) investigated the anatomy and physiology of a group of mouthpart receptors (M.P.R.s) in Homarus gammarus, Panulirus argus, Nephrops norvegicus and Astacus leptodactylus. M.P.R. 1, 2 and 3 are located bilaterally in the mandibular-oesophageal region and their dendrites are associated with a strand of connective tissue extending antero-posteriorly on each side of the oesophagus. M.P.R. 1 is innervated by the outer labral nerve (see Chapter 2 and Figure 2.2). The axons of its receptor cells travel in the inferior oesophageal nerve to the commissural ganglion. MOULINS et al. (1970) suggest that they terminate there, although they state that "The anatomy of the area does not allow us to say if M.P.R. input passes to the commissural ganglion, the oesophageal ganglion, or both.". M.P.R.s 2 and 3 are innervated by the paragnathal nerve and the axons of their receptor cells travel to the suboesophageal ganglion. MOULINS et al. (1970) concluded that M.P.R. 1 comprises predominantly tonic units, whilst M.P.R. 2 and 3 comprise chiefly phasic units. Due to their location all three

receptors can respond to movements of the mandibles, labrum, paragnaths and oesophagus. LAVERACK and DANDO (1968) suggest that M.P.R. 2 is probably activated predominantly by oesophageal movements. MOULINS et al. (1970) conclude that "...it seems very probable that these receptors function to monitor the passage of food in the preoral cavity and the mouth.". Presumably input from these receptors could help to initiate, sustain or even alter the form of the oesophageal rhythm (see part c below). LAVERACK and DANDO (1968) suggest that they may also provide input to the stomatogastric ganglion, "priming" stomach activity for the reception of food.

c. The control of oesophageal peristalsis

Alternating constriction and dilation of the oesophagus pushes food peristaltically up the oesophagus from the mouth to the stomach where it is stored in the cardiac sac region. There is litle information on the control of oesophageal peristalsis. In semi-intact preparations spontaneous, vigorous oesophageal peristalsis is usually observed, even when the stomach has been removed (see for example SPIRITO, 1975). This activity is neurogenic since peristalsis stops on cutting the commissural ganglion nerves. In the semi-intact animal rhythmic motor activity related to oesophageal movements can be recorded in the commissural ganglion nerves (see for example SPIRITO, 1975 in Procambarus clarkii; RUSSELL, unpublished, in SELVERSTON et al., 1976 in Panulirus interruptus; ROBERTSON, 1978 in Homarus gammarus). Activity of motorneurons supplying oesophageal dilator muscles causes a pronounced burst in the superior oesophageal nerve and this may be used to monitor the occurrence and frequency of oesophageal peristalsis (for example ROBERTSON and LAVERACK, 1979).

DANDO' (1969) was the first author to note that in Homarus gammarus "...a commissural ganglion can maintain a discharge indistinguishable from the normal activity for a long time when completely isolated from the central nervous system.". RUSSELL, unpublished, in SELVERSTON et al., (1976) briefly documents evidence that "...each commissural ganglion contains a pattern generator for the [o]esophagus rhythm.". Rhythmic motor activity with a cycle period of about 3-6 seconds can be recorded from the superior and inferior oesophageal nerves for over eight hours (see SELVERSTON et al., 1976, Figure 37). In contrast MOULINS, VEDEL and DANDO (1974) state that in Palinurus vulgaris "...la motricité oesophagienne est programmée au sein d'un réseau neuronique localisé dans le ganglion oesophagien", i.e. oesophageal motor activity is produced by a neural network in the oesophageal ganglion. MOULINS and VEDEL (1977) draw attention to this apparent contradiction with previous work and postulate that the patterned output of the commissural ganglia serves to entrain the oesophageal rhythm produced by the oesophageal ganglion. However, more recently MOULINS and his co-workers consider that the oesophageal pattern generator resides in the commissural ganglia (MOULINS and ROBERTSON, unpublished; M. MOULINS, pers. comm.). At least in Palinurus the oesophageal ganglion is clearly involved to some degree in the production of the oesophageal rhythm (MOULINS and VEDEL, 1977). One neuron, OD1 (oesophageal dilator 1), has a branch in the inferior ventricular nerve and branches in each superior oesophageal nerve, each of which passes through the ipsilateral commissural ganglion to innervate the oesophageal musculature. OD1 has three spike initiating zones : one in each commissural ganglion and one in the oesophageal ganglion. Its membrane potential oscillates slightly at the frequency of the oesophageal rhythm. MOULINS and VEDEL (1977) and MOULINS and NAGY (unpublished) consider OD1 to be of prime importance in the oesophageal network.

Since each right and left commissural ganglion is capable of generating the oesophageal rhythm their activities must be coordinated for efficient oesophageal peristalsis. SPIRITO (1975) claimed that in the crayfish Procambarus clarkii such bilateral coordination is mediated entirely by the post-oesophageal commissure since the lesion of this nerve alone resulted in "...a loss of synchrony and a cessation of rhythmic bursting". Cutting the superior and inferior oesophageal nerves and the circumoesophageal connectives often changed the cycle period but did not affect synchrony.

d. Other foregut rhythms

1. The stomach

Food taken up the oesophagus and stored in the cardiac sac is triturated by the rhythmic action of the three large calcified teeh comprising the gastric mill. Food particles are then sorted by the hair-plates of the posterior pyloric filter, passing to the midgut or digestive glands. Foregut motility in "higher" decapods thus involves four well-defined rhythms : oesophageal, cardiac sac, gastric mill and pyloric filter. There may be other rhythms, for example a labral rhythm or an oral rhythm connecting mandibular to oesophageal activity (ROBERTSON, 1978) but at present this is not clear (see Discussion).

The structure of the stomach and the anatomy of its muscles and nerves have been described in detail for Callinectes sapidus, Homarus americanus and Panulirus argus (MAYNARD and DANDO, 1974). Comparative skeletal structure and musculature have been considered phylogenetically by MEISS and NORMAN (1974 a and b). GOVIND, ATWOOD and MAYNARD (1975) described the innervation pattern and neuromuscular physiology of foregut

musculature in Callinectes sapidus and Panulirus argus. The physiology and ultrastructure of gastric mill muscle fibres and neuromuscular junctions in Callinectes sapidus were investigated by JAHROMI and GOVIND (1976) and ATWOOD, GOVIND and JAHROMI (1977). The role of various neurotransmitters at neuromuscular and nervous synapses in the stomatogastric system, and their ultrastructural correlates, will be briefly discussed in Chapter 4.

Stomach rhythms are produced by alternating bursts of activity in neurons innervating the striated stomach musculature. Patterned rhythmic activity is produced not by large pools of pre-motor interneurons but by the cellular and connectivity parameters of a relatively small number of motorneurons. The gastric mill and the pyloric rhythms are programmed in the stomatogastric ganglion and are relatively well understood. The cardiac sac rhythm is produced by a network distributed between the oesophageal and stomatogastric ganglia.

2. The gastric rhythm

In Panulirus interruptus the gastric rhythm is produced by a network of twelve neurons in the stomatogastric ganglion. These are functionally divided into two coordinated subsets : four motorneurons drive the opening and closing movements of the two lateral teeth and six drive the backwards and forwards action of the medial tooth. In addition two interneurons are common to both subsets. The synaptic organisation of the neurons driving the lateral teeth (MULLONEY and SELVERSTON, 1974a) and the medial tooth (SELVERSTON and MULLONEY, 1974) and the coordination of these subsets (MULLONEY and SELVERSTON, 1974b) have been investigated in detail. MULLONEY and SELVERSTON (1974b) suggest that the connectivity parameters of the lateral teeth subset are such that reciprocal inhibition amongst antagonistic neurons can produce patterned alternating bursts even in the absence of synaptic input. In contrast, the medial tooth subset does not

show such reciprocity between antagonists but exhibits unilateral inhibition of one group of neurons by another. One of the interneurons common to both the lateral teeth and medial tooth subsets, Int 1, is modulated by activity in the lateral teeth subset which may be partly responsible for activity in the medial tooth subset. Thus although the two subsets can operate independently if one is silent, if both are functioning their activities are coordinated.

No single neuron or group of neurons in the gastric mill system has been found to be capable of sustained endogenous bursting. Hence the gastric rhythm is an emergent network phenomenon resulting from the interaction of non-oscillating cells connected primarily by inhibitory synapses. MULLONEY and SELVERSTON (1974b) emphasise the potential importance of the temporal parameters of accomodation and post-inhibitory rebound in determining the duration of the bursts and the repetition rate of the pattern.

3. The pyloric rhythm

The pyloric rhythm causes dilation of the pyloric region of the stomach followed by a caudally-directed wave of contraction. The rhythm is generated by fourteen neurons in the stomatogastric ganglion. Thirteen of these are motorneurons and one, the anterior burster (AB), is an interneuron. These are functionally organised into dilator and constrictor groups supplying antagonistic muscles. The synaptic organisation of the pyloric neuron pool in Panulirus spp. has been characterised by MAYNARD and SELVERSTON (1975). A group of three electrotonically-coupled neurons, two pyloric dilators and the anterior burster (the PD-AB group), exhibits endogenous bursting (see below) and appears to act as a driving force for the pyloric network. Synaptic interactions involving principally inhibition, disinhibition and probably post-inhibitory rebound (SELVERSTON

et al., 1976) account for the phase relationships amongst the other neurons. However, WARSHAW and HARTLINE (1976) demonstrated in computer modelling studies of the pyloric network that the endogenous activity of the PD-AB group is not necessary for production of a patterned output similar to that observed *in vitro*. It appears that such an output can result from the neuronal connectivity pattern alone. Thus the role of the PD-AB group may be primarily one of timing and not of burst production. A recently developed cell inactivation technique involving irradiation of the fluorescent dye Lucifer Yellow (A.I. SELVERSTON, pers. comm.) has shown that the pyloric rhythm can be generated without PD-AB group activity provided that there is input from the commissural ganglia. Furthermore, in the PD-AB group only the AB interneuron is capable of endogenous bursting in the absence of all synaptic input. MAYNARD and WALTON (1975) emphasised the importance of sub-threshold potentials in pre-synaptic neurotransmitter release at inhibitory synapses between pairs of gastric mill neurons and pyloric neurons. Recently RAPER (1979) has shown that graded, non-impulse-mediated chemical synaptic transmission can coordinate neuronal activity in the pyloric system. The role of spikes may be primarily to drive the musculature.

By studying motor output in semi-intact and *in vitro* preparations of *Panulirus argus* HARTLINE and MAYNARD (1975) examined the functional implications of the gastric and pyloric rhythms. POWERS (1973) used chronic electrode implantation techniques in an assessment of gastric mill activity in intact crabs (*Cancer magister* and *C. productus*).

4. The cardiac sac rhythm

In *Palinurus vulgaris* only five motorneurons are involved in the rhythmic dilation and constriction of the cardiac sac : two cardiac dilators (CD1 and CD2), the ventricular dilator (VD) and the inferior

cardiac neuron (IC) which are also involved in the pyloric rhythm and the anterior median neuron (AM) which is also involved in the gastric cycle (MOULINS and VEDEL, 1977). The somata of CD2, VD, IC and AM are located in the stomatogastric ganglion; the soma of CD1 is located in the oesophageal ganglion. VEDEL and MOULINS (1977) emphasise the importance of CD2 which has two spike initiating zones : one in the stomatogastric ganglion and one in the oesophageal ganglion. It can produce bursting and tonic discharges and orthodromic and antidromic spikes. There is an intimate relationship between through fibre (TF) bursts (DANDO and SELVERSTON, 1972 : see below) descending from the brain via the inferior ventricular and stomatogastric nerves and the cardiac sac rhythm. MOULINS and VEDEL (1977) describe TF as the pacemaker of this rhythm.

e. The interaction and modulation of foregut rhythms

It is obvious that the different regions of the foregut, and hence the stomatogastric pattern generators, will be coordinated to maximise the efficiency of the intake of food and its passage down the gut. Furthermore, feeding and the digestion of food may be started, stopped or modulated in response to environmental pressure or to the expression of other parts of the animal's behavioral repertoire. During the past decade much information has accumulated concerning the interaction of the different parts of the stomatogastric system and their modulation and control from higher nervous centres.

There are several direct chemical and electrotonic synapses between neurons in the gastric mill and pyloric systems as well as multisynaptic pathways (SELVERSTON et al.,1976; MULLONEY, 1977). The gastric mill system can be modulated by bursting in pyloric neurons and vice versa,

leading to variable coordination. However, SELVERSTON et al. (1976) emphasise that direct interactions are few, weak and probably secondary in importance to common modulatory input acting on both rhythms. For example, bursts of impulses coordinated with the oesophageal rhythm travel into the stomatogastric ganglion via the stomatogastric nerve. Neuron AB, one of the pyloric pacemaker group, receives trains of excitatory post-synaptic potentials (EPSPs) coordinated with this rhythm, as do the pyloric LP and PY neurons. The gastric rhythm appears to be entrained by the oesophageal rhythm via a more complex pathway involving the commissural ganglion E neurons (see below). The function of the Ex cells in the stomatogastric ganglion is unknown. However, they also receive synaptic input coordinated with the oesophageal rhythm and this may contribute to gastric-pyloric coupling (SELVERSTON et al., 1976). Very recent work (P.S. DICKINSON, pers. comm.) has shown that an oesophageal ganglion neuron can modulate the pyloric network via the inferior oesophageal nerve, commissural ganglion, superior oesophageal and stomatogastric nerves. This modulation is of long latency and duration and involves modification of the membrane properties of several pyloric neurons. The activity of this modulatory cell may be loosely coupled to the oesophageal rhythm.

MOULINS and VEDEL (1977) emphasise the importance of the cardiac sac network in the coordination and modulation of the three other foregut rhythms in Palinurus vulgaris. The involvement of gastric and pyloric neurons in the cardiac sac system is obviously significant. VEDEL and MOULINS (1977) suggest that the cardiac sac neuron CD2 "...might be involved in various motor programs..." and "...could be considered as a "two-way coordinating system"...". MOULINS and VEDEL (1977) showed that CD2 can participate in the oesophageal and pyloric rhythms as well as the cardiac sac rhythm. This multiplicity of function is enhanced by bifunctional muscle action. DANDO and SELVERSTON (1972) investigated two

through fibres (TF) which run in the inferior ventricular nerve and the stomatogastric nerve to the stomatogastric ganglion, possibly with branches in the oesophageal nerves to the commissural ganglia. Stimulation of TF caused frequency-dependent changes in gastric and pyloric output. It is now considered that TF acts as a "command neuron" and as a pacemaker for the cardiac sac network. It is thought to be a common excitor for CD1 and CD2 (the cardiac sac rhythm), OD1 (the oesophageal rhythm) and PD (the pyloric rhythm) and hence is strongly involved in the hierachical control of foregut rhythms (MOULINS and VEDEL, 1977). TF also activates a neuron which sends an efference copy of cardiac sac activity to the brain via the inferior ventricular nerve. OD1 sends an efference copy of oesophageal activity to the brain in the same nerve. The commissural ganglion L cell sends a corollary discharge of total foregut activity to at least the brain (see Chapter 4).

The studies of MORRIS and MAYNARD (1970) on Homarus americanus and those of POWERS (1973) on Cancer spp. using electrode implantation techniques in intact animals emphasised that although the stomatogastric rhythms seen in vitro are similar to those in vivo they are under the modulation of sensory input and interneurons from the central nervous system. The commissural ganglia are important in such inputs. For example, RUSSELL (1977) showed that inclusion of the commissural ganglia with the stomatogastric ganglion in a "combined" preparation caused the gastric rhythm to operate in most preparations; in the isolated stomatogastric ganglion it is active in only about 5% of cases (SELVERSTON et al., 1976). Similarly, RUSSELL (1977) found that inclusion of the commissural ganglia in the preparation enhanced the pyloric rhythm and radically reduced its cycling time. All four oesophageal nerves (two superior and two inferior oesophageal nerves) appear to carry inputs from the commissural ganglia to the gastric and pyloric networks in the

stomatogastric ganglion but in the case of the gastric mill rhythm the major input pathways appear to be the superior oesophageal nerves (SELVERSTON et al., 1976). In particular, in Panulirus interruptus commissural ganglion inputs appear to unmask plateau potentials in both gastric and pyloric neurons, for example in the pyloric PD-AB pacemaker group (RUSSELL and HARTLINE, 1978; RUSSELL, 1979) which may have profound consequences for the operation of the gastric and pyloric networks. Such effects may be mediated by dopamine-containing pathways from the commissural ganglion (see Chapter 4).

SELVERSTON et al. (1976) summarise sources of sensory input to the commissural ganglia which may modulate stomatogastric rhythms. LARIMER and KENNEDY (1966), working on Procambarus clarkii, described a mechanosensory bipolar cell with its soma in the stomatogastric ganglion whose activity is modulated by the movement of gastric ossicles. However, the bursts of impulses that they interpreted as sensory traffic may represent efferent oesophageal dilator bursts emanating from the commissural ganglia. Furthermore, this neuron may not have a counterpart in species such as Panulirus interruptus (SELVERSTON et al., 1976). DANDO and LAVERACK (1969) described proprioceptors in the posterior stomach nerve which respond to gastric mill movements and evoke changes in output from the stomatogastric ganglion. CHANUSSOT and DANDO (1973) and DANDO, CHANUSSOT and NAGY (1974) described the effect of posterior stomach nerve stimulation on gastric and pyloric rhythms in Cancer pagurus. From further studies on this species HERMANN and DANDO (1977) suggested that stimulation of the posterior stomach nerve reflexly activates command fibres derived from the commissural ganglia which act on the pyloric PD pacemakers. In the intact animal this action may cause a protracted opening of the cardio-pyloric valve and a rapid propulsion of food through the pylorus. However, NAGY (1977) found that in Jasus lalandii the first

level of integration of sensory information from the posterior stomach nerve proprioceptors is not in the commissural ganglia but in the suboesophageal ganglion. He considered that the effects induced by stimulation of this nerve do not simply involve the PD pyloric pacemakers but are more widespread in the pyloric system. SIGVARDT and MULLONEY (1977) briefly document the sensory modulation of both gastric and pyloric rhythms in Panulirus interruptus by sensory neurons in the pyloric region of the stomach, which appear to act via the commissural ganglia.

Finally, neurons in the commissural ganglia are involved in qualitatively different ways with the operation of the gastric and pyloric systems. RUSSELL (1976) showed that an E neuron in each commissural ganglion provides phasic excitatory input to gastric neurons. The interneuron Int 1, which is involved in both lateral teeth and medial tooth gastric mill subsets (see part d above), periodically inhibits the E neurons which become active when released from this inhibition. In other words, E neuron activity, which is coordinated with the gastric rhythm, relies on input from the gastric system for its patterning. RUSSELL (1976) suggests that modulation of E neuron activity by C.N.S. and sensory input to the commissural ganglia may adjust the force exerted by the medial tooth. Similarly there are 6-10 P neurons in each commissural ganglion which can participate in the pyloric rhythm (RUSSELL, 1977). Their firing seems to be patterned by the AB pyloric pacemaker and provides excitatory input to certain gastric neurons. Thus modulation of the gastric system by the pyloric system is mediated by neurons in the commissural ganglia.

In contrast, very recently ROBERTSON and MOULINS (unpublished a, b, d and e) have shown that there are neurons in the commissural ganglia which provide phasic input to the pyloric system (the commissural pyloric oscillator : CPO) and to the gastric system (the commissural gastric

driver : CGD) which are not themselves part of the pattern generators. AYERS and SELVERSTON (1977 and 1979) have shown that the pyloric endogenous bursters (PD-AB) can be entrained by excitatory and inhibitory input. The CPO appear to entrain the pyloric pattern generator by trains of EPSPs and the phasic nature of their activity does not rely on input from the stomatogastric ganglion since it continues when the stomatogastric nerve is blocked with a sucrose gap. Similarly, CGD provide phasic input to gastric neurons but do not rely on input from the gastric system for the patterning of their activity. CGD may also be involved in other foregut rhythms and affect the pyloric system by an unknown pathway. CGD spike within a gap of polarisation (ROBERTSON and MOULINS, unpublished b) which has important consequences for their follower neurons. CGD may operate in the commissural pyloric network when in the depolarised non-spiking mode and function as gastric drivers by producing bursts by hyperpolarisation. The importance of these findings must be stressed, since the commissural pyloric oscillators provide the first known example of phasic modulatory input to a pattern generator by neurons whose activity is independent of the pattern generator. Since the gastric rhythm only operates in about 5% of isolated stomatogastric ganglion preparations (see above) the commissural gastric drivers may be considered part of the minimal gastric mill pattern generator which is distributed into the commissural ganglia (see Figure 3.1). This work also underlines the complexity of interaction and hierarchical control of stomatogastric pattern generators. It has recently been demonstrated that the presence or absence of the control of pattern generators by these higher-level oscillators can lead to distinct feeding behaviours (REZER and MOULINS, 1980).

The brief review given above is not intended to be detailed or exhaustive but serves to demonstrate the main principles underlying the

Figure 3.1

Interaction of foregut rhythms

A highly simplified diagram depicting recent work on the modulation and interaction of stomatogastric pattern generators. For clarity only one commissural ganglion is shown.

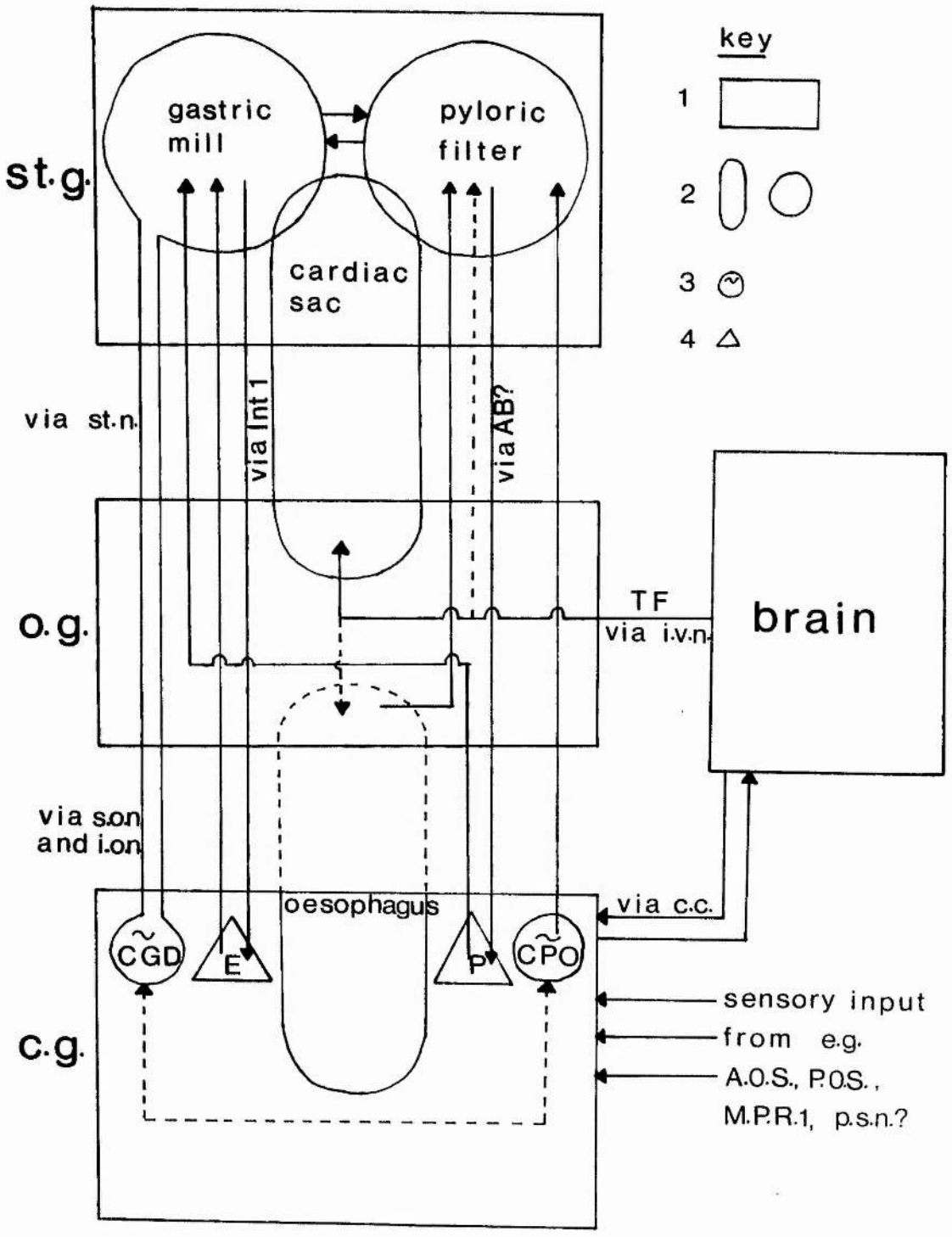
After MOULINS and VEDEL (1977)
MULLONEY (1977)
ROBERTSON and LAVERACK (1979)
ROBERTSON and MOULINS (unpublished a, b, d and e)
RUSSELL (1976)
SELVERSTON et al. (1976)
VEDEL and MOULINS (1977)

Key :-

1. ganglion
2. location of pattern generator
3. commissural ganglion oscillator
4. non-oscillating commissural ganglion neuron

Dashed lines and ? marks indicate problematic pathways.

See Glossary for abbreviations.


key
1
2
3
4
st.g.
gastric mill
pyloric filter
cardiac sac
via st.n.
via Int 1
via AB?
o.g.
TF
via i.v.n.
brain
via s.o.n. and i.o.n.
oesophagus
via c.c.
CGD
E
P
CPO
c.g.
sensory input from e.g. A.O.S., P.O.S., M.P.R.1, p.s.n.?

operation of stomatogastric pattern generators and the importance of the commissural ganglia in their modulation. Figure 3.1 attempts to summarise the most recent work on the modulation and interaction of stomatogastric pattern generators. There are reviews of the stomatogastric nervous system varying in detail and complexity (e.g. MAYNARD, 1972; SELVERSTON, 1974, 1976, 1977) the most comprehensive being that of SELVERSTON et al. (1976).

Materials and methods

a. The preparation

The preparation, usually a desheathed commissural ganglion, was pinned out in a Sylgard-lined Petri dish (see Chapter 2 General Materials and methods part b) and was immersed in chilled, oxygenated saline. It was viewed with transmitted light under a Nikon binocular microscope. The bath was earthed with a silver-chlorided silver wire electrode via the current monitor (see below).

b. Extracellular physiological recording and stimulation

Up to four hand-made suction electrodes were used in each experiment. Changeable plastic tips enabled clear recordings to be made from nerves of different diameters. Each suction electrode was mounted on a Prior manipulator. The output from each electrode was led through a junction box (manufactured in the Gatty Marine Laboratory : G.M.L.) into a differential pre-amplifier with a gain of x2,000 and high-pass and low-pass filters at 80Hz and 1 kHz respectively (manufactured in the G.M.L.). The output from each preamplifier was displayed on one channel of a 4-channel Tektronix 561A cathode ray oscilloscope equipped with a type 3B3 time base unit. Nerve activity was also monitored using an audio amplifier (manufactured in the G.M.L.) connected in parallel with the C.R.O..

A Tektronix 160A power supply drove a 162 waveform generator and a 161 pulse generator linked in series. The square-wave output from the pulse generator was led through a stimulus isolation unit (manufactured in the G.M.L.) to the appropriate suction electrode via the junction box,

which allowed stimulation or recording through any electrode. Often a variety of stimulus parameters was used during an experiment but generally a pulse of 0.5-1.0 msec width was applied at a frequency of 2-4 sec-1 for up to ten seconds. This voltage could be varied from 0 - 50 volts.

c. Intracellular physiological recording

Microelectrodes were pulled from fibre-filled glass (Clark Electromedical Instruments type GC 150F - 15) on an electrode puller (manufactured in the G.M.L.). A microelectrode was filled with the appropriate solution by immersing the barrel to fill the tip by capillary action and then backfilling with solution injected from a hypodermic syringe. The resistance of the microelectrodes varied according to the solution used. Electrodes ranging in resistance from 20 - 80 MΩ(with 2M potassium acetate) and 50 - 100 MΩ(with 5% Lucifer Yellow CH in 1M lithium chloride : see below) were found to be suitable. The tips of the electrodes were examined with a microscope and after filling the resistance was determined with a series voltage divider in the BridgeBAK amplifier (see below).

The microelectrode was held in a rigid perspex electrode holder mounted on a Narishige micromanipulator. A silver-chlorided silver wire inserted in the electrode barrel led to the head stage of a BridgeBAK high-impedance amplifier with a gain of x5 or x10 and a Wheatstone bridge cicuit for balancing DC current passed (manufactured in the G.M.L.). A switch mechanism on the amplifier allowed input to the electrode from one of two sources : either from a DC source supplying a positive or negative current of up to 10 nA to alter the membrane potential of the impaled cell (each neuron penetrated was routinely depolarised and hyperpolarised in this way) or from the Tektronix 161 pulse generator for dye injection

(see below). The output of the BridgeBAK amplifier was displayed on one channel (DC mode) of the Tektronix 561A C.R.O..

d. Permanent records

Extracellular, or extracellular and intracellular records were obtained in each experiment in one of the following ways :-

1. Two extracellular traces could be recorded on a 2-channel Ferrograph AC taperecorder connected in parallel with the C.R.O.. A fast tape speed of 7.5 inches sec-1 aided accurate reproduction.

2. A Nihon Kohden camera was used in conjunction with a 4-channel Tektronix 565 C.R.O. connected in parallel to the 561A display C.R.O.. Records were made directly or from the tape with Agfa Gevaert Oscilloscript 2 film.

3. A 2-channel Devices MX212 heat-sensitive-paper pen-recorder was found to have a sufficiently fast response time to record intracellular activity. Hence one channel was used to record intracellular activity and one extracellular activity.

4. A Tektronix 5103N storage C.R.O. was used in parallel with the 561A display C.R.O. to monitor nerve activity directly or from the tape. Records were made with a Tektronix C-5 oscilloscope Polaroid camera and type 107C Polaroid film.

e. Intracellular dye injection

Intracellular dye injection was used to determine the anatomy of impaled cells. A pronounced tendency for electrode blockage occurred with the injection of cobalt chloride (30 mM). Virtually no such problems were found using Lucifer Yellow CH (STEWART, 1978) which seemed to give a

clear, detailed picture of neuronal anatomy. Hence this dye was used routinely in experiments. 5% Lucifer Yellow CH in filtered 1M lithium chloride solution was used to fill the microelectrode tip and the electrode barrel was backfilled with 1M lithium chloride solution. A negative-going square wave pulse (see part c above) of 5 - 100 nA magnitude and 500 msec duration was applied at a frequency of 1 sec-1 to the electrode for 2 - 30 minutes to eject the negatively-charged dye into the cell. The output of a current monitor (manufactured in the G.M.L.) was led to one channel (DC mode) of the display C.R.O. so that the current passed by the electrode could be checked. Electrode blockage was manifested as a decreased current level and/ or an erratic trace on the negative part of the square wave. The electrode could usually be unblocked by applying a brief high-voltage positive-going pulse to the electrode. As a further check large neurons (> about 30 μm) could usually be seen turning yellow during dye injection (STEWART, 1978).

After injection the preparation was fixed in 4% formaldehyde buffered to pH 7.1 with SORENSEN's phosphate buffer (HUMASON, 1972) for one hour at room temperature. It was then quickly dehydrated in an ethanol series (70%, 90%, 2x 100% : five minutes each) and cleared and mounted in methyl salicylate in a cavity slide. This wholemount preparation was examined with a Zeiss transmission fluorescence microscope equipped with a Wotan HBO 200 W super high pressure mercury lamp, a BG 12 excitation filter and Zeiss 44 and 53 barrier filters. It was photographed with Kodak Tri-X Pan film with an exposure time of 20 - 40 seconds. The preparation was drawn using a Zeiss camera lucida and a light source of variable intensity. This was done as quickly as accuracy permitted since preparations tended to fade considerably after about thirty minutes exposure to U.V. illumination. Both the preparations and the Lucifer Yellow CH solution were stored in the dark.

Results

a. The production of the oesophageal rhythm

An isolated commissural ganglion would usually produce patterned rhythmic activity for over ten hours. This activity would often start, stop or change frequency spontaneously. Rhythmic bursting activity was very similar to that seen in the semi-intact animal (ROBERTSON, 1978). Figure 3.2 shows examples of activity recorded from the superior and inferior oesophageal nerves. Rhythmic activity in the superior oesophageal nerve is characterised by high frequency bursting of dilator units. At least one large unit fires with a declining frequency throughout the burst. There was no clear evidence for a recruitment sequence of units, such as MOULINS and VEDEL (1977) described for OD1, OD2 and OD3 in Palinurus vulgaris. In the semi-intact preparation this burst corresponds to dilation of the oesophagus during peristalsis, thus it is assumed that these are dilator units. Some smaller units which only fired between bursts may be constrictor units. "Extra" bursts occurred occasionally; these appeared to reset the rhythm (Figure 3.2 d). Figure 3.3 shows spontaneous frequency changes in dilator bursts in the superior oesophageal nerve. Such changes can be very pronounced and show no obvious pattern.

In the inferior oesophageal nerve rhythmic activity is not so clearly defined. Studies of many preparations showed that a rather unpronounced burst of small, medium and sometimes large units usually occurred just before the dilator burst in the superior oesophageal nerve (Figure 3.2 c and d). Some cessation of inferior oesophageal nerve firing occurred during the superior oesophageal nerve burst, sometimes leading to almost complete silence in this nerve. Occasionally an inferior oesophageal nerve burst occurred after the superior oesophageal nerve burst (Figure 3.2 a),

Figure 3.2

Rhythmic bursting activity in the superior and inferior oesophageal nerves of an isolated commissural ganglion

a and b. Records from the nerves of a right commissural ganglion at different speeds.

c and d. Records from the corresponding nerves of a right commissural ganglion (from a different preparation to a and b). c and d are continuous.

Note the pronounced burst in the superior oesophageal nerve (a, b, c and d), characterised by high frequency firing of dilator units. At least one large unit fires during the burst and several small constrictor units fire between bursts. In the inferior oesophageal nerve there is relative silence during (a) or at the end of (c and d) the burst in the superior oesophageal nerve, usually preceded (c and d) or followed (a) by an unpronounced burst of small, medium and occasionally large units. In c and d a large unit in the inferior oesophageal nerve fires regularly but is usually suppressed during the beginning of the burst in the superior oesophageal nerve. In a very regularly bursting preparation such as that shown in c and d "extra" bursts occur occasionally which usually reset the rhythms (arrow) (contrast with Figure 3.6 b).

Time scale in a, c and d : 1 sec, in b : 200 msec

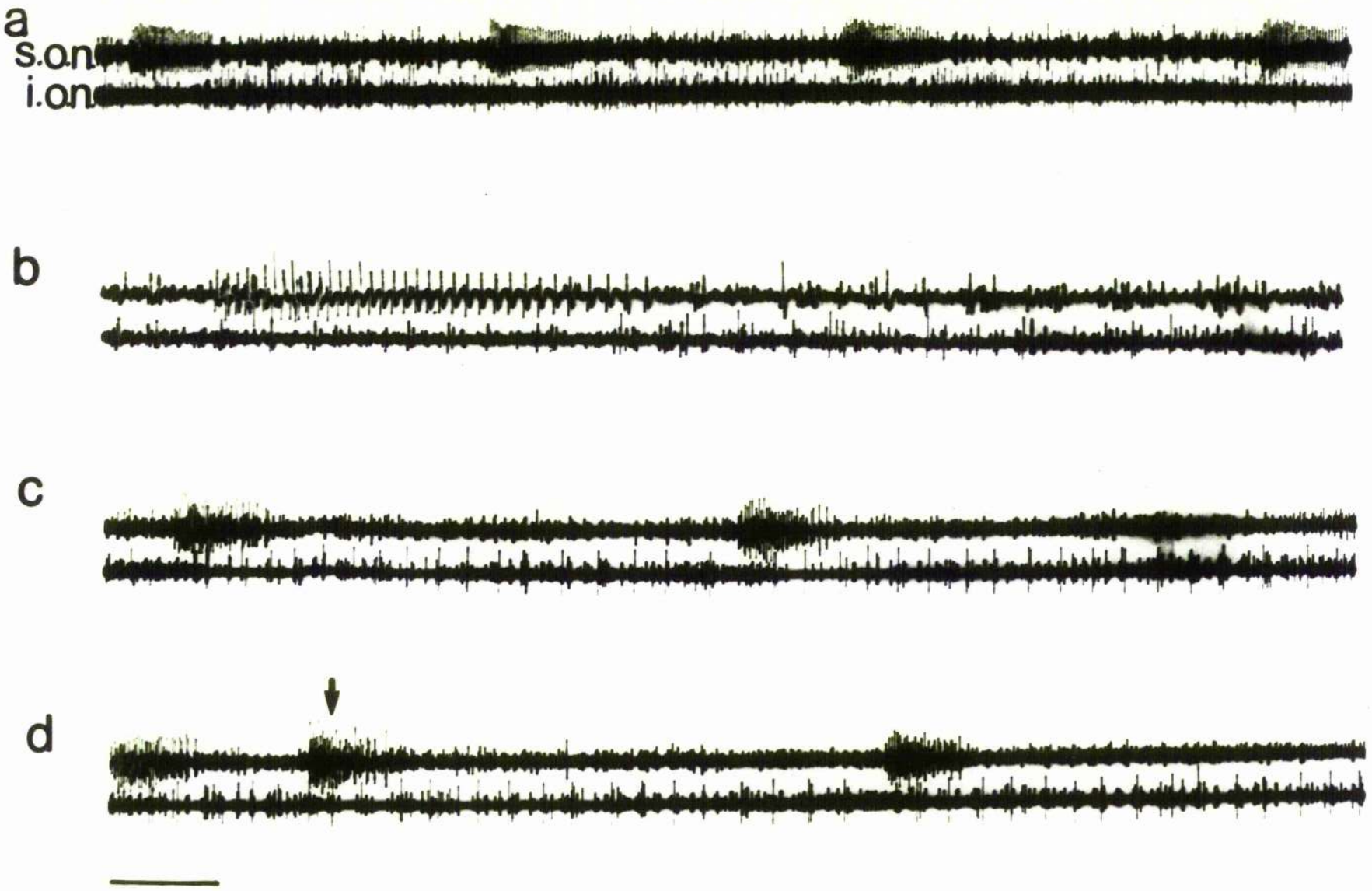

a
s.on
i.on
b
c
d

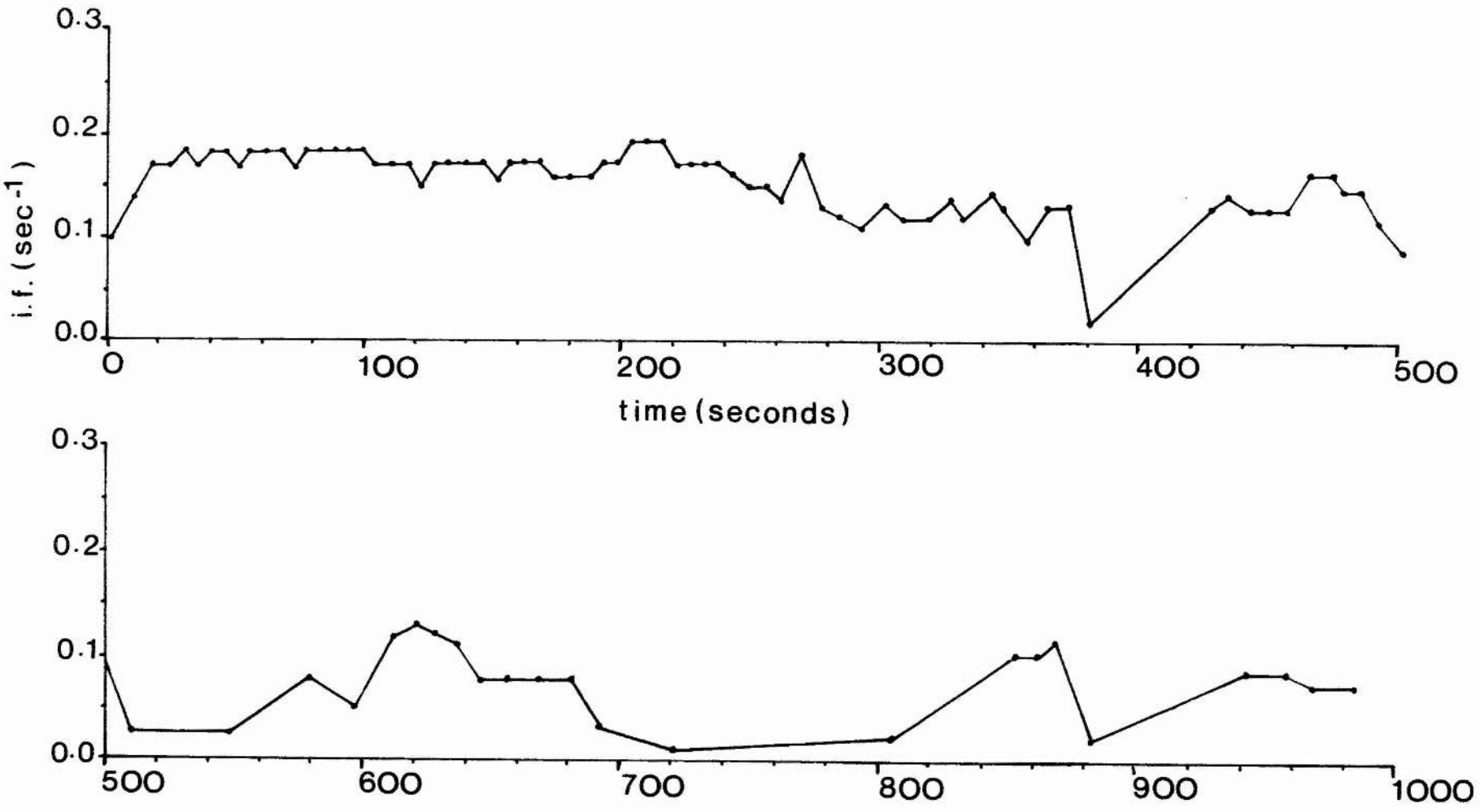
Figure 3.3

Bursting activity in the superior oesophageal nerve

Spontaneous bursting activity in the superior oesophageal nerve of a left commissural ganglion was recorded for 1,000 seconds (over fifteen minutes). The instantaneous frequency was calculated as the reciprocal of the interburst interval, measuring from the start of one dilator burst to the start of the next (ROBERTSON, 1978). Instantaneous frequency (i.f.) is shown as a function of time. The two plots are continuous.

Dilator bursts occur regularly, about every six seconds, for about 200 seconds. Bursting activity then slows and becomes extremely erratic. Such spontaneous frequency changes were seen in most preparations. There was no obvious pattern in the starting, stopping and modulation of the oesophageal rhythm.

Compare with ROBERTSON and LAVERACK (1979) figures 19, 20, 22, 23 and 24 showing oesophageal dilator burst frequency in the semi-intact animal. Note similarities in the frequency range.


0·3
0·2
0·1
0·0
i.f. (sec⁻¹)
0
100
200
300
400
500
time (seconds)
0·3
0·2
0·1
0·0
500
600
700
800
900
1000

as though certain units were released from inhibition by the dilator units.

Rhythmic activity in the ventral-posterior oesophageal nerve is similar to that in the superior oesophageal nerve (Figure 3.4). A burst occurs just before the superior oesophageal nerve burst, as in the semi-intact animal (ROBERTSON, 1978; ROBERTSON and LAVERACK, 1979). At least one unit progressively increases its firing rate between bursts. ROBERTSON (1978) found such a unit in the semi-intact preparation; however, in the in vitro situation there was no clear evidence that it fires 1:1 with a superior oesophageal nerve constrictor unit as ROBERTSON described. There was some evidence of correlation between the very large unit firing in the superior oesophageal nerve burst and a small unit in the ventral-posterior oesophageal nerve and of other common units in the two nerves (Figure 3.4 b).

The minor nerves are small and their branches are variable in disposition (see Chapter 2 Results part c); hence recordings were made from all the minor nerve trunks with one electrode, including both dilator and constrictor branches. Rhythmic activity in the minor nerves is very similar to that seen in the ventral-posterior oesophageal nerve. The firing rate of a prominent unit gradually increases until terminated by a burst involving several units (Figure 3.5). The burst occurs at the same time as, or slightly preceding, the burst in the superior oesophageal nerve. There appeared to be some correlation between minor nerve and superior oesophageal nerve dilator units during the dilator burst but this was not very clear because of the large amount of activity during this period (Figure 3.5).

Rhythmic activities in the various commissural ganglion nerves are thus intimately related and have the same or extremely similar cycle periods. This periodicity exhibited a wider range than that seen in semi-intact preparations (ROBERTSON, 1978) and varied from about

Figure 3.4

Rhythmic bursting activity in the superior and ventral-posterior oesophageal nerves

a. Continuous records from the nerves of a right commissural ganglion. Firing of at least one unit in the ventral-posterior oesophageal nerve progressively increases before being terminated by a burst.

b. Continuous records from the same preparation filmed at higher speed. In several preparations a small ventral-posterior oesophageal nerve unit fired on a 1:1 basis with the very large superior oesophageal nerve unit (▲). There also appeared to be a correlation between other superior and ventral-posterior oesophageal nerve units (e.g. •).

Time scale in a and b : 1 sec, in c and d : 200 msec

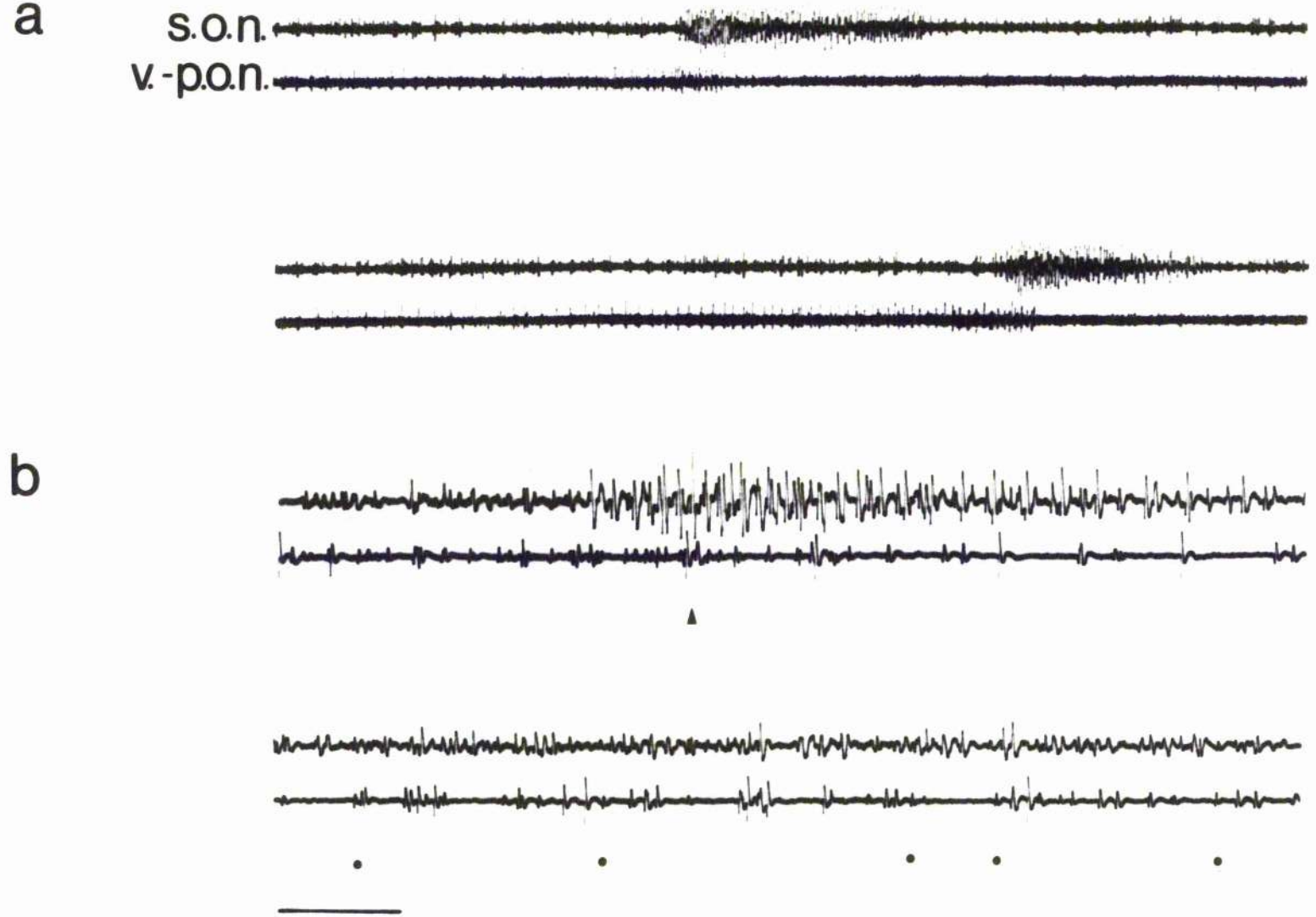

a
s.o.n.
v.-p.o.n.
b

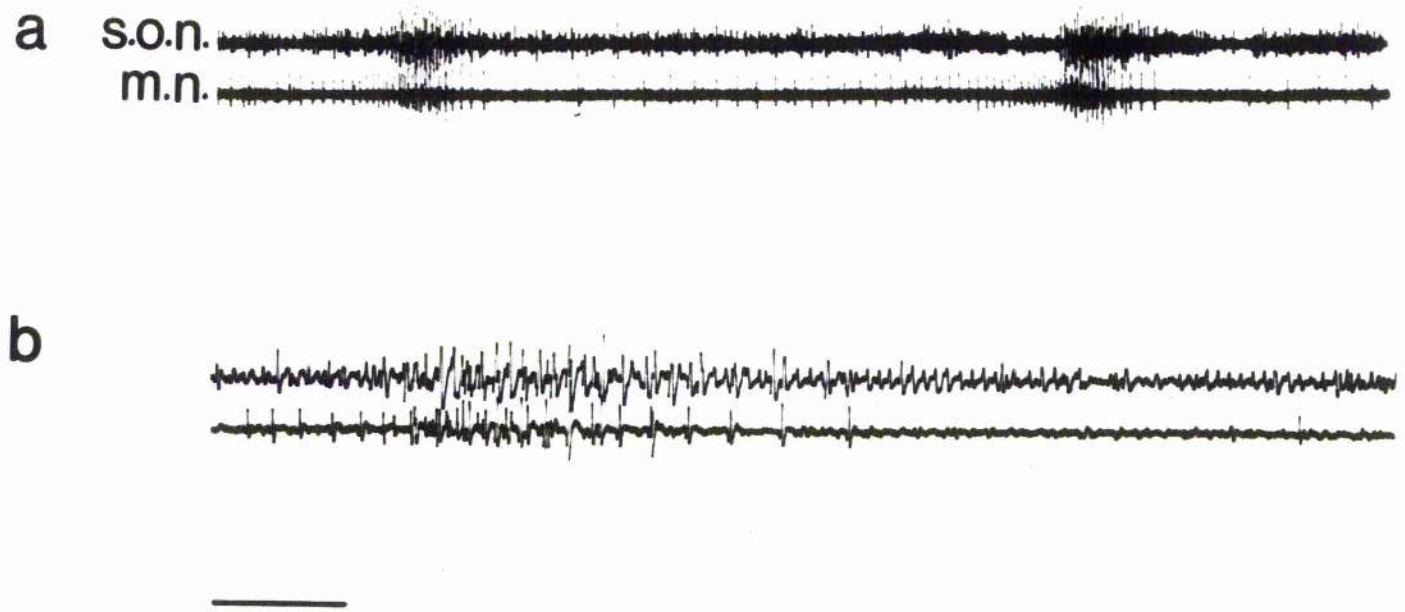
Figure 3.5

Rhythmic bursting activity in the superior oesophageal nerve and minor nerves (all branches)

From a right commissural ganglion. a and b filmed at different speeds.

Firing of one unit in the minor nerves increases progressively before termination by a pronounced dilator burst involving several units. This burst is coincident with or slightly precedes the burst in the superior oesophageal nerve which it resembles. There appears to be some correlation between dilator units in the two nerves but the large amount of activity during the dilator burst makes this difficult to resolve (b).

Time scale in a : 1 sec, in b:200 msec.


a
s.o.n.
m.n.
b

0.05 - 1.0 Hz. There was no clear evidence for rhythmic bursting activity with the same period in the outer mandibular nerve or anterior circumoesophageal connective but pronounced bursting was occasionally seen in the posterior circumoesophageal connective.

In most cases there was some indication of common units in commissural ganglion nerves but this was not clear. Common units in a pair of nerves were looked for during the experiment, by subsequent analysis of tape and film and in some cases by triggering one channel of the storage C.R.O. by large spikes in one nerve, for example dilator units in the superior oesophageal nerve. However, difficulties in these analyses permit only the conclusion that there is some evidence for common units in most pairs of nerves, except between the inferior and ventral-posterior oesophageal nerves. The chief problem was the large amount of spontaneous activity in the nerves and the superimposition of spikes. Other inherent difficulties in such analyses include the limitations of extracellular recording methods in distinguishing small spikes from background noise, the possible presence of different units producing action potentials of the same shape and magnitude and the possibility that units common to two nerves may not be seen because a) two spike initiating zones of a neuron with a bifurcating axon may not fire together or b) tightly coupled cells may usually, but not always, fire in synchrony. Similarly, stimulation of one nerve generally induced some increase in firing in the other nerve but the high level of spontaneous activity obscured any distinction between direct and synaptically-mediated pathways.

No method was found which would consistently elicit bursting in a quiescent preparation. Since activity of the posterior oesophageal sensors (see Introduction part b) activates or accelerates the oesophageal rhythm, stimulation of the ventral-posterior oesophageal nerve should do the same, as ROBERTSON and LAVERACK (1979) found in the semi-intact

animal. However, in over fifteen preparations of the isolated commissural ganglion, employing a wide range of stimulus parameters, ventral-posterior oesophageal nerve stimulation did not elicit or accelerate bursting monitored in the superior and inferior oesophageal nerves.

In four experiments the application of dopamine to the bathing medium to give a concentration of about 200 μM (ANDERSON and BARKER, 1977) produced no clearcut effects on the oesophageal rhythm monitored in the superior, inferior and ventral-posterior oesophageal nerves of a desheathed commissural ganglion. It did not elicit the rhythm in a quiescent preparation nor noticeably accelerate, decelerate or stop it in a bursting preparation.

b. Coordination of the two commissural ganglia

Recordings from the superior oesophageal nerve of a right and a left commissural ganglion linked only by the post-oesophageal commissure showed that generally only one commissural ganglion would produce bursting activity associated with the oesophageal rhythm (Figure 3.6 a). However, in several preparations both commissural ganglia produced bursting activity which was not coordinated, i.e. each assumed its own rhythm (Figure 3.6 b). Only one preparation showed any evidence of phase-locking or synchronous burst production by the two ganglia and this could have been coincidental.

c. Intracellular correlates of the oesophageal rhythm

A number of cells (at least five) widely distributed in the commissural ganglion fired during the interval between oesophageal dilator

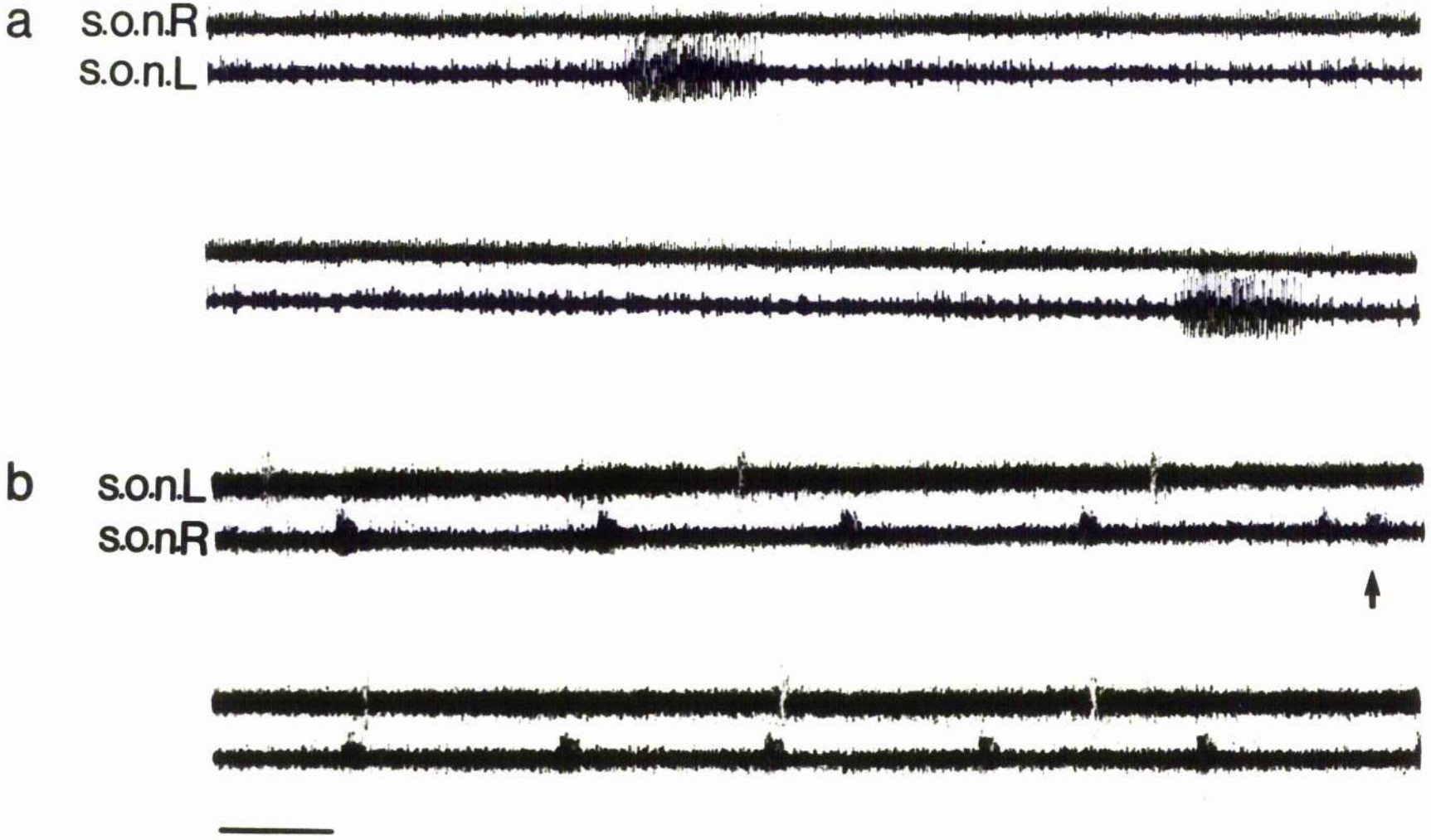
Figure 3.6

Activities of right and left commissural ganglia linked by the post-oesophageal commissure

a. Rhythmic bursting is seen only in the left superior oesophageal nerve. (The two traces are continuous).

b. Continuous traces. Both commissural ganglia show rhythmic bursting but their activities have unrelated cycle periods. Note the "extra" burst produced by the right ganglion (arrow) which does not reset the rhythm (contrast with Figure 3.2 d).

Time scale in a : 1 sec, in b : 4 secs.


a
s.o.n.R
s.o.n.L
b
s.o.n.L
s.o.n.R

bursts monitored in the superior oesophageal nerve and were hyperpolarised during part or all of the burst. These neurons were denoted H cells. Correlating spikes recorded intracellularly from the somata of these cells with extracellularly-recorded spikes in the commissural ganglion nerves and by examining the anatomy of the cells injected with Lucifer Yellow revealed that most of them send a single axon in the inferior oesophageal nerve and may thus be labral motorneurons (Figure 3.8 b and probably d). Some H cells have a bifurcating axon branching into the inferior oesophageal nerve and some other nerve, for example the posterior circumoesophageal connective (Figure 3.8 c). H cells sending an axon in at least the inferior oesophageal nerve tend to increase their firing frequency during the interburst interval, reaching a maximum frequency just before, or at the beginning of, the oesophageal dilator burst. Hyperpolarising input then terminates H cell firing (Figure 3.7 b and d). One H cell did not fire spontaneously during recording (Figure 3.7 c) but when its membrane potential was experimentally depolarised its firing pattern resembled that seen in Figure 3.7 b and d. Oesophageal bursts are occasionally "missed" and no bursting activity is seen in the superior oesophageal nerve at the expected time. In several neurons intracellular evidence could be seen of an "incipient" burst in this situation; for example in Figure 3.7 d the H cell is only partly hyperpolarised at the appropriate time. Only in one experiment was an H cell found which does not send an axon in the inferior oesophageal nerve (Figure 3.7 a). This cell arborises sparsely in the ganglion, in contrast to other H cells. Its activity differed from the other H cells in that its firing frequency did not increase noticeably during the interburst interval and the hyperpolarisation during the burst was slight.

There was some indication that experimental manipulation of the membrane potential of the neuron shown in Figure 3.7 d and 3.8 d altered

Figure 3.7

Cells hyperpolarised during the oesophageal dilator burst (H cells)

a. An H cell which fires tonically during the interburst interval and is slightly hyperpolarised at the beginning of the oesophageal dilator burst (o) monitored in the superior oesophageal nerve. The anatomy of this cell is shown in Figure 3.8 a.

b. A typical example of an H cell whose firing frequency increases during the interburst interval, reaching maximum frequency just before, or at the beginning of, the dilator burst. A pronounced hyperpolarisation occurs during the rest of the dilator burst; the cell then recommences to fire. The anatomy of this cell is shown in Figure 3.8 b.

c. An H cell in a non-firing state showing hyperpolarisations during the dilator burst. Note the less pronounced hyperpolarisations about one second before each dilator burst (arrows); a corresponding slight burst can be seen in the superior oesophageal nerve. This neuron also receives depolarising synaptic input during the interburst interval. On experimental depolarisation of the cell its firing pattern resembled that seen in b and d. The anatomy of this cell is shown in Figure 3.8 c.

d. Continuous records. An H cell exhibiting radical (up to 10 mv) hyperpolarisation during the dilator burst. During the oesophageal rhythm a dilator burst is occasionally "missed" and is not seen in the superior oesophageal nerve (arrow). In this case an "incipient" burst is evidenced by a slight hyperpolarisation in the H cell. The anatomy of this cell is shown in Figure 3.8 d.

Voltage scale in a,b,c and d : 10 mv

Time scale in a,b,c and d : 1 sec.

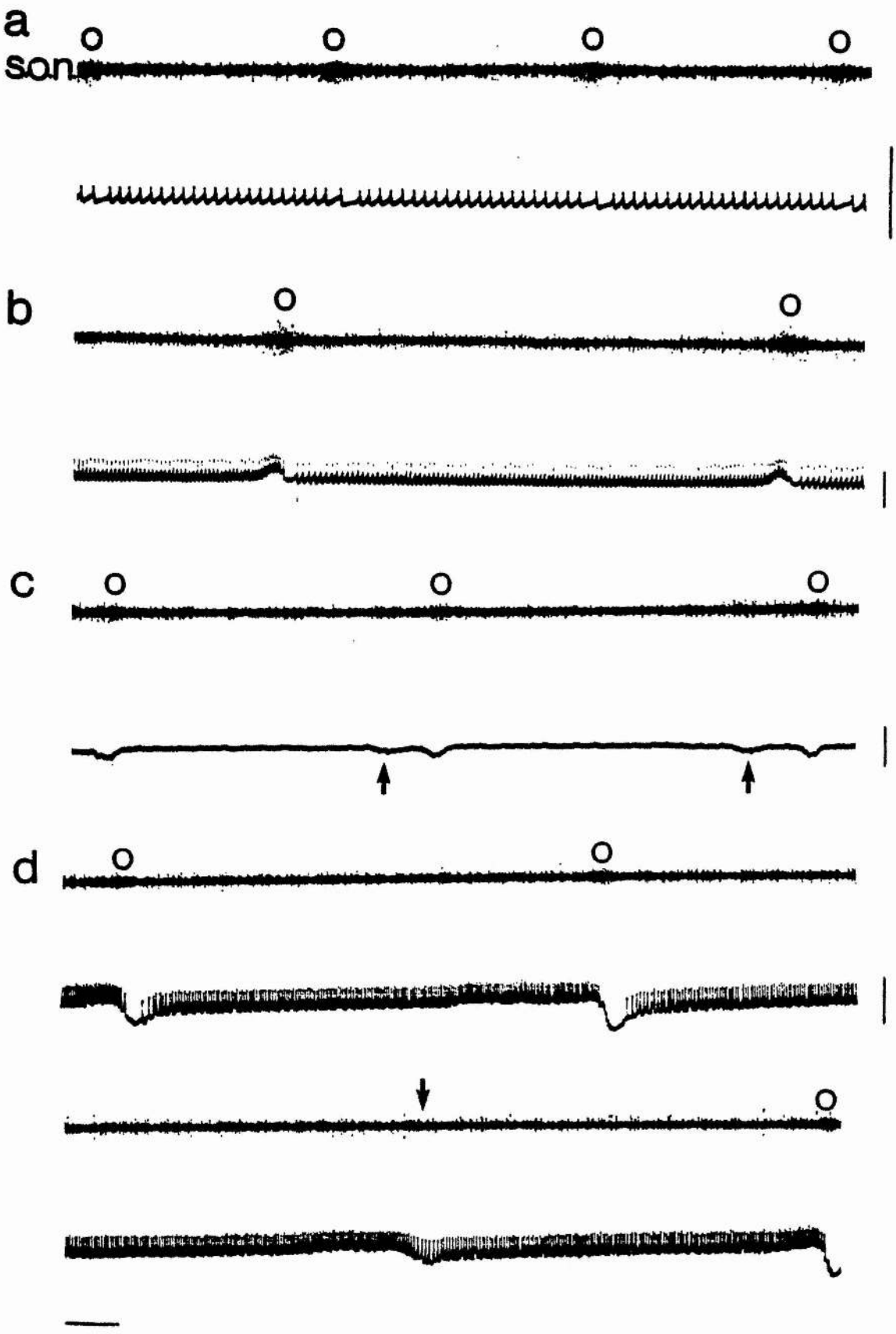

a
son
b
c
d

Figure 3.8

The anatomy of H cells

a. H cell from a left commissural ganglion arborising sparsely in the ganglion and sending a single axon down the posterior circumoesophageal connective towards the suboesophageal ganglion. The activity of this cell is shown in Figure 3.7 a. In this and the following camera lucida drawings a small cartoon depicts the orientation and configuration of the neuron. This cartoon is the same in each drawing, except for the right-left orientation, and is fully labelled in this figure.

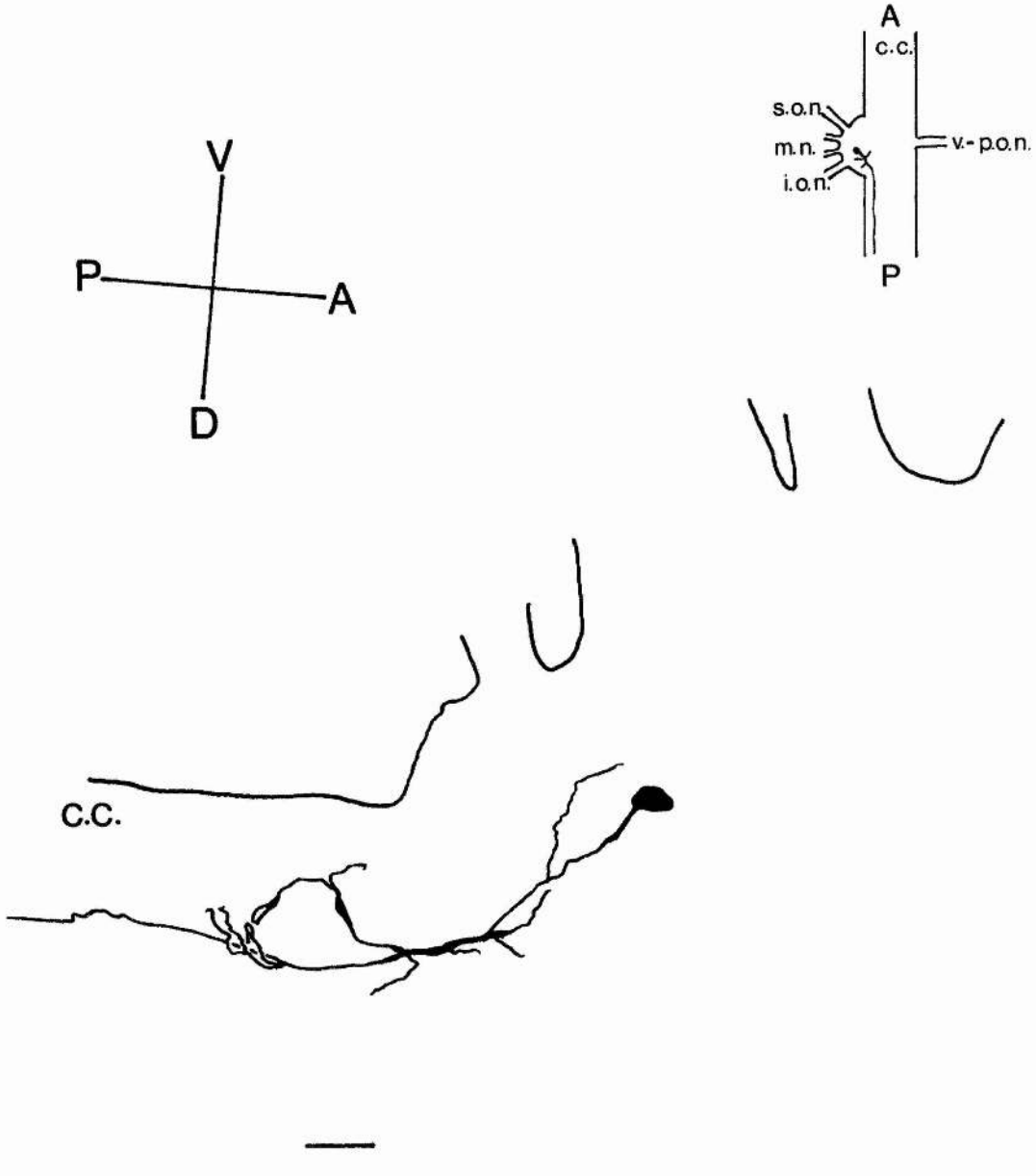
b. H cell from a left commissural ganglion arborising in the ganglion and sending a single axon into the inferior oesophageal nerve. The activity of this cell is shown in Figure 3.7 b.

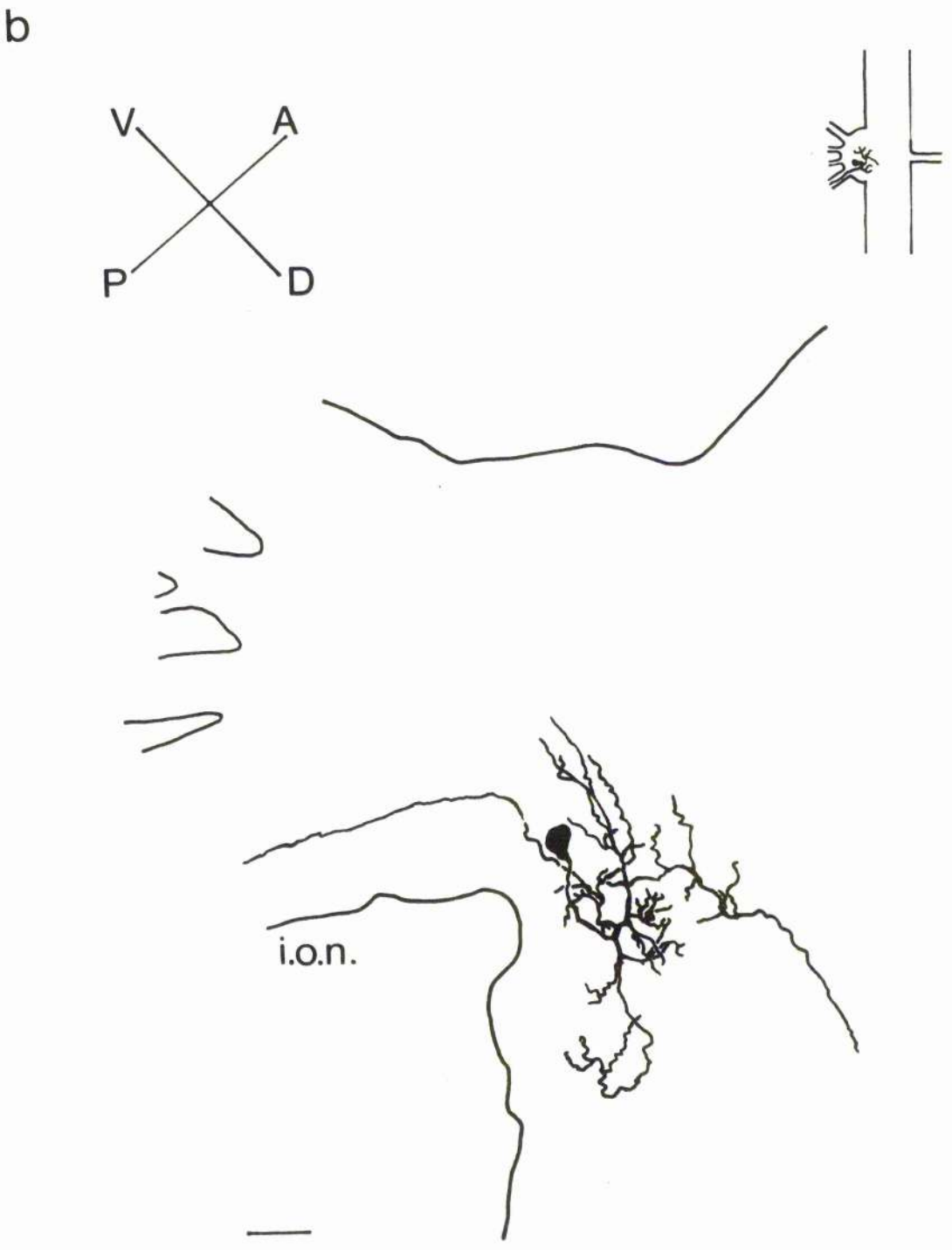
c. H cell from a left commissural ganglion arborising in the ganglion and sending an axon branch in the inferior oesphageal nerve and one in the posterior circumoesophageal connective. The activity of this cell is shown in Figure 3.7 c.

d. H cell from a left commissural ganglion. Dye-coupling occurred and some small somata are filled (arrows). Several axons in the superior, inferior and ventral-posterior oesophageal nerve are filled. Physiologically spikes in this cell appeared to be correlated with spikes monitored extracellularly in the inferior oesophageal nerve and in no other nerve. Hence the axon in this nerve may represent the H cell axon and the other filled axons are due to dye-coupling. The activity of this cell is shown in Figure 3.7 d.

Scale in a,b,c and d : 100 μm

a

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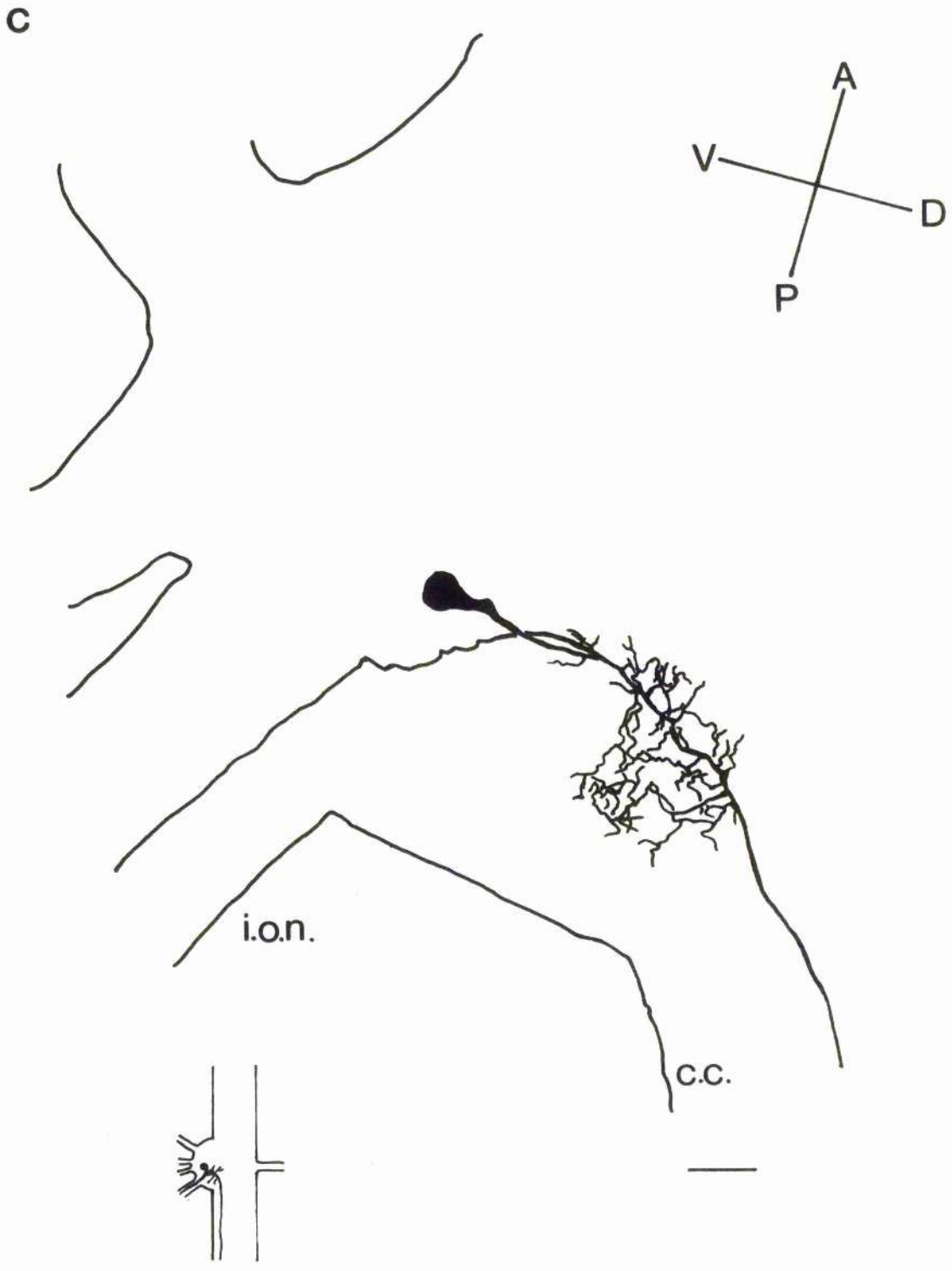
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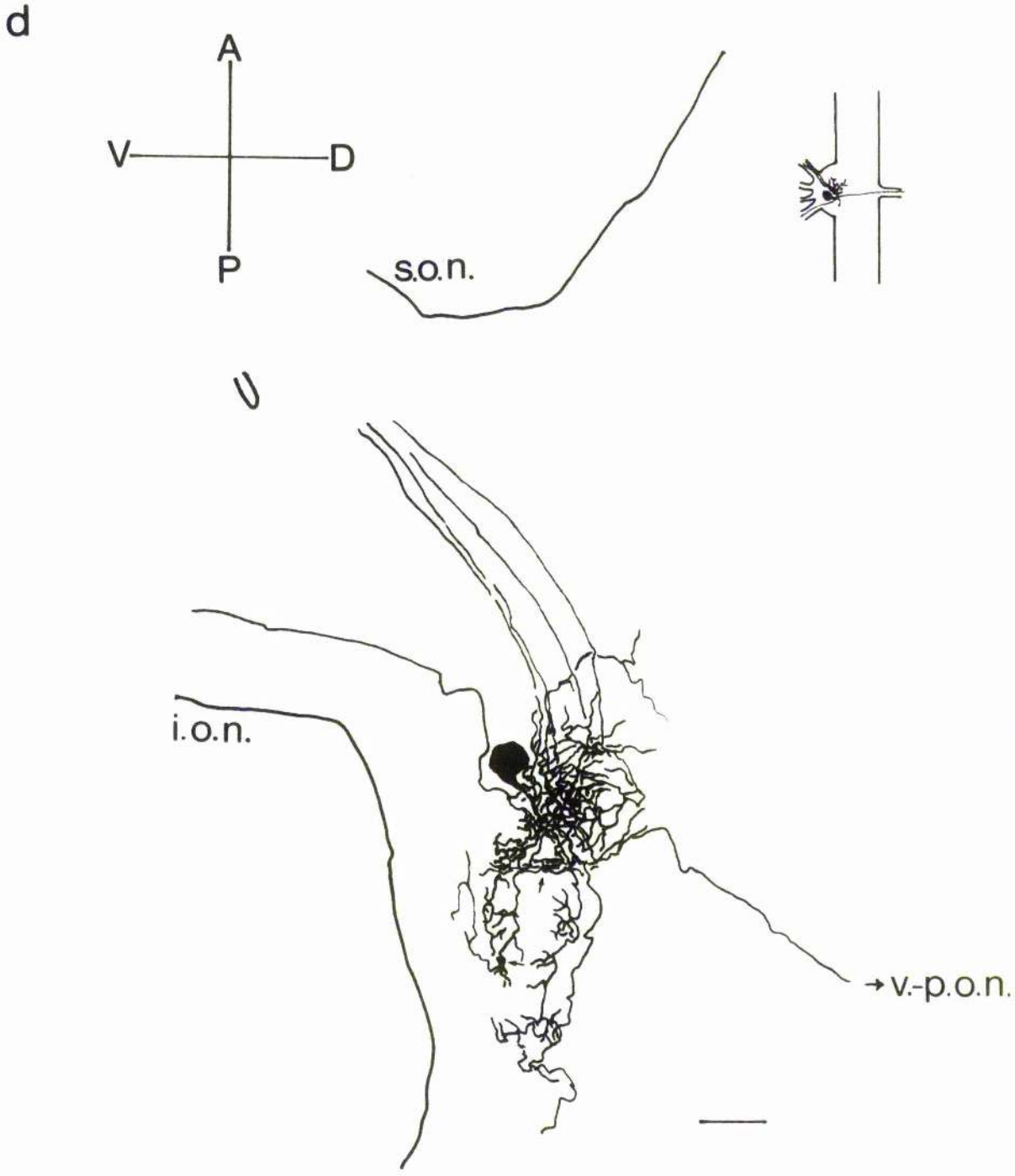
d
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→v.-p.o.n.

the oesophageal rhythm since the frequency appeared to increase slightly on radically depolarising the cell (with 10 nA current) and stop when the cell was hyperpolarised. This effect was not clearly reproducible and in view of the spontaneous frequency changes in the oesophageal rhythm seen in the in vitro preparation these apparent effects are questionable. However, it is interesting to note that this was the only case in which any evidence of the experimental alteration of the oesophageal pattern generator was seen and it was also the only case (out of about fifty dye injections) in which dye-coupling was observed. Lucifer Yellow injected into the soma of this cell also filled other small somata and several axons (Figure 3.8 d).

A protracted search was made for cells which depolarised during the oesophageal dilator burst, for example the units producing the burst seen in the superior oesophageal nerve. The large cells, probably motorneurons, revealed by cobalt chloride backfilling the superior oesophageal nerve (see Chapter 2 Results part d, Figures 2.6 and 2.7) are candidates for such units. However, despite repeated attempts to record from cells in this area of the ganglion these units were not found. At least some cells sending an axon in the superior oesophageal nerve had silent somata, even when depolarised by up to 10 nA current, implying that in these cells the soma is too far from the spike initiating zone to see spikes or subthreshold potentials intrasomatically. Alternatively the current reaching the spike initiating zone was insufficient to cause spiking. In other cells sending an axon in at least the superior oesophageal nerve the firing pattern was not related to the oesophageal rhythm (see parts d and e below). The anatomy of two "silent" neurons sending an axon in the superior oesophageal nerve is shown in Figure 3.9. The structure of these cells is very different, one arborising profusely in the ganglion and one showing very few dendritic arborisations.

Figure 3.9

The anatomy of neurons sending an axon in the superior oesophageal nerve

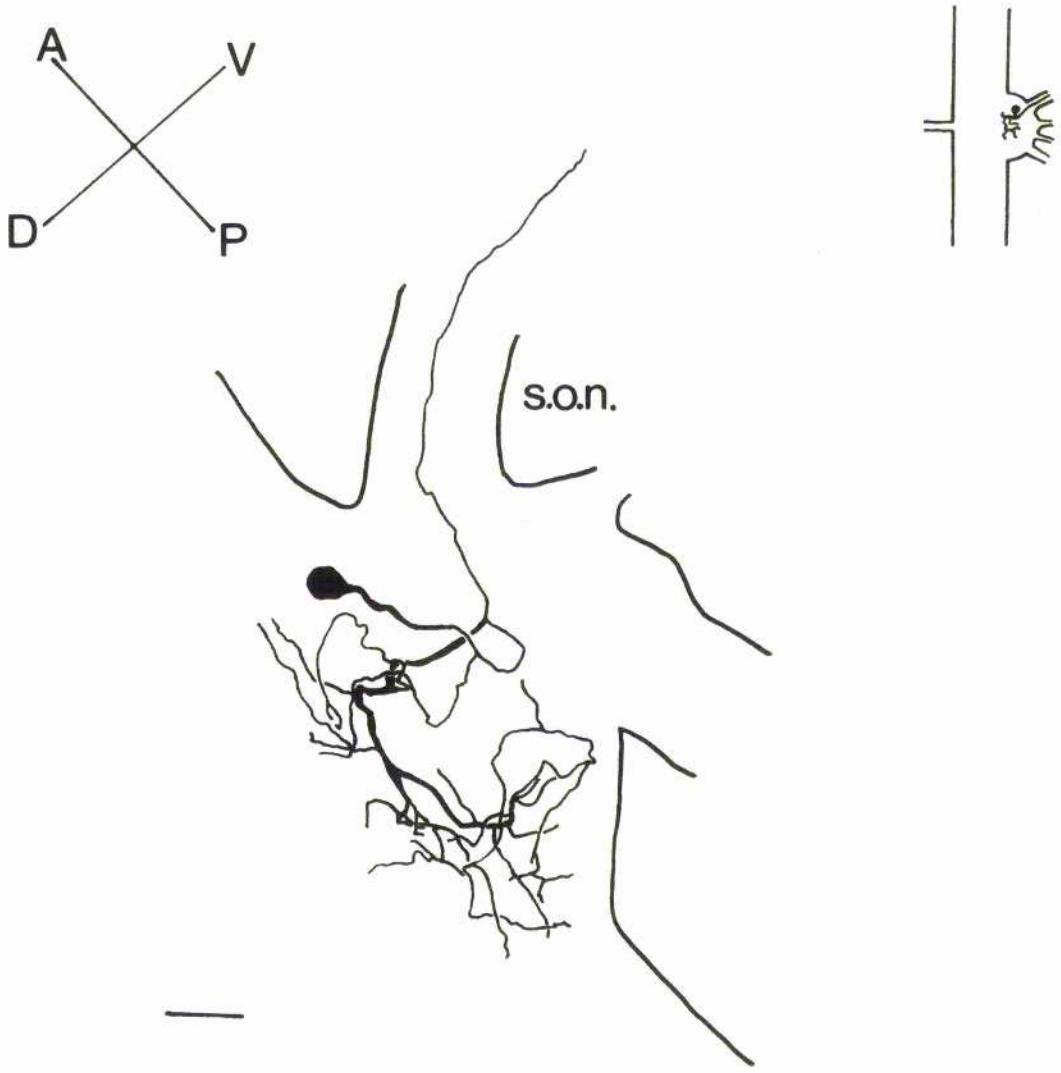
Spikes or subthreshold activity could not be recorded in the somata of these cells (see text).

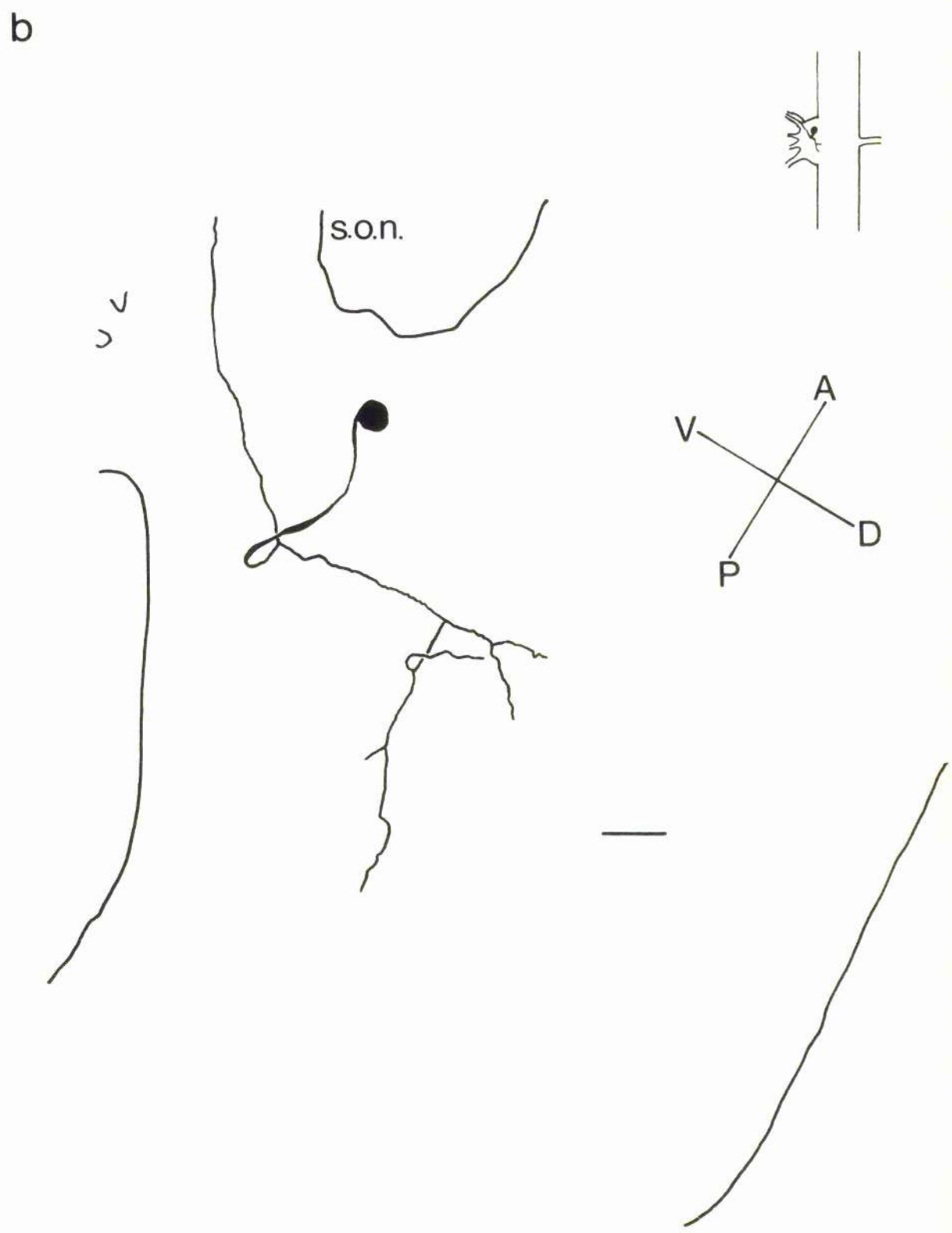
a. A neuron from a right commissural ganglion with a branching axon. One branch gives rise to numerous dendritic arborisations in the ganglion, the other branch travels in the superior oesophageal nerve.

b. A neuron from a left commissural ganglion with a branching axon. One branch travels in the superior oesophageal nerve, the other makes very few dendritic arborisations in the dorsal part of the ganglion.

scale in a and b : 100 µm

a

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Only one cell was found which was depolarised and fired during the oesophageal dilator burst and did not fire during the interburst interval, in contrast to the H cells (Figure 3.10). The large amount of activity during the oesophageal dilator bursts made correlation between spikes in this cell and extracellularly-recorded spikes in the commissural ganglion nerves difficult. Experimental depolarisation and hyperpolarisation of the neuron did not appear to affect the oesophageal rhythm. Lucifer Yellow injection was only effected for a few seconds as protracted penetration of this cell was difficult and it could not be reimpaled. Dye injection only confirmed the position (ventral-posterior quadrant) and size (about 25 μm) of the neuron soma as the dye did not reveal any axonal detail. Hence it was not possible to tell if this cell sends an axon in the superior oesophageal nerve, or in any other nerve.

The L cell is driven by the oesophageal pattern generator; this is discussed in Chapter 4.

d. The production of other rhythms

Complexities in the oesophageal rhythm were sometimes seen, for example "extra" bursts (Figures 3.2 d and 3.6 b), "missing" bursts (Figure 3.7 d) and small bursts occurring before each dilator burst (Figure 3.7 c). However, occasionally rhythms were observed emanating from the commissural ganglion which bore no relation to the oesophageal rhythm monitored by the dilator bursts in the superior oesophageal nerve. This phenomenon was seen infrequently in the superior oesophageal nerve (but see for example Figure 4.3) but more frequently in the inferior oesophageal nerve. Figure 3.11 shows an example of a rhythm in the inferior oesophageal nerve clearly unrelated to the oesophageal rhythm.

Intracellular studies showed that several cells exhibited a regular,

Figure 3.10

A neuron which is depolarised during the oesophageal dilator burst

Recording from the small neuron soma (about 25 μm in diameter) in the ventral-posterior quadrant of a right commissural ganglion.

a. The cell fires small action potentials (1-2 mv) on initial penetration. It is depolarised at the beginning of the dilator burst monitored in the superior oesophageal nerve and is repolarised at the end of the burst.

b. Spikes produced by depolarisation can be seen more clearly on a faster time scale. As the neuron is increasingly depolarised the spikes get smaller and more frequent until hyperpolarising input shuts off the firing.

Voltage scale in a and b : 10 mv

Time scale in a : 4 secs, in b : 1 sec

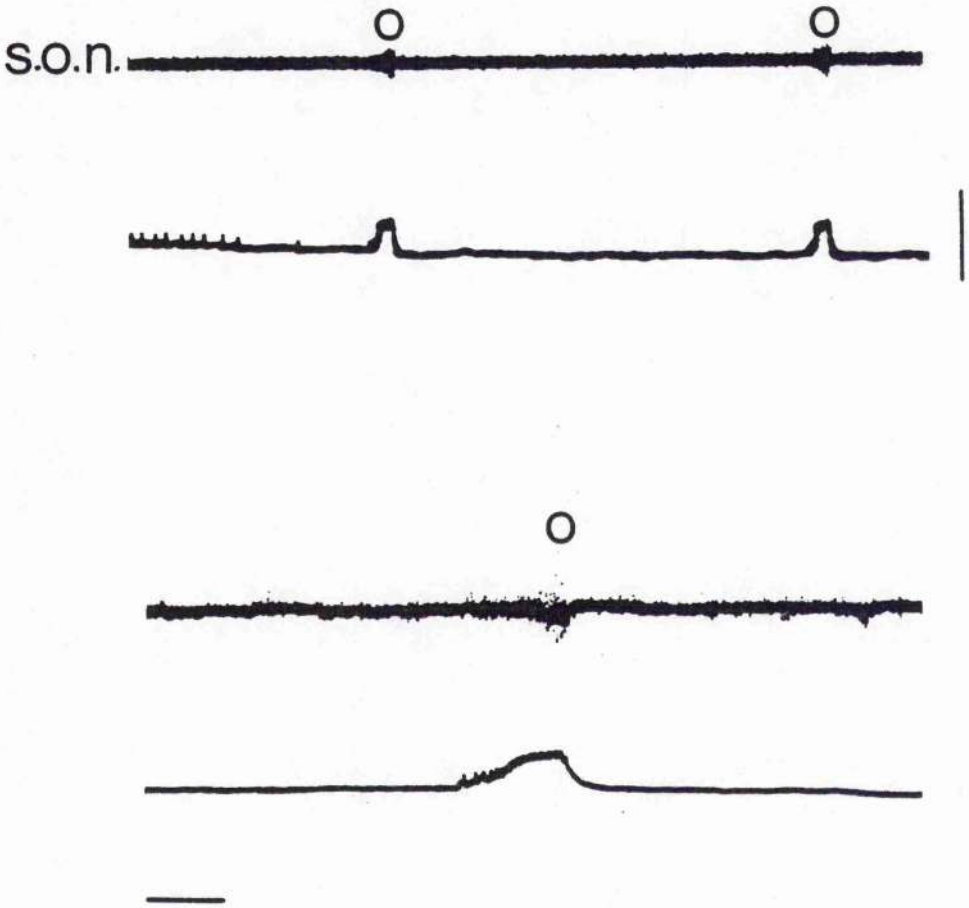
s.o.n.

Figure 3.11

Inferior oesophageal nerve rhythm which is unrelated to the oesophageal rhythm

From a right commissural ganglion. Note the bursts (arrows) in the inferior oesophageal nerve. This rhythm has a different period from the oesophageal rhythm monitored in the superior oesophageal nerve and continues when the oesophageal dilator bursts stop.

Time scale : 4 secs

i.o.n.
s.o.n.

tonic firing pattern apparently not influenced by the oesophageal pattern generator (see part e below). One neuron showed a rhythmic activity only loosely coupled to the oesophageal rhythm. Figure 3.12 shows that the membrane potential of this cell fluctuated sinusoidally, producing rather erratic bursts with a period of about one second. This cycle period did not appear to be altered by experimental depolarisation or hyperpolarisation, implying that the oscillation is not endogenous but is due to cyclic synaptic input. Such experimental manipulation of the membrane potential did not appear to affect the oesophageal rhythm, which had a periodicity of about 15 - 16 seconds. During the oesophageal dilator burst monitored in the superior oesophageal nerve the hyperpolarising phase of its cycle was usually accentuated, implying a loose coupling with the oesophageal rhythm. Spikes in this cell were correlated with spikes recorded extracellularly in the superior oesophageal nerve; Lucifer Yellow injection showed that the axon of this cell branches, one branch travelling in the superior oesophageal nerve and one branch arborising in the ganglion (Figure 3.13).

e. Other features of commissural ganglion cells

1. Tonically firing cells

The activity of several cells (at least eight) which were widely distributed in the commissural ganglion was tonic, i.e. random or unpatterned, and bore no relation to the oesophageal rhythm. This did not appear to be due to injury firing since in all cases the activity continued for the duration of the experiment (over an hour). Several examples are given in Figure 3.14 and the anatomy of these cells is shown in Figure 3.15. Anatomically these cells were of different types : neurons,

Figure 3.12

A neuron whose rhythmic firing pattern is only loosely coupled to the oesophageal rhythm

From a left commissural ganglion. Continuous traces. Sinusoidal oscillations in the membrane potential of this cell produce rather erratic bursts of spikes with a cycle period of about one second. The tops of the spikes have been clipped in recording; the action potentials were about 10 mv in amplitude. The periodicity of the oesophageal rhythm monitored in the superior oesophageal nerve is about 15-16 seconds. The activity of this cell is loosely coupled to the oesophageal rhythm . Usually, but not always, the hyperpolarising phase of its cycle is accentuated during the oesophageal dilator burst (arrows) but pronounced hyperpolarisations also occur at other times (e.g. ●). The anatomy of this cell is shown in Figure 3.13.

Voltage scale : 10 mv

Time scale : 1 sec

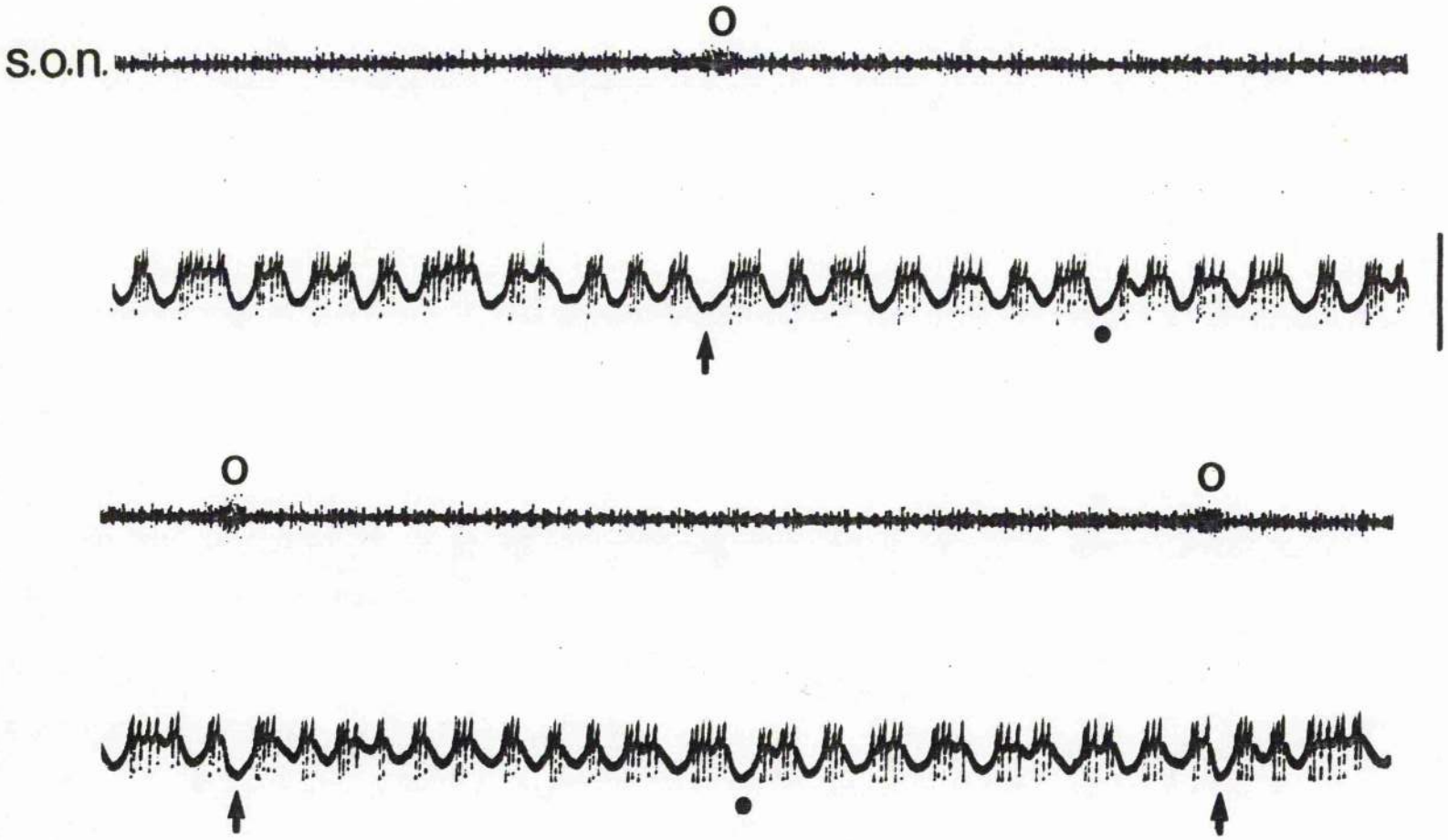
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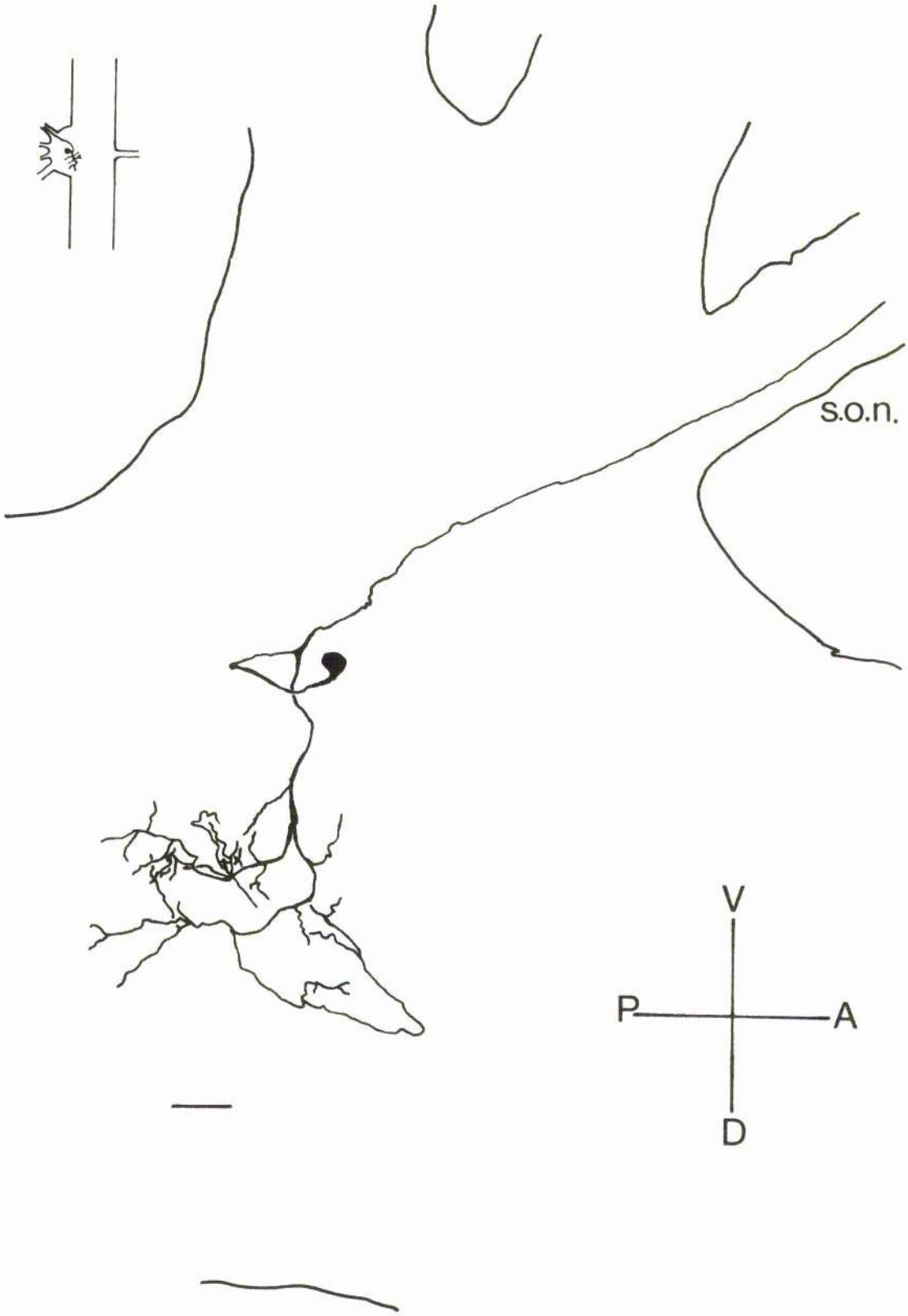
Figure 3.13

The anatomy of a neuron whose rhythmic activity is only loosely coupled to the oesophageal rhythm.

From a left commissural ganglion. The axon of this neuron branches : one branch arborising in the ganglion and one travelling in the superior oesophageal nerve. The activity of this cell is shown in Figure 3.12.

Scale 100 μm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presumably motorneurons, sending an axon into the superior (Figures 3.14 a and 3.15 a) or inferior (Figures 3.14 b and c and 3.15 b and c) oesophageal nerves and interneurons (Figures 3.14 d and e and 3.15 d and e). One tonically firing interneuron (Figures 3.14 e and 3.15 e) sends an axon towards the brain in the anterior circumoesophageal connective; presumably this is one of the large group of interneurons seen on cobalt chloride backfilling the anterior connective (see Chapter 2 Results part d and Figure 2.13). Another tonically firing interneuron appeared to be a local interneuron, not sending an axon in any nerve (Figures 3.14 d and 3.15 d). It is possible that the neuron had an axon but it did not fill with Lucifer. However, in other respects this preparation suggested a "good" fill : the fluorescence was bright and neuron processes had been stained.

2. "Silent" cells

Most commissural ganglion somata penetrated appeared to be silent; no spontaneous action potentials or subthreshold membrane potential changes could be seen or could be evoked by experimental depolarisation of up to 10 nA current. This may imply either that the cell does not produce action potentials, for example non-spiking local interneurons, or that the soma is too far removed from the site of spike initiation to see potential changes intrasomatically or for sufficient depolarising current to reach this zone from the soma. The resting potential of these cells exhibited the range seen in spiking cells (about -30 - -70 mv) and did not appear to be exceptionally high. Some cells, presumably motorneurons, sending an axon in the superior oesophageal nerve, appeared to have silent somata which could not be made to spike (see part c above and Figure 3.9).

Figure 3.14

The activity of neurons whose tonic (random, unpatterned) activity is unrelated to the oesophageal rhythm.

a. From a right commissural ganglion. There appear to be two sizes of spikes in this cell, implying two sites of spike initiation (arrows a and b). The anatomy of this cell is shown in Figure 3.15 a.

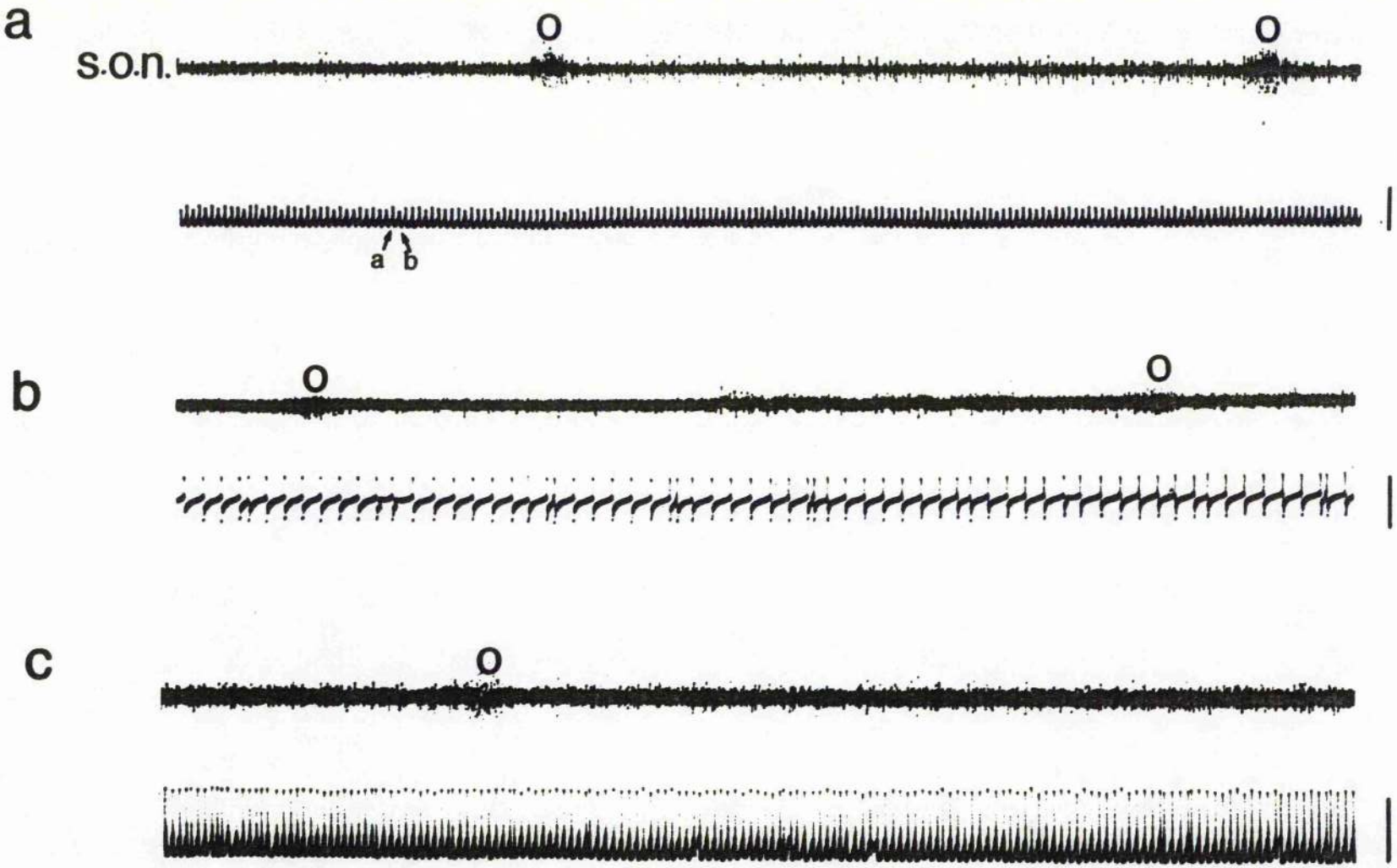
b. From a right commissural ganglion. Note the double spikes which occasionally occur, apparently at random. The anatomy of this cell is shown in Figure 3.15 b.

c. From a left commissural ganglion. The anatomy of this cell is shown in Figure 3.15 c.

d. From a right commissural ganglion. The anatomy of this cell is shown in Figure 3.15 d.

e. From a right commissural ganglion. This neuron spiked erratically, unlike the cells in a-d. Its activity is clearly unrelated to the oesphageal rhythm and continues unaltered when dilator bursts in the superior oesophageal nerve stop. There may be two sizes of spike in this neuron (arrows a and b) implying two sites of spike initiation. The anatomy of this cell is shown in Figure 3.15 e.

Voltage scale in a,b,c,d and e : 10 mv
Time scale in a,b,c and d : 1 sec, in e : 4 secs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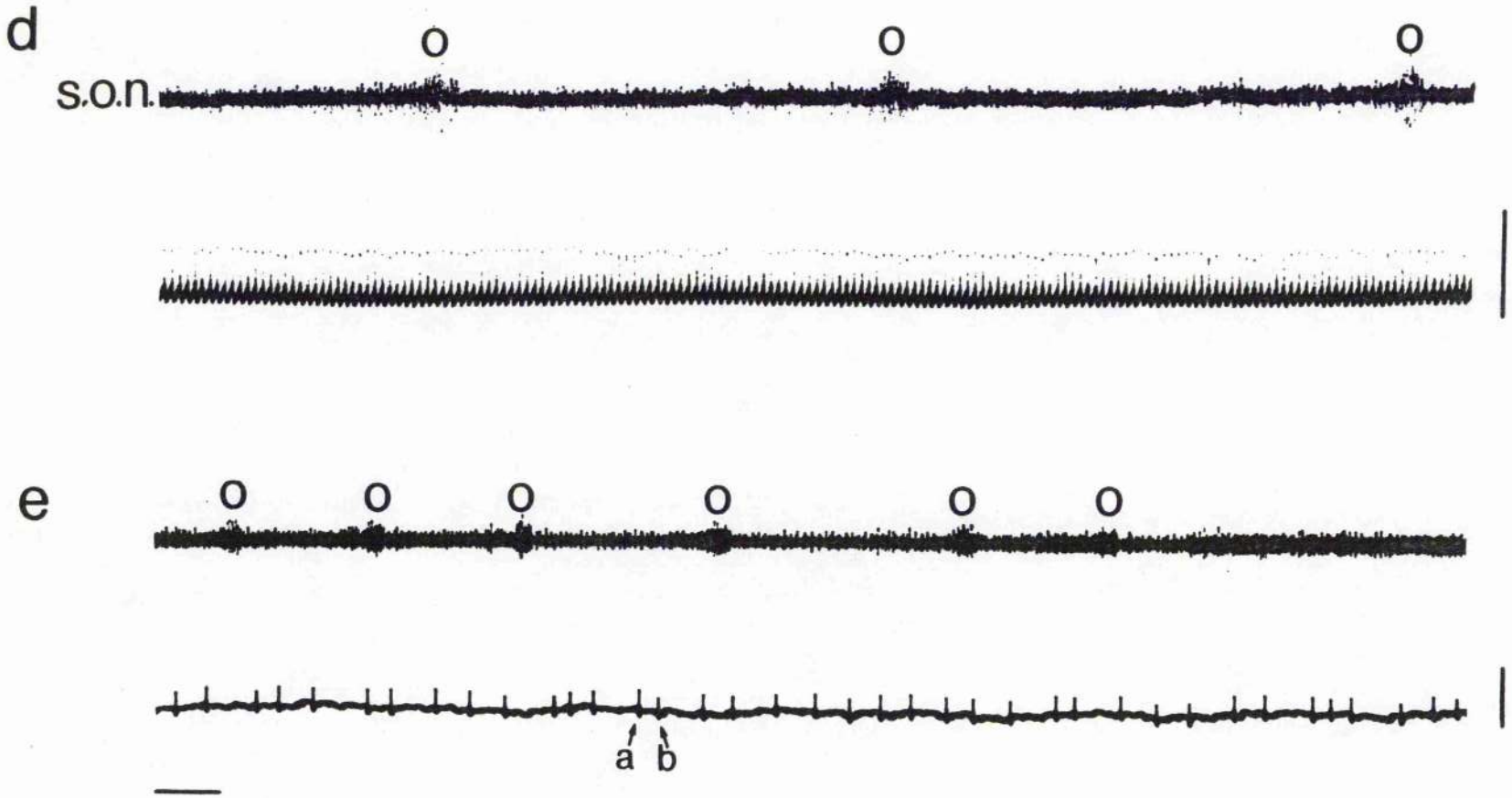
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Figure 3.15

The anatomy of tonically firing cells

a. From a right commissural ganglion. This neuron arborises profusely in the ganglion and sends an axon in the superior oesophageal nerve. The activity of this cell is shown in Figure 3.14 a.

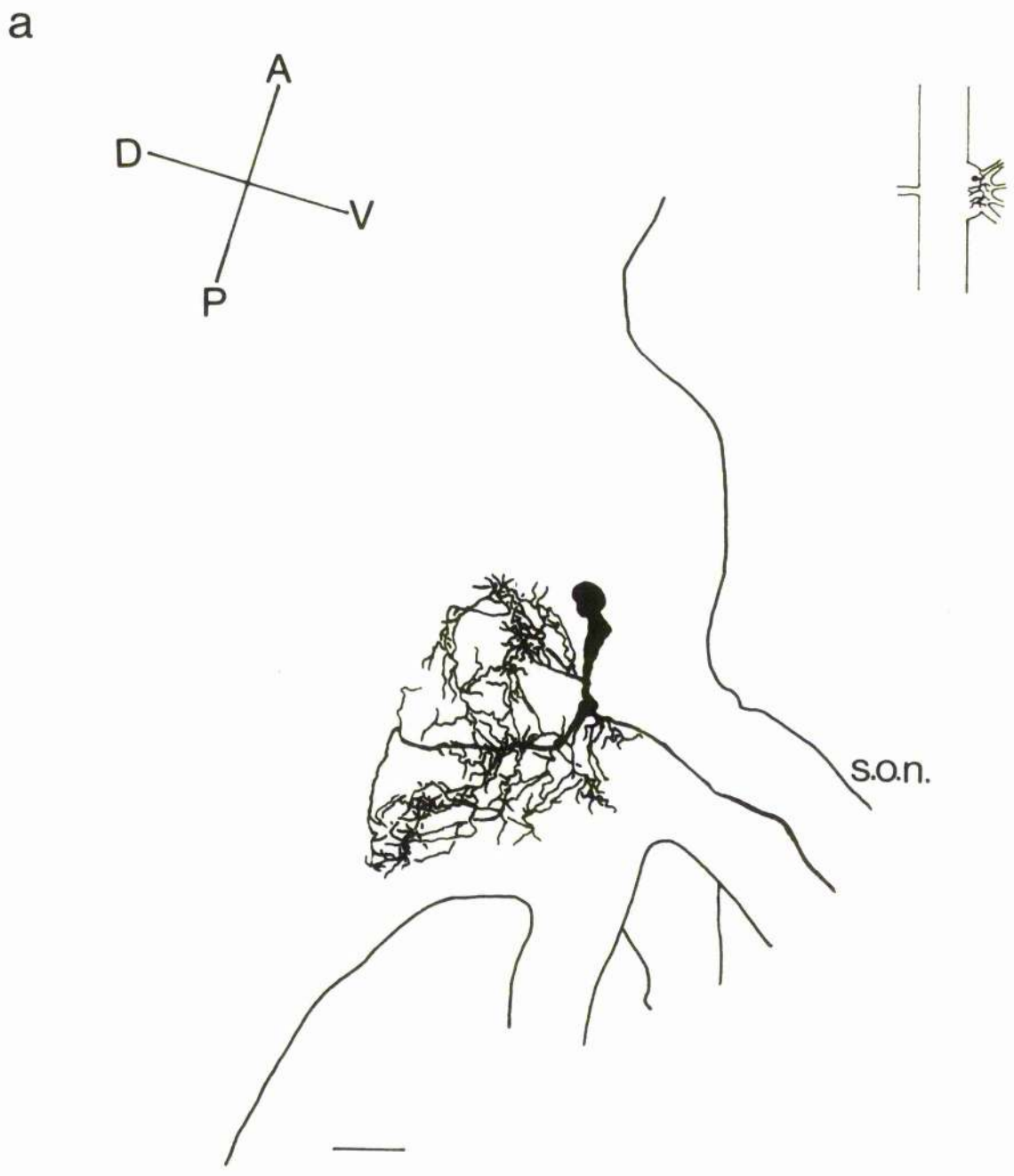
b. From a right commissural ganglion. A neuron arborising sparsely in the ganglion and sending an axon in the inferior oesophageal nerve. The activity of this cell is shown in Figure 3.14 b.

c. From a left commissural ganglion. A neuron arborising extensively in the ganglion and sending an axon in the inferior oesophageal nerve. The activity of this cell is shown in Figure 3.14 c.

d. From a right commissural ganglion. This neuron appears to be a small local interneuron arborising very sparsely in the ganglion. The activity of this cell is shown in Figure 3.14 d.

e. From a right commissural ganglion. An interneuron arborising in the ganglion and sending an axon towards the brain in the anterior circumoesophageal connective. The activity of this cell is shown in Figure 3.14 e.

Scale in a, b, c, d and e : 100 μm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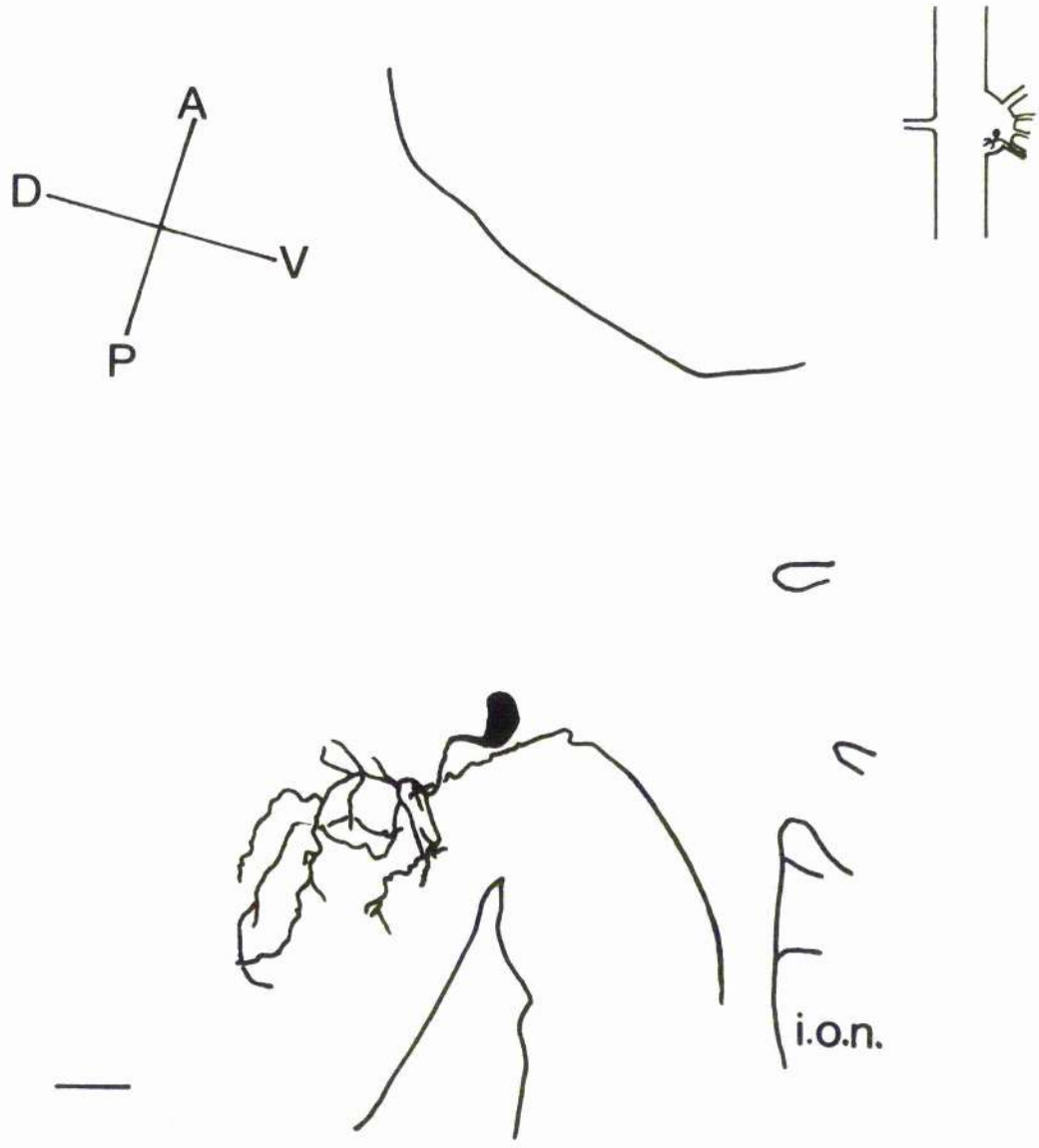


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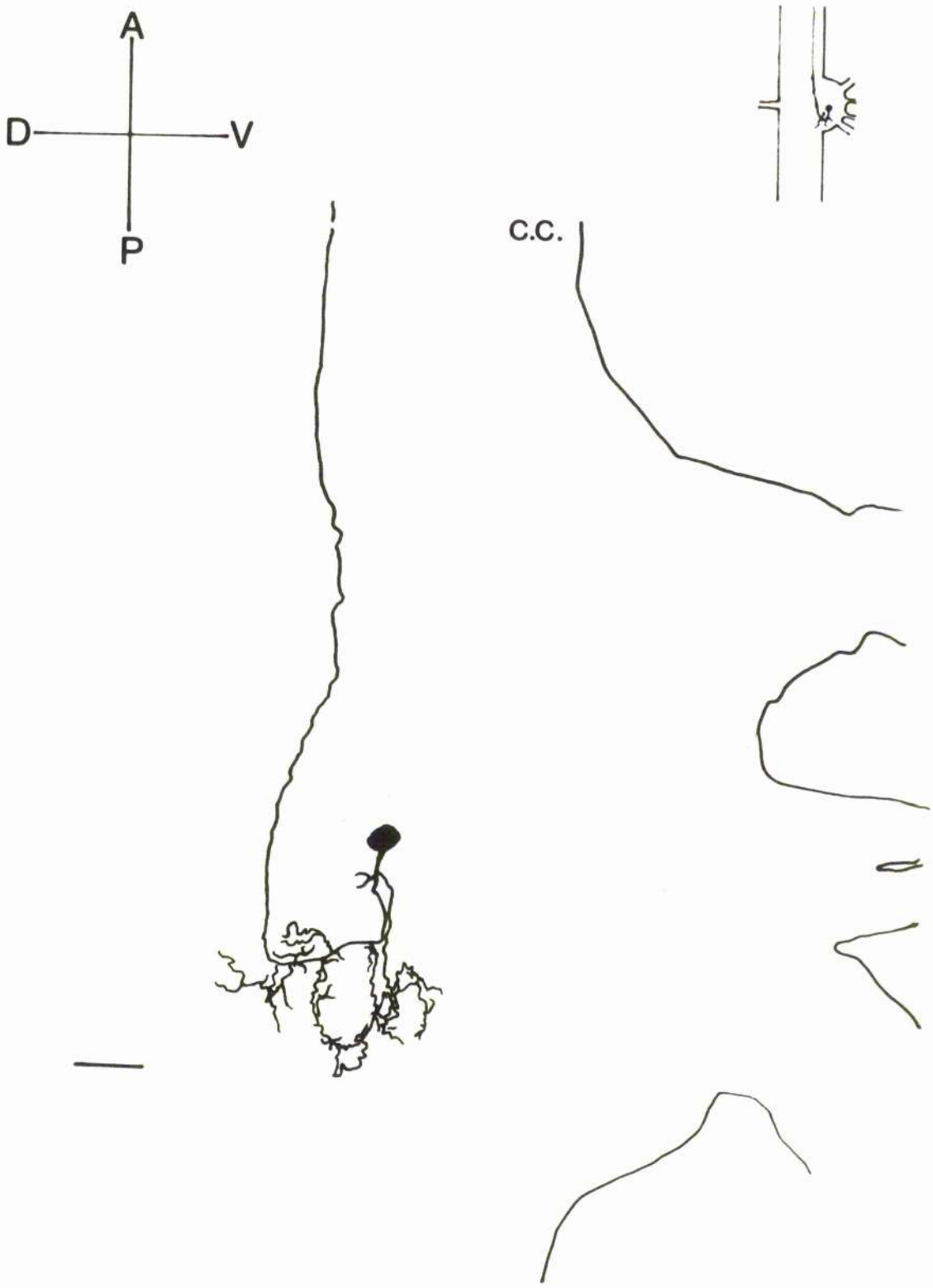


f. Overview of commissural ganglion cells

The maximum number of cells penetrated in one experiment was 47 (excluding the L cell). During this experiment the state of the electrode was constantly monitored, by measuring the resistance using the series voltage divider in the BridgeBAK intracellular amplifier and by frequently impaling the L cell and examining the shape and size (up to 40 mv : see Chapter 4) of the spikes. Artefacts could thus be avoided, for example apparent silence in neuron somata due to electrode blockage. The experiment yielded these results :-

"silent" cells.....33
tonically firing cells.....8
H cells (with an axon in at least
the inferior oesophageal nerve).....5
cells whose rhythmic firing pattern is
unrelated to the oesophageal rhythm.....1
Total.....47

Care was taken not to penetrate the same cell twice, this often entailed avoiding certain areas in the ganglion and introduced some bias in sampling. No attempt was made to record from the ten or so cells on the medial surface of the ganglion (see Chapter 2 Results part d). It was not possible to penetrate many cells. For example the neuron somata are not distributed in a single layer on the surface of the ganglion (see Figure 2.4 b and c) and it was sometimes difficult to penetrate underlying cells. In particular penetration of very small somata (less than about 15 µm in diameter) was usually impossible, for example the large dorsal group of small interneurons sending axons to the brain in the anterior circumoesophageal connective (see Chapter 2 Results part d and Figure

2.13). If there are about 400 or more neuron somata in the commissural ganglion (see Chapter 2), then the cells examined in this experiment represent only about 10% of the total number. Despite these limitations the results give some indication of the proportion of classes of cells in the "recordable" population.

Discussion

The similarities between the in vitro preparation and the semi-intact animal (ROBERTSON, 1978) imply that at least the minimal oesophageal pattern generator resides in the commissural ganglion. However, this does not rule out the possibility of interaction with oesophageal ganglion neurons as the work of MOULINS and his co-workers suggests for Palinurus vulgaris. No consistent anatomical (see Chapter 2) or physiological differences have been observed between right and left commissural ganglia and each is capable of producing the oesophageal rhythm. In Homarus gammarus coordination of the two ganglia is not effected by the post-oesophageal commissure as SPIRITO (1975) claims for Procambarus clarkii. There is some anatomical evidence for common pathways from stomatogastric and oesophageal ganglion neurons to both commissural ganglia (see Chapter 2 Results part d and Figure 2.15). There may also be common pathways from the brain to both ganglia via the circumoesophageal connectives and similar sensory input to both ganglia, for example from both posterior oesophageal sensors. These are ways in which common input may help to coordinate the activity of the two commissural ganglia. Clearly, the problems of bilateral coordination between the ganglia must be viewed in the context of the whole stomatogastic nervous system. It is possible that one role of the post-oesophageal commissure is to mediate the fine detail of such coordination.

There is anatomical evidence (see Chapter 2 Results part d) and some physiological evidence for common pathways between various nerves, for example between the superior and inferior oesophageal nerves, between the superior and ventral-posterior oesophageal nerves and between the superior oesophageal nerve and anterior circumoesophageal connective (see Chapter 2 Discussion). In particular, some neurons have collateral branches in

different nerves, for example in the inferior oesophageal nerve and in the posterior circumoesophageal connective (see Figures 3.7 c and 3.8 c). This suggests a coordinating role since the axon branches to other parts of the nervous system could carry information in the form of an efference copy of motor discharge. In this example labral (via the inferior oesophageal nerve) and mandibular (via the posterior connective collateral to the suboesophageal ganglion) coordination may be effected. Information concerning the oesophageal rhythm is also known to be relayed to the stomatogastric pattern generators via the stomatogastric nerve (SELVERSTON et al., 1976 : see Introduction part e).

Many cells exhibiting activity correlated with the oesophageal rhythm are H cells which are hyperpolarised during the oesophageal dilator burst. Most of these send an axon in at least the inferior oesophageal nerve and are probably labral motorneurons. The activity of these cells partly underlies inferior oesophageal nerve activity recorded extracellularly - an increase in firing and a slight burst before the oesophageal dilator burst and relative silence during this burst. The only observed case of dye-coupling (Figure 3.8 d) was seen in an H cell and this was the only case in which there was a slight suggestion that experimental manipulation of the membrane potential altered the oesophageal rhythm. The H cell was dye-coupled to several small neurons (about 10 μm in diameter) which may suggest electrotonic coupling. However, the correlation between dye-coupling and electrotonic coupling may not be absolute (STEWART, 1978). Nonetheless, it is intriguing to speculate that some of the large population of small neurons may be involved in oesophageal pattern generation. Clearly, much further study is needed to elucidate the neuronal basis of the oesophageal rhythm. This problem has not yet been resolved for any species.

Similarly, the initiation, modulation and cessation of the oesophageal rhythm is poorly understood. It is difficult to comprehend why stimulation of the ventral-posterior oesophageal nerve did not elicit the oesophageal rhythm as ROBERTSON and LAVERACK (1979) found in the semi-intact animal. Studies of the integration of sensory input by the commissural ganglion should clarify this. Since dopamine-containing neurons are found in the stomatogastric ganglion of Homarus gammarus, although not in other species (OSBORNE and DANDO, 1970 : see Chapter 4), it is possible that some inputs to the commissural ganglia from the stomatogastric ganglion may be mediated by dopamine. However, this study suggests that dopamine is not involved in starting, stopping or modulating the oesophageal rhythm.

The commissural ganglion is capable of producing rhythms other than the oesophageal rhythm. For example, bursting activity occasionally observed in the inferior oesophageal nerve may represent a vestigial labral rhythm which occurs rarely since it must normally be gated by sensory input for its expression (R.M. ROBERTSON, pers. comm.). In the in vitro preparation the labral motorneurons (e.g. H cells) appear to be driven by the oesophageal pattern generator and the activity of the inferior oesophageal nerve, which carries the axons of the labral motorneurons, is related to the oesophageal rhythm seen in the superior and ventral-posterior oesophageal nerves carrying oesophageal motorneurons. Presumably common pathways between the various nerves also underlie this integration (see Chapter 2 Discussion) which in turn effects the coordination of the labrum and the oesophagus that has been observed behaviorally (ROBERTSON, 1978). It is possible that the same set of motorneurons are driven by either the oesophageal pattern generator or the labral pattern generator if the latter is called into play by the appropriate sensory input. It has been shown that inferior oesophageal

nerve activity differs from that of the other commissural ganglion nerves in which a very pronounced dilator burst occurs. Furthermore, there is some lability in the relationship of inferior to superior oesophageal nerve activity (see Results part a and Figure 3.2). A very rigid coupling to the oesophageal pattern generator would perhaps not be expected if labral motorneurons can also be driven by another pattern generator. Such neuronal economy is not uncommon in arthropods. For example ELSNER (1974) showed that in the grasshopper Stenobothrus rubicundus motorneurons are common to leg stridulation, wing stridulation and flight. It is also interesting to speculate on the relationship of the two pattern generators - are they represented by separate neural networks or are there common elements ? Some neurons are involved in pattern generation of more than one foregut rhythm, for example neuron AM is implicated in both the gastric mill and cardiac sac rhythms (see Introduction parts d and e).

Certain commissural ganglion neurons exhibit rhythmic activity unrelated to the oesophageal rhythm, for example the commissural gastric driver (ROBERTSON and MOULINS, unpublished a, b and e) and by implication the commissural pyloric oscillator (the activity of this neuron was inferred by monitoring its effects on a follower cell : ROBERTSON and MOULINS, unpublished a and d). Further investigation should clarify the number of commissural ganglion neurons exhibiting such activity and their roles, for example in phasic entrainment of stomatogastric pattern generators.

At least some of the tonically firing neurons in the commissural ganglion may have a function unrelated to the oesophageal rhythm, for example interneurons sending an axon to the brain (see Figures 3.14 e and 3.15 e) may relay information about other aspects of commissural ganglion physiology or about sensory input to the ganglion. It has been suggested that labral motorneurons sending an axon in the inferior oesophageal nerve

may not be rigidly coupled to the oesophageal pattern generator (see above) which may partly explain why some of them exhibit a tonic firing pattern. Tonic activity in oesophageal motorneurons sending an axon in the superior oesophageal nerve is more difficult to interpret. This phenomenon was observed in many preparations and is probably not attributable to damage. A few commissural ganglion neurons may supply the cardiac sac via the superior oesophageal nerve and the postero-lateral nerve (see Chapter 2 Results part d) and their activity may not be related to the oesophageal rhythm. However, it seems unlikely that this could account for all the observed cases of tonic firing in these neurons. Possibly these cells are driven by the oesophageal pattern generator but this effect must normally be gated, perhaps by sensory input, for its expression and will not be seen in the in vitro preparation. Such a mechanism could help to ensure that the oesophagus is only active in the appropriate context, for example when the posterior oesophageal sensors are stimulated by food intake. Observations such as these emphasise the need to study problems of foregut pattern generation in the whole animal as well as in the in vitro preparation.

In Homarus gammarus the "recordable" population of neurons appears to be small. In particular, technical difficulties were experienced in trying to record from very small neurons and their function(s) remain speculative. The large number of neurons with "silent" somata emphasises the need for neuropilar as well as intrasomatic recording. Some of these cells may be non-spiking, for example local interneurons, whilst in others the site of spike initiation may be far removed from the soma, resulting in apparent silence. Dye injection has revealed that commissural ganglion neurons have widely differing anatomies but at our present state of knowledge it is difficult to relate neuronal geometry to function. Moreover, it is not certain how much neuronal detail Lucifer Yellow reveals; markers such as

cobalt do not reveal all branches and specialisations even in high concentrations (STRAUSFELD and OBERMAYER, 1976).

Chapter 4 : The study of a selected neuron

Introduction

a. Dopamine in the stomatogastric nervous system

Several neurotransmitters or neuromodulators are implicated in the operation of the stomatogastric nervous system (SELVERSTON et al., 1976). Cholinesterase has been demonstrated histochemically in the synaptic neuropile of the stomatogastric ganglion of Panulirus argus and Homarus americanus (MAYNARD, 1971b). Acetylcholine acts as an excitatory transmitter at certain neuromuscular junctions in Panulirus interruptus (MARDER 1974 and 1976). MARDER (1976) found that L-glutamate is a transmitter candidate for other motor neurons.

Catecholamines and related compounds have been extensively investigated in the nervous system of "higher" Crustacea. Noradrenalin has not been detected (KERKUT, SEDDEN and WALKER, 1966; COTTRELL, 1967; BARKER, MOLINOFF and KRAVITZ, 1972; BARKER, KUSHNER and HOOPER, 1979). Octopamine plays a significant role in the nervous system (BARKER, MOLINOFF and KRAVITZ, 1972; WALLACE, TALAMO, EVANS and KRAVITZ, 1974; EVANS, TALAMO and KRAVITZ, 1975; EVANS, KRAVITZ, TALAMO and WALLACE, 1976; EVANS, KRAVITZ and TALAMO, 1976). Octopamine has recently been demonstrated in the stomatogastric nervous system of Panulirus interruptus, particularly in the stomatogastric and commissural ganglia (BARKER and HOOPER, 1975; BARKER, KUSHNER and HOOPER, 1979). Evidence suggests that octopamine acts as a neurohormone or modulator in Crustacea (EVANS et al., 1975; EVANS, KRAVITZ and TALAMO, 1976; SULLIVAN, FRIEND and BARKER, 1977; FLOREY and RATHMAYER, 1978) but its role in the stomatogastric system is not yet clear.

The occurrence of dopamine in the crustacean nervous system has been much studied, for example by the FALCK-HILLARP fluorescence technique (see

Materials and methods below) and by radioisotope studies (for example ELOFSSON, KAURI, NIELSEN and STRÖMBERG, 1966; GOLDSTONE and COOKE, 1971; BARKER, KUSHNER and HOOPER, 1979). The importance of dopamine in the stomatogastric nervous system is becoming increasingly recognised. COOKE and GOLDSTONE (1970) and GOLDSTONE and COOKE (1971), using the FALCK-HILLARP technique, described several cells in the commissural ganglia of species including Carcinus maenas, Cardiosoma guanhumi, Cancer borealis and Orconectes virilis which exhibited specific fluorescence probably attributable to dopamine, since it was characteristic of primary catecholamines and noradrenalin is not present (see above). GOLDSTONE and COOKE (1971) attempted to show that these cells are responsible for all the green-fluorescent axons and terminals of the pericardial organ. They performed microspectrofluorometric analyses of green fluoresence in pericardial organs and demonstrated excitation and emission maxima appropriate for a catecholamine. Chromatographic analyses of pericardial organ extracts implied the presence of dopamine. However, these studies do not give direct proof that dopamine is present in the fluorescent cells of the commissural ganglia. Such direct evidence has only been obtained for one commissural ganglion cell, the L cell (see part b below). KUSHNER and MAYNARD (1977) also found several commissural ganglion cells exhibiting specific fluorescence probably due to dopamine in Panulirus interruptus. Fluorescent axons were observed connecting the commissural to the stomatogastric ganglion, via the superior oesophageal and stomatogastric nerves; fluorescent nerves were also observed in the inferior oesophageal nerve. KUSHNER (1977), presented a similar picture of catecholamine-like fluorescence in the crayfish, Pacifastacus leniusculus with the exception that fluorescent nerve fibres were found linking the commissural ganglia and the stomatogastric ganglion via the inferior oesophageal and stomatogastric nerves, a pathway not found in

Panulirus interruptus. OSBORNE and DANDO (1970) claimed that some somata in the stomatogastric ganglion of Homarus vulgaris (H. gammarus) contained dopamine. However, their findings have not been substantiated in other species and the available evidence suggests dopamine-mediated inputs from the CNS (e.g. from the commissural ganglia) to the stomatogastric ganglion and not vice versa.

BARKER and HOOPER (1975) and BARKER, KUSHNER and HOOPER (1979) demonstrated that the distribution of the synthesis of dopamine from radioactive tyrosine in the stomatogastric nervous system in vitro closely matches the areas of catecholamine-like fluorescence. This distribution may also be correlated with that of the type I dense core vesicles observed in the ultrastructural studies of the stomatogastric ganglion (FRIEND, 1976; FRIEND, KUSHNER and MAYNARD, 1975).

Finally, the application of exogenous dopamine or its precursor L-DOPA in "physiological" concentrations to an isolated stomatogastric ganglion activates certain pyloric motorneurons to produce "normal" pyloric output (ANDERSON and BARKER, 1977). This supplements the very brief observation of BARKER and HOOPER (1975) that the application of dopamine, octopamine and 5-HT each affect the isolated stomatogastric ganglion "...in a characteristic fashion...".

b. Previous studies on the L cell

ORLOV (1929) described a large anteriorly-located neuron soma in his methylene blue studies of Astacus commissural ganglia (see Chapter 2 Introduction part d). The large axon of this cell loops posteriorly in the ganglion where it arborises extensively and travels towards the brain in the circumoesophageal connective (see ORLOV, 1929, neuron A in Figure 19; neuron 42 in Figure 16.52 in BULLOCK and HORRIDGE, 1965). This cell

appears to have a homologue in other species (see below) and is denoted the L cell after SELVERSTON et al. (1976).

COOKE and GOLDSTONE (1970) using fluorescence histochemistry technique on the nervous system of Carcinus maenas, the shore crab, (see part a above), showed that the L cell (which in this species is about 90 µm in diameter) exhibits specific fluorescence attributable to catecholamines. It gives rise to a single axon, about 15 µm in diameter, giving off arborisations in the ganglion and travelling to the brain in the circumoesophageal connective. COOKE and GOLDSTONE claim that the axon doubles back from the brain, passes through the suboesophageal ganglion and terminates in the pericardial organ, which is a neurohaemal organ (see COOKE and GOLDSTONE, 1970, Plate 6). In the crayfish, Orconectes virilis, the L cell is similar in size, position and fluorescent properties. Its axon is claimed to follow the same distinctive course (GOLDSTONE and COOKE, 1971). Specific fluorescence apparently due to catecholamines has been demonstrated for the L cell of the crayfish Pacifastacus leniusculus (KUSHNER, 1977) and Panulirus interruptus (KUSHNER and MAYNARD, 1977; BARKER, KUSHNER and HOOPER, 1979). Isolated L cell somata from P. interruptus can synthesise dopamine from radioactive tyrosine (BARKER, KUSHNER and HOOPER, 1979). In all the species studied the L cell appears to be homologous in its size, position and fluorescent nature.

Physiological studies on the L cell are restricted to the very brief observations of RUSSELL (unpublished, in SELVERSTON et al., 1976). In the isolated commissural ganglion of Panulirus interruptus the L cell receives trains of EPSPs causing it to fire in bursts coordinated with the oesophageal rhythm (see SELVERSTON et al., 1976, Figure 37).

Materials and methods

a. Preparation, extracellular physiology, intracellular physiology and dye injection

See Chapter 3 : Materials and methods parts a, b, c, d and e.

b. Fluorescence histochemistry

The FALCK-HILLARP technique for the histochemical demonstration of biogenic monoamines (see for example CORRODI and JONSSON, 1967) was used to study the distribution of catecholamines in the commissural ganglion, particularly in the L cell.

Freeze-drying techniques (see for example COOKE and GOLDSTONE, 1970) followed by wholemount preparation or vacuum-wax embedding were unsuccessful due chiefly to the fragility of the tissue. Phosphorus pentoxide-dried stretched preparations were used successfully. The desheathed commissural ganglion was gently stretched lateral (cell body) side up on a clean microscope slide and the excess moisture blotted. It was placed in a closed vessel containing phosphorus pentoxide and left overnight to dry. The preparation was exposed for 1 or 3 hours at 80 °C to formaldehyde gas generated from paraformaldehyde previously equilibrated to 70% relative humidity (HAMBERGER, MALMFORS and SACHS, 1965). It was mounted in dehydrated liquid paraffin and examined with a Zeiss fluorescence microscope as described in Chapter 3 Materials and Methods part e. Preparations were photographed with Kodak Tri-X Pan film with an exposure time of forty seconds.

As controls preparations were either :

1. Incubated at 80 °C for 1 or 3 hours in the absence of paraformaldehyde

or

2. Not incubated.

They were mounted and examined as described above.

To test the specificity of fluorescence the sodium borohydride test was used. After examination the preparation was immersed in a solution of excess sodium borohydride in 90% propan-2-ol for several minutes. It was then re-mounted and re-examined. This procedure reversibly reduces fluorescent 6,7-dihydroxy-3,4-dihydroisoquinolines (formed on condensation with formaldehyde from primary catecholamines, e.g. dopamine or noradrenalin) and 6-hydroxy-3,4-dihydro-β-carbolines (formed from e.g. 5-HT) to their non-fluorescent 1,2,3,4-tetrahydro- derivatives (CORRODI, HILLARP and JONSSON, 1964). Quenching of the fluorophores could also be carried out by placing the preparation under running water (CORRODI and JONSSON, 1967).

Attempts were made to decrease the autofluorescence in the preparations which tended to obscure details of specific fluorescence. Reduction of the salt content of the tissues was attempted in two ways. Lobsters were kept in decreasing (70%, 50% and 35%) concentrations of sea-water for a minimum of one week for each concentration. Alternatively a dissected commissural ganglion from a normal lobster was washed in a solution of isosmotic sucrose or 10% glycerol in buffered saline with added glucose for one hour prior to phosphorus pentoxide drying. These attempts were not successful in reducing autofluorescence.

Results

a. Physiology

The resting potential of the L cell varied from about -35 - -50 mv. The action potentials recorded in the soma were large, up to 40 mv. In the isolated commissural ganglion the activity of the L cell was usually clearly related to the oesophageal rhythm monitored in the superior oesophageal nerve (Figure 4.1). It fires in bursts coordinated with this rhythm, each burst commencing at the end of an oesophagael dilator burst. The maximum firing frequency was usually at the beginning of an L cell burst, sometimes preceded by a pronounced hyperpolarisation during the superior oesophageal nerve dilator burst, suggesting post-inhibitory rebound firing. Although excitatory and inhibitory post-synaptic potentials could often be distinguished, these appeared to have no simple pattern and could not be related to spikes in the superior or inferior oesophageal nerves.

Experimental manipulation of the L cell membrane potential, depolarising and hyperpolarising the cell with currents of up to 10 nA, did not start, stop or modulate the oesophageal rhythm. Its firing frequency was altered but its cycling time remained the same (Figure 4.2). Hence it appears to be driven by cyclic synaptic input from the oesophageal pattern generator and is not involved in driving this rhythm. In some preparations it was clear that the L cell was not simply driven by the oesophageal network, but exhibited a more complex discharge (Figure 4.3).

Figure 4.1

L cell activity related to the oesophageal rhythm

a, b and c. Records from three different preparations showing that bursting activity in the L cell is clearly related to the oesophageal rhythm monitored by pronounced dilator bursts in the superior oesophageal nerve (o - first burst only labelled in a). Each L cell burst starts at the end of an oesophageal dilator burst, occasionally preceded by an hyperpolarisation (c) and usually commencing with a maximal firing rate (b and c) suggesting post-inhibitory rebound firing. In a the L cell is gradually hyperpolarising after initial penetration but the relationship to the oesophageal rhythm can be clearly seen. Evidence of synaptic input can be seen in c. In b and c the tops of the spikes have been clipped in recording; the action potentials were 15-20 mv in amplitude.

Voltage scale in a and b : 20 mv, in c : 10 mv

Time scale in a : 4 secs, in b and c : 1 sec

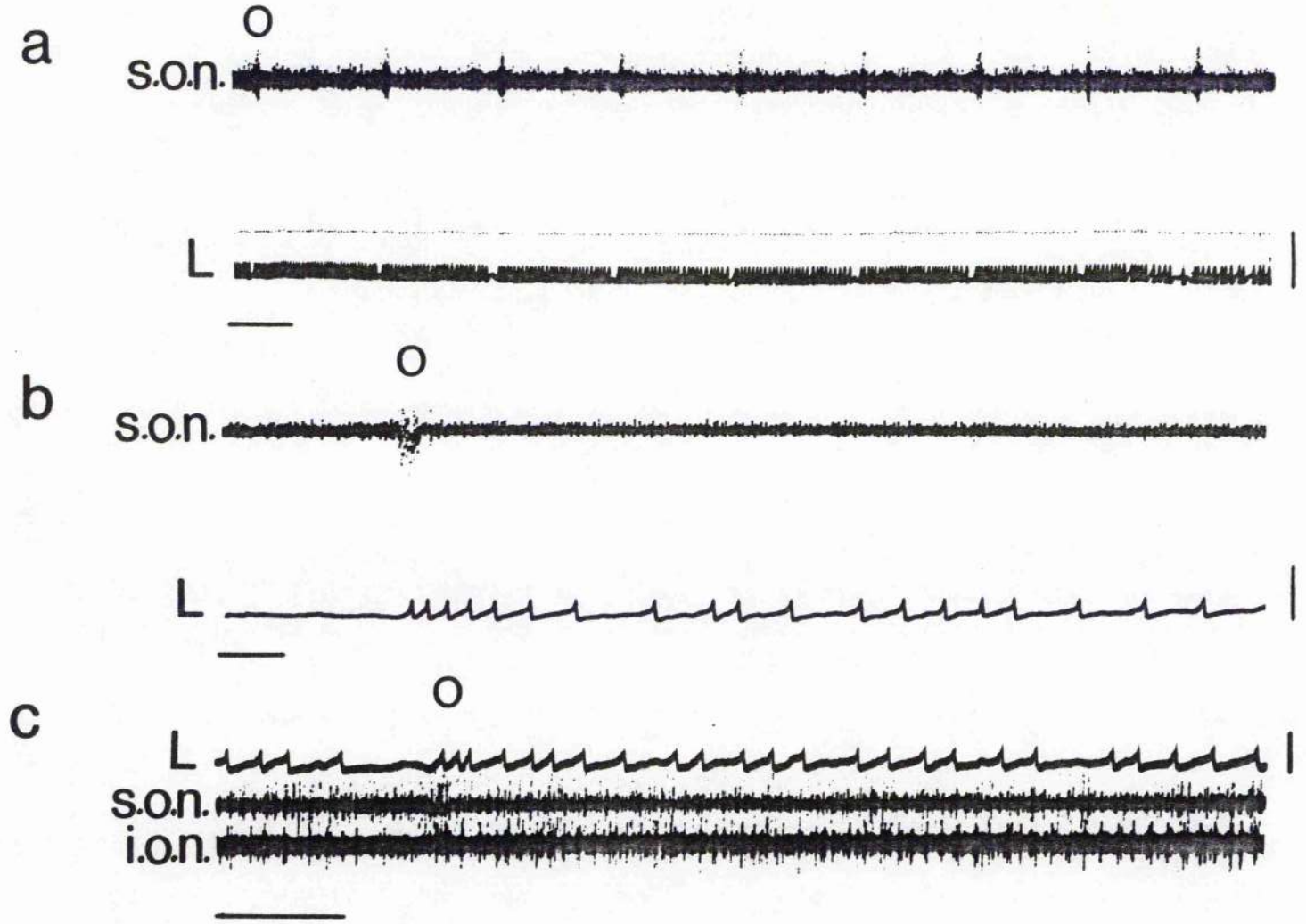

a
o
s.o.n.
L
b
o
s.o.n.
L
c
o
L
s.o.n.
i.o.n.

Figure 4.2

Experimental manipulation of the L cell membrane potential

a. normal

b. depolarised

c. slightly hyperpolarised.

The oesophageal dilator burst (o) monitored in the superior oesophageal nerve was not very clear in these records. It was recorded with the pen-recorder event marker monitoring the CRO screen.

Depolarising (b) or hyperpolarising (c) the L cell membrane potential affects its firing rate but does not alter its cycling period or the the oesophageal rhythm.

Voltage scale in a, b and c : 20 mv

Time scale in a, b and c : 4 secs

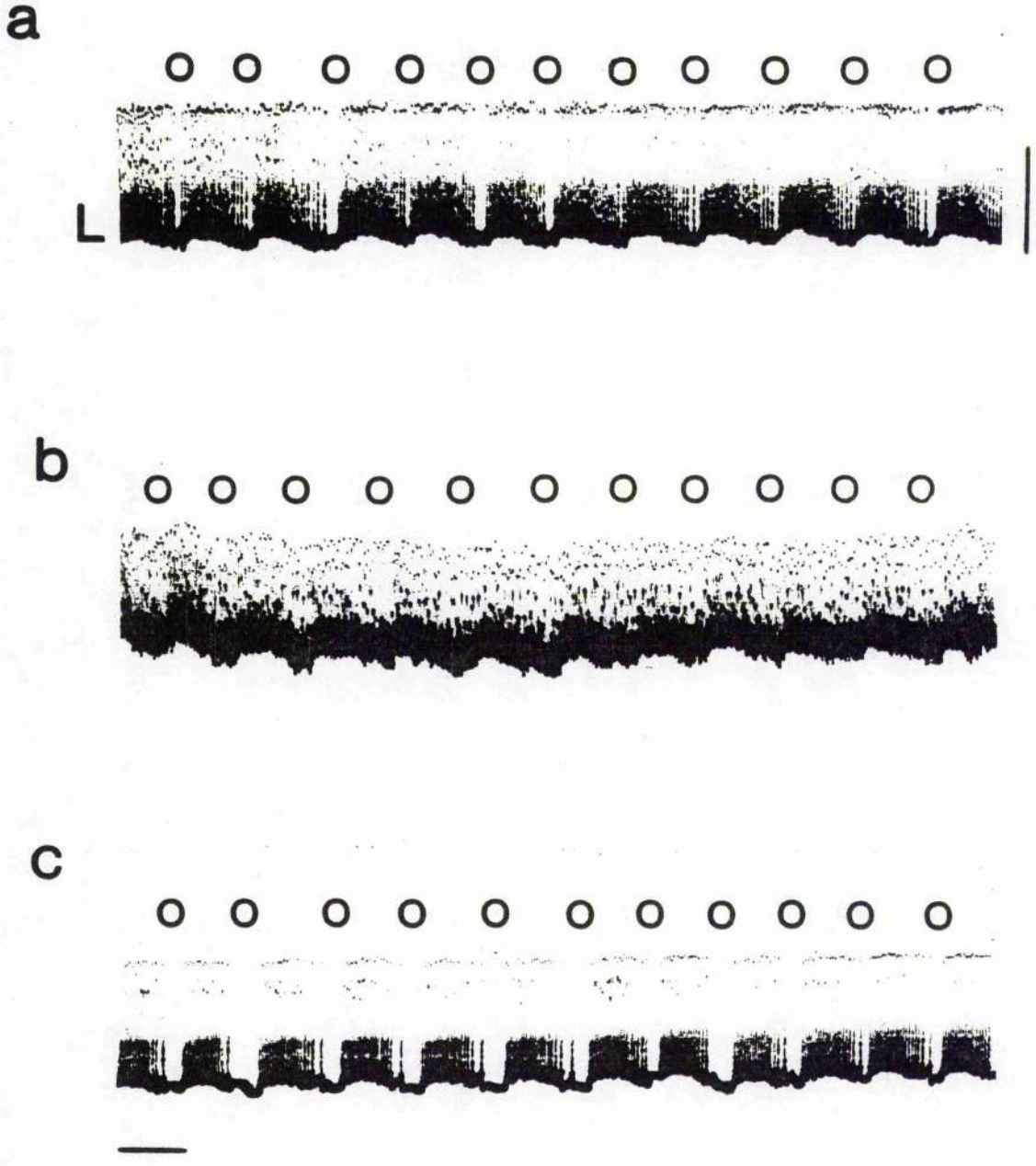

a
L
b
c

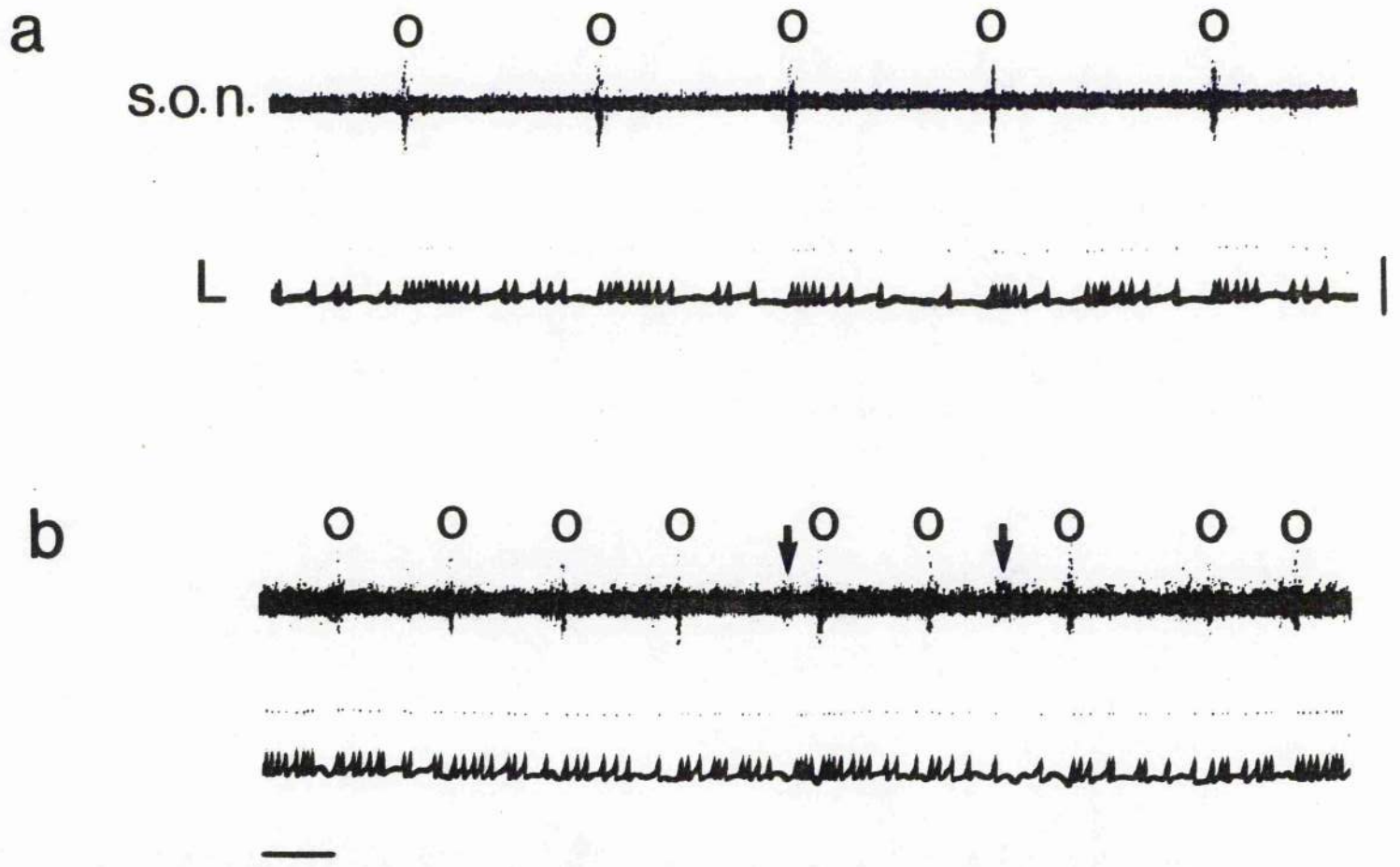
Figure 4.3

Complex L cell firing

a and b. Records from two preparations show that L cell activity is related to the oesophageal rhythm monitored by the dilator burst (o) in the superior oesophageal nerve but is more complex (compare with Figure 4.1). In b there are bursts in the superior oesophageal nerve which are unrelated to the oesophageal rhythm (two examples are arrowed).

Voltage scale in a and b : 20 mv

Time scale in a and b : 4 secs


a
s.o.n.
L
b

b. Structure

Figure 4.4 shows a right commissural ganglion L cell filled with Lucifer Yellow injected into the soma. In different preparations the L cell soma ranges from about 90 - 150 μm in diameter. The course of the axon and some dendritic arborisations on the axon loop can be seen. However, photographs could not adequately demonstrate all the observed detail and camera lucida drawings were used to record structural detail. Four examples from right and left commissural ganglia are given in Figure 4.5. The structure of the L cell was very similar in all preparations examined (about fifteen). The axon loops posteriorly in the ganglion before travelling towards the brain in the circumoesophageal connective. The dendritic arborisations only occurred on the axon loop and were usually profuse. One long posteriorly-going process appeared in most preparations (see Figure 4.5 a,b and c). These findings concur with the results obtained by cobalt chloride backfilling the commissural ganglion from the brain (see Figure 2.13) and with those of ORLOV (see Introduction).

c. Fluorescence histochemistry

After an incubation time of one hour the L cell usually exhibited specific yellow-green fluorescence characteristic of primary catecholamines (Figure 4.6). In fourteen preparations the L cell was visible before stretch-drying, i.e. it had not been lost or damaged during dissection. In twelve (86%) of these preparations the L cell exhibited fluorescence and of these nine (82%) showed reversible quenching of the fluorescence with the sodium borohydride test. Hence in over 70% of preparations (86% x 82%) the L cell showed specific fluorescence. In eleven control preparations, not incubated or incubated in the absence of formaldehyde

Figure 4.4

The structure of the L cell (1)

Photograph of a right commissural ganglion L cell injected with Lucifer Yellow into the soma, showing the course of the axon and a few dendritic arborisations (some are arrowed). Compare with Figure 4.5 b which is a camera lucida drawing of the same preparation.

Scale : 100 μm

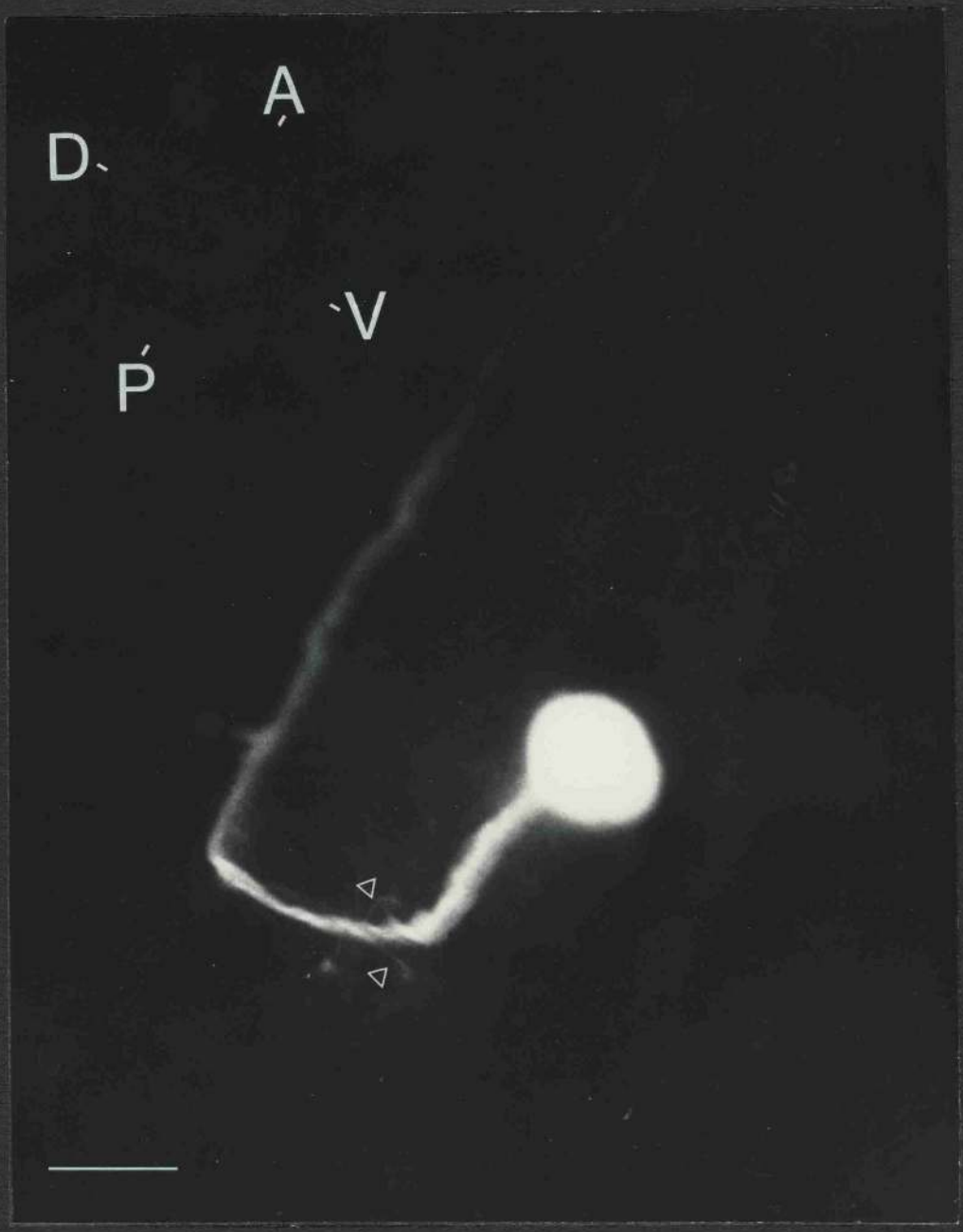

A
D
V
P

Figure 4.5

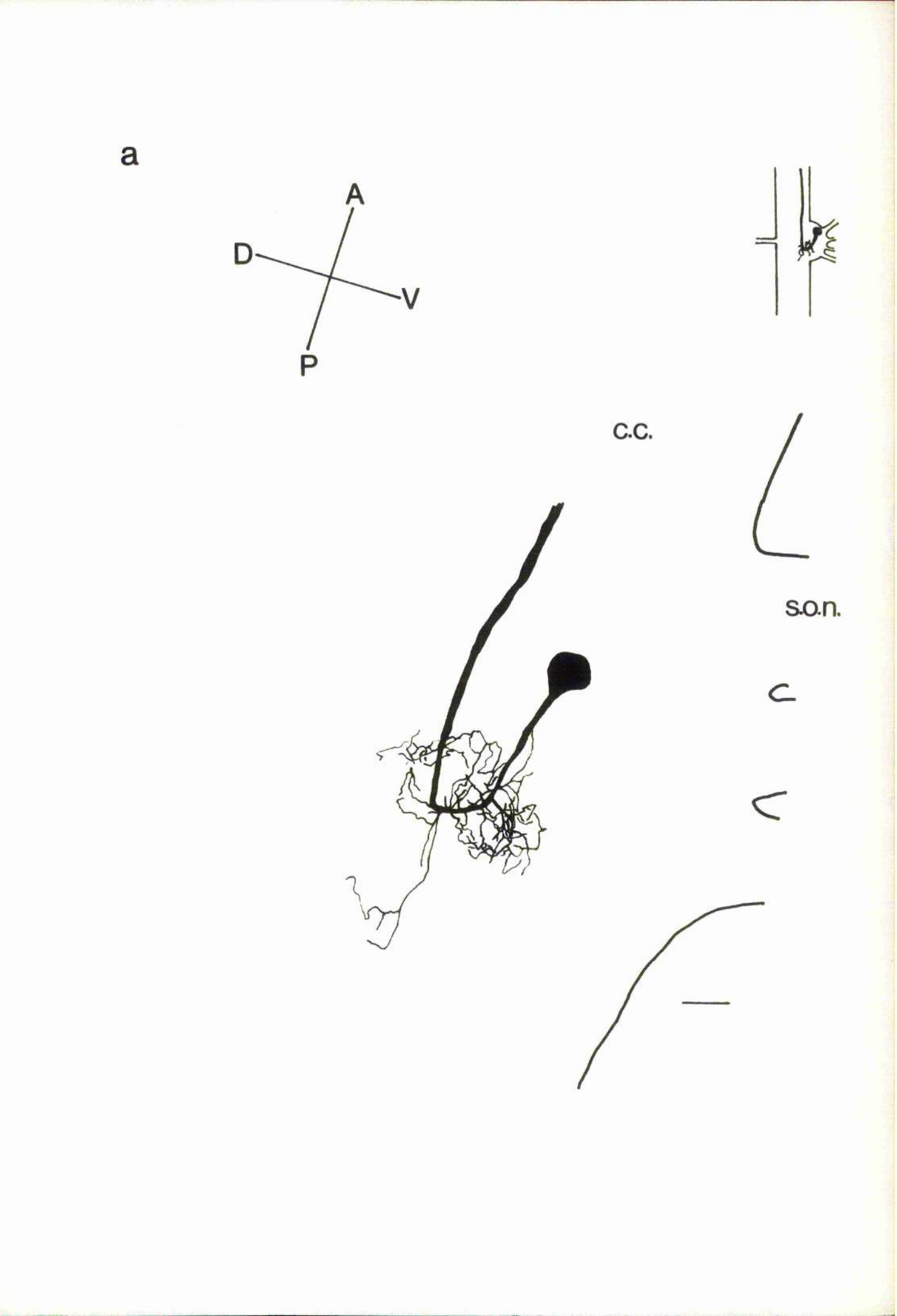
The structure of the L cell (2)

a and b. From right commissural ganglia.

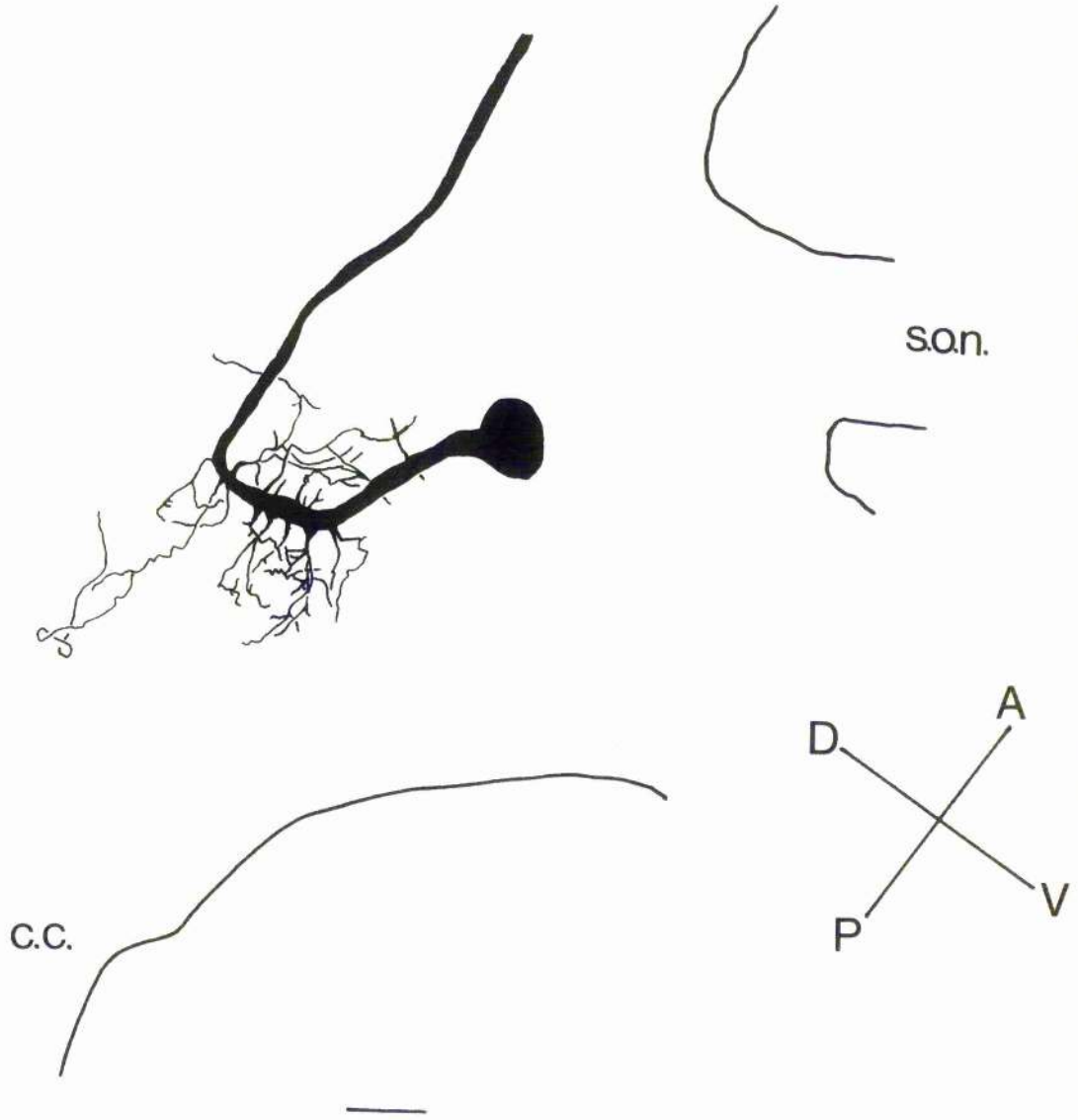
c and d. From left commissural ganglia.

The L cell axon loops posteriorly in the ganglion before travelling towards the brain in the circumoesophageal connective. No axon branch is seen in any other nerve. The L cell makes profuse dendritic arborisations on the axon loop. Note the long posteriorly-going process in a, b and c. Cartoon as in Figure 3.8 a .

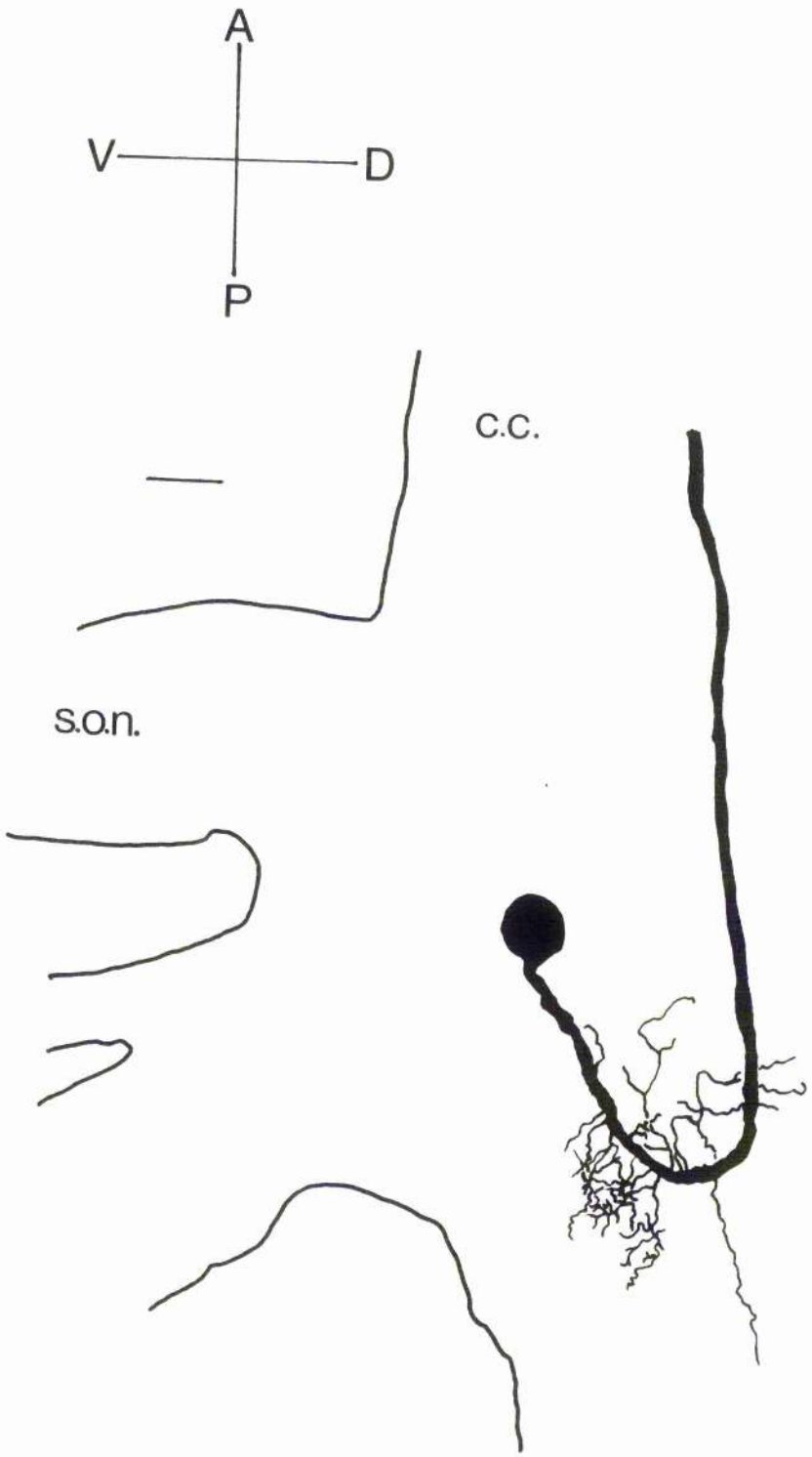
Scale in a, b, c and d : 100 μm


a
A
D
V
P
c.c.
s.o.n.

b



C



d

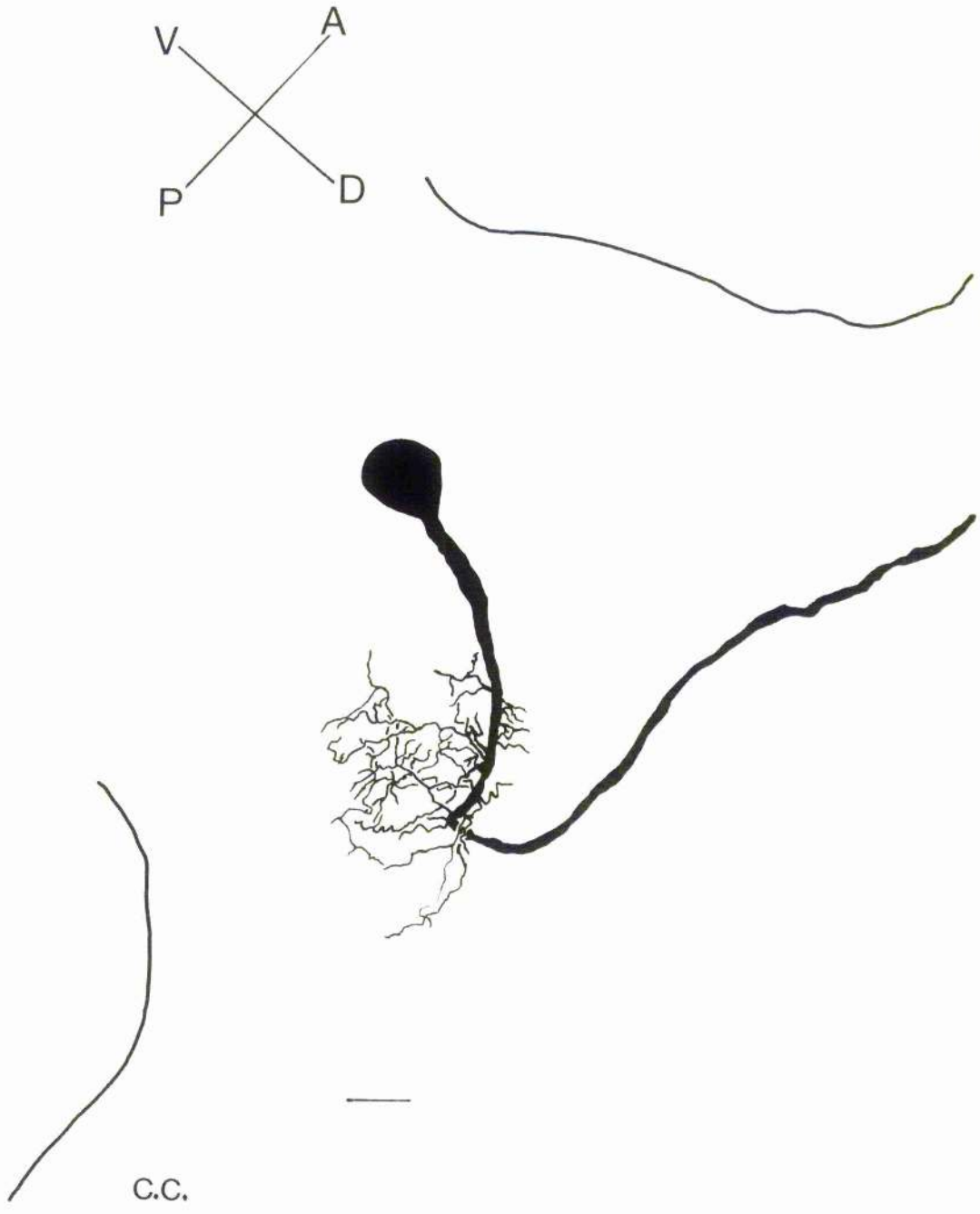


Figure 4.6

Specific fluorescence in the L cell

A left commissural ganglion stretch-dried over phosphorus pentoxide and treated with the FALCK-HILLARP technique for the histochemical demonstration of biogenic monoamines, with an incubation time of one hour. The L cell (L) exhibited pronounced yellow-green fluorescence which could be reversibly quenched with the sodium borohydride test. Note the smaller fluorescent soma dorsal to the L cell and the autofluorescent background.

Scale : 200 µm


V
P-
-A
D
L
c.g.
c.c.

gas, no L cell fluorescence was seen. The specific fluorescence in the L cell faded slowly on prolonged exposure to U.V. light, which is characteristic of primary catecholamine fluorescence (CORRODI and JONSSON, 1967). The nature of the specific fluorescence observed in the L cell suggests that it is due to dopamine as the other primary catecholamine candidate, noradrenalin, has not been found in crustacean nervous systems (see Introduction part a).

After one hour incubation several other commissural ganglion somata often exhibited specific yellow-green fluorescence similar to L cell fluorescence (see Figure 4.6). The level of autofluorescence precluded detailed study of these somata. Autofluorescence was particularly marked in the commissural ganglion, possibly due to its thickness and the perineural tissue and blood sinuses enveloping the ganglion. KUSHNER and MAYNARD (1977) also comment on this.

Axons exhibiting specific fluorescence were occasionally observed travelling anteriorly and posteriorly in the circumoesophageal connective. Only in one preparation could the fluorescent L cell axon be observed looping in the commissural ganglion and travelling anteriorly towards the brain in the connective.

The results of three hour incubations gave no indication of fluorescence due to other monoamines, for example 5-HT.

d. The course of the L cell axon

Since the L cell can be impregnated with cobalt chloride when backfilling from the level of the brain (Chapter 2 Results part d and Figure 2.13) its axon must travel at least this far. COOKE and GOLDSTONE (1970) claimed that in Carcinus maenas the L cell axon loops back from the brain down the circumoesophageal connective, eventually terminating in

the pericardial organ (see Introduction). Attempts were made to see if this is the case in Homarus gammarus. The distance between the commissural ganglion and the brain is about two centimetres. Lucifer Yellow injected intracellularly into the L cell soma would often travel about a centimetre up the anterior connective, but even with extremely large currents (up to 100 nA) and protracted injection times (over 5 hours) it would not travel more than about 1.5 centimetres. The results of cobalt backfilling from the posterior circumoesophageal connective with the brain attached to the anterior end of the connective (as in Figure 4.7 a) were difficult to interpret. Over fifty cells send axons posteriorly down the connective (see Chapter 2 Results part d and Figure 2.14) which presented a complex picture. In most of these preparations, which were incubated for two or three days at 4 C, the L cell appeared as a faintly stained profile. It was not clear if the faintness of the cobalt staining was due to the long distance travelled by the cobalt if going along the looping L cell axon (over four centimetres) or to extracellular, transynaptic or transmembrane cobalt leakage (see Chapter 2 Discussion). Pronounced autofluorescence in the FALCK-HILLARP preparations precluded examination of the course of the L cell axon using this technique.

In the experiment depicted in Figure 4.7 L cell spikes were monitored intracellularly in the soma. Firstly a suction electrode recorded extracellular activity in the posterior circumoesophageal connective (Figure 4.7 a). The connective was then cut at the level of the brain and the suction electrode reapplied to the anterior end (Figure 4.7 b). The L cell soma was reimpaled. If the L cell axon does loop back from the brain it should be possible to observe spikes in the posterior connective correlated with intracellularly-recorded L cell spikes. This was not so (Figure 4.7 a). In the second part of the experiment there was a clear 1:1 correlation between L cell spikes and spikes in the anterior

Figure 4.7

The course of the L cell axon

a. A suction electrode (s.e.) was used to record extracellular activity from the posterior end of the circumoesophageal connective. L cell activity was simultaneously monitored with a microelectrode (m.e.) in the soma. The storage oscilloscope sweep was triggered from the rising phase of the action potentials in the L cell; the trace shows ten superimposed sweeps. Note the absence of correlation between action potentials in the L cell and those in the connective.

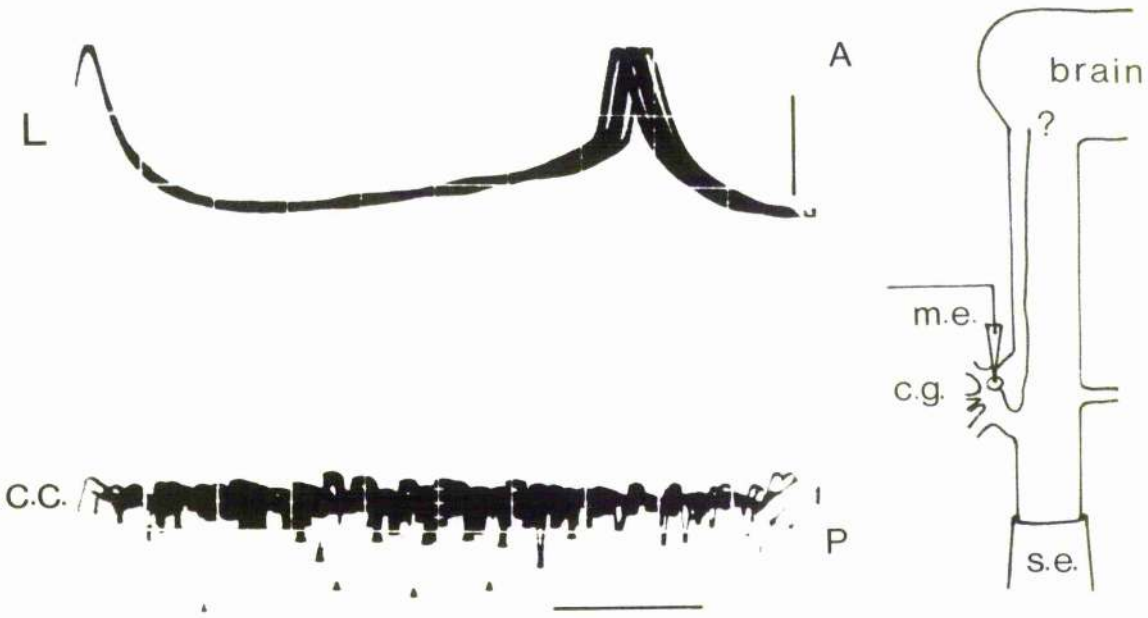
Voltage scale : 20 mv
Time scale : 10 msec

b. After cutting the connective just posterior to the brain the suction electrode was reapplied to the anterior connective. The L cell soma was reimpaled. The trace shows one free-running sweep. Note the large action potentials in the anterior connective which show a 1:1 correlation with those in the L cell.

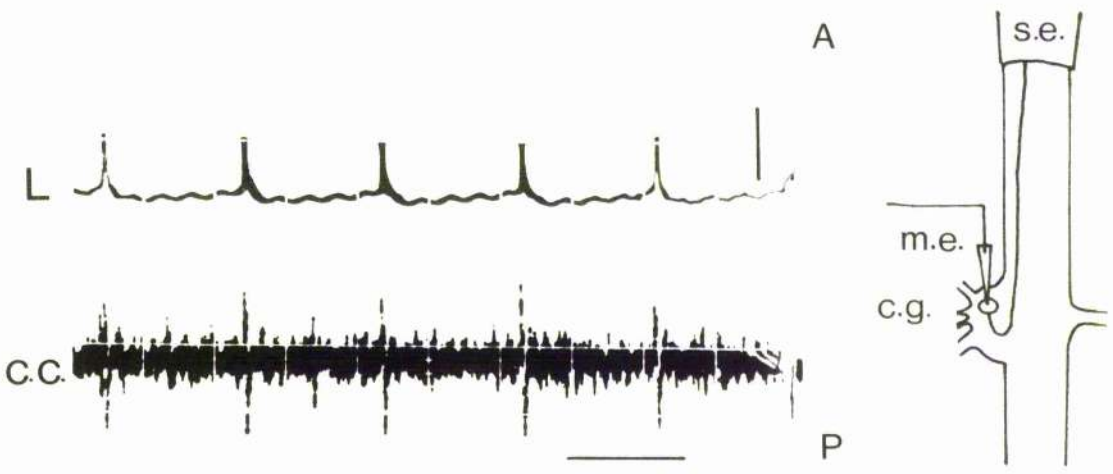
Voltage scale : 20 mv
Time scale : 100 msec

Note :- parts a and b taken from different experiments.

a



b



connective. Fast sweeps enabled the conduction velocity of the L cell axon to be assessed : it was about 1.5 - 2.0 m sec-1.

Discussion

In the isolated commissural ganglion the L cell is usually driven by the oesophageal pattern generator to fire in bursts (Figure 4.1). However, no deductions can be made concerning the individual cells which synapse onto the L cell; identification of the neurons involved in the oesophageal network should clarify this. Hence in the isolated ganglion the L cell acts as a corollary discharge neuron for the oesophageal rhythm, that is its output closely follows the monitored motor activity.

The L cell also receives synaptic input from other sources (Figure 4.3); the profusion of dendritic arborisations (Figure 4.5) underlines this multiplicity of input. ROBERTSON and MOULINS (unpublished c, R.M. ROBERTSON pers. comm.; M.MOULINS pers. comm.) have shown that in the context of the entire stomatogastric nervous system the discharge of the L cell is very complex. However, this discharge can be resolved into oesophageal, cardiac sac, gastric mill and pyloric filter components. Hence in the entire stomatogastric system, and presumably in the intact animal, the L cell is driven by four gut rhythms and acts as a corollary discharge neuron for the action of the foregut, providing an overall picture of foregut activity.

Since the L cell axon is known to travel to the brain it may function to provide an "efference copy" of foregut activity to the brain. Such a pathway may be of fundamental importance in the expresson and modulation of foregut rhythms in the context of the animal's behaviour, for example in this case perhaps coordinating behaviours such as food-searching with the state of the foregut. Corollary discharge neurons of the feeding rhythm of the mollusc Pleurobranchaea mediate a behavioral choice in that during feeding the withdrawal response to a stimulus is lessened (KOVAC and DAVIS, 1980). Perhaps L cell discharge plays a similar role. The

importance of efference copy neurons is becoming increasingly recognised, not only in invertebrates but also in vertebrates. For example, efference copy neurons in the visual system of the goldfish may be responsible for perceptual stability during eye movements (JOHNSTONE and MARK, 1971) and neurons of the ventral spinocerebellar tract are implicated in sending information to the brain concerning the spinal pattern generator for locomotion (LUNDBERG, 1971).

The course of the L cell axon is open to question. The evidence of COOKE and GOLDSTONE (1970) purporting to show that the axon doubles back from the brain down the circumoesophageal connective is equivocal and seems to be overemphasised (for example in ROBERTSON and MOULINS, unpublished c). Physiological experiments (see Figure 4.7) suggest that the L cell axon may terminate in the brain. Alternatively, the axon may loop back down the connective from the brain anatomically but is functionally subdivided by a non-spiking region. Either explanation would account for the absence of correlation between L cell spikes and spikes in the posterior connective (Figure 4.7). If the axon does loop back from the brain and is functionally subdivided, activity in the part of the axon travelling from the brain to the pericardial organ may reflect the integration of the L cell corollary discharge by the brain.

The evidence clearly suggests that the L cell in Homarus gammarus contains dopamine. If the axon loops and terminates in the pericardial organ, dopamine may be released from its terminals into the pericardial cavity in a manner reflecting brain integration of L cell corollary discharge. Dopamine, octopamine and 5-HT are found and synthesised in the pericardial organs of Cancer magister (BARKER and HOOPER, 1975) and Panulirus interruptus (SULLIVAN et al., 1977). At least octopamine can be released by electrical stimulation of pre-terminal nerve trunks (EVANS, KRAVITZ and TALAMO, 1976; SULLIVAN et al., 1977).

Release of dopamine may affect heart action. In at least two crustacean species, Libinia emarginata and L. dubia, the release of peptide neurosecretory material (cardio-acceleratory hormone) stored in the pericardial organ is not detectably affected by the dopamine-containing and 5-HT containing terminals (BERLIND, COOKE and GOLDSTONE, 1970). However, there is some evidence that dopamine has an excitatory effect on the heart in Astacus leptodactylus and Eriphia spinifrons (FLOREY and RATHMAYER, 1978). Hence dopamine released from the pericardial organ when the foregut is active may help to produce an increased level of activity appropriate to feeding.

Furthermore, the stomatogastric ganglion lies in the anterior aorta, directly in the direction of blood flow from the heart. Dopamine released from the pericardial organ by the L cell terminals could participate in a long and indirect feedback loop to the stomatogastric pattern generators, since exogenous dopamine has pronounced effects on at least the pyloric network (ANDERSON and BARKER, 1977). From their histochemical and biosynthetic data BARKER et al. (1979) suggested that dopamine mediates activation of the pyloric network by pathways linking the commissural and stomatogastric ganglia. Furthermore, RUSSELL and HARTLINE (1978) proposed that specific transmitters released by CNS input to the stomatogastric ganglion are responsible for the unmasking of plateau potentials in certain stomatogastric neurons. An obvious candidate for such a transmitter is dopamine. Hence indirect evidence strongly implicates dopamine in an important role in commissural ganglion inputs to the stomatogastric ganglion by nerves linking these ganglia. The possible involvement of the L cell suggests a complementary neurohormonal pathway.

The ideas outlined above represent a tentative attempt to explain L cell activity in the context of the stomatogastric system. The limitations of these suggestions must be considered.

L cell activity is very complex and it may receive input from sources other than the oesophageal, cardiac sac, gastric mill and pyloric filter rhythms (ROBERTSON and MOULINS, unpublished c). For example even in the isolated commissural ganglion it may not simply follow the oesophageal rhythm. Perhaps it monitors other rhythms such as the putative labral rhythm (see Chapter 3 Discussion). Further complexity is evidenced by the phasing of its bursting to the oesophageal dilator burst, which differs in the isolated commissural ganglion (the L cell bursts just after the dilator burst) and the entire stomatogastric nervous system (the L cell bursts before the dilator burst).

The L cell sends an efference copy of foregut activity to at least the brain but the further course of its axon is uncertain. If the axon does loop back from the brain to the pericardial organ and has a non-spiking region then activity in the brain-pericardial organ part of the axon may have no relation to the corollary discharge in the commissural ganglion-brain part of the axon. Moreover, the axon may terminate in the brain. It should be possible to clarify the course of the axon using a tracer such as horseradish peroxidase which is actively transported by the neuron and should travel further than Lucifer Yellow or cobalt chloride.

The possible neurosecretory release of L cell dopamine emphasises the need to consider neurohormonal action in the stomatogastric system. The work of several authors, for example COOKE and GOLDSTONE (1970) and SULLIVAN et al. (1977), has underlined the potential of the pericardial organ to store and secrete biogenic amines. It is possible that the release of these amines has widespread effects which include neurohormonal modulation of the stomatogastric pattern generators, as the work of BARKER and HOOPER (1975) suggests. In particular, future study of dopamine and octopamine in the stomatogastric nervous system must involve considering their possible roles both as neurotransmitters and neurohormones and the integration of these actions.

Chapter 5 : General Discussion

Anatomical investigations have revealed the complex organisation of the commissural ganglia. Each ganglion contains several hundred neurons, there are a large number of input and output pathways to each ganglion and there is strong evidence for numerous interactions between the commissural ganglia and other parts of the nervous system, for example with the brain, suboesophageal ganglion and stomatogastric ganglion. Physiological studies reflect this anatomical complexity, for example the isolated commissural ganglion is capable of producing not only the basic rhythm for oesophageal peristalsis but also other rhythms including a putative labral rhythm. Commissural ganglion neurons exhibit different kinds of activity, for instance in relation to the oesophageal rhythm, and show widely differing structures. The commissural ganglia are in an eminently suitable position to act as an interface between the stomatogastric system and the central nervous system. Since the work of ORLOV (1929) these ganglia have been mooted as coordination centres for the foregut (SELVERSTON et al., 1976). This study reveals that the commissural ganglia have the integratory potential to fulfill this role.

Further research must aim to clarify how far this potential is realised. Many sense organs, for example the anterior and posterior oesophageal sensors, send axons to the commissural ganglia. How much integration of sensory information takes place in the commissural ganglia ? How is this effected ? What implications does such integration have for the modulation and control of foregut rhythms ? These questions emphasise the need to study the function of the commissural ganglia not only in the deafferentiated, in vitro preparation but in the whole animal in order to understand the role of sensory information. The possible function of dopamine and octopamine as neurohormones in the stomatogastric system (see Chapter 4) further underlines the need for whole animal preparations. In contrast to the stomatogastric ganglion the commissural ganglia do not

furnish an accessible *in situ* preparation for intracellular studies. Developing such a preparation will be difficult but ultimately essential. SELVERSTON et al. (1976) say "It is clear that the commissural, [o]esophageal and stomatogastric ganglia should be considered together as a functional entity - the stomatogastric nervous system". This is true, but it should not be forgotten that this system is itself only part of a whole animal.

Other future avenues of research include defining the function of the large number of small commissural ganglion cells. There is a suggestion (see Chapter 3) that some of these neurons may be involved in the production of the oesophageal rhythm. Clearly much work needs to be done to elucidate which neurons are involved in the oesophageal pattern generator, their individual properties and how they are connected. RUSSELL (1977) states rather truistically that "A complete electrophysiological analysis of neurons in the commissural ganglia will probably be difficult". In particular the large number of neurons and the high proportion of small cells impose technical and conceptual difficulties in defining the role of identified neurons and in reaching a comprehensive understanding of commissural ganglion function.

Presumably some commissural ganglion neurons have a coordinating role in the interaction of the commissural ganglia with other parts of the nervous system. At least in one case, the L cell, information is sent to the brain in the form of a corollary discharge reflecting total foregut activity. Further studies should determine if the large number of neurons projecting to the brain and suboesophageal ganglion play a similar role, relaying information about certain aspects of commissural ganglion physiology to other nervous centres. Possibly, as in the case of the L cell, the activity of certain commissural ganglion cells reflects the action of other parts of the stomatogastric nervous system, for example

coordinating and relaying information about stomach activity.

Finally, it is interesting to note that there appear to be pronounced similarities in commissural ganglion organisation amongst different species. This is illustrated by the similarities in L cell structure and physiology in different species (see Chapter 4). The species which have been studied often have very different habits, for example Carcinus maenas (COOKE and GOLDSTONE, 1970) lives intertidally whilst Homarus gammarus is benthic. However, they all have similar feeding habits in that they are all macrophagous omnivores and presumably this is reflected in the similar structure and function of the gut. It would be interesting to investigate the stomatogastric system in herbivorous crustaceans such as the algal-eating xanthids, for example Mithrax and deposit feeders such as Uca. There are radical differences in at least gastric mill teeth morphology in these species (WARNER, 1977). As MEISS and NORMAN (1977b) state, ultimately "...one may be able to suggest hypotheses as to the mechanisms of adaptation of a small motor system in response to selective pressures".

Summary

1. The bilaterally paired commissural ganglia in the lobster Homarus gammarus are situated on the large circumoesophageal connectives which pass each side of the oesophagus to join the brain to the suboesophageal ganglion. Each commissural ganglion gives rise to three major nerves which send branches to innervate the labrum (the inferior oesophageal nerve) and the oesophagus (the superior and the ventral-posterior oesophageal nerves). The superior and inferior oesophageal nerves meet anteriorly in the midline at the level of the oesophageal ganglion and connect to the stomatogastric ganglion via its only input pathway, the stomatogastric nerve.

2. The commissural ganglia are strongly implicated in the action of the stomatogastric nervous system, for example in the integration of sensory input, in the modulation of stomatogastric pattern generators and in the production of the oesophageal rhythm. Despite their importance they have been little studied. This research aimed to make an initial study of the functional organisation of the commissural ganglia.

3. No consistent anatomical or physiological differences were found between right and left commissural ganglia. Each contains the somata of several hundred neurons, most less than 20 μm in diameter. Cobalt chloride backfilling studies revealed that there are about sixty motorneurons supplying the labrum and oesophagus and several hundred interneurons. There are a large number of input and output pathways relating to the commissural ganglia including many sensory fibres in each of the three major nerves. There appear to be common pathways amongst these nerves and much interaction between the commissural ganglia and other parts of the nervous system, for example with the brain, suboesophageal ganglion and stomatogastric ganglion.

4. The isolated commissural ganglion contains at least the minimal pattern generator for oesophageal peristalsis. The in vitro oesophageal rhythm has been compared to that observed in the semi-intact animal.

5. The small size of most commissural ganglion somata prohibits intracellular recording and dye injection. In the "recordable" population, about 10% of the neurons, most somata are silent. The activity of some other cells is related to the oesophageal rhythm, including some labral motorneurons. Intracellular techniques have not revealed the identity of the neurons involved in the oesophageal pattern generator.

6. At least one neuron exhibits rhythmic activity unrelated to the oesophageal cycle. Other rhythms are occasionally observed emanating from the commissural ganglion, one of which may be a labral rhythm. Several neurons exhibit a tonic firing pattern with no apparent rhythmicity; their possible functions are discussed.

7. The large dopamine-containing L cell sends a corollary discharge of foregut activity to at least the brain. The final destination of its axon is uncertain; it may release dopamine neurohormonally from terminals in the pericardial organ, indirectly feeding back to the stomatogastric pattern generators.

8. The function of the post-oesophageal commissure, which contains over a thousand axons, remains enigmatic. It is not involved in bilateral coordination of the two commissural ganglia in oesophageal peristalsis.

9. This study has demonstrated the potential of the commissural ganglia as integratory centres for the foregut. It emphasises the need for further investigation of these ganglia, in particular in the integration of sensory input, the production of the oesophageal and other rhythms and the coordination and modulation of foregut rhythms in the context of the entire stomatogastric nervous system.

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Glossary of abbreviations used in Figures

c.c.	circumoesophageal connective
c.g.	commissural ganglion/ ganglia
i.l.n.	inner labral nerve
i.o.n.	inferior oesophageal nerve
i.v.n.	inferior ventricular nerve
m.n.	commissural ganglion minor nerves
o.g.	oesophageal ganglion
o.l.n.	outer labral nerve
o.m.n.	outer mandibular nerve
o.n.	oesophageal nerve
p.-l.n.	postero-lateral nerve
p.-o.c.	post-oesophageal commissure
s.o.n.	superior oesophageal nerve
st.g.	stomatogastric ganglion
st.n.	stomatogastric nerve
v.-p.o.n.	ventral-posterior oesophageal nerve
L	L cell
O	oesophageal dilator burst

Orientation

A	anterior
P	posterior
D	dorsal
V	ventral
R	right
L	left

Appendix A

Saline derivation

a. Ionic composition

PANTIN's saline (1948) more closely resembles the internal medium of Homarus gammarus than does sea-water. For example, the concentrations of magnesium ions and sulphate ions are much higher in sea-water than in lobster plasma (ROBERTSON, 1939 and 1953). On the advice of J.D. ROBERTSON (pers. comm.) the potassium concentration of PANTIN's saline was lowered since the plasma concentration is equal to or less than that of sea-water (ROBERTSON, 1949).

b. Osmolarity

The osmolarity of sea-water and of marine Crustacea is about 1000 milliosmoles. However, presumably the sea-water in St. Andrews Bay is brackish (see for example LAVERACK and BLACKLER, 1974) and is thus hypotonic to Homarus gammarus plasma. The osmolarity of the saline was raised with glucose to compensate for the effects of colloids, including proteins, in the blood (ROBERTSON, 1939). Approximate calculations (Table a) showed that the osmolarity of the modified saline is the same as that of "normal" sea-water and presumably is isotonic with lobster blood.

c. pH and buffer

The mean pH of the arterial blood of Homarus gammarus from St. Andrews Bay is suprisingly high : 7.8 to 1 s.f. (BUTLER, TAYLOR and McMAHON, 1978 and P.J. BUTLER, pers. comm.). The pH of the laboratory sea-water supply varied between 6.5 and 7.9 but was often less than 7.0. This emphasises its unsuitability as a saline.

Without buffering the pH of the modified PANTIN's saline also varied, often below 7.0. "Natural" buffering by sodium bicarbonate (BURTON, 1975) was not satisfactory. Large amounts (>6mM) were often needed to stabilise the pH at about 7.8 and this caused calcium precipitation. Similar problems occurred with phosphate buffer. Borate/ boric acid has been commonly used in crustacean salines (BURTON, 1975) and was found to be suitable for Homarus gammarus saline although occasionally it did not provide adequate buffering. Both TES and HEPES (BURTON, 1975) should be suitable buffers. Tris is widely used but was avoided in case its effects on molluscan neurons (WILSON, CLARK and PELLMAR, 1977) represent a more widespread phenomenon.

Table a

Modified PANTIN's saline

compound	g litre-1 (to 2 d.p.)
NaCl	28.31
Na2SO4	01.12
KCl	00.72
CaCl2.2H2O	02.12
MgCl2.6H2O	01.39
glucose	02.00

Table b

Comparison of the ionic composition and osmolarity of sea-water (PANTIN, 1948) and modified PANTIN's saline

	ion	sea water	PANTIN	modified PANTIN
M litre-1	Na+	0.457	0.500	0.500
to 3 s.f.	K+	0.00972	0.0140	0.00966
	Ca2+	0.0101	0.0144	0.0144
	Mg2+	0.0526	0.00684	0.00684
	Cl-	0.537	0.540	0.536
	SO42-	0.0277	0.00792	0.00789
approximate osmolarity (osmoles) to 2 d.p.		1.09	1.08	1.09*

*includes glucose

Appendix B

Processing of tissue for routine histology

The procedure refers to fixation with Bouins made up in either sea-water or isotonic sucrose. Using formol-alcohol the tissue was fixed (12 hours) and dehydrated in 85% ethanol (3 hours). It was then treated as in stages 4-11.

stage	procedure	time
1	fixation	24 hours
2	50% ethanol	3 hours
3	70% ethanol + lithium carbonate	overnight
4	90% ethanol	3 hours
5	90% TBA	3 hours
6	100% TBA (58°C)	3 hours
7	1:1 TBA : liquid paraffin (58°C)	overnight
8	4 changes of wax (58°C)	1 hour each
9	cast out	

Appendix C

Processing of tissue for electron microscopy

stage	procedure	time
1	fix in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (see notes a and b below) at pH 7.8 and 4°C	12-15 hours
2	wash three times in 0.1 M sodium cacodylate buffer (see note c below)	15 minutes each
3	post-fix in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at room temperature (see note d below)	1 hour
4	wash three times in deionized water to remove sodium cacodylate which precipitates with uranyl acetate	5 minutes each
5	block stain in 2% aqueous uranyl acetate at 4°C (see note e below)	1 hour
6	wash three times in deionized water	5 minutes each

Different dehydration and embedding procedures were used for the two different embedding media :-

1. Durcupan

7	30%, 50%, 70%, 95%, 2x 100% acetone	5 minutes each
8	1:1 acetone:Durcupan	1 hour
9	1:4 acetone:Durcupan	overnight
10	Durcupan	1 hour
11	vacuum embed and polymerise at 60 °C	2 days

2. Spurr

7	30%, 50%, 70%, 90%, 2x 100% ethanol	5 minutes each
8	1:1 ethanol:Spurr	1 hour
9	1:4 ethanol:Spurr	1 hour
10	Spurr	overnight
11	vacuum embed and polymerise at 60°C	36 hours

Spurr resin was found to give better penetration of the tissue, due to its low viscosity and was easier to section than Durcupan.

Notes

a. 0.2 M sodium cacodylate buffer

To 100 ml 0.4 M sodium cacodylate add sufficient 0.1 M hydrochloric acid (about 5 ml) to obtain the appropriate pH. Make up to 200 ml with distilled water.

b. Glutaraldehyde in cacodylate buffer

To	20 ml 0.2 M sodium cacodylate buffer
add	10 ml 8% glutaraldehyde
and	10 ml distilled water
totalling	40 ml 2% glutaraldehyde in 0.1 M sodium cacodylate buffer

To this volume add 6 g sucrose to adjust the osmolarity to about 1000 milliosmoles.

c. 0.1 M sodium cacodylate buffer

To	20 ml 0.2 M sodium cacodylate buffer
add	20 ml distilled water
totalling	40 ml 0.1 M sodium cacodylate buffer

To this volume add 8 g sucrose to adjust the osmolarity as above.

d. Osmium tetroxide in cacodylate buffer

To	5 ml 2% osmium tetroxide
add	5 ml 0.2 M sodium cacodylate buffer
totalling	10 ml 1% osmium tetroxide in 0.1 M sodium cacodylate buffer

To this volume add 3.5 g sucrose to adjust the osmolarity as above.

e. Block staining in uranyl acetate

This stage can be omitted if only semi-thin sections are required.

Appendix D

Silver intensification : developer base (J.S. ALTMAN, pers. comm.)

citric acid	01.4 g
hydroquinone	00.17 g
sucrose	10.0 g
distilled water	88 ml
25% gum acacia	12 ml

Dissolve the first three ingredients in the distilled water and add the gum acacia solution. Adjust to pH 2.4.