MORPHOLOGICAL AND FUNCTIONAL PROPERTIES OF IDENTIFIED NEURONS IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA

WESLEY T. FRAZIER,* ERIC R. KANDEL, IRVING KUPFERMANN, RAFIQ WAZIRI,* AND RICHARD E. COGGESHALL

Department of Physiology and Psychiatry, New York University School of Medicine, New York City, and Department of Anatomy, Harvard Medical School, Boston, Massachusetts

(Received for publication January 27, 1967)

THE ABDOMINAL GANGLION of the marine gastropod, Aplysia, is a valuable preparation for cellular electrophysiological studies. In large animals, most small nerve cells in this ganglion are comparable in size to the largest neurons in the vertebrate brain (50–100 μ), whereas the largest cells in the ganglion occasionally reach 1 mm in diameter. Furthermore, the majority of the cells are located near the surfaces of the ganglion and can readily be visualized under the dissecting microscope. In a given preparation it is therefore possible to directly visualize and penetrate many cells with micro-electrodes.

The cells within the ganglion vary greatly in appearance and in functional properties. Because of the difficulty in recognizing individual cells or homogeneous cell clusters from preparation to preparation, a great number of studies of this ganglion have been carried out on unidentified cells (7, 34, 41, 42), with exceptions generally limited to the two or three most obvious and constant cells (3, 5, 27, 39, 46). Although this limitation has not restricted biophysical and pharmacological studies on these neurons (18, 44, 47), it has been a major deterrent to studying the organizational and plastic properties of the ganglion. To overcome this difficulty, we have tried to identify all the prominent cells and cell clusters in the ganglion and have tried to relate them to the functional organization of the ganglion as a whole.

The possibility of identifying individual cells in the abdominal ganglion

1 This research was supported in part by grants from the National Institute of Mental Health (MH-12155-01) from the National Institute of Neurological Diseases and Blindness (NB-04550-03 and NB-05980-02) and from the National Science Foundation (GB-3595). During part of the tenure of this research additional support was provided to Eric Kandel by Career Program Award MH-18,558-01, to Irving Kupfermann by the National Institute of Mental Health postdoctoral research training program in Psychiatry (Harvard Medical School), to Rafiq Waziri by a postdoctoral fellowship from the Medical Foundation Inc., and to Richard Coggeshall by Career Program Award 1K3-GM-01, 754-01.

2 Present address: Yerkes Regional Primate Research Center, Emory University, Atlanta, Ga.

3 Present address: Dept. of Psychiatry, University of Iowa Medical School, Iowa City, Iowa.
of *Aplysia* was first recognized by Mme. Arvanitaki and her collaborators (2, 6). They described seven cells in the European species, *Aplysia depilans*, on the basis of position alone. Subsequently they added, as an additional criterion for recognizing three of these cells, their spontaneous firing pattern (3). Although these two criteria are important for distinguishing certain cells in the American species, *Aplysia californica*, they are frequently insufficient. We have therefore included, in addition to 1) position, and 2) spontaneous firing pattern (silent, regular, irregular, or bursting) the following criteria: 3) appearance (size, pigmentation, etc.), 4) spontaneous postsynaptic potential (PSP) pattern, 5) pathway of the efferent axon, 6) synaptic response to orthodromic stimulation, 7) depolarizing (D) or hyperpolarizing (H) response to iontophoretic application of acetylcholine, and 8) connections with other identifiable cells. These cells have also been examined by both light and electron microscopy. In many cases the cells that can be identified physiologically also have distinct morphological characteristics, and these characteristics can then serve as further identifying criteria for certain cells.

By using these multiple criteria we have been able to identify almost all of the large cells (30 cells) and cell clusters (8 clusters) visible on the dorsal and ventral surfaces of the ganglion. Seventeen cells can be clearly recognized as unique individuals which can be differentiated from each other and from the surrounding cells. An additional 13 cells can be identified but not yet as individuals. Eleven of these are the rostral white cells. Since each ganglion seems to contain a fixed number of these cells, we have assigned individual labels (R3–R13) to them, even though we cannot presently distinguish one rostral white cell from another. The remaining two cells (L8 and L9) are the most prominent representatives of two identifiable cell clusters (LB and LC). Finally, there are identifiable clusters consisting of homogeneous cells of undetermined number. Since in these clusters individual cells could neither be identified nor numbered we have assigned a label to the whole cluster (clusters LA, LB, LC, LD, RA, RB, RC, and RD).

As a beginning in the study of the organization of the ganglion we have utilized the identified cells and the cell clusters in the following ways: 1) to examine the relationships between electrophysiological function and morphological fine structure; 2) to examine several organizational features of the ganglion, e.g., to map the topographical distribution within the ganglion of cells that have similar physiological, pharmacological, and morphological properties; 3) to study the synaptic organization resulting from direct, common, and indirect connections among different identifiable cells, and, as will be reported in later work; 4) to study the relationship of the identified cells to peripheral sensory and motor structures and the participation of these cells in certain types of neural plasticity. The present paper will describe the morphological and physiological characteristics of the 30 individual cells and 8 clusters. The following paper will describe some direct and common connections between and among the identifiable cells.
METHODS

These experiments were carried out, over a 2-year period, on several hundred specimens of Aplysia californica supplied by Dr. Rimmon C. Fay of Pacific Bio-Marine Corporation, Venice, California. The animals were kept in natural or artificial (Marine Magic or Instant Ocean) sea-water aquaria with the temperature maintained at 14 C. The animals survived equally well in both media without feeding, for several weeks.

For dissection, the animal was firmly pinned to a wax dish in a fully extended position, and an incision was made along the full length of the animal. The abdominal ganglion, with its main peripheral nerves (the siphon, genital pericardial, and branchial nerves) and two connectives, was dissected from the animal, pinned by the edges of the connective tissue capsule to the paraffin base of a Lucite chamber, and covered with sea water. The chamber contained five pairs of Ag-AgCl electrodes for nerve stimulation. The left connective, the right connective, the branchial nerve, the combined genital-pericardial nerve, and the siphon nerve were each pinned over separate pairs of electrodes. The chamber was slowly perfused with sea water. The experiments were carried out both at room temperature (18-22 C) and at 14 C (the temperature was controlled by means of a thermoelectric cooling unit designed by Cambion). The identifying characteristics to be described were qualitatively the same at both temperatures.

Microelectrodes filled with 2 M potassium citrate were led through conventional unity-gain cathode followers to the differential d-c amplifiers of a multibeam oscilloscope. The electrodes had resistances of 5-10 megohms. A simple Wheatstone bridge (25) was used for simultaneously recording and passing current through the microelectrodes. A submerged coil of chlorided silver wire served as ground. The anatomical techniques for light and electron microscopy used in these studies were described in the previous paper (15).

A major technical problem was to insert microelectrodes into the cells even though they were overlain by thick connective tissue. In previous experiments this was generally accomplished by slicing the connective tissue with a microscalpel (41). However, this procedure invariably caused the exposed cells to pop out, resulting in distortion of the ganglionic architecture and loss of topographical relationships among the cells. In order to avoid this difficulty, the microelectrode was pressed against the undissected connective tissue overlying a given cell, and then the micromanipulator was gently tapped so that the connective tissue was pierced and the underlying cell penetrated. This procedure worked well, and with some practice it was possible to tap two independent electrodes into the same cell or into neighboring cells. In some cases, localized dissection of the capsule (27) was of additional help.

Orthodromic responses were determined by stimulating the nerves and connectives with threshold or slightly greater than threshold intensity. Very strong stimulation often produced complicated responses, only some of which will be described in the present report. Antidromic responses were distinguished from orthodromic ones by the appearance of typical, constant-latency, A or AB spikes which could be blocked by direct hyperpolarization of the cell body. Responses to ACh were determined by means of iontophoretic injection using micropipettes filled with 5%, w/v ACh in distilled water.

In some cases cells were marked by passing a dye iontophoretically through the recording microelectrode in order to secure a morphologic identification.

RESULTS

I. GENERAL STRUCTURE OF THE GANGLION

The ganglion is organized into an anterior neuropil which is continuous with the connectives and peripheral nerves, and an outer cortex of ganglion cells (Fig. 1A). The basic structure of each of these regions has been discussed in the previous paper (15).

The ganglion is represented schematically in Fig. 1A as two approximately spherical masses of cells, joined by a commissure and covered by a connective tissue capsule. The connectives, which run between this ganglion
FIG. 1. Simplified drawings of the abdominal ganglion. A: schematic horizontal section through the ganglion showing 1) the two rostral clusters of bag cells, 2) the left and right hemiganglia, 3) the pleuroabdominal connectives, and 4) the major nerves (to the periphery). The axons of the nerves, connectives, and ganglion cells enter the right and left neuropil which are the synaptic regions of the ganglion. The two neuropils are joined by the commissure. B: a schematic representation of the ventral surface of the ganglion. Shown in black are the identifiable cells which lie near the circumference of the ganglion and which can therefore be seen and impaled from either the dorsal or the ventral surface. The identifiable cells on the ventral surface are shown in white. Areas in which one can impale cells belonging to identifiable clusters (LB, LC, LD; RB, RC, RD) are stippled. The major portions of these clusters are on the dorsal surface.

and the pleural ganglia, and the major nerves, which go to the periphery, are also shown.

A useful first step in examining the ganglion is to pin it by the five nerves and the connective tissue sheath, thus stretching and flattening the ganglion. When flattened, the ganglion more closely approximates a dorsal and a ventral monolayer of cells separated by a central layer of neuropil. Most of the cells on a given surface then tend to appear in the same plane of focus, thus making it easier to identify cells (Fig. 2, A and B). The flattening, however, tends to obscure the fact that the cells at the periphery of the
dorsal and ventral surface are immediately adjacent to one another at the lateral edge of the ganglion. This point is of considerable importance because a slight shift in position of a cell located at the circumference can move it from the dorsal to the ventral surface, or vice versa (Fig. 1B).

Figure 2, A and B, is a closer approximation to what one sees when viewing the dorsal and the ventral surfaces of the ganglion through a dissecting microscope. The typical position of each of the identified cells is indicated. For descriptive purposes the left and right hemiganglia have been subdivided into rostral and caudal quarter-ganglia. Although the division...
of the ganglion into hemiganglia is natural, division into quarter-ganglia is arbitrary. The dorsal or ventral surface of each quarter-ganglion can be considered as a quadrant.

On the dorsal surface, the arbitrary boundary between the right rostral quarter-ganglion (RRQG) and the right caudal quarter-ganglion (RCQG) is a transverse line that bisects the ganglion (Fig. 2A). In smaller ganglia this line frequently lies at the caudal pole of R2, the largest and most easily identifiable cell in the ganglion. On the dorsal surface, the boundary between the left rostral quarter-ganglion (LRQG) and the left caudal quarter-ganglion (LCQG) is an arbitrary line separating the six large rostral cells from smaller more caudal cells. The left end of this boundary passes between L1 and L7 (Fig. 2A). The arbitrary lines which divide the dorsal surface can be extended to the ventral surface. A line passing just caudal to the rostral white cells separates the RRQG from the RCQG. A line passing just caudal to L5 separates the LRQG from the LCQG (Fig. 2B).

A few identified cells (lying near the circumference) can be seen either from the dorsal or ventral surface (Fig. 1B). Furthermore, some clusters of cells consistently extend over the circumference.

With the exception of two of the identified clusters (RC, RD) and one identified cell (R2) all identified cells and identified clusters consistently lie within one of the quarter-ganglia.

II. ASSUMPTIONS AND NOMENCLATURE

In attempting to develop a system of identifying cells in the ganglion, it was very useful to assume that the main cells are relatively constant in location and in morphological and functional characteristics. These assumptions were supported by the fact that many cells could be identified and that, as our experience with a cell or a cell group increased, it could be found with increasing ease and precision. However, the location as well as some morphological and functional characteristics of certain cells vary, at times considerably, from ganglion to ganglion. Thus, this study must present a simplified and idealized picture of the ganglion in which the identified cells are characterized by their most common location and by their typical physiological characteristics. Common deviation from the idealized position of each cell will be considered below. Some of the structural deviations from this idealized pattern of fixed cells seem to be accounted for by rotation of all or part of one of the quarter-ganglia. The functional deviations are more difficult to account for at present, although diurnal, seasonal, and maturational variables are likely to be involved.

In devising a nomenclature for the identifiable cells of the abdominal ganglion, we realized that our descriptions would probably be incomplete, and that new cells would be identified in the future. The following system is “open ended” and relatively simple. The cells were each assigned: 1) a letter (R or L) indicating the hemiganglion in which it is found (right or left), as viewed from the dorsal surface, and 2) an arbitrary number, begin-
ning with "1" on each side. It should be pointed out that cells with the same number in the right and left hemiganglia (e.g., L3 and R3) do not necessarily bear any special relationship to one another. The identifiable cell clusters were assigned: a) a letter (R or L) indicating the hemiganglion in which it is found, and b) an arbitrary letter beginning with "A" on each side.

III. GROUPS OF IDENTIFIABLE CELLS

In the following description of the identifiable cells we will be guided by two frameworks: A, the arrangement of cells into topographical groups and B, the arrangement of cells into functional groups as indicated by their physiological and morphological properties.

The topographical groups will be considered in the following order: 1, the bag cell clusters (RA and LA); 2, the right rostral quarter-ganglion; 3, the right caudal quarter-ganglion; 4, the left rostral quarter-ganglion; 5, the left caudal quarter-ganglion; 6, the symmetrical cells (L1 and R1) and the giant cell R2; and 7, the remaining identified clusters of cells (RB, RC, RD, LB, LC, LD).

The functional groups will be considered in the following order: 1, the cells sending axons into the connectives; 2, cells sending axons to the periphery; 3, cells categorized according to spontaneous firing patterns; 4, cells showing D or H response to local application of ACH\(^4\) and 5, granule-producing cells.

Since a large number of identified cells contain granules, it should be pointed out that the granules in all granule-containing neuronal perikarya are of the same shape (round) and the same size (1,000–4,000 Å in diameter with the majority being between 1,500 and 2,500 Å). The granules in different cells can be distinguished by 1) their frequency of occurrence, 2) the electron density of their cores, and 3) whether or not they undergo a morphological change as they pass from the perikaryon to the cell process. These differences will be discussed when the individual topographical groups are considered.

A. Topographical groups

1. Left and right cluster of bag cells (RA, LA). The bag cell bodies are located in two clusters at the junctions of the left and right connectives with the ganglion (Fig. 2). In relatively large animals (60 g or more) the pocket in the connective tissue sheath that holds the bag cells has a considerable volume and is easily visualized from either the dorsal or the ventral surface.

\(^4\) Tauc and Gerschenfeld (47) have shown that the cells in this ganglion can be categorized into two groups according to their response to acetylcholine: cells which responded with hyperpolarization (H cells) and cells which responded with depolarization (D cells). ACh appears to be the inhibitory transmitter in H cells (which receive noncholinergic excitation) and the excitatory transmitter in D cells (which receive no inhibitory input). More recently Gerschenfeld and Tauc (20) have encountered D cells with inhibitory inputs and have therefore subdivided the D category into three: 1) D cells (no inhibitory input), 2) DIN1 cells (D cells with noncholinergic IPSP's), and 3) DILDA cells (D cells with inhibition of long duration). These various distinctions will be discussed below as the responses to ACh of the identified cells are considered.
The pocket is shaped like a bag with its mouth opening perpendicularly onto the axons of the connective and its closed end extending back into the sheath. Although all bag cells appear alike in any one animal, they undergo striking changes as the animal grows.

In the smallest animals studied (2 g) there are probably less than 100 small (less than 10 μ) bag cells in each cluster. These cells are not yet attended by glial cells and their processes extend only a slight distance from the perikaryon. In contrast to the bag cells in larger animals, there is no morphological evidence that these cells produce granules.

In these small animals, the bag cells are too small to be studied with the light microscope. However, if thin sections of the ganglion-connective junction are examined, many small cells are seen. A large number of these are the glial cells typical of the connective as evidenced by their tonofilament-filled cytoplasm and numerous long processes which surround the axons (15). At the periphery of the connective, however, there are some small cells which are clearly different from the glial cells. These cells, which are found only at the ganglion-connective junction, possess few tonofilaments and their processes do not extend between the axons. Instead, the cytoplasm of this second cell type contains a prominent Golgi apparatus, many cisternae of the granular endoplasmic reticulum, and occasional lysosome-like bodies. Because of their location and cytologic characteristics, we regard them as immature bag cells. Since the bag cells cannot be distinguished from the glial cells in the light microscope, they cannot be accurately counted, but in thin sections they are relatively sparse and it is unlikely that there are more than 100 in each cluster.

In slightly larger animals (5–20 g) the bag cells are more numerous (125–150 cells in each cluster, Table 1 in ref. 15). These cells differ from those found in the smallest animals in a number of ways. First, the cell bodies have become unipolar with a perikaryal diameter of approximately 20 μ (Fig. 3A). Second, the cell bodies and the initial segments of their processes are now attended by glial cells. Third, the processes of the bag cells have thickened and lengthened and now follow a complex path to their destination in the sheath (Fig. 3A). Fourth, there is morphological evidence that granules are now being produced in the perikarya and transported down the axons (Figs. 3B, 4).

<table>
<thead>
<tr>
<th>Cells</th>
<th>Size and Shape</th>
<th>Frequency</th>
<th>Electron Density of Core</th>
<th>Transformation of Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bag cells</td>
<td>Round, 1500–2500 A</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>White cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3–R13</td>
<td>Round, 1500–2500 A</td>
<td>Many</td>
<td>Dense</td>
<td>No</td>
</tr>
<tr>
<td>R14</td>
<td>Round, 1500–2500 A</td>
<td>Many</td>
<td>Dense</td>
<td>No</td>
</tr>
<tr>
<td>R15</td>
<td>Round, 1500–2500 A</td>
<td>Few in small animals, many in large animals</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>LRQG cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2, L3</td>
<td>Round, 1500–2500 A</td>
<td>Few</td>
<td>Light</td>
<td>No</td>
</tr>
<tr>
<td>L4, L6</td>
<td>Round, 1500–2500 A</td>
<td>Many</td>
<td>Moderate</td>
<td>No</td>
</tr>
</tbody>
</table>

* See footnote 6 in text.
FIG. 3. A: a drawing of the edge of a bag cell cluster. The bag cells send their processes toward the longitudinally oriented axons of the connectives. When the processes meet the axons in the connective, they wrap around them. From this point the bag cell processes either turn laterally into the sheath or they turn rostrally and travel between the axons of the connective and the sheath, and then laterally. The bag cell processes end within the sheath and no morphological evidence for functional contact between these processes and other axons or muscle cells within the sheath has been obtained. B: a diagram of the ganglion with the area shown in A outlined. C: the nucleus and perinuclear cytoplasm of a bag cell body. The nucleolus in the bag cell nucleus is remarkable in that many of the particles
The bag cell processes in animals larger than 5 g end within the sheath without showing morphological evidence of functional contacts with other axons or with effector cells. It has also been demonstrated that arterial blood is emptied directly into the interstices of the sheath and there presumably comes in contact with the granule-filled bag cell processes (15). No morphological evidence for functional contacts between bag cell processes and other axons or effector cells in the sheath has been found. The demonstration that granule-filled neuronal processes end in a vascularized sheath without showing morphological evidence of synaptic contact with other axons or with effector cells suggests that the sheath and the neuronal processes which end within it form a neurohemal organ (cf. ref. 10). The bag cells thus fulfill the morphological criteria for neurosecretory cells.

In relatively large animals (25–200 g) there are no obvious changes in the basic cytologic organization of the bag cells and the destination of their processes remains the same (see below). Nevertheless, the quantitative changes are impressive. The perikarya increase in size to 50 μ or more and they increase in numbers to 400 or more per cluster (15). The individual cells are now large enough to be seen with the dissecting microscope. There is also an enormous increase in the number of bag cell processes in the sheath, so that in animals of 150 g or more, the cross-sectional area of the bag cell processes at the ganglion-connective junction may exceed that of the axons of the connectives.

As the animals enlarge, the space in which the bag cell perikarya are found also enlarges and the bag cells come to be surrounded by an increasing number of glial cells. These glial cells do not resemble the nearby glial cells of the connective. Rather they are similar to the glial cells that surround neuronal perikarya elsewhere in the ganglion. Their origin is not clear in this material.

Each bag cell in large animals sends a single stout process inward toward the axons of the connectives (Fig. 3A). When the bag cell processes reach the axons in the connective, they wrap around them and form a cuff or collar which surrounds the longitudinally oriented bag cell processes then turn rostrally toward the pleural ganglia and travel between the axons of the connective and the fibrous sheath. Thus as one proceeds from the ganglion-connective junction rostrally, the circularly oriented cuff of the bag cell axons gives way to a cuff of longitudinally oriented bag cell axons that run parallel with, but superficially to, the axons in the connective. At frequent intervals these longitudinally oriented bag cell processes turn laterally into the sheath (Fig. 4B). Most processes enter the sheath within a centimeter of each bag cell cluster and all have entered at a point midway between the pleural and abdominal ganglia. The processes may travel some distance in the sheath before they end. Many pass back over the bag cell cluster to end in the sheath over the rostral part of the ganglion. Others pass forward in the sheath that surrounds the axons of the connectives but all of these end before the midpoint between the abdominal and pleural ganglia is reached.

In animals weighing more than 5 g, a major cytologic reorganization accompanies the change in bag cell size and extent. In these animals the perikarya become filled with granules which are round and have moderately electron-dense cores (Fig. 3B). As the granules are traced into the bag cell processes, the cores become smaller and less electron-
Fig. 4. A: at one point in their course, the bag cell processes wrap around the axons in the connective. This is a cross section of the connective at a point where the bag cell processes are found and it can be seen that the periphery of the connective consists of bag cell and glial processes. The axons of the connective are located centrally (not shown). The granules in the bag cell processes here differ from those in the cell body (Fig. 3B) by having ruffled, crenated and sometimes broken membranes, and by having less electron-dense cores. \( \times 39,000 \). B: from their position surrounding the axons of the connectives, the bag cell processes turn laterally into the sheath and there end. In this picture, which is an al-
dense, and the membrane of each granule becomes ruffled or crenated and is frequently broken (Fig. 4, A and B). These findings probably indicate that the granular contents are being released either into the sheath or into the extracellular space at the side of the connective, although the possibility that the content of the granules changes and becomes less amenable to fixation cannot be definitively excluded. The changes which the granules undergo as they pass down the bag cell processes distinguish these granules from those in the other granule-containing neurons in this ganglion.

In the largest animals (300–900 g) the bag cells are approximately 50 μ in diameter and can be impaled if they are first exposed by carefully slitting the surrounding connective tissue. The exposed cells exhibit some of the same opaque whiteness that characterizes the RRQG white cells, a second presumed neurosecretory cluster to be described below.

The bag cells have remarkably similar electrophysiological properties. Upon impalement the bag cells are usually silent, but they can be made to generate an action potential in response to intracellular current pulses. Moreover, stimulation of the connectives frequently causes a sustained discharge of the bag cells. When the bag cells are recorded from two at a time (Fig. 5), it is evident that all the bag cells on the same side invariably fire in synchrony (30). Underlying the synchronization is a prepotential (Fig. 6) which is similar and simultaneous in all of the bag cells on the same side. The mechanism of this synchronization has not yet been worked out but it appears to be mediated either by an electrical connection among bag cell processes or by an interneuron which innervates all 400 bag cells in the cluster. Attempts to define the possible areas of electrical interaction or innervation by morphological means have yet not revealed any areas of specialized contacts.

2. Right rostral quarter-ganglion. The identified cells of the RRQG are the rostral white cells (R3–R13), and R1 and R2 (Fig. 2, A and B).

Because R1 and R2, along with L1, constitute an unusual structural and functional group, they will be described in section 6.

One of the most striking characteristics of the rostral white cells (R3–R13) is that they have an opalescent appearance when examined with transilluminating light. This appearance contrasts strongly with the translucence and orange color of all other ganglion cells except R14 and R15. Under reflected light, the contrast is even more striking, for R3–R15 are white and the other cells are orange or brownish red.

With reflected light, L5, the bag cells, and some unidentified cells also have a whitish appearance although they are considerably less striking than the rostral and caudal “white”...
Fig. 5. A: simultaneous records from two ipsilateral bag cells (cluster LA) which have been set into activity by stimulating the left connective. B: at higher amplification to better illustrate the close synchrony of the two cells.

Fig. 6. Prepotentials in bag cells in response to stimulation of the ipsilateral connective. These records were made from a cell that had previously been set into activity by a stimulus train to the ipsilateral connective. At the time this record was made, spontaneous activity had ceased, and the cell could not be set into repetitive activity. A: a single shock produced an all-or-none prepotential. B: when the frequency of stimulation was increased, the prepotentials appeared to summate and trigger a spike. C: shows that the prepotential could disappear in an all-or-none fashion during constant-intensity repetitive stimulation of a connective. Although this prepotential resembles an EPSP, all attempts to alter the amplitude of the prepotential by changing the membrane potential of a bag cell have been unsuccessful; we therefore cannot distinguish between a remote EPSP or an electrotonically propagated axonal spike.
cells. Also, L2, L3, L4, and L6 occasionally have a faint tinge of white. The cytologic characteristic which distinguishes all of these cells from the pigmented cells in the ganglion is their content of granules, the rostral and caudal white cells having many granules per unit of cytoplasm and the rest having fewer.

The number of white cells that can be seen in the dissecting microscope is usually 11 (Fig. 2A and B), but in some ganglia as few as 6 can be clearly seen. The dorsal and ventral members of the cluster are continuous over the rostral margin of the ganglion, near the midline. Thus, slight movements of the rostral white cell cluster can result in significant shifts in the number of white cells visible on either the dorsal or ventral surfaces of the ganglion. Typically, the dorsal white cells, including R14 and R15, are large (500 μ or more) and can often be seen with the naked eye. The ventral white cells are smaller and usually form a characteristic rosette (Fig. 2B).

The rostral white cells have common morphological and physiological properties. They are morphologically distinctive because of 1) the presence of myriad characteristic granules (Figs. 7B, 8, 9), 2) the relative absence of pigment bodies (15), 3) the destination of their cellular processes (Fig. 7A), and 4) the numerous deep infoldings of their plasma membranes (15).

When examined under the electron microscope the white cells were found to be filled with granules which differ from those found in most other granule-containing cells by the marked electron density of their cores (Figs. 7B, 8, 9). These granules resemble the “elementary neurosecretory granules” described by Rosenbluth (35) and Simpson et al. (37) in unidentified cells in the ganglion.

As in the other granule-containing cells in the nervous system, the granules seem to be formed in the Golgi complexes (Fig. 8A), but they are distributed evenly throughout the white cell perikaryon and its processes. In occasional animals, however, parts of the processes become swollen with very tightly packed granules (Fig. 7B). These granule-packed swellings are similar to the Herring bodies seen in the vertebrate supraoptic-neurohypophyseal system (8).

The rostral white cells send processes into the sheath that surrounds the ganglion (Fig. 7A). The number of these processes is correlated with the size of the animal, and thus with the size of the white cell. In an animal weighing 2 g, only a few processes filled with the characteristic granules can be seen in the sheath, while in a 100-g animal, there may be several hundred processes in the sheath. Despite the great proliferation of white cell processes as the animal grows, the number of white cells (in contrast to the marked change in the number of bag cells) does not change; rather, each white cell emits more processes. The white cell processes intermingle with the bag cell processes in the sheath (Fig. 4B) and, in any animal larger than 5 g, there are about 10 times as many bag cell processes as white cell processes. Serial sections show that the white cell processes also end within the sheath without morphological evidence of functional contact with other axons or effector cells. Thus, as with the bag cells, the rostral white cells fulfill the morphological requirements for neurosecretory cells.

The relationship of whitish appearance to the neurosecretory morphology described above was carefully examined in many ganglia in which prominent white cells seen grossly could readily be identified in both light- and electron-microscopic sections. In addition, two rostral white cells were marked by passing dye iontophoretically from recording microelectrodes. In all instances, the cells which were grossly white contained the characteristic granules when examined with the electron microscope. However, the reverse was not true.
Some cells which contained granules were not visualized as white under the dissecting microscope. As indicated above, only from 6 to 11 rostral white cells can be seen with the dissecting microscope. In serial histological sections, however, 12 cells (the number varies from 11 to 14) containing the characteristic granules and processes are consistently seen. The reason that some neurosecretory cells were not seen as white cells in the dissecting microscope is not clear. In some cases white cells were clearly hidden under larger pigmented cells and could not be seen from the surface. But it is also possible that some neurosecretory cells do not appear white because they do not contain a high proportion of granules (see discussion of R1b).

Each of the white cells also sends a single stout axon into the neuropil (Figs. 7A, 9). The axons of the rostral white cells gather within the neuropil and travel, in a characteristic bundle, down the medial side of the neuropil of the right hemiganglion to emerge in the branchial nerve. From an occasional axon a collateral separates and crosses the midline to emerge in the genital-pericardial nerve. The peripheral destinations of these fibers are not yet known. Although the white cell axons can be traced throughout the neuropil and many thin sections of these axons have been taken at regular and irregular intervals, no morphological evidence that a white cell axon is presynaptic to another axon has been obtained. Furthermore, at all levels of the neuropil, very few synaptic boutons seem to end on the white cell axons, a finding consistent with the physiological observations that these cells are relatively insensitive to neural input (Fig. 9). Even when vesicle-filled axonal swellings do abut on a white cell axon, the typical cytoplasmic thickenings and enlarged synaptic clefts are not seen (Fig. 9).

The vesicle- or granule-filled neuronal profiles which end on the white cell axons can be categorized as follows. Approximately half are filled with granules which are approximately 1,000 Å in diameter and possess very electron-dense, small cores. An occasional profile is filled with small clear vesicles, 400-500 Å wide, which resemble the synaptic vesicle found at cholinergic neuromuscular junctions. Some profiles have both small clear vesicles like those described above, and granules that are approximately 1,200 Å in diameter, and have a diffuse moderately electron-dense interior.

The electrophysiological properties of the rostral white cells are quite uniform. The cells usually fire spontaneously at a highly regular and slow rate of about 0.5–1/sec (Fig. 10). The high degree of regularity appears to result from a regular endogenous pacemaker rhythm in the cell, rather than from a regular synaptic input. The endogenous nature of the pacemaker rhythm was established by 1) failing to demonstrate rhythmic synaptic potentials underlying the spikes, even when the cell is markedly hyperpolarized.

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**FIG. 7. A:** A drawing of two white cells. Note the processes that extend into the sheath and the single axon that passes through the neuropil to enter the branchial nerve. The processes in the sheath, the cell bodies, and the axons all contain large numbers of granules that are characteristic for these cells. **B:** In certain animals, either the white cell processes that enter the sheath or the axons that enter the branchial nerve develop localized swellings that are packed with granules. The function of these granule-filled swellings is not known, but they resemble the Herring bodies seen in the mammalian supraoptico-neurohypophysial system. ×9,000.
or depolarized; and 2) resetting the rhythm of the bursting cell by interjecting spikes, which should not alter the time of arrival of synaptic potentials produced by another cell.

The action potentials of the rostral white cells have a duration of about 20–25 msec, which is two or three times that of most neighboring cells. The spike is usually followed by a relatively small hyperpolarization, which differs from the larger after-hyperpolarization typical of neighboring non-neurosecretory cells and of most spontaneously firing identifiable neurons in the ganglion (Fig. 10B). All of the rostral white cells, without exception, show antidromic response to stimulation of the branchial nerve, confirming the morphological evidence that a neurosecretory tract emerges in that nerve (Fig. 10C).

The rostral white cells gave relatively weak responses to stimulation of major pathways into the ganglion (Fig. 10, A and B). This finding is consistent with the morphological finding of a paucity of terminal boutons ending on white cell axons in the neuropil. Single shocks delivered to the two connectives, or to the genital-pericardial or siphon nerves, usually had little or no effect on the spontaneous firing rate. Stimuli to the branchial nerve produced a slowing which seemed in large part attributable to the hyperpolarizing afterpotential following the antidromic spike since it could be simulated by directly initiated spikes. Trains of stimuli (6/sec for 1 sec), particularly those to the peripheral nerves, did produce changes in firing rate in a number of white cells, consisting of a slight increment followed by a slowing (Fig. 10, A and B). In clear contrast to the weak response obtained in the white cells, nerve and connective stimulation always produced large and distinct synaptic responses in all the other identified cells in the ganglion and in the majority of unidentified pigmented cells, even those which border on the white cells (Fig. 10A). A weak response to nerve stimulation was, however, also characteristic of a few small pigmented cells which lie within the white cell cluster on the ventral surface. Whether these latter are small pigmented neurosecretory cells, similar in other respects to the larger white cells, or whether they are a different class of cells which have a weak synaptic input, can only be decided by marking the cells with microelectrodes and examining them morphologically. This has not yet been done.

The rostral white cells were hyperpolarized by ACh and therefore fall

Fig. 8. A: the perikarya, processes, and axons of the white cells, R3–R14, contain many granules. These granules are round, have an average diameter of approximately 2,000 Å and, in comparison with the granules of other cells in this ganglion, have very electron-dense cores. This is a picture of the perikaryon of R14. The granules in these white cells, as well as those of other granule-containing cells, take shape in the Golgi complex (G.C.). In the Golgi cisternae localized regions of electron-dense material accumulate (unlabeled arrows) and are eventually pinched off, forming the final granule. ×42,000. B: this is a white cell axon in a peripheral nerve. Note that the granules are essentially unchanged from those illustrated in the perikaryon. ×26,000.
into the category of II cells. These cells also occasionally show hyperpolarization following the turning on of the substage illumination.

3. Right caudal quarter-ganglion. The identified cells of the RCQG are the caudal white cells (R14 and R15) and R16 (Fig. 2, A and B). In addition, the RCQG contains the identified cluster RB and parts of clusters RC and RD. The clusters will be considered in section 7.

The right hemiganglion has, in addition to the rostra1 cluster of white cells, two caudal white cells: R14 and R15 (Fig. 2A). Both cells are always seen on the dorsal surface; occasionally an edge of either cell can also be seen on the ventral surface (Figs. 1B and 2B). R14 usually is in line with the entrance of the branchial nerve, but at times it is medial or lateral to the entrance. R15 is the largest cell near the midline on the dorsal surface of the RCQG. It usually lies at the mediocaudal tip of the hemiganglion but occasionally it is found more rostrally.

Cell R14 is similar to the rostra1 white cells: it has the same type of granules, its processes end within the sheath, and it is a neurosecretory cell by the same criteria.

The major morphological difference between R14 and the rostra1 white cells is the extent of its axonal tree. As its axon passes into the neuropil, one branch invariably enters the branchial nerve to join the axons of the rostra1 white cells. The other branches are more variable. The most common pattern is as follows: 1) one branch enters a small nerve that exits from the caudal pole of the midline and goes to a tributary of the dorsal aorta, 2) another branch passes rostrally in the right neuropil to emerge in a small nerve that leaves the ganglion just caudal to the right bag cell cluster, 3) a large branch frequently crosses the midline and exits in the genital nerve; occasionally this branch bifurcates so that another process emerges in the siphon nerve. In approximately one out of three ganglia one or more of the above branches cannot be found.

Physiologically R14 resembles the rostra1 white cells except that it frequently shows, as expected from the morphological data, an antidromic spike from stimulation of the siphon and genital-pericardial nerves, in addition to its invariable antidromic response from the branchial nerve. Also R14 often has no spontaneous spike activity.

R15 also contains granules, but it differs markedly from the other white cells in that: 1) it is distinctly white only in large animals, 2) its granules have

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Fig. 9. Possible synaptic terminals on the white cell axons are sparse. When they do occur, they lack the cytoplasmic and intercellular specializations that characterize the vertebrate synapse and some of the synapses in this nervous system (15). Three such possible terminals on the white cells are shown here. In A the possible presynaptic element (arrow) is filled with small vesicles. In B and C the possible presynaptic elements (arrows) contain moderately large granules with a small very electron-dense core. It is not known whether such junctions are functionally active. A, ×33,000; B and C, ×41,000.
less electron-dense cores, 3) it sends no processes into the sheath, 4) it fires in bursts, and 5) it has a strong synaptic input.

The gross appearance of R15 varies considerably with the size of the animal. In animals that weigh less than 15 g, R15 is translucent and orange colored. In animals that weigh from 15 to 100 g, the cell is sometimes translu-

Fig. 10. Responses of rostral white and pigmented cells to stimulus trains (8/sec for 1 sec) applied to afferent nerves and connectives. The duration of the train is indicated by the arrows. A: a simultaneous recording from two rostral white cells. B: a simultaneous record from a white cell in the rostral cluster on the dorsal surface (lower trace) and from a neighboring pigmented cell (upper trace). The pigmented cell is probably a member of identified cluster RD. Note that the stimulus trains produce only weak responses in the white cells (A and lower trace of B) and brisk responses in the pigmented cell (upper trace). The responses of the white cell ranged from practically nothing (upper trace in A and lower trace in B), to a slight or moderate slowing (lower trace in A). C: a simultaneous recording from two white cells showing the antidromic action potentials which follow stimulation of the branchial nerve. The voltage calibration at the beginning of the sweep in A and B is 10 mv. The time calibration in B applies to both A and B and is 2.5 sec. The calibrations for C are 20 mv and 40 msec.
IDENTIFIED NEURONS IN *APLYSIA*

... and orange and sometimes opaque and white, but usually it appears turbid or milky with a faint orange tinge. In animals larger than 100 g, the cell is distinctly opaque and white and looks like the other white cells, R3–R14.

Since the visible appearance of R15 varies strikingly in different sized animals, this cell provides an excellent opportunity for correlating the relative numbers of cytoplasmic granules with the gross appearance of the cell. In small animals where the cell appears translucent and orange under the dissecting microscope, the granules are thinly scattered throughout the cytoplasm (Fig. 11A). In the 15- to 100-g animals, where the cell usually appears turbid, the granules are somewhat more concentrated and in the largest animals, where the cell appears white even to the naked eye, the granules are very closely packed. It is difficult to escape the conclusion that the relative number of cytoplasmic granules is responsible for the gross appearance of the cell. These findings would indicate that the gross appearance of a cell can be used as a rough measure of the number of intracytoplasmic granules.

The axon of R15 passes ventrally into the neuropil and then turns rostrally and travels forward on the medial side of the right hemiganglion until it reaches the commissure that joins the two hemiganglia. The axon crosses in the commissure and then turns caudally and appears to enter the genital-pericardial nerve. Occasionally the axon splits, and one branch also enters the siphon nerve.

The axon of R15 receives more “terminals” than do the axons of the rostral white cells and R14. Many terminals are filled with relatively large vesicles having a diameter of approximately 1,200 A. Another common type of terminal is filled with granules that range from 1,000–1,200 A in diameter, and whose interiors are only slightly more electron-dense than surrounding axoplasm (Fig. 12A). Finally, an occasional terminal is filled with large (approx 1,200 A) granules containing electron-dense cores. In occasional places the axons of R15 appear to be presynaptic to some small unidentified neuronal processes (Fig. 12B).

When studied with intracellular microelectrodes R15 was usually spontaneously active, showing a burst pattern of 15–20 spikes occurring fairly regularly every 5–20 sec, with silent periods between the bursts (Figs. 13 and 14; also see ref. 3, 5, and 39 for earlier descriptions of this cell). In confirmation of the findings of these earlier workers, our data indicate that the burst rhythm is endogenous, and not the result of a rhythmic synaptic input.

In most preparations no rhythmic synaptic potentials which could account for the bursting rhythm can be seen in this cell, even when it is markedly hyperpolarized or depolarized. In addition, the time of onset of the burst can be altered by interjecting spikes by means of brief intracellular depolarizing pulses. Further evidence that the rhythm in this cell is endogenous has now been provided by Alving (personal communication) who in one instance demonstrated a persistence of the bursting rhythm in a successfully isolated soma of R15.

Although the rhythm of R15 is endogenous, it can be altered by a number of factors. The cell receives synaptic inputs, which on occasion can be rhythmic, and which appear to interact with the endogenous rhythm of the cell to produce the final rhythm. One source of spontaneous excitatory synaptic potentials comes from L10 (see Figs. 6, 8, 9 in ref. 24).

In addition to the synaptic input from this and other interneurons, R15
IDENTIFIED NEURONS IN APLYSIA shows a significant synaptic response to the stimulation of nerves and connectives. Stimulation of the right and left connectives produces small EPSP's. In addition, stimulation of the right connective produces a large unitary and presumably monosynaptic EPSP. Stimulation of the branchial nerve produces a complex diaphasic synaptic potential consisting of a brief depolarization and a prolonged hyperpolarization (Fig. 14). The prolonged hyperpolarization usually lasts 10-60 sec and resets the bursting rhythm of R15. At times the hyperpolarization may last a number of minutes and may completely suppress burst generation for this period. This complex synaptic potential resembles the inhibition of long duration first described in unidentified cells by Tauc (43) as a sequela to an excitatory synaptic potential. We propose to refer to this synaptic complex as "inhibition of long duration following excitation (ILD-E)" in order to distinguish it from "inhibition of long duration following inhibition (ILD-I)," another synaptic process which occurs in L10, and was described by Chalazonitis and Arvanitaki (13) in an unidentified cell.

Studies by Arvanitaki and Chalazonitis (4) and Chalazonitis (12) indicate that the rhythm of spontaneously active cells in the ganglion can be altered by ionic factors and by exposure to light. We have not investigated ionic factors, but we have observed that light onset tends to hyperpolarize R15 and slow the burst rhythm. In some instances the hyperpolarization is sufficient to arrest the bursting pattern completely (Fig. 13).

R15 responds with a very brisk depolarization to iontophoretic application of ACh. Since it also exhibits ILD it does not qualify as a classic D cell but falls into the category now called DILDA (D cell with ILD) by Gerschenfeld and Tauc (20).

R15 is almost certainly homologous to the "Br" cell identified by Arvanitaki and Chalazonitis (3, 5) and is the "parabolic burster" identified by Strumwasser in A. californica (39). Our findings on the endogenous nature of the rhythm of this cell are in complete agreement with Strumwasser's earlier and more complete study on this cell. However, unlike Strumwasser, who suggested that synaptic input produced only minimal modulation of the endogenous rhythm, we are impressed with the potential efficacy of the synaptic input, as well as that of direct light, in modulating the burst rhythm of this cell.

In view of the striking change in the number of granules which R15 con-

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**Fig. 11.** A: cell R15 is translucent and pigmented in small animals and opaque and white in large animals. This figure illustrates the relative paucity of cytoplasmic granules when the cell is translucent and orange colored. When the cell is white, the granule concentration approaches or equals that of the other white cells such as illustrated in Fig. 8A. Note that although the granules are of the same size and shape as in R3-R14, their cores are considerably less electron dense (arrows). ×23,000. B: the axon of R15. Note that the cytoplasm of this axon contains occasional granules (unlabeled arrow) and innumerable neurotubules. The high concentration of neurotubules is not characteristic for the axon of any particular identified cell, rather in a few ganglia many axons have comparable numbers of tubules, whereas in other ganglia no axons have many tubules. The axonal swellings which indent R15 in this picture are filled with various types of granules. ×24,000.
Fig. 13. Cell R15 response to substage illumination. A–E: a continuous record. When the light was turned on (B) the cell hyperpolarized, and the burst activity was completely abolished (A, C, and D); when the light was turned off (E) the cell depolarized and burst activity was reinstated.

In addition to the white cells, the right lower quarter-ganglion contains another identifiable cell, R16. This cell is small (about 100–150 μ) and lies on the caudal pole of the ventral surface near the midline (Fig. 2B). It has not been examined with the electron microscope and so its granular content is not known. It shares common EPSP’s with neighboring unidentified cells (see Fig. 12. A: this is a “typical” synaptic ending on the axon of R15 (arrow) as evidenced by 1) the high concentration of vesicles in the presumed presynaptic terminals, 2) the slightly widened intercellular gap, and 3) the narrow layers of electron dense cytoplasm that are applied to the plasma membranes on either side of the widened gap. It is presumed that these junctions, which are much rarer than those illustrated in Figs. 9, A–C and 11B, are functional synapses. X26,000. B: at isolated points in the axoplasm of R15, vesicles cluster at the side of the axon. It is possible that R15 is presynaptic to small unidentified neuronal processes at these points (arrow). Similar clusters of vesicles have been seen in the axon of L6. X38,000.
below) and receives a large excitatory synaptic potential from L10 (Fig. 15A). It sends its axon into the genital-pericardial nerve. The properties of R16 are very similar to the cells which make up the RB group.

4. Left rostral quarter-ganglion. The identified cells of the LRQG are L1–L6. L1 will be considered along with R1 and R2 in section 6.

The six identified cells in the left rostral quarter-ganglion are large and can be located grossly in the following way (Fig. 2, A and B). If the left connective is followed into the ganglion, the most rostral large cell in line with the connective on the dorsal surface is L2. Lateral to L2 is L1, which frequently appears smaller and often has a distinctive dark pigment spot in the cell body. On the medial side of L2 is L3. Directly caudal to L2 is L4, and caudal to L3 is L6. L5 is on the ventral surface of the ganglion near the midline; it lies caudal to L3 and rostral to cell L14 (see section 5b, below). One of these cells, L1, sends its axon into the left connective. Of the remaining five cells, L2, L4, and L6 are usually visible only on the dorsal surface, L5 is usually visible only on the ventral surface, and L3 can often be seen on both surfaces. L6 is sometimes deep in the midline and cannot be seen from either surface.

Perhaps the most frequent problem in identifying these cells grossly is that one or another of them cannot be visualized. Often this is due to the fact that the connective tissue sheath is thick and the cell body is hidden beneath it. In other instances, there is a rotation of the cells so that, for ex-
ample, L2 lies medial to the entrance of the connective, and L3 and L6 are deep in the septal region. If the rotation is in the opposite direction, L1 is on the lateral surface rather than the dorsal surface of the ganglion. Sometimes one or several of these large cells cannot be found grossly, but with experience they can all be demonstrated in the great majority of ganglia.

The most distinctive feature of the cytoplasm of L2–L6 is the presence of granules, which seem to be formed in the perikaryon and are passed down the axon (Figs. 16, 17). L5 differs from the other four cells, however, in that the granules are more concentrated and have denser cores (Fig. 17). As might be expected from the example of R15, this difference is apparent under the dissecting microscope, where L5 often has an opaque and gray appearance which is transitional between the bright whiteness of the white cells and the translucent orange color of most other cells.

![Figure 15](http://jn.physiology.org/)

**Fig. 15.** A: cell R16. Simultaneous recordings from R16 (bottom trace) and L10 (top trace) showing excitatory connection between L10 and R16. Each spike in L10 produces an EPSP in R16. L10 is firing spontaneously, the brief bursts are produced by intracellularly applied current pulses. B: cell L14. Simultaneous recordings from L14 (top trace) and L10 (bottom trace) showing absence of synaptic connection between L10 and L14.

The axons of L2–L6 pass caudally and travel in the medial and ventral part of the left neuropil until they emerge in the genital-pericardial nerve. Collaterals have been seen occasionally, but are small and difficult to follow. The axons form a characteristic bundle that can be recognized in cross sections of the ganglion. Unfortunately, they interweave so that, with the exception of the axon L5, which contains characteristic granules, it is impossible to identify any particular axon without tracing it back to the cell body.

The axon of L5 also differs from the axons of the other four cells in that it seems, at intervals, to be presynaptic to the small fibers which flank it. The presumed presynaptic regions are characterized by the accumulation of vesicles, 1,000–1,200 A in diameter, at the side of the axon or in one of the small processes of the axon that penetrates for a short distance into the neuropil. The larger, denser granules that characterize this axon are also seen among the presumed presynaptic vesicles, but they are no more concentrated here than they are elsewhere in the axon.

The terminals that end on these five axons are filled with granules that have diameters of 1,000–1,200 A and diffusely dense interiors, or with vesicles that are usually 800–1,000 A wide.
The most distinctive electrophysiological property which L2, L3, L4, and L6 share, and which sets them apart from all other identifiable cells with the exception of R15 (see section 3, above), is the ability to generate a regularly occurring burst of spikes (Fig. 18). Although bursting is the most frequent pattern of spontaneous spike activity, these cells can be silent or they can fire continuously. If silent, these cells can sometimes be made to burst by depolarization; if they are firing continuously, they can often be made to burst by hyperpolarization. Indeed, with two independent electrodes for recording and passing of current, it can be shown that the burst onset interval is a continuous function of the polarizing current (Fig. 18, B–E). This finding as well as other data are consistent with the endogenous nature of this rhythm.

The endogenous nature of the rhythm is supported by the following findings (51): 1) These cells receive their major synaptic input from an inhibitory interneuron (L10) which modulates their burst activity. When L10 is impaled and hyperpolarized, the modulating effect is removed but burst generation continues. 2) Direct hyperpolarization of the bursting cells fails to reveal PSP’s which could account for this rhythm, and 3) intracellularly applied pulses react the rhythm. The bursts are followed by a hyperpolarization which decays with time. This decay constitutes the “pacemaker” depolarization (PD). As the PD brings the membrane voltage to threshold a new burst is triggered. The hyperpolarization has the properties of an afterpotential; its amplitude and duration are functions of the number of spikes in the burst. Slope conductance during the PD is about two times higher at maximum hyperpolarization than just prior to burst onset. This conductance change is largely voltage-dependent and anomalously rectifying, increasing with hyperpolarization and decreasing with depolarization (29). From these findings it appears that the properties of these neurons may be similar to those of Purkinje muscle fibers (49), and like Purkinje fibers the bursting cells are assumed to have a high resting Na+ conductance. The pacemaker potential could represent either a time- and voltage-dependent decrease of the high conductance (perhaps to K+), which is turned on following the burst of spikes, or the activation of an electrogenic Na+ pump (1, 32).

The endogenous rhythm in these cells is modulated by synaptic input. In the absence of nerve stimulation, the main modulating input in the isolated ganglion is an IPSP which occurs synchronously in all four cells (Fig. 19). As will be shown in the following paper (24), this IPSP is mediated by different branches of a single indentifiable interneuron. In addition to this PSP, all these cells receive a common EPSP from an as yet unidentified interneuron. This EPSP is best activated by light offset, and is most evident in cell L2 and L4 (Fig. 20B). All cells also receive EPSPs from all the peripheral nerves and connectives (Fig. 20A), and show antidromic responses to stimulation of...
FIG. 18. LRQG bursting cell. Response of a bursting cell to polarizing current. Two independent electrodes have been placed in the cell, one for recording and the other for stimulating. The upper trace is the intracellular record and the lower trace indicates the current through the stimulating electrode. A, with no polarizing current, shows the cell firing regularly without bursts. In B, the cell is hyperpolarized and bursts are generated in a regular fashion. C–E show that with increasing amount of hyperpolarization, the steady-state burst onset interval is progressively prolonged. With further hyperpolarization (not indicated), the bursting can be completely suppressed.

The genital-pericardial nerve. Not infrequently a branch also emerges in the siphon nerve, and on occasion, in the brachial nerve as well. Cells L2, L3, L4, and L6 all respond with hyperpolarization to acetylcholine and therefore are H cells.

Although cells L2, L3, L4, and L6 are quite similar, there are some empirical rules which are helpful in distinguishing between them. These are: 1) Cells L3 and L6 are most likely to burst spontaneously, L2 is intermediate and, L4 least likely. 2) The EPSP's (either spontaneous or evoked by light) which are common to the four cells are largest in L2, smaller in L4,
Fig. 19. Synchronous IPSP's in three bursting cells of the LRQG. A, 1–3: simultaneous low-gain records of three bursting cells showing that there is a synchronous IPSP in all three cells. Although it is not well seen at this low gain there is also a synchronous EPSP which is largest in L2, smallest in L6, and intermediate in L3. B and C are at progressively higher gain to illustrate the coincidence of the IPSP's in the three cells.

and smallest in L3 and L6 (Fig. 20B). 3) The EPSP's evoked by electrical stimulation of the connectives are largest in L2, smaller in L4, and smallest in L3 and L6 (Fig. 20A). Thus, those cells that show the greatest tendency to spontaneous bursting have the smaller EPSP's.

Cell L5, as indicated above, is one of the most distinctive cells of the left rostral quarter-ganglion. It is almost always a silent cell with a high resting

Fig. 20. Comparison of size of EPSP's in cells L1 and L3. A, 1–3: stimulation of connectives and a peripheral nerve result in a compound EPSP which is synchronous in the two cells; the amplitude of the EPSP in L1 being considerably larger than in L3. B: synchronous EPSP's in L1 and L3 following light offset. The large size of the EPSP in L1 causes it to fire, while the EPSP in L3 is barely detectable and exerts no significant control over its bursting rhythm.
membrane potential. However, by means of sustained depolarizing current, it can sometimes be made to fire in a burst pattern. It also receives IPSP’s from L10, but they are usually inverted and appear as depolarizing PSP’s due to its high resting potential. EPSP’s can be evoked by connective and nerve stimulation but these inputs are not large and do not trigger bursts. The axon of this cell emerges in the genital-pericardial nerve. Iontophoretic application of ACh causes a depolarization when L5 is in the resting state. However, the ACh effect is inhibitory and merely reflects the high resting potential of this cell which is beyond the equilibrium level for the H response. When depolarized to near the firing level, the ACh response is hyperpolarizing and therefore this cell is classified as an H cell.

5. Left caudal quarter-ganglion. a. Dorsal surface of the LCQG. The dorsal surface of the LCQG contains four identifiable cells (L7, L8, L9, and L11) (Fig. 2A) and the major parts of three identifiable clusters (LB, LC, and LD). These cells are not remarkable in the light microscope but their fine structural characteristic have not yet been determined.

Cell L7 is generally located at the periphery of the ganglion below L1. It usually is pale yellow or gold in color, and in animals weighing 50–300 g L7
ranges in size from 150–250 \( \mu \) in diameter. It is spontaneously active with a basic rhythm of 1–3 spikes/sec. The spikes are interspersed with EPSP's and IPSP's which cause considerable irregularity in the basic rhythm (Fig. 21). The IPSP's are especially prominent, ranging between 3 and 4 mv in amplitude (Fig. 21, A1–A4). Stimulation of the connectives or the nerves produces a direct EPSP and an increase in the frequency of IPSP's (Fig. 21A1). This IPSP "recruitment" may last from a few seconds to several minutes (17) and can completely inhibit the spontaneous spike activity. Cell L7 shows characteristic bursts of spontaneous IPSP's. These range from slow bursts (Fig. 21, A2 and A3) to a very distinctive high-frequency IPSP burst (Fig. 21, A4 and B). The axon of L7 most commonly exits in the branchial nerve. The response of L7 to ACh is neither pure D nor H but is a diphasic D–H response (see 50).

Cell L8 (the most prominent member of the LB cluster) is usually lo-
cated next to L7, but may be found as far medial as the midline. It often has a stippled orange-red appearance. L8 is similar to L7 with respect to spontaneous rhythm, spontaneous and evoked PSP’s, IPSP recruitment, and ACh response (Fig. 22 and 23, A–C). It differs from L7 in its location, the size of the PSP’s (smaller in L7), the path of its efferent axon (into the genital-pericardial nerve), and in its response to ACh (H instead of D–H).

Cell L9 (the most prominent member of the LC cluster) has a stippled orange-red appearance and is usually located rostral to L11. It is one of the smallest of the identifiable cells on the dorsal surface. Cell L9 is quite similar to L7 and L8 with respect to spontaneous rhythm, spontaneous and evoked PSP’s and IPSP recruitment (Fig. 23). It differs from L7 and L8 in two ways: 1) its axon enters the siphon nerve and 2) it has a depolarizing response to ACh, and is therefore a D cell (Fig. 23 D). Since D cells were originally described as lacking any inhibitory inputs, this cell deviates from the traditional D cell and resembles the DIN1 cells recently described by Gershenfeld and Tauc (20).

Cell L11 is usually the largest cell on the dorsal surface of the left caudal quarter-ganglion, ranging in size from 200–400 μ in diameter. It is usually located at the periphery of the LCQG near the exit of the siphon and genital-pericardial nerves, but at times it is more central and reaches the rostral mar-
gin of the caudal quarter-ganglion. L11 is pale with little pigmentation. Its spontaneous activity is fairly regular, and usually it fires at a rate of 2–4/sec. Connective stimulation produces large direct IPSP's (Fig. 24, A1–4), and also an increase in frequency and amplitude of the spontaneously occurring IPSP's and EPSP's. The prominent IPSP's following connective stimulation are particularly useful for distinguishing this cell, since no other identifiable cell on the dorsal surface shows large direct IPSP's on connective or nerve stimulation. Cell L11 also shows a high-frequency burst of spikes that recurs periodically (Figs. 24B and 25D). Cell L11 sends axons into the genital-pericardial and siphon nerves. It gives an H response to ACh. Cell L11 is probably homologous to cell “Gen.,” described by Arvanitaki and Chalazonitis (3).

The most striking characteristic of these identifiable cells on the dorsal surface of the LCQG (L7, L8, L9, and L11) is a synchronous event which is also shared by the cells in the surrounding identified cell clusters (LB, LC, LD), as well as by L10. This event consists of a high-frequency burst of IPSP's in L7, L8, and L9, and a high-frequency burst of EPSP's in L11 which triggers a burst of spikes (Figs. 21B, 22B, 23B, 24B, and 25D). In some preparations this phenomenon occurs spontaneously every 2–4 min. It can sometimes also be produced, after a long latency, following connective or nerve stimulation. When records are made simultaneously, one from L11 and the other from L7, L8, or L9, one can see that the train of spikes triggered by the EPSP burst in L11 occurs simultaneously with a high-frequency burst of IPSP's in the other cells (Fig. 32B).

b. Ventral surface of the LCQG. The identified cells on the ventral surface of the LCQG are L10, L12, L13, and L14 (Fig. 2B). Parts of the identifiable
IDENTIFIED NEURONS IN APlysia

Fig. 25. Cell L10. A: stimulation of the left connective (indicated by arrows) with a 1-sec train (6/sec) results in a train of brief IPSP’s followed, after the stimulus, by a long-lasting hyperpolarization; this phenomenon is referred to as ILD-I. B: spontaneous occurrence of long-lasting hyperpolarization without preceding brief IPSP’s; this phenomenon is referred to as ILD. C: simultaneous record from L10 and L8 to illustrate that the spontaneous ILD in L10 is synchronous with the high-frequency IPSP burst in L8. D: simultaneous record from L10 and L11 to illustrate that the spontaneous ILD in L10 is synchronous with high-frequency burst of spikes in L11. E: simultaneous record from L10 and L3 to illustrate the direct inhibitory connection which L10 makes with cells of the LRQG.

clusters L.R, I.C, and I.D are also found here. These clusters will be taken up in section 7.

It is difficult to see cells on the ventral surface. This is partly because 1) the connective tissue is thicker; 2) the branchial, siphon, and genital-pericardial nerves exit primarily from this surface and their exit zones cover about a fifth of the total surface; and 3) the ventral cells tend to be smaller than the dorsal cells. However, once the ganglion is pinned and the connective tissue stretched, considerable cellular detail is visible. Usually the largest cell seen on the ventral surface of the LCQG is L10. The other large cells of this region can best be identified and described in relation to L10.

Cell L10 is a lightly pigmented cell, 200–400 μ in diameter. Its most common position is just medial to the insertion of the siphon and genital-pericardial nerves. The distinctive feature of L10 is the distribution of its axonal tree. All other identifiable cells emit one stout process into the neuropil which begins to branch only at some distance from the cell body. By con-
trast, L10 usually emits, along with a single relatively large process, many smaller branches either from the cell body or from the proximal parts of the main axon. Unfortunately, those smaller processes are so fine that they cannot be traced for any distance in the neuropil.

Cell L10 is either silent or shows moderately regular spike activity. It has two distinct types of spontaneous inhibition: 1) brief IPSP’s, which last about 150–300 msec; and 2) a type of inhibition of long duration (ILD), which lasts 10–30 sec (Fig. 25, A–D).

The inhibition of long duration in L10 is different from that following excitation described by Tauc (43) for unidentified cells, and from the ILD-E seen in R15. When the inhibition of long duration in L10 occurs spontaneously it consists of a prolonged and large hyperpolarization without any preceding depolarization (Fig. 25, B, C, D). Therefore, we refer to this prolonged synaptic potential as ILD. A prolonged inhibition can also be produced by repetitive stimulation of the connectives in which case it follows the brief IPSP’s produced by the stimulus (Fig. 25A). We propose to refer to this prolonged inhibition as “inhibition of long duration following inhibition” (ILD-I). ILD and ILD-I are similar, and the basic mechanism underlying both may be the same. Moreover, ILD in L10 occurs synchronously with the ILD-E in R15, with the high-frequency IPSP burst in L7, L8, and L9 and with the high-frequency EPSP burst in L11 (Fig. 25, C and D). For this reason we believe that a single interneuron might mediate all these effects, but it has not yet been identified (ref. 24 and unpublished observations).

Cell L10 shows brief, direct IPSP’s following connective stimulation. The IPSP’s following trains of stimuli to the connectives are followed by three subsequent events: 1) ILD-I; 2) an increase in frequency of the spontaneous IPSP’s (IPSP recruitment); and 3) occasionally, a slight increase in spontaneous EPSP’s.

Cell L10 sends an axon into the genital-pericardial nerve and less commonly, into the siphon nerve. Its response to ACh is hyperpolarizing.

Cell L10 is one of two identified interneurons in the ganglion (24). Its many branches synapse on a number of identified cells in both the left and right hemiganglia (Fig. 25E, and Fig. 13 in ref. 24).

Cell L12 is rostral and usually lateral to L10, and L13 is caudal and usually lateral to L10. Cells L12 and L13 are large (200–350 μ in diameter), stippled, orange-red cells, which are similar in their physiological activity and their pharmacological properties and can only be distinguished by their position. On penetration, both cells have a fairly regular frequency of 4–6 spikes/sec (Fig. 26A). Both L12 and L13 are H cells. They receive powerful excitatory inputs from the connectives and nerves, which cause them to generate bursts of spikes (Fig. 26B). Both cells send their axons into the genital-pericardial nerves. Both cells receive an inhibitory branch from L10, which produces very small IPSP’s (Fig. 26D) and they also possess synchronous EPSP’s from an unidentified interneuron (Fig. 26, C1 and C2).

Cell L14 is rostral and medial to L12, usually lying directly caudal to L5.
It is a pigmented cell which, on impalement, is silent and has a high resting potential of about 70 mv. It has a few spontaneous EPSP’s, but shows large EPSP’s in response to stimulation of the connectives and nerves. It sends its axons into the branchial and siphon nerves. This cell receives no IPSP’s from L10 (Fig. 15B). Iontophoretic application of ACh on this cell causes a depolarization which is inverted to a hyperpolarization (H response) when the membrane potential is brought to the firing level.

6. Symmetrical cells (L1 and R1) and the giant cell (R2). Only two of the identifiable cells in the abdominal ganglion are topographically symmetrical. These are L1 and R1 (Fig. 2A). They are located on the dorsal surface of the rostral quarters of the left and right hemiganglia just lateral to the entrance of the appropriate connective. Cell L1 lies lateral to L2, which is in direct line with the connective, and rostral to L7. Cell L1 frequently has a prominent dark-brown pigment spot which facilitates its recognition. Cell R1 is adja-
Fig. 27. A: a cross section of the LRQG. There are six large cells in the LRQG (L1-L6), five of which are illustrated here. Although L6 is located midway between the dorsal and ventral surfaces of the ganglion in this plane of section, in more caudal parts of the ganglion, L3 will have ended and L6 will be visible from the dorsal surface. Note that the cytoplasm of L1 is much lighter than that of the other identified cells. This is due in part to the absence of cytoplasmic granules in L1 and in part to the presence of cytoplasmic regions that are filled only with a fine filamentous material. ×50. B: a cross section of the RRQG. Note that R1, which is located on the lateral aspect of the dorsal surface of the RRQG, is symmetrical with L1 (Fig. 27A) in both position and cytological appearance. None of the large dorsal white cells are visible in this section, but two smaller white cells (W.C.) are seen on the ventral surface of the ganglion. ×50. C: a cross section through the middle of the ganglion. Note the large and prominent "giant" cell, R2, on the right dorsolateral margin of the ganglion. ×50.
cent and lateral to the rostral white cells, R3–R13. Cell R2 is frequently found just caudal to R1.

The giant cell, R2, is the largest and most deeply pigmented cell in the ganglion (Figs. 2A, 27C). In Aplysia depilans and in small specimens of A. californica, this cell covers a large part of the dorsal surface of the RRQG. In larger specimens of A. californica, its position is more variable, sometimes being in the RRQG and sometimes in RCQG. Because of its size and coloration, however, there is almost never any difficulty in recognizing it. An edge of R2 can frequently be seen from the ventral surface (Fig. 1B).

Cells L1, R1, and R2 have a number of common properties. 1) They do not possess granules in their cytoplasm,6 2) they all send axons into the connectives, 3) they are silent, and 4) they have common interneurons.

Cell R2 is the well known "giant cell" of the abdominal ganglion (36, 44). It is homologous to cell "A" described by Arvanataki and Chalazonitis in A. fasciata (3), and to the "right upper quadrant giant cell" of Kandel and Tauc in A. depilans (26, 27).

The axon of R2 usually arises from the posterior part of the cell, dips into the neuropil, and emerges in the right connective. It can be easily recognized in the neuropil and in the connective because it is the largest axon present. There is an interesting cytologic difference between this axon and the other large axons in the connective. In the light microscope, the axon of R2 appears distinctly more basophilic than the other large axons, and in the electron microscope this basophilia is shown to result from a tighter packing of all the usual cytoplasmic organelles (mitochondria, agranular endoplasmic reticulum, glycogen, etc.) (Fig. 28A). The function of this tight packing is not known, but it bears a striking resemblance to the increased basophilia that is found on one side of the septum of the giant axon in the earthworm, Lumbricus (14, 38).

Within the neuropil, the axon emits numerous slender branches that penetrate deeper into the neuropil for a distance of 5–40 μ. In serial sections for the light microscope, these processes seem to end blindly, but it is possible that the processes just narrow suddenly and so are lost to view. Synaptic endings are found both on these processes and on the main axons. Although they are more numerous on the slender branches, the type of synapses on the branches and on the parent axon accm similar. Three synapses are illustrated in Figs. 28B and 29.

Four examples have been found within the neuropil in which the giant axon seems to be presynaptic to one of the smaller adjacent axons. The presumed presynaptic terminals were characterized by numerous, 400- to 600-A vesicles that cluster at the side of the giant axon. However, the membrane specializations and the widened intercellular gap that characterize typical vertebrate synapses have not been accm (see Fig. 11B for a similar junction).

Several distinct synaptic endings that resemble vertebrate synapses have been seen on the giant axon. These are few in number when compared with the numerous vesicle- and granule-filled profiles which do not have obvious cytoplasmic or intercellular specializations. In the neuropil of the giant cell, as well as in that of other cells, one frequently finds

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6 Recently, with improved glutaraldehyde fixation we have found that R1, L1, and R2 also possess granules. However these granules are few in number, small (600 A in diameter), and have moderately electron-dense cores. The cores do not fix well with buffered osmic acid alone.
vesicle- and granule-filled profiles contacting each other, in addition to contacting the giant axon, without the interposition of glial cells. If vesicle- and granule-filled processes represent functional synapses such contacts between presynaptic elements might account for the presynaptic interactions which have been postulated for heterosynaptic facilitation in the giant cell (27).

The vesicles that are found in either the distinct synaptic endings or in the other "terminals" are about 800 Å in diameter. There are two types of granules, the first about 1,000 Å in diameter with a rather diffuse, not very electron-dense core, and the second also about 1,000 Å in diameter with a small, extremely electron-dense core.

The physiological properties of R2 are generally similar to those described for the right upper quadrant giant cell of A. depilans (27).

Cell R2 is almost always silent. All inputs produce large EPSP's. The cell can be stimulated antidromically from the right connective (Fig. 32). In addition, a small antidromic A spike is produced by stimulation of the branchial nerve (Strumwasser, personal communication). If the cell is fired orthodromically its action potential can be readily recorded by gross electrodes placed on the right connective. Cell R2 is hyperpolarized by acetylcholine.

The homologous cell in A. fasciata (called "A" by Arvanitaki) was described by her as being spontaneously active. In A. Californica, spontaneous activity was rare and attributable to injury or to the transient discharge of an excitatory interneuron.

Cells L1 and R1 are cytologically identical, and can be recognized in thick plastic sections because they are much less basophilic than the large neighboring cells, all of which contain granules (Fig. 27, A and B).

Since the cell bodies are adjacent to the connectives, the axons are in the neuropil for only a short distance before they turn cranially and enter the ipsilateral pathway. In that limited space, the "synaptic" endings on these axons are relatively sparse. The endings contain either 800-1,000 Å wide vesicle, or granules, 1,000-1,200 Å wide and filled with diffuse, not very electron-dense cores.

The axons of L1 and R1 can be traced into the connectives. The axon of L1 is the largest fiber in the left connective, the axon of R1 is the second largest (after that of R2) in the right connective. Despite their being smaller, axons of L1 and R1 both conduct more rapidly than does the axon of R2 (Fig. 32). The reason for this disparity between fiber diameter and conduction velocity is obscure but similar observations have previously been made by a number of workers (21, 23, 27). Two fast fibers, one in each connective, have been described in extracellular recordings by Goldman (21) in A. depilans.

**Fig. 28.** A: in light-microscopic sections, the axon of R2 is distinctive because of its large size. This axon is also more deeply stained than any of the neighboring axons. If examined with the electron microscope, this deep or intense staining is seen to be the result of a tighter packing of the usual axoplasmic organelles (cf. R2 with the other axons). Also note that the axon of R2 is deeply indented by glial processes (Gl. pr. and arrows). B: an axon containing small granules with very electron-dense cores (arrows) lies adjacent to the "giant" axon, R2. This junction lacks the cytoplasmic and intercellular specializations that characterize the vertebrate synapse. It is not known whether this arrangement represents a functional synapse. ×40,000.
caifornica and by Hughes and Tauc (23) in A. depilans. These fibers produce distinctive small spikes which conduct more rapidly than the spike of R2. Kesselman and Kupfermann (unpublished observations) have recently shown that the small fast spike of the right connective is produced by the axon of R1. The axon of L1 produces a similar small spike in the left connective but often it cannot be distinguished from a similar spike produced by an unidentified cell.

In addition, Kandel and Tauc (27) found that stimulation of either the right or left connectives consistently produced a monosynaptic EPSP in R2 (the right upper quadrant giant cell) of A. depilans. These EPSP's were presumably also mediated by fibers which, although smaller, conduct more rapidly than the axon spike of R2. In A. californica monosynaptic EPSP's which appear before the antidromic spike of R2 are rarely seen. This is one of the few clear differences in the properties of R2 in the two species.

As is true for both their positions and their axonal projections, the organization of the afferent input to L1 and R1 is also symmetrical (Fig. 30). The ipsilateral input (siphon N. for L1; branchial N. for R1) is of shorter latency and greater efficacy than the contralateral one, whereas the input from the genital-pericardial nerve produces approximately the same effects in both cells. In these features L1 and R1 resemble the symmetrical metacerebral cells of the snail (28).

Both cells respond to ACh with a depolarization. However, the ACh effect is inhibitory and merely reflects the high resting potential of these cells. Thus both these cells show at their resting level a depolarizing variant of an ACh inhibitory response as do L5 and L14, and are classified as H cells.

Cells L1, R1, and R2, receive input from at least two common excitatory interneurons (Fig. 31, A and B). One of these is common to all three; the other, which responds briskly to light offset, is common only to R1 and R2.

7. Identifiable cell clusters. a. The right dorsal hemiganglion. In addition to the large identifiable cells found in the dorsal right hemiganglion, there is an unknown number of smaller cells that are also easy to penetrate. The majority of these cells fall into three distinct classes, although there is some variability of the cell properties from preparation to preparation, within a given preparation the cells of a single class are remarkably uniform.

<image>

Fig. 29. A: a small finger projects from the main body of the axon of R2 toward the upper right corner of the picture. At the base of the figure is a possible presynaptic terminal (left arrow). Note that this terminal seems to contain 1) small vesicles; 2) granules with small, relatively electron-dense cores; and 3) granules with larger, not very dense cores. The functional implication of the various granules and vesicles in a presumed presynaptic terminal is not clear. On the right-hand side of the figure (right arrows) are two presumed presynaptic terminals ending on an unidentified neuronal process. x33,000. B: this terminal deeply indents the axon of R2 and seems to contain only one type of granule. Its connections are presumably with R2 at the left-hand arrow and with a small unidentified process at the right-hand arrow. x45,000.
Fig. 30. Simultaneous records from symmetrical cells, L1 and R1. A–D: show EPSP's following single shocks to the siphon nerve (A, D), genital-pericardial nerve (B), and branchial nerve (C). The EPSP is larger in the cell ipsilateral to the nerve stimulated. In D the sweep speed has been increased to show the shorter latency, as well as the larger amplitude of the EPSP in the cell ipsilateral to the stimulated nerve. In A–D the cells were hyperpolarized in order to prevent a spike. E and F show the antidromic response following stimulation of the connectives. Cell L1 sends its axon into the left connective (E), while R1 sends its axon into the right connective (F).

The largest class of cells (RB) is typically found in the caudal part of the dorsal surface of the right hemiganglion (Fig. 36). These cells frequently form a ring around R15, some of whose properties they share. These cells range from 150 to 200 μ in diameter. They are usually spontaneously active, showing large compound EPSP's and irregularly occurring spikes that average 2/sec (Fig. 33). They receive compound EPSP's from the nerves and connectives, and with strong stimulation they show ILD-E. Upon threshold stimulation of the connectives, especially the right one, they frequently exhibit a unitary, elementary EPSP (Fig. 33B). This elementary PSP may not be monosynaptic. Its latency can be variable and it occurs spontaneously, suggesting that it comes from an interneuron. By recording from cells in the RB group two at a time, both the evoked and spontaneous elementary PSP's
can be shown to be synchronous (Fig. 33, A and B). These cells usually send their axons into both the branchial and siphon nerves. They all give a brisk depolarizing response to ACh (Fig. 33C), and since they can show ILD-E they are DILDA cells. Cells of the RB cluster are very likely homologous to the P1 cells studied by Tauc (45) in A. depilans and A. punctata.

A group of cells having properties similar to the cells in the dorsal RB group is found in the caudal part of the ventral surface of the right hemiganglion. Since these cells are contiguous with those on the dorsal surface, we believe that they represent a ventral extension of the RB group. The ventral cells in this group can be shown to receive a direct EPSP from L10 similar to that received by the immediately adjacent R16 and R15. We have not been able to impale L10 and a dorsal cell simultaneously in order to see whether this connection is also found in the dorsal cells as well.

The second largest class of cells, the RC group, is generally found rostral to the RB group (Fig. 36). The RC cells fire irregularly and are characterized by a long-lasting hyperpolarization, with (ILD-E) or without (ILD) a preceding depolarization, in response to stimulation of any input to the ganglion (Fig. 34, A–D). When the hyperpolarization is preceded by a depolarization, it resembles the ILD-E seen in L10. The ILD, but not the ILD-E, is sometimes seen spontaneously and often can be triggered by turning off the sub-

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**Fig. 31.** Simultaneous records from the three identified cells (L1, R1, R2) that send their axons into the connectives. A2 shows the same cells as A1, but at higher gain. The records show that some of the most prominent spontaneous EPSP's are synchronous in all three cells.
stage illumination (Fig. 34F). The RC cells send their axons into the branchial and the siphon nerves, and they are all H cells (Fig. 34G).

The RD group contains the fewest cells. The cells show neither spontaneous IPSP's nor ILD but rather, gave a large burst of spikes upon stimulation of connectives and nerves (Fig. 35). They send their axons into any of the three peripheral nerves but most frequently into the branchial and siphon nerves. They give H responses to ACh.

b. Left dorsal hemiganglion. In addition to the large identifiable cells in the left dorsal hemiganglion, a number of medium sized cells can be categorized. We have examined in detail the cells of the left caudal quarter-ganglion and these cells appear to fall into three groups (LB, LC, LD, Fig. 36). Each group seems to share the properties of one of the identifiable cells of the dorsal surface of this quarter-ganglion. The LB group has properties

![Figure 32](http://jn.physiology.org/)
which are indistinguishable from L8. These cells send their axons into the genital-pericardial nerve, show spontaneous IPSP's, and give an H response to ACh. The LC group has the properties of L9. These cells send their axons into the siphon nerve and give D responses to ACh. They receive IPSP bursts which are synchronous with the IPSP bursts in the cells of the

![Diagram](image-url)

**Fig. 33.** Some characteristic electrophysiological features of cells in identifiable cluster RB. A: a simultaneous record from two RB cells, illustrating close synchrony of spontaneous EPSP activity. A2 shows the same two cells as A1, but at twice the gain. B: a simultaneous record from two RB cells, illustrating simultaneous unitary EPSP's evoked by a shock to the R connective. C: a D response in an RB cell, following iontophoretic ACh application. The duration of the current pulse is indicated by the arrows.

![Diagram](image-url)

**Fig. 34.** Some characteristic electrophysiological features of cells in identifiable cluster RC. A–E: shows inhibition of long duration (ILD-E) elicited by a single shock to the left connective (A), right connective (B), branchial nerve (C), genital-pericardial nerve (D), and siphon nerve (E). F: shows ILD elicited by turning off the substage illumination at the calibration signals. G: shows an H response following iontophoretic ACh application.
LB group. The LB and LC groups account for most of the medium-sized cells in the LCQG.

The LD group consists of a small number of cells which share the properties of L11 and show a high-frequency burst of spikes which is synchronous with the IPSP bursts in the LB and LC groups.

Fig. 36. Schematic drawing of the dorsal and ventral surfaces indicating the location of the identified cell clusters. Clusters LA and RA are the bag cell clusters, cluster LB is indicated in black (identified cell L8 is its most prominent member), LC is stippled (L9 is its most prominent member), LD is marked with v's to show its resemblance to L11. Cluster RB is marked with horizontal stripes, RC with vertical lines, and RD cells are crosshatched.
Although the cell groups tend to cluster around the identifiable cell whose properties they share, they can be widely distributed and there is an intermingling of cells from the three classes. Some of these cells also appear to extend to the ventral surface where they form a cluster on the caudal pole. Finally, there are some cells which show a behavior transitional between that of the cells of the LC and LD groups.

Fig. 37. Distribution of cells sending axons into the connectives (A) and into the branchial (B) and genital-pericardial (C) nerves. Cells sending axons into connectives or given nerves have been stippled.
B. Functional groups

1. Cells sending axons into the connectives. Of the 30 identified cells, only 3 send an axon into a connective: the two symmetrical cells, L1 and R1, and the giant cell R2 (Fig. 37A). These cells seem to represent a functional group (section A6), and they are the only identifiable cells (including the many cells in the identifiable cell clusters) capable of sending information to the higher ganglia.
2. Cells sending axons to the periphery. All of the identified cells with the possible exception of Ll and Rl have one or more axons which go to the periphery (Fig. 37, B and C and Fig. 38A). Although most cells send axons to more than one peripheral nerve, there is some lateralization in the distribution of the most constant axons. For example, almost all identified cells in the right hemiganglion send an axon into the branchial nerve (Fig. 37B), whereas almost all identified cells in the left hemiganglion send an axon into the genital-pericardial nerve. Many cells in the left hemiganglion send a less
### Table 2. Identified cells and identified cell clusters

<table>
<thead>
<tr>
<th>Identified Cell</th>
<th>Firing Pattern</th>
<th>Anti(^1)</th>
<th>Ortho(^2)</th>
<th>Spont.(^4)</th>
<th>ACh Cell Class(^5)</th>
</tr>
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<tbody>
<tr>
<td>L1</td>
<td>Silent</td>
<td>1</td>
<td>E (1-5)</td>
<td>E</td>
<td>H(^*)</td>
</tr>
<tr>
<td>L2</td>
<td>Bursting</td>
<td>4 (3, 5)</td>
<td>E (1-5)(^3)</td>
<td>I-E</td>
<td>H</td>
</tr>
<tr>
<td>L3</td>
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<td>E (1-5)(^3)</td>
<td>I-E</td>
<td>H</td>
</tr>
<tr>
<td>L4</td>
<td>Bursting</td>
<td>4 (3, 5)</td>
<td>E (1-5)(^2)</td>
<td>I-E</td>
<td>H</td>
</tr>
<tr>
<td>L5</td>
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<td>E (1-5)</td>
<td>I-E</td>
<td>H(^*)</td>
</tr>
<tr>
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<td>Bursting</td>
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<td>H</td>
</tr>
<tr>
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<td>Irregular</td>
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<td>E, I (1-5)</td>
<td>I-E burst</td>
<td>I-D H(^6)</td>
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<td>E, I (1-5)</td>
<td>I-E</td>
<td>I burst</td>
</tr>
<tr>
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<td>E (1 5)</td>
<td>I-E</td>
<td>I burst</td>
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<td>Irregular with burst tendency</td>
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<td>I-LD-I (1,2)</td>
<td>I-LD, I</td>
<td>H weak</td>
</tr>
<tr>
<td>L11</td>
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<td>I (1,2)</td>
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<tr>
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<td>E</td>
<td>H(^*)</td>
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<tr>
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<td>4</td>
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<td>I</td>
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**Identified cluster**

| LA, RA | Silent, activated by orthostimulation | None | 1, 2 | None |
| LB     | Irregular                         | 4 (3) | E, I (1-5) | I-E I burst | H |
For description of cells, see text. 1 Left connective = 1; right connective = 2; branchial n = 3, genital-pericardinal n = 4; siphon n = 5. Nerves which could be stimulated to produce an antidromic spike are indicated, with the less common variants in parentheses. 2 Most prominent or characteristic responses to stimulation of the nerves (1-5) are indicated: $E =$ EPSP; $I =$ IPSP; ILED-I (ILED) = inhibition of long duration following inhibition (excitation). 3 Cell L2, L3, L4, and L6 tend to show different sized orthodromic and spontaneous PSP’s. See text for details. 4 Most prominent and characteristic spontaneous activity is indicated. 5 Following Tauc and Gerschenfeld (47) and Gerschenfeld and Tauc (20) cells have been classified as “H” (hyperpolarizing response to ACh application), “D” (depolarizing response to ACh in cells showing no IPSP’s), “DIN1” (depolarizing response to ACh in cells showing inhibition of long duration). $H^*$ indicates that cell can show a depolarizing response that is inhibitory. See text for an elaboration of these points. 6 Double nature of the response of L7 is described elsewhere (50).

constant axon into the siphon nerve (see Table 2). Only one cell L8, sends its axon only into the siphon nerve (Fig. 38A). There is also some crossing over: for example, R14, R15, and R16 send branches into the genital-pericardial and siphon nerves, whereas L7, L9, and occasionally other left-sided cells, send axons into the branchial nerve (Fig. 37, B and C).

3. Cells categorized according to spontaneous firing patterns. The identified cells can be classified by their firing patterns into four groups: 1) silent cells, 2) cells with highly regular firing rate, 3) cells with irregular firing rates modulated by PSP’s, and 4) bursting cells. Figure 39 gives the distribution of these cell types in the ganglion. Six cells are silent (L1, R1, R2, L5, L14, R16) and the rest are spontaneously active. The rostral white cells have a highly regular rate, whereas the cells of the left caudal quarter-ganglia have a somewhat irregular rate due to the presence of EPSP’s and IPSP’s. Four of the five bursting cells are in the LRQG. The other bursting cell (R15) is in the RCQG.

4. Location of cells according to their response to ACh. Twenty-two of thirty identifiable cells give hyperpolarizing (H) responses to ACh, seven give depolarizing (D) responses, and one (L7, see ref. 46) gives a diphasic D–H response (Fig. 38B) Of the seven giving D responses four cells with high resting potentials (R1, L1, L5, and L14) show D responses which invert to H responses when the membrane potential is brought toward the firing level. In these cells the membrane potential is higher than the equilibrium level for
the ACh response. Thus despite their depolarizing response to ACh these cells are inhibited by ACh and should be considered H cells. From this it is clear that the terminology D and H does not always satisfactorily express the functional response (excitatory or inhibitory) to ACh, because some observed D responses are inverted H responses. An observed D response must be further investigated to clarify the position of the equilibrium level for ACh.

One cell (L9) shows a D response to ACh which is excitatory, but this cell has spontaneous IPSP's and thus falls into the category of D cells with inhibition of long duration (DINI) described by Gerschenfeld and Taut (20) for Helix and for A. depilans. Cell R15 shows a D response which is excitatory and it has no spontaneous or evoked brief IPSP's. But this cell shows ILD-E. Such cells were previously considered D cells by Tauc and Gerschenfeld but more recently, D cells with ILD have been placed in a special category called DILDA. The term D cell is now restricted to cells having no inhibitory input of any kind. According to these criteria only R16 is a pure D cell.

All of the cells in the clusters LB, LD, RC, and RD give H response to ACh. The cells in cluster LC can be categorized as DINI, those in RB as DILDA.

In summary, the vast majority of identified cells (26/30) show an inhibitory response to ACh and can be categorized as H cells. Three identified cells respond with depolarization. One of these is a DIN1 cell, one a DILDA cell, and one a D cell. One identified cell (L7) cannot be simply categorized as giving either D or H response; this cell has a diphasic, D–H response to ACh (50).

5. Location of granule-containing cells. Most of the granule-containing cells are in the rostral part of the ganglion (Fig. 38C). These are the bag cell clusters (RA and LA), the white cells in the right rostral quarter-ganglion (R3–R13), and five of the large cells in the left rostral quarter-ganglion (L2–L6). The only identified granule-containing cells in the caudal ganglion are R14 and R15.

Discussion

In this paper we have attempted to describe the identifiable features of 30 cells and of 8 cell clusters in the abdominal ganglion of Aplysia californica (Table 1). The importance of identifying cells in this ganglion was first appreciated by Arvanitaki and her collaborators (2, 3). In several recently published maps, Arvanitaki and Chalazonitis (4, 5) and Chalazonitis (12) show 5–8 cells in the abdominal ganglion of Aplysia fasciata. We can find unequivocal homologics to 3 of their cells (R2 = A, R15 = Br, L11 = gcen). The comparison with the remaining cells in A. fasciata is difficult because of the lack of sufficient data in the European species. Strumwasser (personal communication) has also formulated a map of 10 cells which he has identified in the abdominal ganglion of A. californica. This work is still in progress, and, with some exceptions (cell 1 = R2, cell 2 = R14, and cell 3
(PB) = R15), we cannot determine the correspondence of most of these cells to those described in the present paper.

In the discussion which follows we will try to use the identifiable cells as a vantage point for considering certain embryological, functional, and organizational features of the ganglion.

I. EMBRYOLOGICAL CONSIDERATIONS

In the primitive opisthobranch nervous system, six ganglia are arranged in a ring around the esophagus, and five ganglia are located along the paired connectives that run the length of the animal. These five ganglia are the paired pallial ganglia, the supra- and subintestinal ganglia and the visceral ganglion. In Aplysia, a higher opisthobranch, only the abdominal ganglion is found along the connectives, and it is generally considered that this ganglion represents a fusion of the five primitive ganglia (10). However, gross subdivisions that might correspond to the five primitive ganglia have not been consistently described (10, 22).

All authors agree that the abdominal ganglion consists of two hemi-ganglia joined by a commissure. From our data, we can distinguish three other gross anatomical subdivisions of the ganglion: the two clusters of bag cells that surround each ganglion-connective junction and the cluster of rostral white cells which, together with some pigmented cells, forms a distinct elevation in the right rostral quarter-ganglion. It is tempting to homologize the five primitive opisthobranch ganglia with the five divisions of the abdominal ganglion in Aplysia: the two bag cell clusters, the rostral white cell region, the remainder of the right hemiganglion and the left hemiganglion. But, such a correlation is based almost entirely on gross anatomical considerations and is therefore highly speculative. Further embryological and comparative histological data are much needed.

II. FUNCTIONAL CONSIDERATIONS

The morphological and functional properties of the identifiable cells may serve, at this stage of the investigation, as useful guides to an understanding of the integrative function of the ganglion. Of the several features we have considered, three are particularly worthy of comment: a) the neurosecretory function of the ganglion, b) the relationship of spontaneous firing and synaptic activity to the transformation of information, and c) the relationship of the ganglion to the periphery and to the rest of the central nervous system.

A. Granule-containing cells and their relationship to the neurosecretory function of the ganglion

Among the identified cells, there are two subdivisions of granule-containing cells: those that fulfill the morphological requirements for neurosecretory cells and those that do not. The bag cells and the white cells R3–R14 are considered to be neurosecretory. They send granule-filled pro-
cesses into the vascularized sheath, where the processes end without appearing to make functional contact with other axons or effector cells.

These cells seem to be related to the growth or maturation of the animal since the number of granule-filled processes in the sheath increases markedly as the animal ages (15). Beyond this, the function of the two types of neurosecretory cells is not known.

Although the bag cells and the white cells, R3-R14, have common properties that provisionally establish them as neurosecretory, there are striking differences between the two cell types. First, the granules in the two types differ (see Table 1). Second, the white cells send axons that go into the neuropil and then emerge in a peripheral nerve, whereas the bag cells do not. These findings suggest that the white cells may have a relatively specific action at the periphery in addition to the proposed release of granular contents into the sheath. Third, as the animal enlarges, the cells use different mechanisms to increase the number of processes they send into the sheath. The major mechanism for the bag cells is an increase in the number of cells, whereas the major mechanism for the white cells is the increasing number of processes each cell sends into the sheath. Although the demands on both the white cells and the bag cells presumably increase with increasing size of the animal, as evidenced by increasing number of processes in the sheath, the number of white cells does not change. This finding emphasizes the invariant nature of the large identified cells. Fourth, the two types of cells differ physiologically. The bag cells are silent although they can be activated by stimulating the connectives. By contrast, the white cells fire regularly, and little synaptic modulation of their firing is possible.

The remaining identifiable cells that contain granules (L2-L6 and R15) are not neurosecretory by the criteria outlined above, since they do not send processes into the sheath. Nevertheless, they cannot as yet be regarded as nonneurosecretory until the peripheral destinations of these cells are found. In contrast with the rostral white cells, these cells have a large and effective synaptic input. It is interesting that all of the bursting identified cells (L2, L3, L4, L6, and R15) are within this group.

The important morphological characteristics of the granules in the identified cells are summarized in Table 1.

The next major step in the study of the granule-containing cells is to correlate the morphology of the granules with their chemistry and function.

The white or bluish-white color of neurosecretory cells and organs is well known, and this appearance is usually ascribed to the presence of innumerable cytoplasmic “neurosecretory” granules (31, 33, 48). In order to provide further evidence for this conclusion, it would be desirable to show that a cell appears translucent and pigmented when it has few or no granules and white and opaque when it has many granules. Cell R15 offers a unique opportunity for this correlation because both the appearance and granular content of the cell change as the animal enlarges. This cell is translucent and pigmented in small animals and its granular content is low. In larger animals, it is opaque and white and its granule content is high. At intermediate levels of
granule content, the cell is turbid or milky. Thus, by following this identified cell through time, it is possible to provide further evidence that the elementary neurosecretory granules are responsible for the opaque white appearance of the cell. Furthermore, these findings suggest that it may be possible to get crude quantitative estimates of a cell's content of granules by examining the cell under the dissecting microscope.

B. Relationship of spontaneous firing and synaptic activity to the transformation of information

Most of the identifiable cells (24/30) are spontaneously active. In some of the identifiable cells, in particular the rostral white cells (L3-L13), the rhythm is highly regular and lacks modulating synaptic input. In other cells (L7, L8, L9, L10, and L11) the natural rhythm is continuously modulated by EPSP's and IPSP's produced by the activity of a number of interneurons. Finally, there are five cells in the ganglion (L2, L3, L4, L6, and R15) with bursting rhythms, and in each of these cells the rhythm is again modulated by the synaptic activity generated by a few interneurons.

Spontaneous neural activity has been recorded in the isolated nervous systems of invertebrates. It has often been assumed that such activity arises from endogenously active cells but, with the exception of direct spike resetting in some arthropod neurons, direct evidence was lacking in most preparations. In Aplysia a number of cells have been examined in detail, and there are several lines of evidence that indicate that the activity of many of the spontaneously active cells in the abdominal ganglion is of endogenous origin (5, 39, 51; and Alving, unpublished observations).

The evidence for an endogenous source of spontaneous spike activity is particularly strong for the bursting cells (L2, L3, L4, L6, and R15) and for the regularly firing cells (the rostral white cells R3-R13). The other spontaneously firing cells often have a great deal of synaptic activity, but most of these cells also seem to have an underlying endogenous pacemaker potential. The prevalence of endogenous pacemaker activity in this ganglion suggests that a major method for information transformation in the ganglion involves the modulation of an endogenous rhythm by synaptic input. This formulation for many cells in the abdominal ganglion of Aplysia is thus essentially different from a recent view of vertebrate central neurons which regards cells as being silent unless affected by synaptic input (11, 17).

There are at least two advantages that endogenous neuronal activity can provide for a nervous system. One, it serves as a constant excitatory bias which makes a cell's output sensitive to change in inhibitory as well as excitatory drive. Two, spontaneous rhythms provide an additional mechanism for neural plasticity. It can be shown that it is possible to produce long-term changes in the firing pattern of an endogenously active cell either by alterations in the endogenous rhythm (19, 39) or by alterations in the activity of interneurons which modulate the cell synaptically (19; and unpublished observations).
C. Relationship of the ganglion to the periphery and to the rest of the central nervous system

Our data add little to the few available findings as to the precise functions of the abdominal ganglion. A first step in the resolution of this problem is to relate the identifiable cells to peripheral sensory and motor structures and to the rest of the nervous system. Stimulation of various nerves leading from the ganglion results in the modulation of the movement of the organs (heart, genital organs, ctenidium, siphon) innervated by the nerves (9; and unpublished observations). Preliminary data indicate that direct stimulation of certain identified cells can also produce some of these effects (unpublished observations). All the identifiable cells send axons out of the ganglion, and most cells send at least one, and sometimes as many as three, axons to the periphery. In general, cells in the right hemiganglion send an axon into the branchial nerve while those in the left hemiganglion send an axon into the genital-pericardial nerve. However, there is some crossing over, particularly by cells which send out more than one process to the periphery.

By contrast to the many cells which send axons to the peripheral nerves only three identifiable cells L1, R1, and R2, send their axons into the connective. These are therefore the only identifiable cells capable of sending information to the higher ganglia. It is of further interest that these cells are silent in the isolated ganglion. The functional significance of this type of arrangement is obscure, but it suggests that while the abdominal ganglion receives a great deal of information from the supraesophageal ganglia, it may transmit only a small amount of information to them. These three cells thus seem to provide an excellent experimental vantage point from which to examine the output of the ganglion which goes to the rest of the nervous system.

III. ORGANIZATIONAL CONSIDERATIONS

In the beginning of this study we were forced to assume that there is a constancy in the number, position, and location of the cell bodies and a high degree of specificity in their interconnections. It may be profitable, even at this preliminary juncture, to examine these assumptions. The strongest finding against the assumption is that the number of neurons increases as the animal matures (15). In order to see whether certain classes of cells were responsible for the increase in cell number, ganglia from different-sized animals, presumably at different stages of maturation, were examined. Even in the ganglia of very small animals, the large identified cells can be seen and they are particularly prominent because the smaller cells are significantly lower in number. These findings suggest that the identified cells may be early and invariant elements in the organization of the ganglion. Consistent with this idea is the observation that in response to an apparent functional demand which calls forth an enormous increase in the number of white cell processes in the sheath, the white cells remain constant in number. By con-
trast, the bag cells, a cluster of smaller cells, respond to an apparently similar demand by increasing their number fourfold. Furthermore, the identified cell clusters consist of cells with some properties similar to those of the nearby large identifiable cell. This is especially obvious for the cells surrounding L7, L8, L9, L11 (clusters LB, LC, LD, and transitional cells) and, on the right side, for R2 (cluster RD) and R15 and R16 (clusters RB and RC). It may therefore be possible to view the ganglion, in a highly simplified and speculative way, as being made up of a constant number of large cells many of which have been identified, and a variable number of smaller cells. Since each cluster appears to relate to the nearby identified cells, the ganglion may be made up of a constant number of functional units consisting of a fixed but small number of large cells and a large but variable number of small cells.

**SUMMARY**

By the use of multiple criteria we have identified almost all of the large cells (30 cells) and cell clusters (8 clusters) in the abdominal ganglion of *Aplysia californica*. The criteria for identification of a cell included: location of the cell in the ganglion, peripheral distribution of its efferent axon, its synaptic input, spontaneous activity, synaptic connections with other identifiable cells, response to ACh, and its appearance under the light and electron microscope.

We have tried to use the identified cells and cell clusters as a vantage point for considering certain functional and organizational features of the ganglion. The following generalizations have emerged: 1) many cells in the ganglion contain elementary granules. The granule-containing cells fall into two groups: those that fulfill the morphological requirements for neurosecretory cells, and those that do not. 2) Among the neurosecretory cells, two classes can be distinguished: the bag cells and the white cells R3-R14. These two groups show striking morphological and electrophysiological differences. 3) Most of the identifiable cells (28/30) and all the cells in the identifiable clusters send one or more axons into the peripheral nerves. Only three identified cells (L1, R1, and R2) send axons into the connectives. These three are the only identified cells capable of sending information to the higher ganglia. The three cells have other common morphological, and physiological properties. They have common interneurons, lack granules, and are not spontaneously active. By contrast, the majority of the identified cells (24/27) that do not send axons into the connectives show spontaneous activity, which in many cases can be demonstrated to be an intrinsic property of the cell. The modulation of endogenous rhythms by synaptic input appears to be a major mode of information transformation in the isolated ganglion.

**ACKNOWLEDGMENT**

The authors express their thanks to E. J. Furshpan for suggesting the dye, Chicago blue, used in marking the rostral white cells, and for his many helpful suggestions and discussions.
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