IMPULSE IDENTIFICATION AND AXON MAPPING OF THE NINE NEURONS IN THE CARDIAC GANGLION OF THE LOBSTER HOMARUS AMERICANUS

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The isolated cardiac ganglion of the lobster is an autonomous, integrating system containing only nine neurons. Its physiology was first investigated by Welsh & Maynard (1951) and has been reviewed by Hagiwara (1961). Its component cells, when isolated from synaptic input, are usually either quiescent or fire nerve impulses at a uniform low frequency but, when connected normally in the ganglion, they periodically fire short, high-frequency trains of impulses. The periods of activity in all cells are approximately coincident, producing what is collectively termed the ‘burst’, which is followed by a quiescent period in all units (Maynard, 1955). Evidence so far accumulated indicates that the four posteriorly located ‘small cells’ or ‘pacemaker cells’ control the burst timing, one of them initiating the normal burst. These interact strongly with the five anterior ‘large cells’ or ‘follower cells’, producing firing in them. Output from these goes to the heart muscle, causing heart contraction. Pacemaker-pacemaker interactions are presumed but not rigorously demonstrated; follower-follower interactions via impulses are present but weak (Otani & Bullock, 1959); long-lasting potential changes in followers affect pacemaker firing and intracellular potentials of other followers (Watanabe & Bullock, 1960). As a step toward the more exact determination of the means by which the over-all ganglionic behaviour derives from the synaptic interactions of its component cells, the paths of the axons of the large and small cells within the ganglion have been determined physiologically, and a means for identifying the nerve impulses produced by each during a burst has been developed.

ANATOMY

Alexandrowicz (1932) has described the general form of the lobster cardiac ganglion and its associated nerves. Terminology used here attempts to follow his. The gross anatomy and the location of the large-cell bodies in the cardiac ganglion of Homarus americanus is relatively constant. Aberrant forms, though not used to determine main results, often showed physiological characteristics expected from the nature of their aberration, and thus proved a nice verification of the theory.

The normal ganglion (Fig. 1) is a ‘Y’-shaped structure composed of a medial trunk with right and left lateral trunks. The term ‘trunk’ in contrast to ‘nerve’ is applied to the portions of nervous tissue where the cell bodies and synaptic regions are normally found. In a lobster weighing 0.5 kg the medial trunk, from the posterior tip to the anterior bifurcation of the ‘Y’, is about 1 cm long and 0.3–0.5 mm wide over
much of its length; the lateral trunks are 2–3 mm long. Forming the anterior peripheral bounds of the lateral trunks are two large-cell somata. These were designated 'Cell 1' (right side of animal) and 'Cell 2' (left side). At the level of these somata, on either side, the dorsal nerve (D.n.) from the central nervous system enters the ganglion.

A connective, the anterior commissure, (Ant.comm.) leaves at this point and connects at the same level on the opposite side. Two anterior nerves (Ant.n.), which travel anteriad, close to the dorsal mid-line, are given off either from the commissure or close to its junction with the ganglion. As the branches of the 'Y' proceed beyond Cells 1 and 2, they become the large anterolateral nerves (A-l.n.). Half way between the anterior bifurcation and the posterior tip of the ganglion, dividing the medial trunk into the 'anterior trunk' and 'posterior trunk', paired posterolateral nerves (P-l.n.) are given off. As they progress peripherally, the posterolateral and anterolateral nerves communicate via the lateral commissures (Lat.comm.) which are probably homologous to the 'circular trunks' described by Alexandrowicz (1932) in *Cancer*. The anterior trunk contains three large-cell somata. Cell 3 is located at the anterior
bifurcation, Cell 5 where the posterolateral nerves come off, and Cell 4 usually half way between, often where a prominent nerve twig leaves the trunk. Several other such twigs are given off from the ganglionic trunk, especially the posterior trunk. The four small-cell somata are spaced along the posterior trunk (Alexandrowicz, 1932). From anterior to posterior I have numbered them Cells 6 to 9 inclusive. Large-cell somata, but not small-cell somata, can be seen in live preparations.

**MATERIALS AND METHODS**

The heart of a specimen of the lobster *Homarus americanus* weighing 0.5–0.8 kg was removed through a window cut in the dorsal carapace. It was pinned ventral side up in a dish of perfusion fluid (Cole, 1941), the ventral wall was slit longitudinally, and the sides were pinned out exposing the ganglion, which adheres to the inside of the postero-dorsal wall. In most experiments the ganglion was isolated completely from the heart muscle, leaving several efferent nerves intact for a few millimetres. In several experiments designed to determine what effects the isolation process had on ganglionic physiology, a semi-isolated preparation was studied before completing the isolation. In this preparation only certain of the efferent nerves (usually the posterolateral and anterolateral nerves of both sides) were freed and drawn up into oil for recording. The ganglionic trunk, with its numerous small nerve twigs, was disturbed as little as possible. In the isolated preparation the posterior end of the ganglion and cut ends of both anterolateral nerves were clamped in fine forceps, and several pairs of fine silver-wire hook electrodes (inter-electrode spacing ca. 1 mm) were positioned at various points beneath the ganglion and along the efferents. The ganglion and electrodes were lifted into mineral oil which had been equilibrated with perfusion fluid. Temperature (about 22°C) did not usually vary by more than 2°C during an experiment. Such a preparation survived well for 6 hr or more, though various changes in its activity were observed with time. Electrical activity recorded by the electrode pairs was amplified differentially and displayed on a multibeam oscilloscope. Electrical stimulation could be given through the same electrodes.

**Identification of efferent axons**

About thirty-five ganglia were studied sufficiently to identify most, if not all, efferent axons. Electrical stimulation of any of the various nerves leaving the ganglion typically elicits a compound action potential recordable in several pairs of recording electrodes. This action potential is composed usually of three or four components, each having a constant wave-form, each elicited by a stimulus voltage above a specific sharp threshold, and thus each taken to represent a nerve impulse in one specific axon. The nerve impulse from a given axon can be recorded by electrodes in certain regions of the ganglion and is absent from other regions. By systematically recording from all regions a physiological map of the excitable parts of an axon within the ganglion can be made. Except near cut ends, where depolarization blockage is expected, the wave-form of the nerve impulse is typically biphasic wherever it appears, except in one region. As it progresses into this one region it becomes monophasic, decreases in amplitude, and disappears. This is taken to indicate the approach of the impulse to the cell body, for nerve impulses are known not to invade the soma, as will be
Practically without exception, if an impulse from a given axon was recorded in an efferent, stimulation of that efferent could elicit an impulse in the same axon.

Impulses fired during the normal spontaneous burst of the ganglion by these same axons can be identified by their presence in the same combinations of electrodes as the corresponding antidromic impulses and by the identity of their wave forms to the antidromic ones, allowance being made for polarity inversions due to the opposite direction of impulse propagation past some electrodes. The identity of wave-form is further evidence that each sharp threshold of electrical stimulation represents but a single axon.

The region where the orthodromic impulse originated (trigger zone) for a given unit was determined by the direction in which its nerve impulse crossed the various electrode pairs, and the relative times of occurrence in each. Impulses were found to originate at a distance of 2–3 mm. from the cell body and to propagate primarily away from it (distally). Electrodes proximal to the origin would record a reversed polarity (distal electrode initially negative) potential in some cases. When such a potential was biphasic it was interpreted as an actively propagating, proximally directed impulse. When it was monophasic, however, it could represent either a propagating impulse that did not reach the more proximal of the two electrodes of the pair or the proximal electrotonic spread of more distal events.

The application of the method outlined above is best illustrated by the analysis of a typical experiment as shown in Fig. 2 (refer to the inset at the lower right for labelling of electrode positions). Frames F1, F2 and F3 show the three components elicited successively in response to stimulation of the right posterolateral nerve (E) as the stimulating voltage was increased. The first threshold encountered (frame F1) was for an impulse present only as a small monophasic potential in position C, located at the anterior end of the anterior trunk. It was absent from both branches of the 'Y', that is, from traces A', A, B' and B. Thus the impulse travelled in the stimulated axon from E up into the anterior trunk (solid line, diagram D1) but died out on approaching its cell body, being incapable of invading the soma (broken line, diagram D1). This behaviour identifies the axon as belonging to the cell situated at the anterior bifurcation, Cell 3, and owing to its presence in the anterior trunk, it is designated 3C. On increasing the stimulus voltage, a second threshold was reached (F2), this for an axon with an impulse biphasic in C, B, and B', developing a characteristic large slow monophasic form on approaching the Cell 1 soma in A, and absent from A' (D2). Thus this axon belonged to Cell 1. The third threshold was for a unit similar to Cell 1 reversing the right and left branches of the 'Y'; this is Cell 2 (F3 and D3).

Fig. 2. Efferent axon identification. This figure illustrates the technique used for determining the physiological anatomy of efferent axons, the somata from which they arise (all large cells, it turns out), and the occurrence of their impulses during the burst. The inset at the lower right shows, on a diagrammatic ganglion, electrode position nomenclature. Frames F1–F10 show compound action potentials elicited by stimulation of various efferent nerves. Diagrams D1–D11 schematically show corresponding deductions concerning the course of active axon (solid lines), inactive axon (heavy broken lines) and corresponding somata (filled-in circles) with the remainder of the ganglion represented by light broken lines. Records R1 and R2 show two spontaneous bursts with the occurrences of certain impulses circled and identified. See explanation in text. Scale: 0.1 sec.; 1 mV. for A', A, B', C, E; 400 μV. for B. Upward deflexion for posterior electrode negative.
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Fig. 2. For legend see facing page.
Having established these, the stimulation was shifted to the A' electrodes. Three thresholds were found: first, an impulse present in A and slightly in C, the A branch of a truncated Cell 4, thus designated $4_A$ (see below for discussion of truncated forms); second, one absent only from E, the Cell 5 axon; and third, one present in E, absent from B', with the characteristic large monophasic wave in B. This is Cell 2, stimulated this time via its A' branch. Similarly three thresholds to B' stimulation were found: first, Cell 5, characterized by being present in the opposite branch of the 'Y' and the anterior trunk but absent from the posterolateral nerves; secondly, a second axon belonging to Cell 3, designated $3_B$ and characterized by absence everywhere except as a small disappearing wave in B; and third, Cell 1 stimulated via its B' branch. The B branch of Cell 4 was not stimulated in B' probably because insufficient voltage was used, as evidenced by the small size of its B' spike; however, it was stimulated in a small efferent branch as shown in F10 and D10. It is distinguishable from $3_B$ by its large, propagating, biphasic wave in B. Cell $3_A$ (D11) was not seen in the stimulation experiment because, as is apparent from the record of the spontaneous burst, it is barely present in A at all in this preparation.

Having established the identification of the axons of various cells, their presence in or absence from electrode positions A', A, B', B, and E, and wave-form of their impulses at each of these positions, records R1 and R2 of the spontaneous burst recorded by these electrodes can be analysed. Units found in the burst can be identified by comparison with those identified by stimulation. Starting with the unit labelled 'a' (bottom of R1), it can be seen that this unit is present in E, B, B', and as a large monophasic slow wave in A. Comparison with F2 and F9 shows it to occur in the same combinations of electrodes and to have the same amplitude and shape as those components due to Cell 1. Two occurrences of impulses in Cell 1 are labelled in R2. Unit 'b' is present only in E, which, of the three axons stimulated in E, is behaviour to be expected only of $3_C$. Unit 'c' on the far right is present in E, B, A and A' but is absent from B'. In most traces it is seen to be identical with the antidromically stimulated impulse in Cell 2 (F3 and F6), though (probably due to an alteration of recording conditions) the size of the monophasic wave in B is smaller. Units 'd' and 'e' are similar in that each is restricted to one branch of the 'Y' yet they have anteriorly propagating (initially upward) impulses in B and A. Comparison of wave-form with F10 and F4, allowing for the inversion of polarity caused by the posterior direction of propagation of the antidromic spikes, shows them to be the $4_B$ and $4_A$ axons. Although their firing is not synchronized at the beginning of the burst, it is at the end ($4_{AB}$ in R1), thus representing a case of 'semi-truncation' (see below). Finally, unit 'f' is seen to be present in A', A, B' and B, but absent from E, and comparing to F5 and F7, again allowing for polarity inversion, it is found to be Cell 5. Other occurrences of these impulses are identified in R2 along with a $3_A$ and a $3_B$ impulse. With sufficient patience most of the occurrences of most of the units can be identified in each burst using two additional observations (first noted by Maynard, 1955): first, firing in each unit tends to be fairly regular, steadily declining from a high frequency at the beginning of the burst; secondly, the firing pattern for each unit strongly tends to be the same from burst to burst, so that for example the units 'a', 'd' and 'f' in R1 correspond to the firings of units 1, $4_B$ and 5 labelled in R2. Small impulses in A and B are from small-cell axons.
Identification of small cells

Axons identified by stimulation of efferents all belonged to the five large cells. Small-cell axons had high thresholds to electrical stimulation, small impulses easily obscured by impulses in other axons, and very similar courses in the ganglion, precluding study by electrical stimulation. Instead, they were identified during the burst on the basis of how far posteriorly their impulses appeared and on the parallel positioning of their trigger zones. The most posteriorly occurring impulse and trigger zone were assumed to belong to Cell 9, the next more anterior to Cell 8, and so on. Figure 3 illustrates this. The burst shown was recorded from several pairs of electrodes placed along the ganglionic trunk at positions indicated by the diagram. The most posteriorly occurring impulse, which was thereby assumed to belong to Cell 9 (circled impulse labelled '9') was present in the most posterior electrode pair (D) as an initially upward deflexion. Its small rapid form in this pair probably indicates that it arose between the two electrodes of the pair, somewhat closer to the posterior one. The impulse travelled anteriorly in all of the other electrodes. A posteriorly propagating component is not seen in this record as it occurred posterior to D. The only other small-cell impulse found in D is initially downward and hence triggered anterior to the Cell 9 trigger zone. Since it reached the next to most posterior level and had the next to most posterior trigger zone, it is identified as Cell 8. The Cell 7 impulse was absent from D and triggered between C" and C*. The Cell 6 impulse failed to reach C" and triggered between C and C*. Having established the impulse characteristics of each small cell, other impulses from them can be classified. As with the large cells, utilization of the regularity of the discharge and its constancy from burst to burst often allows the identification of almost every small-cell impulse in the burst.

RESULTS

I. Large cells

On the basis of their region of origin and direction of propagation, Maynard (1955) identified the large rapidly propagating impulses seen during the burst with the large cells and the small slowly propagating ones with the small cells. My results confirm this assignment and show that only large-cell impulses are present in efferent nerves. The physiologically determined anatomy of these axons (which unless stated is identical for ortho- and antidromic impulses) and their trigger zones during the burst are presented below. Figure 4 summarizes these results diagrammatically.

Cell 1 antidromic impulses are biphasic in the two posterolateral nerves, the anterior trunk, the left lateral trunk, and the left anterolateral nerve. They become monophasic and disappear as they progress into the right lateral trunk approaching the Cell 1 soma. They are the only impulses with the latter characteristic, hence their identification as belonging to the Cell 1 axon. A branch of the axon enters the anterior commissure from the left lateral trunk (i.e. the lateral trunk contralateral to the cell body). In some ganglia, at least, this crosses via the commissure into the ipsilateral anterolateral nerve, whereupon it proceeds peripherally. A small impulse is often present in the posterior trunk. Orthodromic impulses originate close to the anterior bifurcation and propagate away from it in three directions. During the burst, Cell 1
Fig. 3. Small-cell identification. Using several pairs of electrodes spaced along the ganglionic trunk (diagram), the four small-cell impulses can be distinguished by wave-form and posterior extent of occurrence (see text for details). Several occurrences of each impulse are labelled, as well as one example of each of the large-cell impulses present in these electrodes. The pacemaker cell, which initiates the burst, is Cell 7. In most preparations it was found to be either Cell 7 or Cell 6.

Scale: 0.1 sec; 400 μV., A, C, C', C''; 1 mV., D.
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Impulses are best identified by their characteristically large slow monophasic form in the right lateral trunk and by the fact that they are one of only three impulse types seen in the posterolateral nerves.

Cell 2 is the mirror image of Cell 1. Its major axonal branches as determined by its impulses correspond exactly to those of Cell 1 reversing right for left. One preparation had two axons with these properties and indeed proved histologically to have an extra cell body, located in the left lateral trunk.

![Diagram of cells](image)

Fig. 4. Physiologically determined anatomy and trigger-zone locations for neurons in Homarus cardiac ganglion. The normal course of active axon in the ganglion and its efferents is represented diagrammatically for each of the nine neurons. Inactive axon, which does not carry a regenerative impulse, is represented by a broken line. Normal trigger zones and directions of impulse spread are indicated with arrows. Cell 4 is represented as non-truncated.

Cell 3. Three functionally independent axons have been assigned to this cell, since antidromic impulses travelling back along all three become monophasic and disappear as they approach the anterior bifurcation, where the Cell 3 soma is located. All three fire orthodromic impulses independently during the burst.
The antidromic impulse in the axon designated '3A' is biphasic in the right anterolateral nerve, becoming monophasic as it passes Cell 1 to enter the right lateral trunk of the ganglion. The orthodromic impulse originates at about the level of Cell 1 and propagates peripherad. A small monophasic reversed-polarity potential (anterior electrode negative) is recordable proximal to the origin. This serves best to identify the unit during the burst.

The axon '3B' is the exact mirror image of 3A exchanging right for left.

The antidromic impulse in 3C is biphasic in both posterolateral nerves and in the posterior part of the anterior trunk. It becomes monophasic as it proceeds into the anterior region of the anterior trunk. A small impulse is often present in the posterior trunk. Orthodromic impulses trigger in the anterior trunk and travel posteriorly and in certain ganglia, at least, a short distance anteriorly as well. In the burst they are best identified by their presence in the posterolateral nerves and their absence from both branches of the 'Y'.

Cell 4 axons occur in two forms. In the 'non-truncated' form antidromic impulses are biphasic in both lateral trunks, in both anterolateral nerves and are often seen to enter the anterior commissure from both sides. They become monophasic and disappear a short distance down the anterior trunk. Orthodromic impulses originate near the bifurcation and propagate three ways. They are best identified by their synchronized appearance in both anterolateral nerves and their absence from the posterolateral nerves, but they must be distinguished from Cell 5 impulses, which have similar properties but which propagate further posteriorly in the anterior trunk.

In the 'truncated' form, antidromic impulses elicited in one anterolateral nerve only propagate into the lateral trunk of the same side and do not spread far into the medial trunk or contralateral branch of the 'Y'. The term 'truncated' is thus used to describe a condition in which an apparent blockage restricts the regions into which an antidromic impulse can spread compared with the 'non-truncated' condition. The occurrence of the two forms is discussed below. In the burst either there is a single trigger zone at the bifurcation or in one lateral trunk with the orthodromic impulse spreading to both branches of the 'Y' (semi-truncation), or there may be two independent trigger zones, one in each lateral trunk, with the impulse confined to the side where it originated (complete truncation). In the latter case the two independent axons are designated '4A' (right side) and '4B' (left side). They are similar to 3A and 3B impulses in being restricted to one branch of the 'Y' but usually originate more posteriorly than these, often producing an anteriorly directed biphasic impulse in the lateral trunk electrodes instead of the posteriorly directed small monophasic potential typical of 3A and 3B (see R2, Fig. 2). In some cases, however, they closely resemble 3A and 3B impulses, making identification by present means impossible.

Cell 5 physiological anatomy is the same as the non-truncated form of Cell 4 with the exception that impulses propagate further posteriorly in the anterior trunk, often beyond the Cell 4 soma. Orthodromic impulses usually originate in the anterior trunk and propagate anteriad, splitting at the bifurcation and continuing peripherad. When looked for they were observed to propagate posteriorly from the trigger zone as well. Sometimes they would originate at the bifurcation and propagate three ways. In the course of one experiment, the trigger zone shifted from anterior trunk to bifurcation. Truncated forms were found only occasionally.
II. Small cells

The physiological anatomy of the small cells, as explained under Methods, was determined from orthodromic impulses fired during the burst. The usual anatomy and trigger zones are as follows (see Fig. 4):

Cell 6 impulses originate in the middle of the anterior trunk. They travel posteriorly for a short distance before disappearing, and anteriorly to the anterior bifurcation. Here they split and propagate into both lateral trunks but disappear before they reach the somata of Cells 1 and 2.

Cell 7 impulses trigger at about the level of the Cell 5 soma. Like Cell 6 impulses, they travel a short distance posteriorly, and anteriorly into the lateral trunks.

Cell 8 impulses trigger in the anterior portion of the posterior trunk. Other characteristics are the same as for Cells 6 and 7.

Cell 9 trigger zone is in the middle portion of the posterior trunk; otherwise Cell 9 resembles the other small cells.

Small-cell impulses appear to be confined to the ganglion. As they travel anteriorly they split at the bifurcation in agreement with the histological evidence of Alexandrowicz (1932), but they are of decreasing size as they proceed into the lateral trunks and disappear at about the levels of the somata of Cells 1 and 2. They were never seen in the anterolateral nerves, nor in any other efferent nerves, including some branches arising from the posterior trunk. Perhaps this answers the long-standing question of the destination of their axons, although injury in efferent branches cannot be excluded. As Maynard (1953) noted, small cells can be stimulated electrically in the ganglionic trunk. The lowest-threshold axon stimulated in the posterior trunk is often identifiable as Cell 9, but as explained in Methods, impulses in other axons prevent such identification of other small cells.

III. Truncated large cells

Frequently large-cell axons were found whose antidromic impulses seemed to encounter a block at the anterior bifurcation, preventing them from spreading to all parts of the cell expected. If this excluded region contained an efferent segment that could be stimulated, impulses in it were usually excluded from the first region. Identification of these 'truncated' axons could generally be made on the basis of their replacing the non-truncated form, sometimes supported by circumstances under which such a unit could be de-truncated. An example of a truncated Cell 4 is shown in Fig. 2, where stimulation of its axon in either side of the 'Y' elicited an impulse restricted to that side rather than spreading to both as in non-truncated cases. In cases termed 'complete truncation', the truncated cell, during the burst, would have two independently firing axons separated by the apparently blocked region. In other cases, termed 'semi-truncated', the firings of the two axons would be synchronized for several impulses, at least, in spite of the block. In thirty-five ganglia, there were twenty-six cases of truncation of Cell 4, twelve of Cells 1 and 2, and three of Cell 5. Truncation of Cells 1 and 2 has been produced by stretching the bifurcation, but in seven semi-isolated preparations (see Methods section) where disturbances to the ganglion and bifurcation were minimal, six examples of truncated Cell 4 occurred and
one of non-truncated Cell 4. No major changes in any cells were observed on completing the isolation (5 cases). Somewhat rough treatment of this and other cases of non-truncated Cell 4 did not produce truncation.

DISCUSSION

Validity of cell identifications

The assignment of a given large-cell nerve impulse to a particular cell body was based on the observation that the impulse would become monophasic, decrease in amplitude and disappear as it approached that cell body. In the lobster cardiac ganglion such behaviour is expected of an impulse approaching its cell body, for it is known from the work of Hagiwara & Bullock (1957) on Panulirus and of Cooke (1966) on Homarus that the soma of a large cell in the cardiac ganglion is not invaded by the active spike. In Homarus, in fact, the electrotonic attenuation of the spike by the time it reaches the soma is so extreme that it is often difficult or impossible to identify it in an intracellular record. This agrees with the present observation that the disappearance of ortho- and antidromic impulses occurred a few millimetres from the soma.

There were cases where more than a single soma lay proximal to the point of disappearance of the impulse, and soma assignment for these requires justification. Cell 5 impulses and non-truncated Cell 4 impulses, when both were present, ended in the anterior trunk, sometimes both ending short of the Cell 4 soma. The thought might be entertained that they belong instead to small-cell axons, but the large size of the impulses, plus the fact that there are sufficient distinct small impulses to account for all small cells argues against this. The distinction of Cell 5 from Cell 4 was based on the assumption that the Cell 5 impulse was the one which propagated further down the trunk. In many cases it disappeared posterior to the Cell 4 soma, making its identification more certain. Even when it disappeared before reaching Cell 4, distinction based on the stated assumption seems the most reasonable, especially in view of the observation noted above that the impulses disappear at a considerable electrical distance from the soma. In the case of a truncated Cell 4 one might question whether sometimes it is not Cell 5 instead which is truncated. Where the antidromic Cell 5 impulse disappears posterior to Cell 4 this is not a problem. Where it stops short of Cell 4, in most cases it stops close enough to violate the large-electrical-distance observations.

The identification of the axons assigned to Cell 3 should also be examined, for there are three such independent axons compared with the usual one, or (in the case of a completely truncated cell) two, for the other cells. The antidromic 3C impulse sometimes propagates beyond (anterior to) Cell 4, making it unlikely that the impulse is carried by a posterior process of that cell. Both Cells 1 and 2 are adequately accounted for, so it seems unlikely that the 3C axon belongs to any but Cell 3. For anteriorly directed axons, Cells 4 and 5 are adequately supplied, so 3A and 3B are unlikely to belong to them. Cells 1 and 2 might have anteriorly directed processes, but they would be unlikely candidates since antidromic impulses in 3A and 3B are usually detectable posterior to their somata, and in some cases, at least, orthodromic 3A and 3B impulses originate posterior to them. In one preparation the Cell 3 soma was placed abnormally, part way out along one branch, and its trigger zones in that branch of the
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Y' (there were two—see below) were also displaced peripherally. Preliminary intracellular studies confirm the presence of electrotonically spread potentials in the Cell 3 soma corresponding to ortho- and antidromic impulses in 3A and 3B axons. Histological observations by Alexandrowicz (1932) indicate three processes arising from the cell, one in each branch of the ganglion. The observation that a single neuron in the cardiac ganglion can have more than a single functionally independent axon has been made previously by Otani & Bullock (1959), using intracellular electrodes, on Panulirus.

The number of small impulses seen during a burst corresponds exactly to the number of small cells. The somata of these cells are located in the posterior trunk (Alexandrowicz, 1932), yet one and usually two small-cell impulses have trigger zones in the anterior trunk, and at least one impulse is normally excluded completely from the posterior trunk. Thus a considerable length of axon not invaded by the impulse must be present between at least some and probably all, small-cell somata and their active axons. The spread of soma locations seen histologically and the spread in posterior extent of impulse penetration and corresponding trigger-zone location suggests the identification of successively located levels of penetration and trigger zones with successively positioned small-cell somata. If this is incorrect, there must be a grossly uneven distribution of inactive axon among the small cells.

In general the physiological anatomy presented here is consistent with the histological findings of Alexandrowicz (1932). Although the basis for soma assignment is not absolutely rigorous, these results show that there exists a set of units having well-defined mutually distinct characteristics, identifiable reliably from burst to burst and from ganglion to ganglion.

Truncated units

The impulse blockage at a branch point in truncated units might stem from a decrease in safety factor for impulse propagation (due to increased axonal surface at a branch point) making it especially susceptible to damage. It could be due to some other mechanism increasing susceptibility. On the other hand, results from the semi-isolated ganglion suggest that some truncated units do occur naturally. A fairly simple explanation for the natural occurrence of truncation might be proposed. All of the large cells have a rather extensive length of apparently inexcitable axon adjoining the cell body and extending for a few millimetres distally, which is incapable, under normal conditions, of sustaining a nerve impulse. If the bifurcation of a cell's axon and its vicinity were included in this inexcitable region a truncated cell would result. In the case of a non-truncated Cell 4, for example, an antidromic impulse is often in the process of running into inexcitable axon, that is of becoming monophasic, immediately posterior to the bifurcation. In such a case only a slight anterior extension of the inexcitable region to include the bifurcation would be needed to produce a truncated Cell 4. Considerable support for this theory was added by relating the distance of a soma from the bifurcation, when known, to the presence of truncation. Abnormal closeness was invariably accompanied by truncation in Cells 1, 2, 4 and 5. Incidence was reduced though not completely eliminated with increased distances. Thus it seems reasonable to view most cases of truncation as the natural result of the overlap of normal inactive axon with a branch point, the probability of this being
increased by proximity of the soma to the branch. A very interesting further confirmation of this was found with the Cell 3 soma mentioned above which was abnormally displaced into one of the lateral trunks. Its inactive axon apparently encountered a branch of the anterolateral nerve where the lateral commissure came off. Consequently it had two independent trigger zones on that side of the ganglion making a total of four independent Cell 3 axons.

The results presented here of themselves have implications concerning ganglionic physiology. The sequence: soma, inactive axon, trigger zone, active axon, is apparently present in all cells, large and small. It suggests an underlying unity of physiological structure perhaps related to the unity of morphological structure. In addition, the techniques developed allow a more thorough study to be made of the patterns of activity and synaptic interactions present in the ganglion. These will be the subject of a further communication.

**SUMMARY**

1. Simultaneous recording from several pairs of electrodes placed along the ganglion and certain efferent nerves, during stimulation of other efferents, allows the course of antidromic impulses in each stimulated axon to be mapped.

2. These impulses disappear as they approach their somata, being incapable of invading them, a fact which permits identification of a particular efferent axon with a particular soma.

3. By these means the courses of all such efferent axons, and their corresponding somata, have been determined. These all belong to the five large cells.

4. The impulses from each such axon occurring during the spontaneous burst can be identified, as can impulses from each small cell.

5. Each large-cell axon appears to be inexcitable until it is a few mm from the soma.

6. If the axon branches within this inexcitable region, the branches tend to fire impulses independently.

7. The technique of cell identification opens the way to a more complete analysis of the ganglion's activity and the synaptic interactions which produce it.

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