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PARTICIPATION OF AN UNPAIRED MOTOR NEURONE IN THE BILATERALLY ORGANIZED OESOPHAGEAL RHYTHM IN THE LOBSTERS *JASUS LALANDII* AND *PALINURUS VULGARIS*

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SUMMARY

1. The main oesophageal motor neurone (OD1) of the rock lobster is an unpaired bifurcating nerve cell. The cell body is located in the oesophageal ganglion and the left and right axonal branches pass through the left and right commissural ganglia to innervate all the oesophageal dilator muscles.

2. Three types of potentials are recorded in the cell body *in vitro*; each type is associated with an extracellular spike recorded from the nerves connecting the ganglia.

3. Comparison between the three types of potentials (and the extracellular spikes) and collision experiments shows that all three are spikes.

4. Spontaneous collisions can sometimes occur and it is concluded that one spike is generated in the oesophageal ganglion (somatofugal *a*-spike) while the other two are generated in the left commissural ganglion (somatopetal *b*-spike) or the right commissural ganglion (somatopetal *c*-spike).

5. Each spike initiating zone is synaptically driven.

6. The commissural zones fire short phasic bursts; each burst is composed of only one type of spike (*b*- or *c*-). The oesophageal (*a*-) zone gives a tonic discharge interrupted when the other zones are firing. Finally, combined firing of the spike initiating zones can generate three different patterns of discharge.

7. OD1 participates in the oesophageal motor rhythm produced by two oscillators (one in each commissural ganglion) which fire alternated series of bursts.

8. It is concluded that the three axonal spike initiating zones enable the motor neurone (1) to follow the oesophageal motor rhythm at any time regardless of which oscillator is in operation and (2) to co-ordinate phasic and tonic activation of the oesophageal dilator muscles.

INTRODUCTION

The ability to work on identified neurones (i.e. to ask questions of the same neurone from one experiment to another) has been very fruitful in the last ten years (Fentress, 1976; Hoyle, 1976). This possibility considerably increases the chance of discovering new or unusual properties of a nerve cell. When this nerve cell is integrated into a

network the characteristics of which are known, any new property can be understood in functional and, sometimes, in behavioural terms.

Invertebrates provide most of the preparations in which it is possible to identify individual neurones from one animal to another. Among these the stomatogastric nervous system of Crustacea (which governs the motor rhythms of the foregut) is one of the most promising (Maynard, 1972; Selverston *et al.* 1976; Moulins & Vedel, 1977). This system consists of the two commissural ganglia and of the unpaired oesophageal and stomatogastric ganglia which are connected to each other by the stomatogastric nerve (Maynard and Dando, 1974). This system is active *in vitro*, and the spontaneous activity of the neurones can be routinely recorded by intracellular and extracellular methods. Numerous neurones are now identified in the stomatogastric (Maynard, 1972; Mulloney & Selverston, 1974; Selverston & Mulloney, 1974) and oesophageal ganglia (Vedel & Moulins, 1977). The roles of many of these neurones in the four networks which organize the motor activity of the foregut are quite well known (Moulins & Vedel, 1977). Nevertheless there is a need to know more about the properties of each identified neurone, since with this preparation the functional meaning of any cellular property has a real chance to be understood (Russell & Hartline, 1978).

In rock lobsters, the rhythmic motor behaviour of the oesophagus is organized by a neuronal network which is bilaterally distributed between the paired commissural ganglia and the unpaired oesophageal ganglion (Spirito, 1975; Moulins & Vedel, 1977). These three nervous centres are anatomically distinct and oesophageal motor activity can be routinely recorded *in vitro*. This preparation is thus particularly suited for the study of the involvement of a single motor neurone in the activity of a bilaterally distributed network.

The nerve cell under investigation is the main dilator neurone of the oesophagus (OD1; Moulins & Vedel, 1977). It is an unpaired motor neurone with a branching axon innervating both left and right oesophageal dilator muscles. Simultaneous recording from the cell body (in the oesophageal ganglion) together measurements with several extracellular electrodes along the nerve trunks connecting the three ganglia demonstrates that OD1 possesses a spike initiating zone in each ganglion. This unusual property allows the neurone to fire, under synaptic drive, from each ganglion and to follow the activity of the two commissural oscillators which interact to produce the oesophageal motor rhythm (see Nagy & Moulins, 1980). Finally, by considering the functional characteristics of the oesophageal network and the intrinsic properties of the OD1 spike initiating zones, it is possible to understand how the combined firing of the three zones can impose several different patterns upon the oesophageal rhythm. The occurrence of these patterns seems to vary between lobster species and so we have studied OD1 in *Jasus lalandii* and *Palinurus vulgaris*.

A preliminary report of this work has been published elsewhere (Moulins, Vedel & Nagy, 1979).

MATERIALS AND METHODS

Experiments were performed on male and female rock lobsters, *Jasus lalandii* and *Palinurus vulgaris*, obtained from commercial suppliers and held in running sea water tanks in the laboratory.

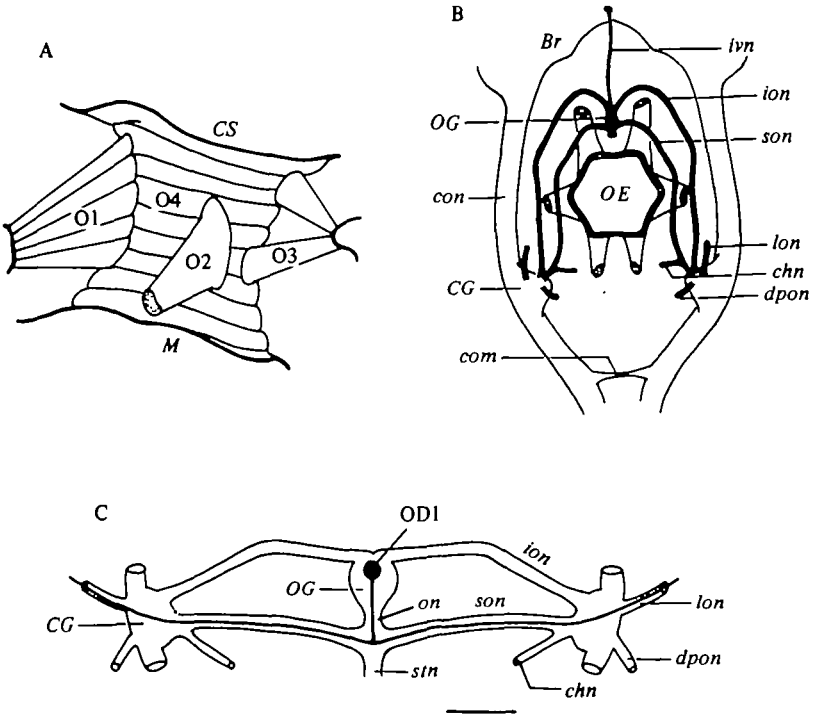

Fig. 1. Anatomy. (A) Lateral view of the oesophagus with its muscles. (B) Dorsal schematic view of the oesophageal region of the stomatogastric nervous system. (C) The *in vitro* preparation with OD1 in black. *Br*, brain; *CG*, commissural ganglion; *chn*, chemoreceptor nerve; *com*, ventral commissure; *con*, connective; *CS*, cardiac sac; *dpon*, dorsal posterior oesophageal nerve; *ion*, inferior oesophageal nerve; *ivn*, inferior ventricular nerve; *lon*, lateral oesophageal nerve; *M*, mouth; O1, dorsal dilator muscles; O2, lateral dilator muscles; O3, ventral dilator muscles; O4, constrictor muscles; OE, oesophagus; *OG*, oesophageal ganglion; *on*, oesophageal nerve; *son*, superior oesophageal nerve; *stn*, stomatogastric nerve. Calibration: 5 mm.

For semi-intact preparations, the animal was opened dorsally and laterally to expose the anterior part of the foregut. The antennal extrinsic muscles, the green gland and the hepatopancreas were removed. Recordings were made from the main oesophageal nerves (*son*, *lon*, see Fig. 1 B) with suction electrodes and from oesophageal muscles fibres with glass microelectrodes filled with 3 M-KCl and having resistances in the range of 16–20 MΩ.

For *in vitro* preparations, the anterior part of the stomatogastric nervous system was dissected out and pinned in a Petri dish. In this situation it is possible to record simultaneously intracellularly from the cell bodies in the ganglia and extracellularly with metal electrodes along the nerves. The dorsal part of the foregut was exposed and the inferior ventricular nerve and circumoesophageal connectives were cut posterior to the brain and anterior or posterior to the ventral commissure (see Fig. 1 B). The foregut was then transferred to a dissecting dish and opened ventrally, allowing us to flatten it in the saline. The oesophageal ganglion, the commissural ganglia and the oesophageal nerves were carefully removed from the oesophageal wall and transferred to a Sylgard lined Petri dish (Fig. 1 C). Most frequently the stomatogastric nerve was cut and the stomatogastric ganglion discarded. Finally the dorsal

sheath overlying the cell bodies of the oesophageal ganglion was removed. Platinum wire electrodes were placed along the nerves in a bipolar or monopolar configuration and used for extracellular recording via conventional 10000 gain a.c. amplifiers. The nerve-wire junction was covered with vaseline. The same electrodes could be used for electrical stimulation of the axons via a switch box. The OD1 cell body, seen with transmitted light, was penetrated with a glass micropipette electrode filled with 3 M-KCl (resistance range; 20–35 MΩ). Intracellular potentials were recorded using a WPI electrometer which also allowed us to inject current into the cell body. Results were directly filmed with a Grass camera or stored on a Schlumberger tape recorder and displayed later for film on a Tektronix oscilloscope. The saline used was artificial sea water. Experiments were performed at room temperature (20–22 °C).

RESULTS

(1) *Functional anatomy of the oesophagus and identification of the main oesophageal dilator motor neurone (OD1)*

In rock lobsters the oesophagus is a short tube opening anteriorly at the mouth between the mandibules and posteriorly in the cardiac sac. Extrinsic dilator muscles (O1, O2, O3) and intrinsic constrictor muscles (O4) produce the rhythmical movements of the oesophagus (Fig. 1 A).

These muscles are innervated by the oesophageal nerves connected to the anterior part of the stomatogastric nervous system (Fig. 1 B) (i.e. to the oesophageal ganglion and to the two commissural ganglia). The oesophageal ganglion lies on the anterior (dorsal) wall of the oesophagus; it is connected to the brain by the inferior ventricular nerve (*ivn*) and to the commissural ganglia by the superior and inferior oesophageal nerves (*son*, *ion*). Laterally to the oesophagus the commissural ganglia are attached to the connectives between the brain and the suboesophageal ganglion. They receive the lateral oesophageal nerve (*lon*) which innervates the O2 dilator muscle, the dorsal posterior oesophageal nerve (*dpon*) which innervates cardiac sac muscles and oesophageal constrictor muscles and the so-called chemoreceptor nerve (*chn*).

In semi-intact preparations, a suction electrode on the *son* records a bursting activity (Fig. 2 A) which can be also recorded *in vitro*, in the isolated nervous system (Fig. 2 B). Muscle recordings from these preparations show that each burst of large spikes in the *son* is correlated with the activation of the oesophageal dilator muscles. In the two species under investigation, the first neurone to fire in each burst innervates all the dilator muscles (Fig. 2 C, D, E) and for this reason has been called the main oesophageal dilator neurone (OD1) (Moulins & Vedel, 1977).

The cell body of the above neurone is located in the oesophageal ganglion (Fig. 1 C) which contains only twelve somata. The axon travels the length of the oesophageal nerve (*on*), then bifurcates to send an axon in each *son*. These branches pass through the commissural ganglia to give a lateral branch in each *lon*. The position of OD1 cell body is variable among the 12 neurones of the oesophageal ganglion and is located by random penetrations. Once penetrated, OD1 can be distinguished from other oesophageal neurones by correlating spontaneous intracellular potentials with the first extracellular spikes occuring in each oesophageal dilator burst on the *son* and *lon* (Fig. 2 B). This identification was confirmed by: (1) current injection into the cell bod

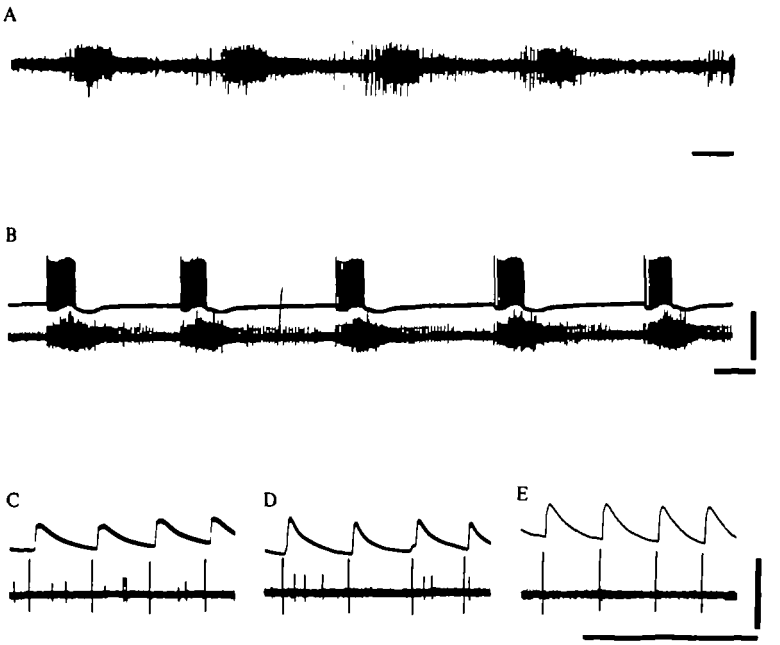

Fig. 2. OD1 is the main dilator motor neurone of the oesophagus. (A) Oesophageal bursting activity recorded on the *son* in a semi-intact preparation. In each burst, OD1 fires first and gives the larger spikes (*Palinurus vulgaris*). (B) Oesophageal bursting activity recorded *in vitro* from the OD1 cell body (first trace) and on the *son* (second trace) (*Jasus lalandii*). (C–E) Intracellular activity of fibres of the three oesophageal dilator muscles (O1 in C, O2 in D, O3 in E; see Fig. 1A) recorded on a semi-intact preparation. Each muscle junction potential (first trace) is time-locked with OD1 spike recorded extracellularly on the *son* (second trace) (*Palinurus vulgaris*). Calibrations: horizontal bars, 1 s; vertical bars, 20 mV.

(which caused the neurone to fire spikes recorded at the extracellular electrodes on the *son* and *lon*) and (2) antidromic stimulation by one of the extracellular electrodes on the *son* and *lon*, (which gave an antidromic spike in the cell body). Furthermore the 12 neurones of the oesophageal ganglion are now quite well known and only a few possess a shape comparable with the shape of OD1. Among these OD1 is the only one, as will be shown, to exhibit several types of intracellular potentials (Fig. 3B) which are, as always in Arthropods, attenuated potentials recorded in an inexcitable cell body. The existence of three types of potentials allowed us to confirm the identification.

(2) *Different types of OD1 potentials*

With *in vitro* preparations two types of intracellular potentials can be recorded in the OD1 cell body (Fig. 3B). The first (Fig. 3B(i)) arises from a slow depolarizing phase; the second type (Fig. 3B(ii)) is a fast rising potential which appears without the slow depolarization and can arise from any membrane potential. The amplitude of the two types of potentials, measured from the onset, is about the same (20 mV in *J. lalandii*; mV in *P. vulgaris*); the after potential level reached by each type of potential is also the same (—60 mV for *J. lalandii*; Fig. 3B). The first type of potential always

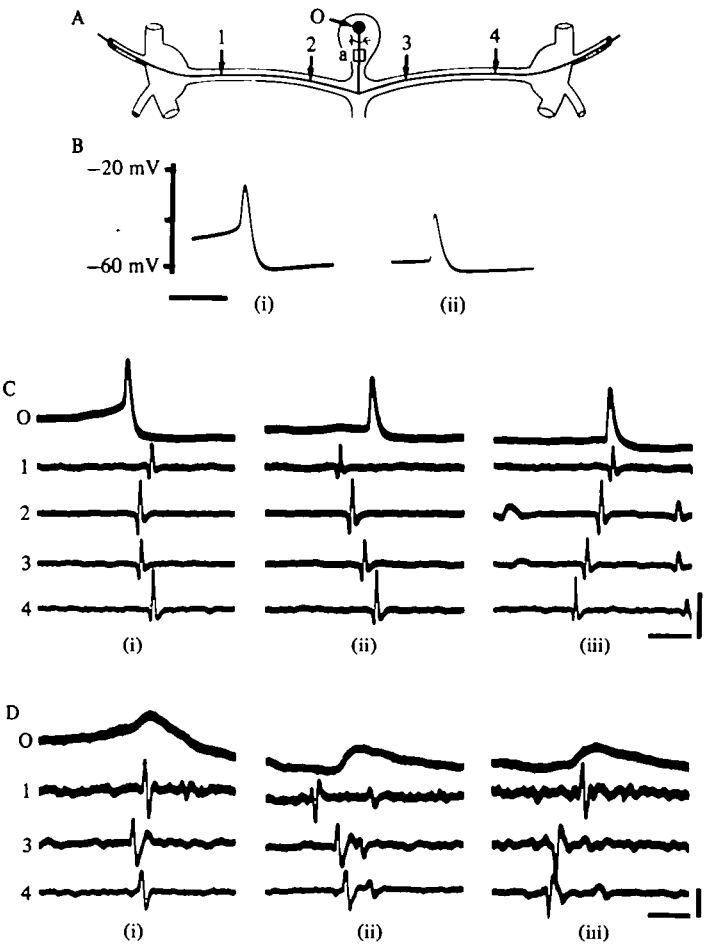

Fig. 3. OD1 potentials. (A) Schema of the experimental procedure. O, intracellular recording from OD1 cell body; 1, 2, 3, 4, extracellular wire electrodes along the two *son*'s (monopolar configuration in C, bipolar configuration in D). (B) The two types of potentials recorded in the OD1 cell body (*Jasus lalandii*). (i) somatofugal *a*-spike; (ii) fast rise time potential (*b*- or *c*-potential). (C) Correlation between spontaneous intracellular potentials of OD1 and extracellular spikes recorded on the *son* in *Jasus lalandii*. In C(i), OD1 exhibits a somatofugal *a*-spike which simultaneously invades the left (electrodes 2 and 1) and right (electrodes 3 and 4) branches of the axon in the *son*'s. In C(ii) the soma potential (*b*-potential) recorded in O is associated with an extracellularly recorded spike coming from the left commissural ganglion. This spike invades successively the left *son* (electrodes 1 and 2), the *on* to give the OD1 *b*-potential (electrode O) and the right *son* (electrodes 3 and 4). In C(iii) the OD1 *c*-potential recorded in O is associated with an extracellularly recorded spike coming from the right commissural ganglion. This spike invades successively the right *son* (electrodes 4 and 3), the *on* to give the OD1 *c*-potential (electrode O) and the left *son* (electrodes 2 and 1). (D) Correlation between spontaneous intracellular potentials of OD1 and extracellular spikes recorded on the *son* in *Palinurus vulgaris*. D(i) is a recording of a somatofugal *a*-spike which simultaneously invades the left (electrode 1) and the right (electrodes 3 and 4) *son*'s. In D(ii) and D(iii), a *b*- and *c*-potential are respectively recorded in the cell body. The *b*-potential is associated with a spike coming from the left commissural ganglion (chronology 1–3–4); the *c*-potential is associated with a spike coming from the right commissural ganglion (chronology 4–3–1). Calibrations: horizontal bars, 10 ms; vertical bars, 20 mV in C, 2 mV in D.

possesses a low firing rate which can be modulated by current injection in the cell body; the frequency of the second type of potentials can be high (see Fig. 10B for comparison) and cannot be modulated by current injection in the cell body.

It is easy to show that the first type of potential is a somatofugal spike. In *J. lalandii* when this potential occurs in the cell body (Fig. 3C(i)) it is recorded extracellularly, first at electrodes 2 and 3 and later at electrodes 1 and 4 (see Fig. 3A). This means that it is a spike which originates in the oesophageal ganglion, near the cell body, and invades simultaneously, from the bifurcating point of the axon, the right and left branches in the right and left *son*'s. On each side, the spike goes through the commissural ganglion via the axon which runs in the *lon* (see Fig. 4B, electrode 5). The situation is exactly the same for *P. vulgaris* (Fig. 3D(i)); the only difference between the two species lies in the amplitude and time course of the intracellular spike (compare Fig. 3C(i) and 3D(i)). This somatofugal spike will be called the *a*-spike as it comes from the *a*-spike initiating zone (see Fig. 3A). In our experimental conditions, i.e. *in vitro*, spontaneous *a*-spike are commonly recorded in *P. vulgaris*; they are quite rare in *J. lalandii* but are easy to induce by soma depolarization.

In both species two different types of fast rise time potentials (Fig. 3B(ii)) can be distinguished and will be called *b*- and *c*-potentials. They have, in the same species, the same shape and the same amplitude but can be identified by the observation that they are associated with two different extracellularly recorded spikes. The *b*-potential is associated with a spike coming from the left commissural ganglion; the *c*-potential is associated with a spike coming from the right commissural ganglion. The extracellular spike associated with a soma *b*-potential appears first at electrode 1, and successively at electrodes 2, 3 and 4 (Figs. 3C(ii), 3D(ii)). This spike, which comes from the left commissural ganglion, invades first the left *son* and then, simultaneously, the *on* (to give the OD1 soma *b*-potential) and the right *son* to reach the right *lon* through the right commissural ganglion (see electrode 5 in Fig. 4C). In contrast, the extracellular spike associated with a soma *c*-potential appears first at electrode 4 and successively at electrodes 3, 2 and 1 (Figs. 3C(iii), 3D(iii)). In both species, the extracellularly recorded spike comes from the right commissural ganglion, invades first the right *son* and then simultaneously the *on* (to give the OD1 soma *c*-potential) and the left *son* to reach the left *lon* through the left commissural ganglion. The extracellular spike associated to a soma *b*-potential invades also, from the left commissural ganglion, the left *lon* while the extracellular spike associated to a soma *c*-potential invades, from the right commissural ganglion, the right *lon* (see Fig. 8C).

There are two hypotheses to explain the nature of the intracellular *b*- and *c*-potentials and of their associated extracellular spikes:

(1) *b*- and *c*-potentials are EPSP's and the associated spikes belong to two presynaptic neurones, one from the left commissural ganglion and one from the right commissural ganglion;

(2) *b*- and *c*-potentials are OD1 somatopetal (antidromic) spikes coming respectively from a spike initiating zone situated in the left commissural ganglion and from another spike initiating zone situated in the right commissural ganglion. In other words, in this hypothesis, the 'associated spikes' would occur in axons of OD1.

These two hypothesis will be now considered and it will be shown that *b*- and *c*-potentials are OD1 somatopetal spikes.

(3) *b- and c-potentials are OD1 somatopetal spikes*

(*a*) *Comparison between the a-spike and the spike associated with b- (and c-) potential*

By considering only the extracellular recording from the *son and lon* it can be shown that the *a*-spike and the spike associated with *b*- (or *c*-) potentials have common features.

In the experiment shown in Fig. 4, the sweep of the oscilloscope was triggered by the intracellular potential recorded in the cell body during a series of *a*-spikes (Fig. 4B), during a series of *b*-potentials (Fig. 4C) and during a series of *a*-spikes and a series of *b*-potentials (Fig. 4D). The extracellular recordings were made with monopolar electrodes. The *a*-spike (Fig. 4B) is recorded, with a constant delay and a constant shape, at about the same time at electrodes 2 and 3, later at electrode 4 and still later at electrode 5 (see Fig. 4A). The extracellular spike associated with the *b*-potential and coming from the left commissural ganglion (Fig. 4C) is recorded, with a constant delay and a constant shape, at electrode 4 and then at electrode 5. It appears at electrode 2 before the triggering of the sweep and at electrode 3 with the triggering of the sweep (only its later phase is recorded at this electrode).

These records show that at a given extracellular electrode the *a*-spike and the spike associated with a *b*-potential are identical in shape and amplitude (see electrode 4 or electrode 5 in Fig. 4D). This is also true for the *a*-spike and the spike associated with a *c*-potential (compare Fig. 3C(i) and (iii)). Such similarity would be difficult to explain if the three extracellular spikes did not belong to the same axon, i.e. to the axon of OD1. Furthermore, when the recording is made with bipolar electrodes (Fig. 3D) the similarity between extracellular spikes at the same electrode remains only when the different spikes are propagated in the same direction. For example, in Fig. 3D the *a*-spike (i) and the spike associated with the *b*-potential (ii) are identical at electrode 4 where they are travelling in the same direction (to the right). When the spikes are propagated in opposite directions, their shapes are reversed, i.e. their successive negative and positive components are similar but occur in inverse order. This appears for example in Fig. 3D for the *a*-spike (i) and the spike associated with a *c*-potential (iii) at electrode 4 where the spikes are travelling in opposite directions (to the right for the first and to the left for the second). Again such peculiarities in the shapes of the different spikes would be difficult to explain if these spikes did not belong to OD1.

Another argument in favour of this hypothesis is the observation that conduction velocities of the somatofugal *a*-spike and the spike associated with the *b*- (or *c*-) potential are the same (i.e. 2, 75 m s^{-1}). For example, the conduction time between electrode 3 and electrode 5 (a distance of more than 2·5 cm) is exactly the same for an *a*-spike (Fig. 4B) and for a spike associated with a *b*-potential (Fig. 4C).

(*b*) *Collision experiments*

The most convincing evidence to demonstrate that the different types of spikes belong to OD1 has been obtained by collision experiments (Fig. 5). A single electrical shock delivered to the left *son* (see Fig. 5A) induces, with a constant delay, a de- polarizing fast potential (*s*-potential, Fig. 5B(i)) in the cell body. The OD1 *s*-potenti-

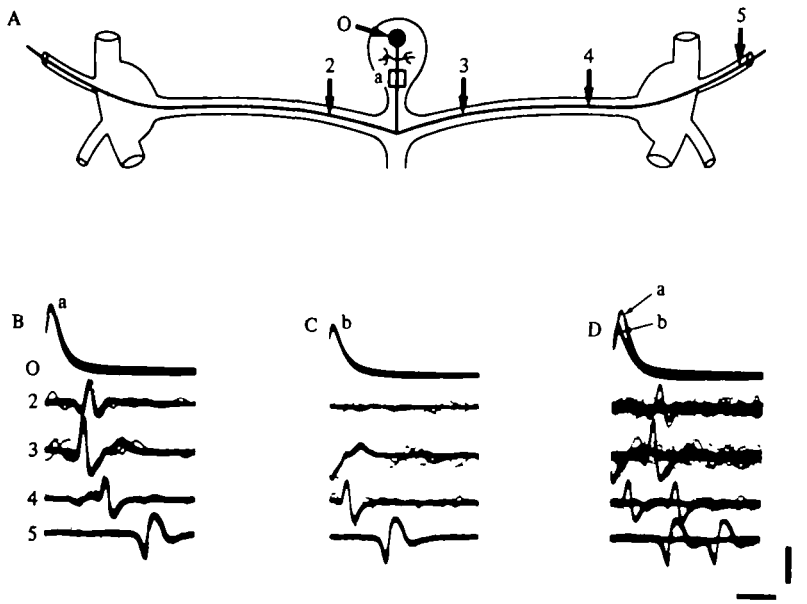

Fig. 4. Comparison between the somatofugal *a*-spike and the spike associated with *b*-potential (multiple recordings triggered by the intracellular potential) (*Jasus lalandii*). (A) Disposition of the monopolar recording electrodes. The oscilloscope sweeps are triggered by the intracellular OD1 potential in the cell body (O) during a burst of spontaneous *a*-spikes (B), during a burst of spontaneous *b*-potentials (C) and during two bursts, one of *a*-spikes and one of *b*-potentials (D). In B, the *a*-spike appears at extracellular electrodes with a constant delay first in 2 and 3, later in 4 and still later in 5. In C, the extracellular spike associated with the *b*-potential appears, also with a constant delay, first in 3 (falling phase of the spike), later in 4 and later in 5. This spike which is coming from the left commissural ganglion appears in 2 before the triggering of the sweep and so is not recorded. The two types of spikes have, at the same extracellular electrode, the same shape and same conduction velocity. See text for further details. Calibrations: horizontal bar, 4 ms; vertical bar, 20 mV.

is not an EPSP obtained by activation of a presynaptic pathway but an antidromic spike. It is possible to get collision between an *s*-potential and a somatofugal *a*-spike. In Fig. 5B(ii) a spontaneous *a*-spike occurs in the cell body just before the electrical stimulation: in this case the *s*-potential is not recorded in the cell body (arrow, Fig. 5B(ii)); the *a*-spike and the *s*-potential collide somewhere between *a* and *s* (see Fig. 5A). The same events appear in Fig. 5B(iii) for the two sweeps in which an *a*-spike appears just before and just after the electrical stimulation; in both cases the *s*-potential is suppressed in the cell body. This suppression of the *s*-potential by a somatofugal spike cannot be explained by considering that the *s*-potential is an induced EPSP which cannot appear in the cell body during the period of total refractoriness induced by the somatofugal *a*-spike because the *s*-potential can be suppressed by an *a*-spike which appears in the cell body more than 10 ms earlier than the *s*-potential would appear (Fig. 5B(ii)). The *s*-potential is therefore an antidromic spike.

If it is possible to get a collision between an *s*-spike and a spontaneous spike associated with a *b*- or *c*-potential, this would demonstrate that this associated spike is also an OD1 spike. Such a collision was obtained for the *c*-potential by stimulating

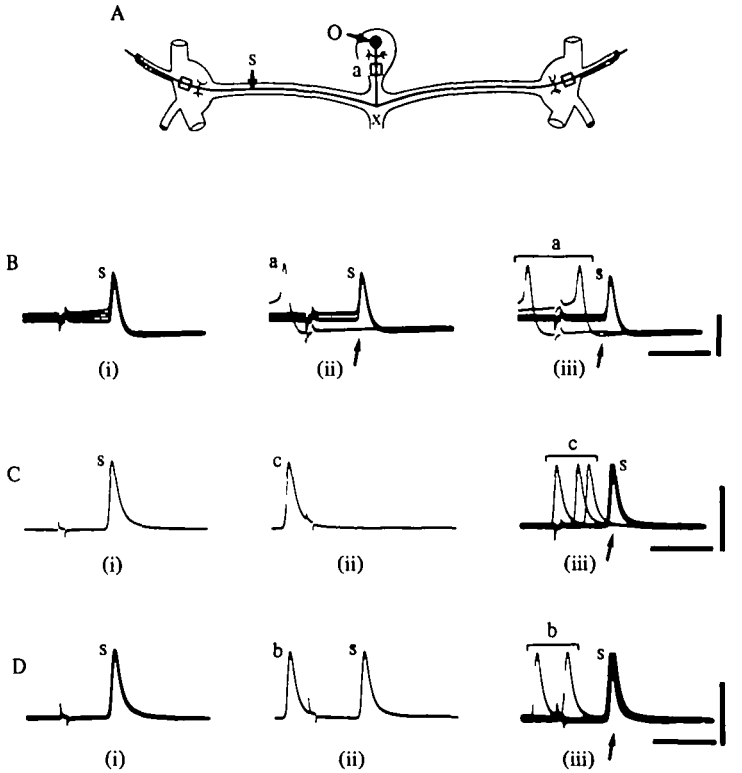

Fig. 5. An induced antidromic spike can collide with the somatofugal *a*-spike and with the spike associated with an OD1 *c*-potential. (A) Schema of the experimental procedure. Recordings are from an intracellular electrode in the cell body (O); electrical stimulations of the *son* at *s* trigger the oscilloscope sweep and induce antidromic *s*-spikes and *s*-potentials in OD1 soma. (B) Collision occurs between the *s*-spike and the spontaneous somatofugal spike (*a*-spike). The *s*-spike obtained by antidromic stimulation (B (i)) does not appear in the cell body (B (ii)) (→) when an *a*-spike appears at the beginning of the sweep: it has been suppressed by collision with the *a*-spike between *s* and *a* (see A). Collision also occurs when the *a*-spike appears later: in B (iii) for the two sweeps in which an *a*-spike appears the *s*-spike has been suppressed (→). (C) Collision also occurs between the *s*-spike and the spontaneous spike associated with a *c*-potential. The *s*-spike obtained by stimulation (C (i)) does not appear in the cell body when a spontaneous *c*-potential occurs in the cell body at the beginning of the sweep (C (ii)) (single sweep); it has been suppressed by collision with the spike associated with the *c*-potential between *s* and *x* (see A). In C (iii) successive stimulations are delivered at *s* during a burst of *c*-potentials: collisions occur for the three sweeps during which a *c*-potential appears in the cell body. (D) In this experimental situation the *s*-spike obtained by stimulation (D (i)) is not suppressed when a *b*-potential occurs in the cell body at the beginning of the sweep (D (ii); single sweep). The spike associated with the *b*-potential and the *s*-spike are travelling in the same direction and collision is impossible. This is confirmed in D (iii) where successive stimulations are delivered in *s* during a burst of *b*-potentials and collision does not occur for the two sweeps during which a *b*-potential appears in the cell body (→). Note that *b*-, *c*-potentials and *s*-spikes have exactly the same shape. Calibrations: horizontal bars, 10 ms; vertical bars, 20 mV.

the left *son* (Fig. 5C). When a spontaneous *c*-potential appears in the cell body just before (Fig. 5C(ii)) or just after (Fig. 5C(iii)) the electrical stimulation which induces the *s*-potential (Fig. 5C(i)), this last does not appear in the cell body. The *s*-spike and the spike associated with the *c*-potential collide somewhere between the branching point of the axon (*x*) and *s*. Thus, this spike associated with the *c*-potential is also an

OD1 spike. The same result can be obtained for the spike associated with the *b*-potential, in this case by stimulation of the right *son*. If, however, a *b*-potential appears in the cell body just before (Fig. 5D (ii)) or just after (Fig. 5D(iii)) an electrical shock to the left *son* (as in Fig. 5A), the induced *s*-spike is still recorded in the cell body. This result is easy to understand; the *s*-spike and the spike associated with the *b*-potential, both of which are OD1 spikes, are propagated in the same direction (at least between *s* and the cell body) and the *s*-spike cannot be suppressed by a collision with the spike associated with the *b*-potential. This experiment also shows that the refractory period of OD1 cannot explain the results of figures 5B and C. Indeed, in Fig. 5D(iii), a *b*-spike which appears in the cell body after the stimulation does not suppress the *s*-potential.

It now seems clear that *b*- and *c*-potentials are not EPSP's but OD1 spikes. OD1 possesses three spike initiating zones, one in the oesophageal ganglion (*a*), one in the left commissural ganglion (*b*) and one in the right commissural ganglion (*c*). The *b*- and *c*-spikes will be now called somatopetal spikes for convenience. Nevertheless, the *b*-spike is somatopetal only in the left *son* and in the *on* (and somatofugal in the right *son*, right *lon* and left *lon*); the *c*-spike is somatopetal only in the right *son* and in the *on* (and somatofugal in the left *son*, left *lon* and right *lon*).

(4) *Spontaneous collisions between the three types of OD1 spikes*

Knowing that spike collisions can be induced, we wished to see if they could also occur spontaneously. Such spontaneous collisions would provide further evidence that the *b*- and *c*-potentials are in fact OD1 spikes. In general, when one of the three spike initiating zones is firing the others are not (see below). Nevertheless, in some experiments two spike initiating zones can fire at the same time and careful examinations of long term recordings allow us to observe occasional spontaneous collisions, especially in *P.vulgaris*. Such collisions are illustrated in Fig. 6 and 7. Because of the low recording speed, drawings are given (Figs. 6C, 7B) to emphasize the shape of the spikes and their chronology at the different electrodes.

Collision can occur between spontaneous somatofugal and somatopetal spikes (Fig. 6). In Fig. 6B, the first spike (i) is a somatofugal spike. This is shown by its shape in the cell body (O) (compare with Fig. 3D(i)) and by its time of occurrence at the extracellular electrodes: first in 3 (medial right *son*) and later in 1 and 4 (lateral right *son* and lateral left *son*). The third spike is a somatopetal *c*-spike: it appears first in the right *son* (4–3) and later in the left *son* (1). In (ii) the spike recorded in the cell body (O) and at electrodes 1 and 3 is a somatofugal *a*-spike as shown by comparison of time of occurrence and shape with (i). But the time of occurrence and shape of the spike recorded at electrode (4) in (ii) suggest that it is not a somatofugal *a*-spike. By its shape it is a somatopetal *c*-spike. It must be assumed that in (ii), a collision between a somatofugal *a*-spike (which does not appear at electrode 4) and a somatopetal *c*-spike (which does not appear at electrodes 3, 1 and O) has occurred somewhere between electrodes 3 and 4.

Collision can also occur between spontaneous *b*- and *c*-somatopetal spikes (Fig. 7). In the recording of Fig. 7A (from the same experiment as Fig. 6), the two commissural spike initiating zones are firing. For *b*-spikes the chronology at the recording

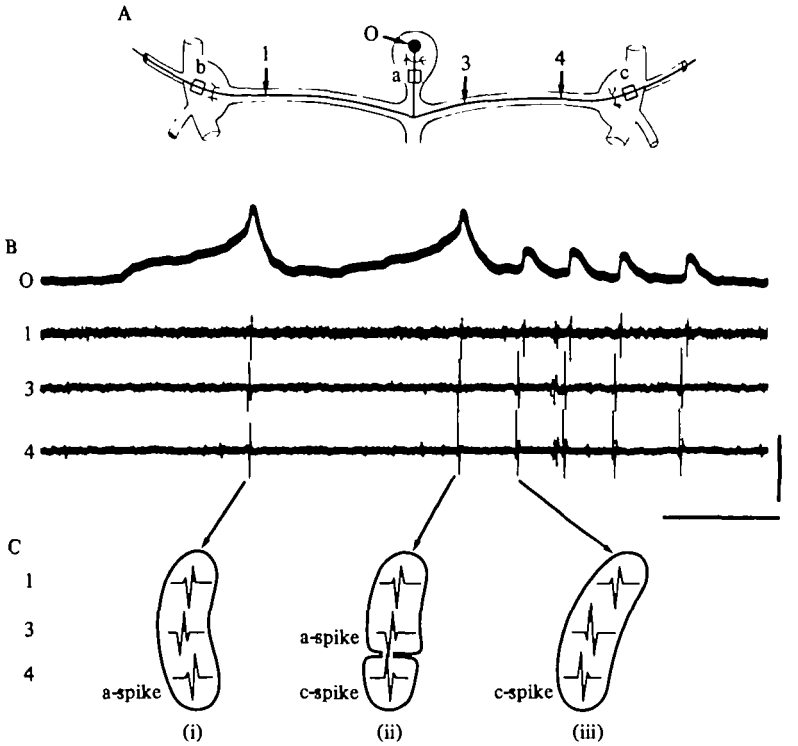

Fig. 6. Collision between spontaneous somatofugal and spontaneous somatopetal spikes (*Palinurus vulgaris*). (A) Diagram showing positions of the recording electrodes. In B the spontaneous activity of OD1 is recorded intracellularly from the cell body (O) and extracellularly from the axon in 1 (left branch) 3 and 4 (right branch) (see A for disposition of the bipolar recording electrodes along the nerve). (C) Drawing to emphasize the relative time of occurrence and the shape of each extracellular spike at the different recording electrodes. Examination of the shape of intracellular spikes in O shows that the two first are somatofugal spikes and that the rest are somatopetal spikes. We can confirm that the first (i) is an *a*-spike by considering the time of occurrence of the spike at different extracellular electrodes: the spike appears first in 3 and later (about the same time) in 1 and 4. The same observation shows that the third (iii) is a *c*-spike which appears first in 4, later in 3 and still later in 1. In (ii) the situation is more complex. The spike recorded in (ii) at electrodes 1 and 3 is, as in O, an *a*-spike: the delay between the two potentials is the same as for the *a*-spike recorded in (i). On the other hand, by its time of occurrence and its shape, the spike recorded in (ii) at electrode 4 is clearly not an *a*-spike, but is a *c*-spike identical to that recorded in (iii) at electrode 4. So in (ii) a collision occurs between the *a*-spike recorded in 1 and 3 (which is not recorded in 4) and the *c*-spike recorded in 4 (which is not recorded in 3 and 1). Calibrations: horizontal bar, 100 ms; vertical bar, 5 mV.

electrodes is 1–3–4 (see (i)) while for *c*-spikes the chronology is 4–3–1 (see (ii)). In (iii) the spike recorded at electrodes (1) and (3) is a *b*-spike, as shown by comparison with (i). The timing of the spike recorded at electrode (4) indicates that is clearly not a *b*-spike. Its shape is that of a *c*-spike (compare with (ii)). It must be assumed that in (iii) collision has occurred between a *b*-spike (which does not appear at electrode 4) and a *c*-spike (which does not appear at electrodes 3 and 1) somewhere between electrodes (3) and (4).

It is not possible to find another interpretation of these recordings, which again show that *b*- and *c*-potentials are somatopetal OD1 spikes and cannot be considered as EPSP's in the cell body.

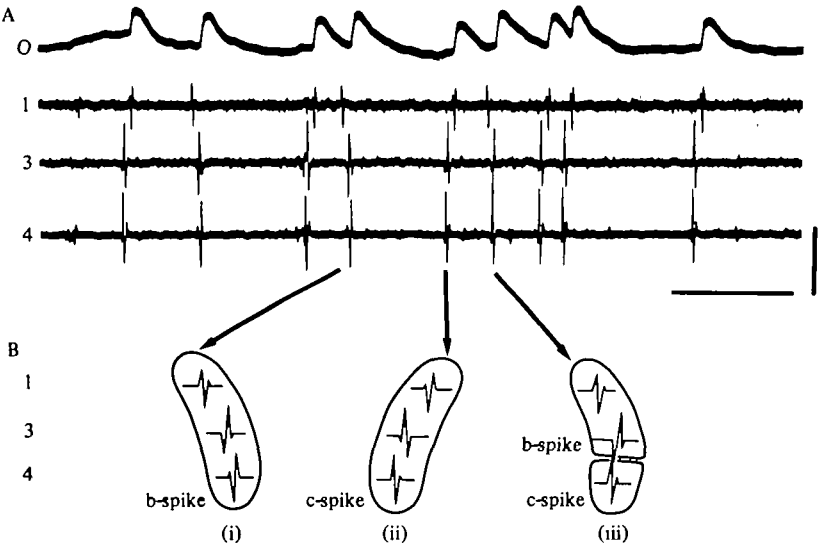

Fig. 7. Collision between spontaneous somatopetal *b*- and *c*-spikes (*Palinurus vulgaris*). In A the spontaneous activity of OD1 is recorded from the cell body (O) and from the axon in 1, 3 and 4 (see Fig. 6A for disposition of bipolar recording electrodes along the *son*). (B) Drawing to emphasize the relative time of occurrence and the shape of each extracellular spike at the different recording electrodes. The first spike (i) is a *b*-spike (chronology: 1–3–4); the second spike (ii) is a *c*-spike (chronology: 4–3–1). The spike recorded in (iii) at electrodes 1 and 3 is a *b*-spike (compare with the *b*-spike recorded in (i) at the same electrodes); the spike recorded in (iii) at electrode 4 is not a *b*-spike (compare with the *b*-spike recorded in (i) at the same electrode) but a *c*-spike (compare with the *c*-spike recorded in (ii) at the same electrode). So in (iii) a collision occurs between a *b*-spike recorded in 1 and 3 (not recorded in 4) and a *c*-spike recorded in 4 (not recorded in 3 and 1). Calibrations: horizontal bar, 100 ms; vertical bar, 5 mV.

(5) *Synaptic drive of the three spike initiating zones*

The preceding results have shown that OD1 possesses three distinct zones of spike initiation. In our experiments (i.e. *in vitro*), in the two species each zone can be spontaneously active, suggesting that each zone could be synaptically driven. This is of importance because, if true, it means that each zone might be associated with a receptive field in which integration can occur.

By looking only at the intracellular activity recorded in the cell body it is possible to see that the *a*-spike initiating zone receives synaptic activation. For example, in the experiment of Fig. 11C the *a*-spike initiating zone is firing tonically and, in the soma, EPSP's appear regularly in bursts which periodically accelerate the ongoing somatofugal spiking activity. It must be again noticed that *b*- and *c*-potentials recorded in the cell body cannot be EPSP's, as is confirmed by comparison between Fig. 11B and 11C. In Fig. 11C, the *b*-potentials, which appear regularly, do not increase the somatofugal discharge (as do the EPSP's in Fig. 11C); instead this discharge stops during the *b*-potential burst and for a short time afterwards.

Thus far, it has not been possible to penetrate the OD1 axon with a microelectrode in the commissural ganglia (i.e. near the *b*- and *c*-spike initiating zones). So it has not been possible to demonstrate directly, by recording PSP's, that these two regions receive synaptic input. This can be achieved indirectly by stimulation of the *dpon*

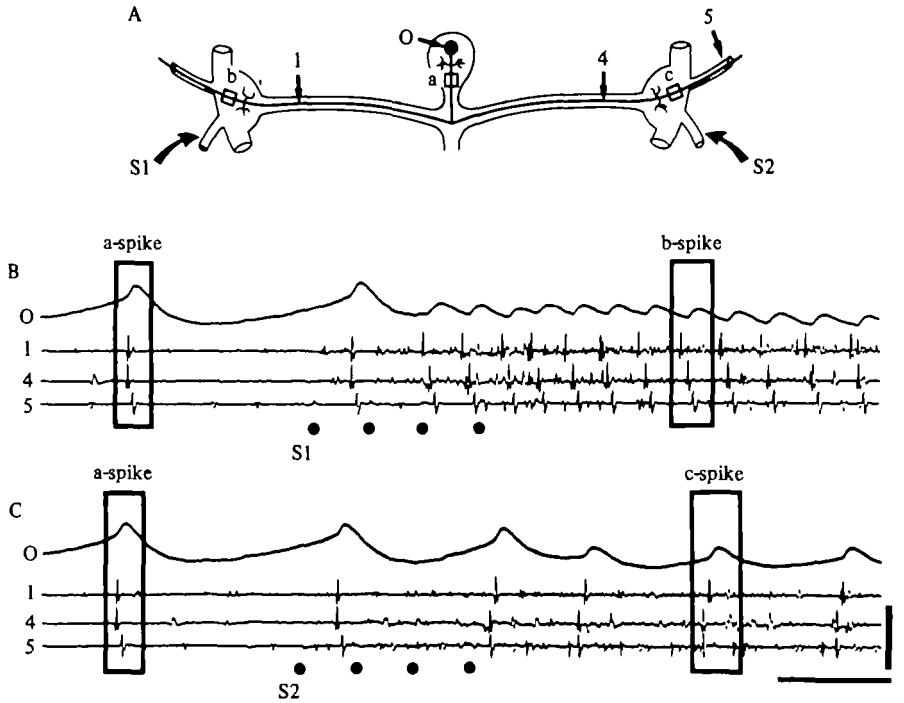

Fig. 8. Synaptic activation of the commissural spike initiating zones (*b–c*) of OD1. (A) Experimental scheme. (B, C) During a spontaneous somatofugal burst (*a*-spikes) an electrical stimulation is delivered to the left *dpon* (S1, in B) or the right *dpon* (S2, in C). S1 stimulation activates the left commissural zone and produces *b*-spikes; S2 stimulation activates the right commissural zone and produces *c*-spikes. Each spike can be identified by looking at its time of occurrence at each recording electrode (*a*-spike: 1 and 4 at the same time, 5 later; *b*-spike: 1 first, 4 later and 5 still later; *c*-spike: 4 and 5 at the same time, 1 later) (*Palinurus vulgaris*). Calibrations: horizontal bar, 100 ms; vertical bar, 10 mV.

(Fig. 8). This nerve does not contain an OD1 axon branch, yet its electrical stimulation activates the OD1 commissural spike initiating zone of the same side. For example, in *P. vulgaris*, the electrical shocks delivered on the left *dpon* when OD1 is firing only somatofugal spikes (pattern 3, Fig. 11C) induce a burst of *b*-spikes (Fig. 8B). The same result can be obtained in the same situation (i.e. when the somatopetal spike initiating zones are silent) for the *c*-spike initiating zone by stimulation of the right *dpon* (Fig. 8C).

It is also possible to show morphologically that OD1 possesses an arborization in each commissural ganglion. This has been done by cobalt migration from the cut end of the *lon*, a nerve which contains only a few axons, the largest of which is that of OD1 (at least in *P. vulgaris*). Nevertheless it is difficult to be absolutely sure that the observed arborization belongs to OD1 because cobalt migration from the cut end of the *lon* to the cell body (previously identified by electrophysiology in the oesophageal ganglion) more than 2·5 cm away, is rarely achieved. In any case, physiological results show that *b*- and *c*-spike initiating zones can be independently driven by synaptic input and therefore must be, in this respect, equivalent to the oesophageal *a*-zone associated with the cell body.

(6) *Temporal organization of the activity of the three spike initiating zones*

It has been shown that OD1 is a bursting motor neurone involved in the oesophageal motor rhythm (Fig. 2). The questions which arise now are how a bursting pattern of firing can be organized in a neurone by three spike initiating zones and whether such a neurone is able to exhibit several patterns of firing.

(*a*) *Each burst is composed of only one type of somatopetal spike*

The first thing to emphasize is that in our *in vitro* experiments most bursts are composed of somatopetal spikes having the same origin (i.e. only of *b*-spikes (*b*-bursts, Fig. 9B) or only of *c*-spikes (*c*-bursts, Fig. 9C)). The origin of each spike in a burst can be determined by looking at the shape of the intracellular spike (to distinguish between somatofugal and somatopetal spikes) and at the time of occurrence of the extracellular spike at the different electrodes (Fig. 9C, 9E) to distinguish between *b*- and *c*-spikes. Another way to show that each burst includes only one type of spikes is to trigger the oscilloscope sweep by the spike at one of the electrodes and to record during a single burst. If the analyzed burst contains only spikes of one origin, a multiple sweep recording gives only one spike at each electrode (Fig. 9F and G) and the spike initiating zone which is firing during this burst can be identified (*b*-zone in Fig. 9F; *c*-zone in Fig. 9G). If the analyzed burst contains spikes of two different origins, the same multiple sweep recording gives two different spikes at each electrode (except, of course, at the triggering electrode) (Fig. 9H). The scarcity of such composite bursts explains the observation that spontaneous collisions between somatopetal spikes are infrequent.

(*b*) *Temporal relationships between somatofugal and somatopetal spiking*

If a spontaneous burst of somatopetal spikes occurs when the somatofugal *a*-spike initiating zone is spontaneously firing (or is induced to fire by soma depolarization), the somatofugal firing immediately stops. In Fig. 10B and C the firing of the somatopetal zone (*a*-spikes) is interrupted when a burst of somatopetal *c*-spikes occurs; the same result is obtained when a burst of somatopetal *b*-spikes occurs (Fig. 10D and E). In each case, the somatopetal burst is followed by a silent period, after which the somatofugal firing reappears slowly and with an increasing frequency, to be interrupted again by the next somatopetal burst (Fig. 10B). The mechanism by which the OD1 somatofugal firing is interrupted when a somatopetal burst occurs is not completely clear. Nevertheless it must be pointed out that the somatofugal as well as the somatopetal spikes present a pronounced after-potential (Fig. 3B). From this after-potential, a long lasting depolarizing phase occurs until the cell reaches again the firing threshold and produce a somatofugal spike. This might explain the observation that the somatofugal spike initiating zone fires only at low frequencies; it also explains how the somatofugal firing is interrupted when a somatopetal burst occurs. If a *b*- or *c*-spike, whose occurrence in the cell body is independent of the soma membrane potential, occurs after an *a*-spike, the depolarization is interrupted, the after-potential is reset and the next *a*-spike is delayed. Moreover, as *b*- and *c*-spike initiating zones always fire at high frequency, the *a*-spike initiating zone can never reach the firing

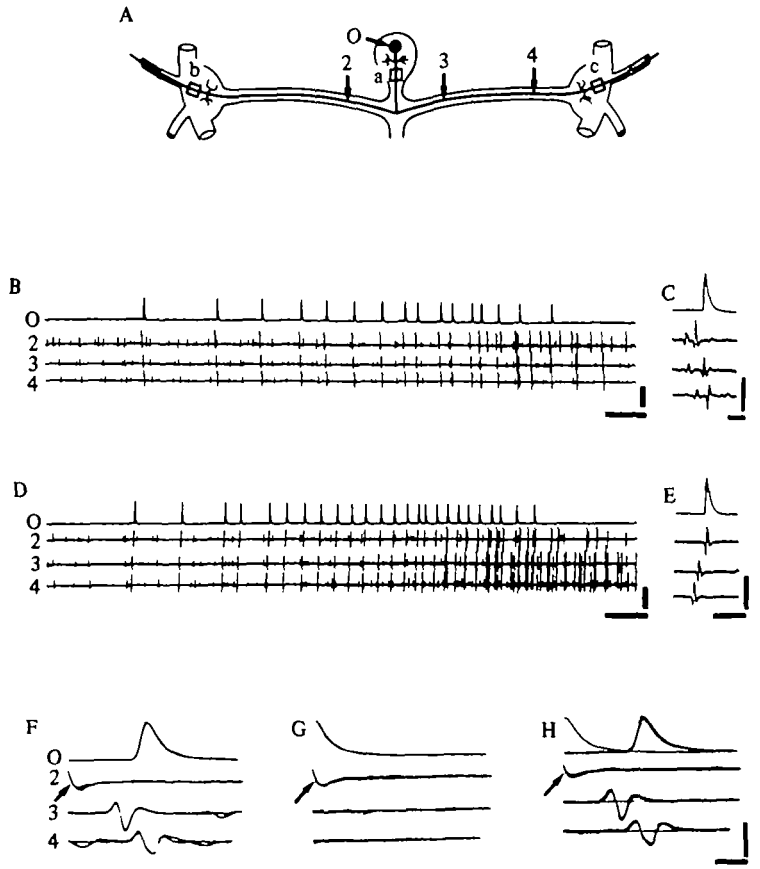

Fig. 9. Each burst is composed of only one type of spike (*Jasus lalandii*). (A) Experimental scheme. (B) Spontaneous burst of spikes originating in the left commissural ganglion (*b*-spikes), as shown in the high-speed recording (C). (D) Spontaneous burst of spikes originating in the right commissural ganglion (*c*-spikes) as shown in E. In F, G, H the oscilloscope sweep is triggered by the spike at electrode (2) during a burst of *b*-spikes (F), during a burst of *c*-spikes (G) and during an uncommon burst composed of *b*- and *c*-spikes (H). During the *c*-burst, the spike appears at electrodes 4 and 3 before the triggering of the sweep (and is not recorded). Calibrations: horizontal bars, 100 ms in B and D, 10 ms in C and E, 2 ms in F, G and H; vertical bars, 20 mV.

threshold during such firing, and it is thus kept silent. This does not, however, explain why the *a*-spike initiating zone remains silent for a short time after a somatopetal burst (Fig. 10). It is difficult to think that this silent period might represent an hyperpolarizing after-potential as known in bursting pacemaker neurones: in many cases this silent period does not immediately follow the somatopetal burst and a depolarizing phase can be intercalated (sometimes with somatofugal spikes) between the burst and the silent period (Fig. 10B). Such complex firing can only be partly understood by looking at the cell itself and to fully explain such aspects of OD1's firing one must bear in mind that this neurone is part of a network, and that each of its spike initiating zones receives distinct synaptic input (P. Dickinson, F. Nagy and M. Moulins, in preparation).

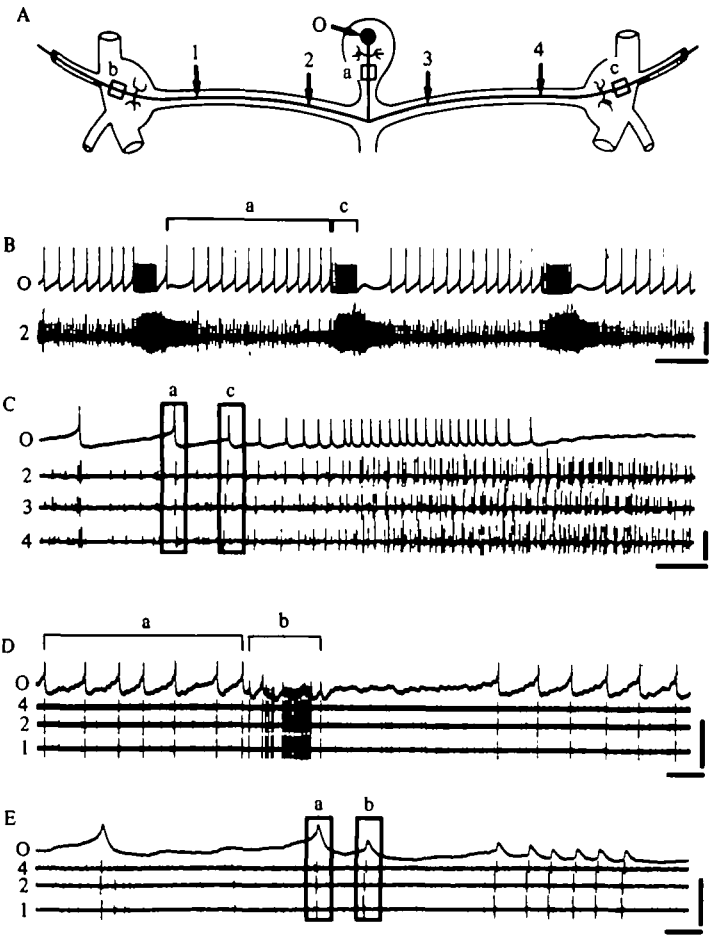

Fig. 10. Hierarchy between somatofugal (*a*) and somatopetal (*b–c*) spikes of OD1. (A) Experimental scheme. In B (*Jasus lalandii*) the cell body has been slightly depolarized and the somatofugal (*a*) spike initiating zone fires. This firing is interrupted by rhythmic spontaneous bursts of *c*-spikes followed by a silent period. (C) Same experiment as in B; *a*- and *c*-spikes are easy to identify by looking at the chronology of the spike at the different recording electrodes. In D (*Palinurus vulgaris*) the spontaneous somatofugal (*a*) activity is interrupted by a burst of *b*-spikes followed by a silent period. (E) Same experiment as in D; *a*- and *b*-spikes can be identified by looking at the chronology of the spike at the different recording electrodes. Calibrations: horizontal bars, 1 s in B and D, 100 ms in C and E; vertical bars, 20 mV in B and C, 10 mV in D and E.

(*c*) *Different patterns of OD1 firing*

In our experimental conditions, OD1 exhibits three different bursting patterns of firing (Fig. 11).

Pattern 1 (Fig. 11A): This phasic pattern consists of regular periodic bursts of somatopetal spikes separated by silent periods. In each burst, the spikes appear at high frequency and all come from the same commissural spike initiating zone (*b*- or *c*-). It is this pattern which frequently occurs in *J. lalandii*, but has never yet been observed in *P. vulgaris*.

Pattern 2 (Fig. 11B): This is a phasic-tonic pattern which can be considered identical to pattern 1, but in which the somatofugal *a*-spike initiating zone is also firing.

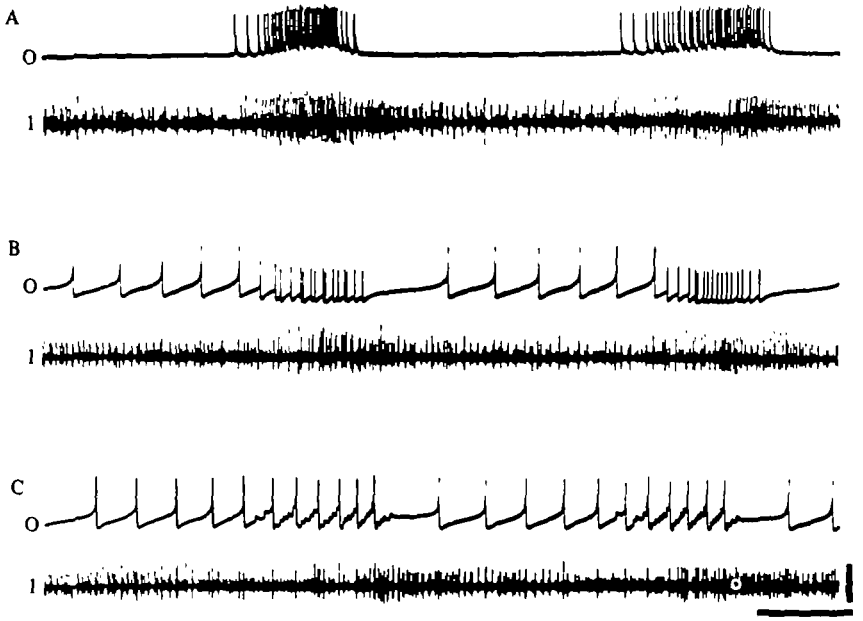

Fig. 11. The three patterns of firing of OD1 (from the same experiment). O intracellular recording from the cell body of OD1; 1, extracellular recording on the *son*. (A) Pattern 1; phasic firing organized by a somatopetal spike initiating zone alone. (B) Pattern 2; phasic-tonic firing organized by the somatofugal spike initiating zone and a somatopetal spike initiating zone. (C) Pattern 3; tonic firing organized by the somatofugal spike initiating zone alone. (For further details, see text.) (*Janus lalandii*). Calibrations: horizontal bar, 1 s; vertical bar, 20 mV.

As seen above, this somatofugal firing is interrupted at each somatopetal burst. The somatofugal spike initiating zone remains silent during the somatopetal burst and for a short time after this burst, then starts again with a slightly increasing frequency (see also Fig. 10). In other words, this pattern is characterized by composite long bursts separated by short silent periods. Spontaneous collision occasionally occurs at the beginning of the somatopetal firing (see Fig. 6). This pattern is sometimes observed in *J. lalandii* and in *P. vulgaris*; in *J. lalandii* it can be induced from pattern 1 by depolarization of the cell body.

Pattern 3 (Fig. 11 C): This tonic pattern is characterised by a continuous firing of the somatofugal (*a*-) spike initiating zone while the somatopetal (*b*- and *c*-) spike initiating zones are silent. The firing frequency of the somatofugal spike initiating zone is periodically increased by a short burst of EPSP's after which the frequency decreases. This somatofugal firing is comparable to that of pattern 2, but lacks the somatopetal firing. It is the most common pattern in *P. vulgaris* and it is quite rare in *J. lalandii*.

(7) *Participation of OD1 in the oesophageal motor rhythmic behaviour*

It is possible to understand how these different patterns of OD1 firing are formed by considering the functional properties of the network which organize the oesophageal motor rhythm. This motor rhythm consists of periodic discharges of dilator neurones followed by discharges of constrictor neurones (Fig. 2B, 12, 13).

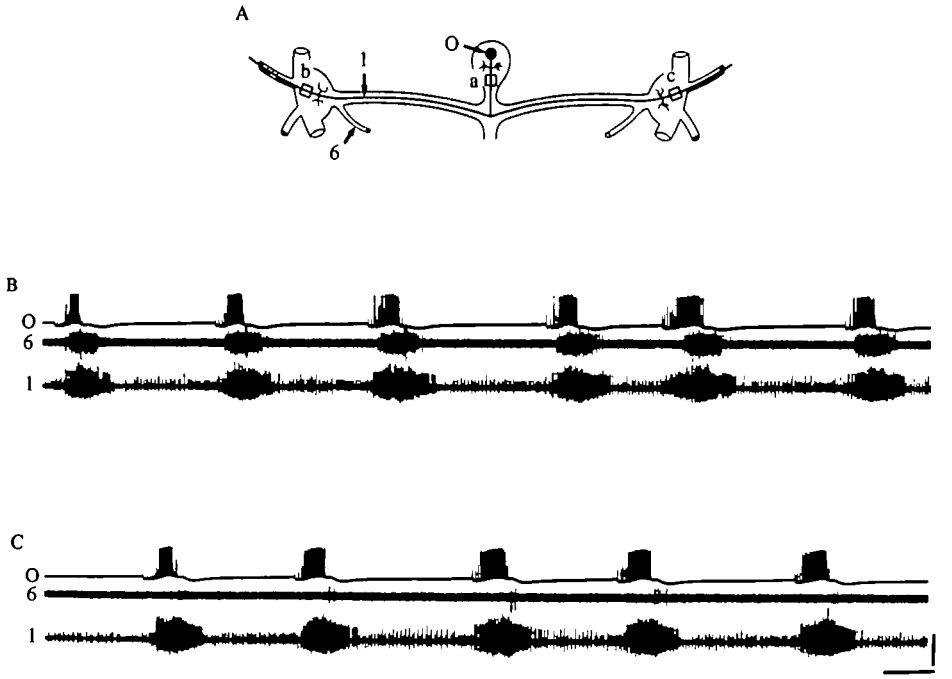

Fig. 12. When one somatopetal spike initiating zone of OD1 is bursting, the other is silent (*Jasus lalandii*). (A) Experimental scheme. (B, C) Recording in cell body (O), on the *son* (1) and from the left chemoreceptor nerve (6) in which some unidentified units fire only with the ipsilateral spike initiating zone of OD1. In B, the *b*-spike initiating zone is bursting (and the *c*-spike initiating zone silent); in C the *c*-spike initiating zone is bursting (and the *b*-spike initiating zone silent). Calibrations: horizontal bar, 1 s; vertical bar, 20 mV.

J. lalandii each commissural ganglion is able to produce such a rhythm in total isolation (i.e. the oesophageal network is distributed between the two ganglia and each one possesses an oesophageal oscillator).

In our preparation, when the two oscillators are connected, if one is firing, the other one is silent. This can be shown by recording from neurones which are driven by only one of the two oscillators. This is the case for some unidentified dilator neurones whose bursting activity can be recorded in the chemoreceptor nerve (*chn*) (see Fig 1 B and C). These neurones are driven by the ipsilateral oesophageal oscillator and are silent when the contralateral oscillator is bursting. In the experiment of Fig. 12, the oesophageal motor rhythm is recorded on the *son* (electrode 1). In the first recording (Fig. 12B) the motor neurones of the *chn* (electrode 6) are bursting in synchrony with the oesophageal rhythm. In the second recording (Fig. 12C) the same motor neurones do not fire and thus do not follow the oesophageal rhythm. This can be interpreted to mean that in the first recording the left oscillator drives the oesophageal rhythm while in the second recording it is the right oscillator which drives the rhythm.

It is important to note that OD1 (electrode O on Fig. 12) is driven by the left oscillator (Fig. 12B) *and* by the right oscillator (Fig. 12C). When the left oscillator is bursting OD1 gives *b*-bursts (Fig. 9C); when the right oscillator is bursting, OD1

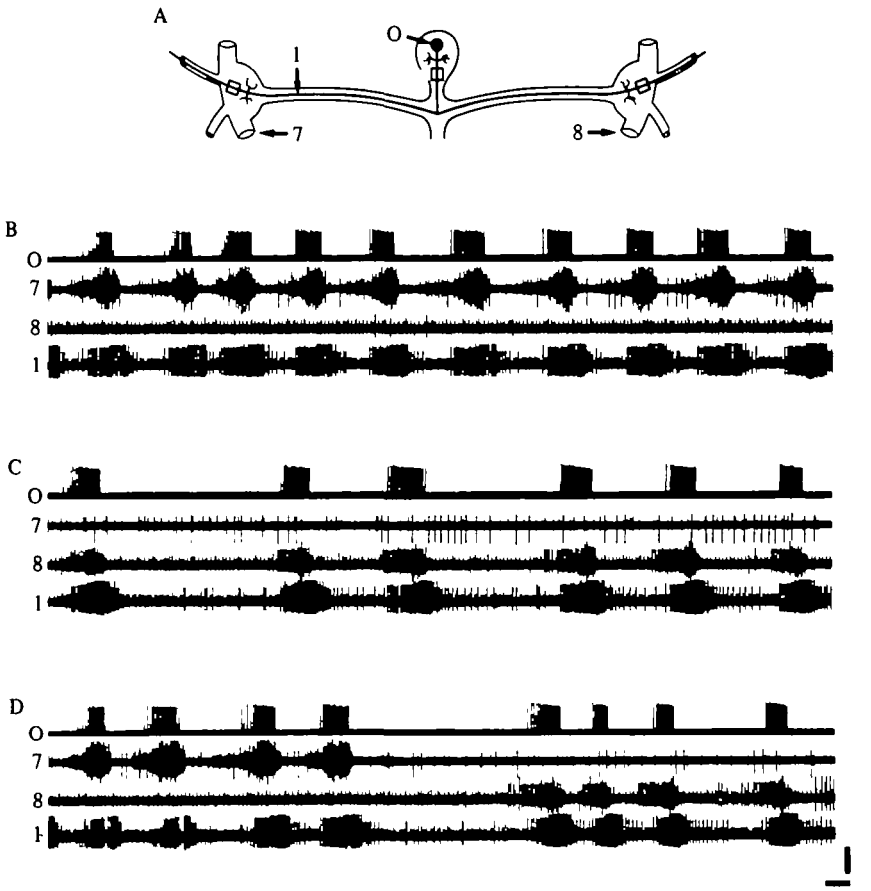

Fig. 13. The two somatopetal spike initiating zones are alternately active (*Jasus lalandii*). (A) Experimental scheme. (B–D) Recording in the cell body (O), from the *son* (1) and from a selected bundle of fibers in the two connectives (7, 8) which fire only with the ipsilateral spike initiating zone of OD1. In B, the left (*b*-) spike initiating zone is firing while in (C) it is the right (*c*-) spike initiating zone which is bursting. In (D) the left spike initiating zone, which is bursting at the beginning of the recording, stops and after a short silent period the right spike initiating zone starts firing. Calibrations: horizontal bar, 2 s; vertical bar, 20 mV.

gives *c*-bursts (Fig. 9D). In other words OD1 is driven by the two oesophageal oscillators through its two axonal (*b*- and *c*-) spike initiating zones. Since only one oscillator fires at a time, each somatopetal burst of OD1 consists of somatopetal spikes having the same origin.

These results can be confirmed by recording from a selected bundle of interneuronal fibres in the connectives at the rear of the commissural ganglia (Fig. 13A). The bundle of fibres in the left connective fires with the oesophageal rhythm when the left oscillator is bursting (Fig. 13B); the bundle of fibres in the right connective fires with the oesophageal rhythm when the right oscillator is bursting (Fig. 13C). OD1 is again driven when the left oscillator is bursting (Fig. 13B) *and* when the right oscillator (Fig. 13C) is bursting. Long term recordings show that *in vitro* there is generally a regular alternation between series of bursts originati

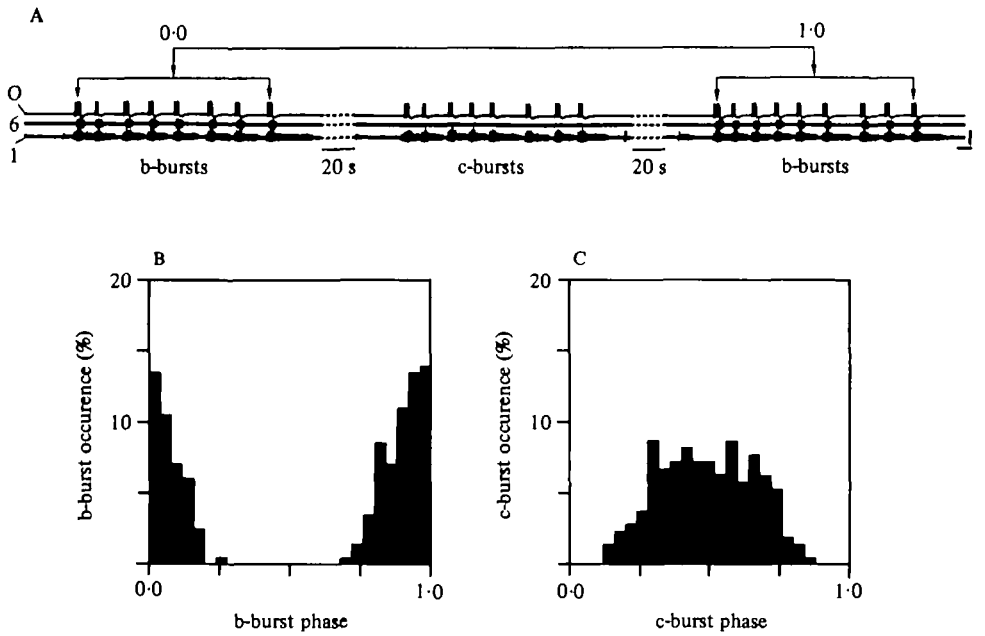

Fig. 14. Long-term temporal organization of the activity of the two somatopetal spike initiating zones of OD1 (*Jasus lalandii*). (Experimental procedure as in Fig. 12A). Long term recording (A) shows that the OD1 activity (O) is organized in burst series (bouts) separated by silent periods. In a bout all the bursts come from the same spike initiating zone, which can be identified by recording from one of the chemoreceptor nerves (6) (see Fig. 12). As can be shown by looking at the *b*-burst phase (B) and the *c*-burst phase (C) in the reference period defined in A there is a regular alternation between the activity of the *b*-spike initiating zone and the activity of the *c*-spike initiating zone. Calibrations: horizontal bar, 2 s; vertical bar, 20 mV.

in the right and left oscillators reflected in an alternation of *b*- and *c*-bursts coming from the two oscillators (Figs. 13D, 14A). The histograms in Fig. 14B and C quantify the phase of OD1 *b*- and *c*-bursts in the reference period defined in Fig. 14A and show how regular the alternation between *b*- and *c*-burst series can be.

In conclusion, by looking at the cooperation of the two commissural oscillators in generating the oesophageal rhythm it is possible to understand how OD1 generates its somatopetal pattern of firing (pattern 1, Fig. 11A). With regard to what we have called pattern 2 (Fig. 11B), in which the (*a*-) somatofugal spike initiating zone is also firing, we have shown that the intrinsic properties of the somatofugal zone of electrogenesis (i.e. its long repolarizing phase) can explain why the superimposed somatofugal spiking is interrupted by each somatopetal burst. To understand pattern 3 (Fig. 11C), when the (*a*-) somatofugal spike initiating zone is firing alone, it must be assumed that the commissural oscillators also project onto the OD1 *a*-spike initiating zone in the oesophageal ganglion. Indeed, this pattern results from periodic depolarizations induced by bursts of EPSP's which are time locked with the oesophageal rhythm. Thus the oesophageal oscillators can also govern the OD1 somatofugal firing when the *b*- and *c*-spike initiating zones are kept below threshold.

DISCUSSION

The main dilator motor neurone of the oesophagus, OD1, is an unpaired Y-shaped neurone in which the two bars represent the two lateral axon branches and the vertical bar the short unpaired axon connected to the cell body in the oesophageal ganglion. The two lateral axon branches go to the periphery through the left and right commissural ganglia. In this paper it is shown that, in addition to the spike initiating zone in the oesophageal ganglion (*a*) near the cell body, OD1 also possesses a spike initiating zone in each commissural ganglion (*b* and *c*). The study of the spontaneous activity of this neurone shows that its firing is organized from the three spike initiating zones according to a defined pattern generated by a bilaterally distributed oesophageal network.

Our results have been obtained *in vitro*, after peripheral axotomy, and it has been demonstrated in other arthropods that in such conditions some neuronal properties can be modified (Pitman, Tweedle & Cohen, 1972; Goodman & Heitler, 1977) especially in unpaired motor neurones (Heitler & Goodman, 1978). Nevertheless it is unlikely that the axotomy performed in our experimental procedure is responsible for the appearance of new localized axonal spike initiating zones. Indeed, (1) the modification of neuronal properties (mainly changes in ionophore composition of the membrane) occurs only several days after axotomy; (2) we have shown that each spike initiating zone is synaptically driven and gives a stereotyped well organized discharge, which could be difficult to understand for newly differentiated spike initiating zones; (3) the organized OD1 firing is directly comparable to the one which can be recorded in semi-intact preparations without any axotomy; (4) we know of some unpaired motor neurones in the stomatogastric nervous system which, in the same experimental conditions (after axotomy), do not fire from multiple axonal spike initiating zones.

(1) *Multiple axonal spike initiating zones*

Multiple sites of spike initiation have now been demonstrated in a variety of invertebrate and vertebrate central neurones. Most of these studies have concerned neurones with multiple *dendritic* triggering zones as, for example, alligator Purkinge cells (Llinas *et al.* 1968; Llinas & Nicholson, 1971), crab-eye stalk motor neurones (Sandeman, 1969*a*, *b*) and crayfish fast flexor motor neurones (Zucker, 1972). In these neurones the same dendritic arborization possesses multiple spike-initiating zones but the dendritic spikes generated by each zone are only electrotonically propagated to the single axonal spike initiating zone near the cell body. Such dendritic spikes contribute only to subthreshold activation of the neurone and are functionally equivalent to EPSP's. In other words in these neurones the dendritic triggering zones are participating in a unique integration process which is finally expressed by a single axonal spike initiating zone.

The situation is completely different for OD1 which possesses three *axonal* spike initiating zones, each zone being independently synaptically driven (see Nagy & Moulins, 1980). Separate integration occurs for each region and is expressed independently at the corresponding axonal spike initiating zone, (i.e. the neurone exhibits separated compartments of integration). Until now, such unusual properties have been

demonstrated for only three identified neurones in the central nervous system of arthropods: the crayfish multisegmental tactile interneurone (MTI) (Hughes & Wiersma, 1960), the locust lobular giant movement detector interneurone (LGMD) (O'Shea, 1975) and a lobster cardiac sac dilator motorneurone (CD2) (Vedel & Moulins, 1978).

However, neurones with separated compartments of integration are perhaps more common and not only a curiosity found in invertebrates with ladder-like nerve cords (Horridge, 1968). Indeed there is now accumulating data which suggest that, even in vertebrates, a single dendritic arborization can be functionally compartmentalized. First, there are several examples of neurones in which dendritic spikes may sometimes have access to the axon (Purpura, 1967; Llinas *et al.* 1968; Llinas & Nicholson, 1971). Furthermore Calabrese and Kennedy (1974) have shown that the crayfish MIT, which possesses an axonal spike initiating zone in each ganglion of the ventral cord, also possesses functionally separated spike initiating zones in the same ganglion (at least in the last abdominal ganglion). Finally, in fish oculomotor neurones there is good evidence that the axon is invaded by two different discharges (Kriebel *et al.* 1969; Korn & Bennett, 1971, 1975). One of these discharges controls the fast phase of the nystagmus by somatic impulses; the other discharge controls the slow phase of the nystagmus generated in dendrites. Each phase is governed by different synaptic inputs; thus the neurone seems to be involved in two different behaviours by means of two separate zones of integration.

(2) *Neuronal economy and properties of each spike initiating zone*

It is well known that in arthropods the number of motor neurones innervating each muscle is very low. In such a situation it can be expected that each neurone has to assume several functions. This can be achieved by neurones with multiple axon branches and with independent discharges in the different branches. For example, the five large motor neurones of the cardiac ganglion give twelve independent axons (Friesen, 1975) and each axon branch probably carries independent spikes. A comparable situation is known for the dorsal unpaired median neurone of the extensor tibial muscle (DUMEti) in the locust (Hoyle *et al.* 1974; Hoyle & Dagan, 1978) which is, at least under controlled stimulation, able to carry independent spikes in its two axon branches (Heitler & Goodman, 1978). In other words, such neurones exhibit compartmentalization for conduction.

In this respect, OD1 is not comparable to these compartmentalized neurones; in our experimental conditions, each spike, regardless of its origin, invades the axon branches in the *son*'s. Nevertheless the existence of these separate spike initiating zones can be understood in terms of neuronal economy if the properties of each zone are considered. We have shown that the somatofugal *a*-spike initiating zone of OD1 fires only at a low frequency. In contrast, the commissural somatopetal (*b*- and *c*-) spike initiating zones fire at high frequency and generate short phasic bursts. In other words, if we assume that each spike invades all the axon branches, OD1, by means of its two types of spike initiating zones (somatopetal and somatofugal), is able to produce two qualitatively different patterns of firing (phasic and tonic). It would be difficult for a single neurone to achieve such clear cut different muscular activation

from the same spike initiating zone. So it can be suggested that a single motor neurone with qualitatively different spike initiating zones could behave as a phasic motor neurone *and* a tonic motor neurone. This possibility remains to be investigated at the neuromuscular junction.

(3) *Participation in a bilaterally organized behaviour*

The significance of a central neurone able to fire from multiple axonic spike initiating zones can be completely understood only if this neurone is well identified and if the behaviour in which this neurone is involved is known. It is probably for this reason that a full explanation has never been proposed for such interneurones as the crayfish MTI (Hughes & Wiersma, 1960) which can fire from each abdominal ganglion under tactile sensory inputs or for the locust LGMD (O'Shea, 1975) which integrates separately two qualitatively different (visual and auditory) sensory inputs.

It is certainly easier to answer this question where motor neurones are concerned. We have already shown that a single motor neurone (CD2), which innervates the dilator muscles of the cardiac sac of the rock lobster stomach, can participate, by means of two axonal spike initiating zones, in two different motor rhythms generated by two neuronal networks located in two different nervous centres (Moulins & Vedel, 1977; Vedel & Moulins, 1978). In other words CD2 is clearly a bifunctional motor neurone able to participate in two completely different motor behaviours through two different spike initiating zones.

In the present paper we show that multiple spike initiating zones can also allow a motor neurone to participate in a rhythmic motor behaviour organized by a distributed network. This network consists mainly of two oscillators, one in the left commissural ganglion and one in the right. At least in our experimental conditions, the two oscillators are alternately active (i.e. the oesophageal rhythm is alternately controlled by each oscillator). Through its two somatopetal spike initiating zones, OD1 is driven by each oscillator and so is always participating in the oesophageal motor behaviour. This is not surprising if we remember that OD1 innervates all the oesophageal dilator muscles (Moulins & Vedel, 1977) and is in this way certainly the most important motor neurone in the 'return stroke' phase of the rhythm.

In bilaterally organized animals many rhythmic behavioural activities probably result from the co-operation of paired oscillators and, at least in arthropods, unpaired motor neurones are known. In the thoracic ganglia of insects, for example, there is a group of dorsal unpaired neurones (DUM) (Hoyle *et al.* 1974). One of these, which innervates the left and right extensor tibiae muscles (Eti) ,is known as DUMEti, and has been extensively studied. Recently it has been shown by electrical stimulation that this bifurcating neurone possesses 3 separate spike initiating zones, one near the soma and one in each axon branch in the ganglion (Heitler & Goodman, 1978). Unfortunately it has not yet been possible to drive these spike initiating zones synaptically and to understand what their functional significance might be. Nevertheless, it is possible that DUMEti and OD1 might be comparable. Organization of firing of unpaired bifurcating neurones from right and left axonal spike initiating zones may thus be widespread.

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