Further work is needed to pinpoint the nature and mediation of granulation tissue contraction in situ during wound healing. However, our results support the conclusion that fibroblasts, under certain conditions, are capable of modulating toward a cell type that is structurally and functionally close to smooth muscle; for these cells the name "myofibroblast" may be appropriate.

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Bilateral Symmetry and Interneuronal Organization in the Buccal Ganglia of Aplysia

Abstract. *Principles of functional organization of the bilaterally symmetric buccal ganglia of* Aplysia *were studied in 20 identified cells used as a reference population. Four of the identified cells (two in each ganglion) are multiaction interneurons, each of which innervates six identified ipsilateral follower cells, mediating cholinergic excitation to one cell and cholinergic inhibition to five others. Bilateral coordination is effected by common inputs to all four interneurons. Ipsilateral pairs of interneurons are electrotonically coupled and produce identical synaptic actions on their common follower population. This apparent redundancy of interneuronal action leads to feed-forward summation, eliciting amplified synaptic output from each interneuron pair.*

The buccal ganglia of the marine mollusk *Aplysia californica* share with the better known abdominal ganglion several advantages for neurophysiological studies (*1*). Chief among these is the presence of large neuronal cell bodies that are easily penetrated by microelectrodes, thus permitting the electrophysiological identification of individual cells. In addition, the two buccal ganglia are symmetric and provide an opportunity for studying the principles of organization of a bilaterally symmetric structure. I have identified 20 cells, including four multiaction, presumably cholinergic, interneurons, in the two buccal ganglia and have used these cells as a reference population for describing the functional interconnections of these ganglia (*2*). The two interneurons in each ganglion receive common inputs and produce identical synaptic actions on their common follower cell population. This arrangement permits neural information to be conveyed through either of two parallel channels. In addition, information can be conveyed through both channels simultaneously, because electrical coupling between the two interneurons tends to cause them to fire synchronously in response to a large common input. Moreover, activity in one interneuron tends to initiate activity in the other.

The two mirror-image buccal ganglia are linked by a commissure and are located on the caudal surface of the pharyngeal bulb (buccal muscle). The ganglia innervate the buccal musculature, the radula, the esophagus, and the salivary glands (*3*). Each of the ten identified cells in one ganglion has a symmetric mate in the other ganglion. The symmetric cell pairs display similar properties, including common synaptic input (*1*). The symmetric cells are not directly interconnected, although functional connections do exist between ganglia. Six of the ten identified cells on each side are innervated by two interneurons (BL_4 and BL_5 on the left, or BR_4 and BR_5 on the right). The actions of each interneuron appear to be confined ipsilaterally; I have not yet found contralateral follower cells.

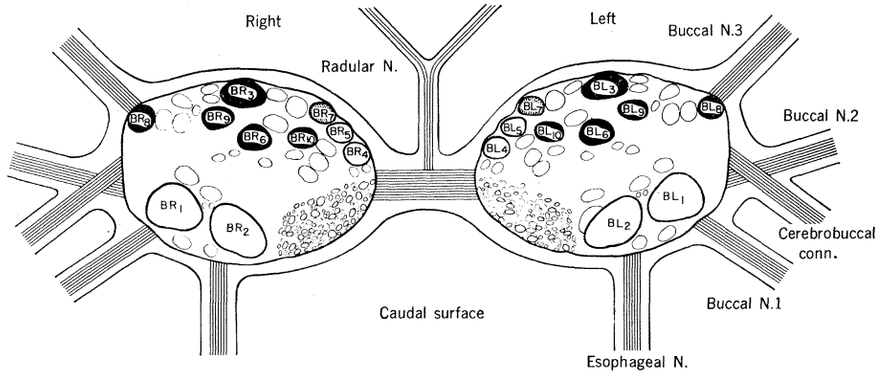

Fig. 1. Schematic drawing of the caudal surface of the buccal ganglia of *Aplysia californica* indicating typical positions of 20 identified cells. Cells are designated by the letters *BL* or *BR*, which indicate location in the left or right buccal ganglion, followed by a one- or two-digit number. Anatomically and functionally symmetric cells are assigned the same number, otherwise the numerical designations are arbitrary. Cells BL_4, BL_5, BR_4, and BR_5 are multiaction interneurons. Each mediates inhibition to five ipsilateral cells, shown outlined in black, and excitation to one ipsilateral cell, shown stippled.

Within each ganglion, the pair of identified interneurons mediate identical actions upon a common follower cell population. Each interneuron mediates inhibition to five identified follower cells and excitation (*4*) to the sixth identified follower cell (Fig. 1). These inhibitory and excitatory connections appear to be monosynaptic (Fig. 2A). The postsynaptic potentials (PSP's) followed the action potentials one-for-one without failure at frequencies greater than 50 per second, and the latencies of both the excitatory PSP (EPSP) and the inhibitory PSP (IPSP) were short (3 to 4 msec) and constant. Furthermore, the latencies were essentially unchanged when the ganglia were bathed in a solution of artificial seawater containing 60 m*M* Ca^{2+}. These high concentrations of Ca^{2+} increase the neuronal threshold by a factor of 3 to 4, and thereby tend to suppress polysynaptic actions (*5*).

The chemical transmitter released by each of the interneurons appears to be acetylcholine (ACh). Iontophoretically applied ACh simulates the action of the natural transmitter released by the interneurons on all members of the follower cell population. For example, cell BL_7, which receives EPSP's from interneurons BL_4 and BL_5, displayed a depolarizing excitatory response to ACh (*4*), whereas BL_3, which receives IPSP's, responded with hyperpolarization (Fig. 2, A and C). Bathing the ganglia in seawater containing *d*-tubocurarine (Fig. 2B) reversibly blocked both EPSP's and IPSP's. A similar cholinergic multiaction interneuron has previously been identified in the abdominal ganglion (*6–8*).

The interneurons on one side are electrically interconnected. An action potential in one interneuron gives rise to a small biphasic coupling potential in the other interneuron. This potential is not mimicked by ACh or blocked by curare. It appears to be due to nonrectifying electrical coupling between the interneurons because a current pulse injected into one produces an attenuated potential change in the other (Fig. 3A). The average attenuation ratio is 10 : 1. There is no significant coupling between symmetric mates. There is also some electrical coupling between each interneuron and several of its follower cells.

Ipsilateral interneurons are innervated by several spontaneously active, unidentified cells. The lower and middle traces of Fig. 3, B1, show the similarity of the PSP's recorded simul-

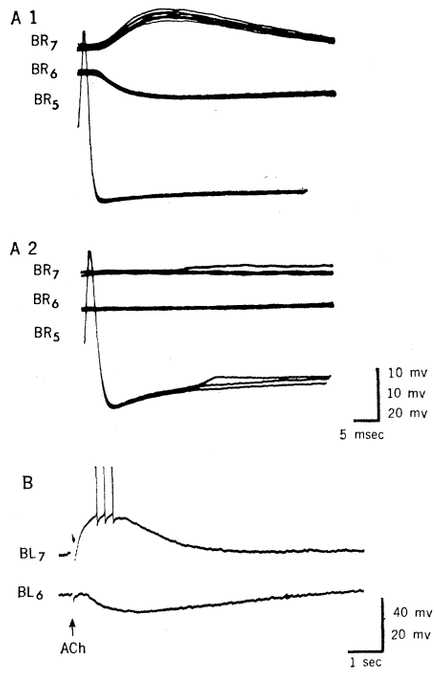

Fig. 2. (A) Intracellular recordings from interneuron BR_5 and two of its follower cells, BR_7 and BR_6. The oscilloscope sweeps were triggered by action potentials in the interneuron. Several sweeps were superimposed. The ganglion was bathed in seawater containing 60 m*M* Ca^{2+}, to suppress polysynaptic activity and to increase the amplitude of unitary PSP's. (A1) Action potentials in BR_5 produce depolarizing PSP's in BR_7 and hyperpolarizing PSP's in BR_6. Both PSP's are all-or-none and of constant and short latency. (A2) The application of *d*-tubocurarine (10^{-4} g/ml) blocks both PSP's. (B) Iontophoretic application of acetylcholine (ACh) to the cell bodies of two follower cells of interneurons BL_4 and BL_5. Upon application of ACh, the excitatory follower BL_7 is depolarized and the inhibitory follower BL_6 is hyperpolarized, thus mimicking, in each case, the synaptic actions produced by the natural transmitter.

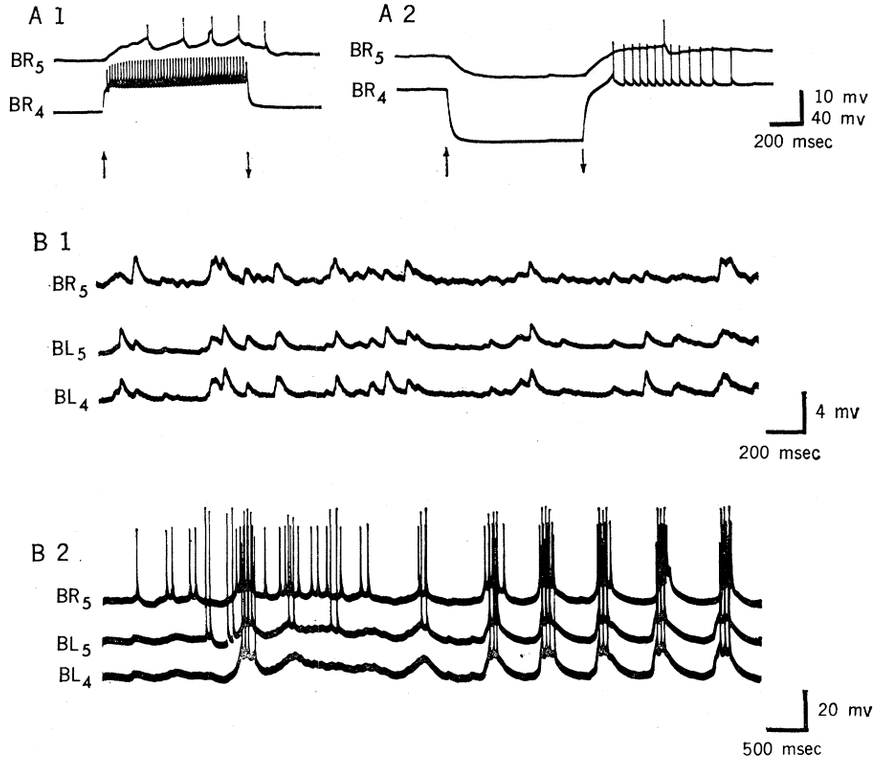

Fig. 3. (A) Bidirectional electrotonic coupling between an ipsilateral pair of interneurons (BR_4 and BR_5). A double-barrel electrode was inserted into BL_4 for recording and injecting current, and a single electrode was inserted into BL_5. In each case the arrows mark onset and termination of direct-current pulses delivered to BR_4. The absolute amplitudes and durations of the two pulses are not equal. (A1) A depolarizing pulse injected into BR_4 produces an attenuated depolarizing electrotonic potential in BR_5 that triggers action potentials. (The spikes in BR_5 are cut off by photography.) (A2). A hyperpolarizing pulse in BR_4 produces an attenuated hyperpolarizing electrotonic potential in BR_5. After the release of hyperpolarization, rebound excitation leads to action potentials in BR_4 and BR_5. (B) Simultaneous recordings of spontaneous activity in three of the four identified interneurons. In each set of records, the middle and lower traces record activity in an ipsilateral pair of cells; a contralateral cell is shown in the top trace. (B1) Fast, high-gain records show common inputs received by the interneurons from several higher-order unidentified cells. (B2) Slower, low-gain records show synchronous firing patterns of the interneurons.

taneously in an ipsilateral pair of interneurons. The upper trace indicates an impressive amount of common synaptic input to a contralateral interneuron, which suggests that much of this input is distributed bilaterally to all four interneurons. The EPSP's produced by stimulation of nerves and connectives appear with shorter latency and greater amplitude in the ipsilateral than in the contralateral interneurons. As a consequence of common synaptic inputs and ipsilateral electrical coupling, all four interneurons display synchronized firing patterns. Figure 3, B2, illustrates the especially tight synchrony between spikes in an ipsilateral pair, and a somewhat looser synchrony in a symmetric pair.

Every follower cell which receives synaptic input from one of the interneurons in a ganglion invariably receives similar input from the other interneuron. The PSP's produced in the follower cells by each of the two ipsilateral interneurons are practically indistinguishable in shape, amplitude, and latency (Fig. 4A). Since the two interneurons often fire synchronously, simultaneous action potentials give rise to large, summated PSP's which appear elementary (Fig. 4B).

Thus the symmetric follower cells in each ganglion are innervated by the ipsilateral members of a symmetric network of four interneurons. The two interneurons in each ganglion receive common inputs, are electrically coupled, and mediate identical excitatory and inhibitory synaptic actions to a common population of follower cells. The system therefore provides an example of bilateral symmetry as well as of an apparent ipsilateral redundancy.

Coordination between symmetric neural elements may be effected in either or both of two basic ways. Symmetric cells may be directly connected to one another (*9*), or they may share common inputs from one or more bilaterally projecting higher-order interneurons. My findings in the buccal ganglia support the common input mechanism and are in agreement with those previously reported for symmetric cell pairs in mollusks. Strumwasser (*1*) has demonstrated common synaptic input rather than direct connections as the mechanism of synchrony in a symmetric cell pair in this preparation, as did Kandel and Tauc (*10*) for the symmetric metacerebral giant cells of the snail. Common inputs from the periphery have been observed to favor ipsilateral cells of symmetric pairs in snails (*10*) and in the abdominal ganglion of *Aplysia* (*7*).

Either of the two multiaction interneurons on one side may serve as the channel for transmitting information from their common inputs to any member of their common population of followers. This arrangement provides an example of redundant parallel pathways at the cellular level. However, redundancy is not the only consequence of this scheme. Each interneuron receives some inputs which are not shared by its neighbor, and each can fire independently. The two interneurons with their separate inputs thus provide two partially distinct channels for converging neural information from higher-order elements upon the follower cell population. Both ipsilateral interneurons may also fire simultaneously, producing summated PSP's which are larger, and therefore more effective, than those produced by either interneuron alone.

This facilitatory scheme therefore provides a second example of functional interconnection of multiaction interneurons. In the abdominal ganglion there exists a pair of multiaction interneurons (interneurons I and II) which share a common follower cell population. There is strong indirect evidence for reciprocal inhibitory connections between interneurons I (L10) and II, providing feed-forward substitution of the actions of one interneuron for the actions of the other upon the follower population (*8*, *11*). Some of these substitutions are equivalent whereas others are not. Moreover, some follower cells of one interneuron receive no input from the other and do not participate in the substitution process.

In the buccal ganglia I have shown that another pattern for interconnecting multiaction interneurons predominates—common inputs to, and recip-

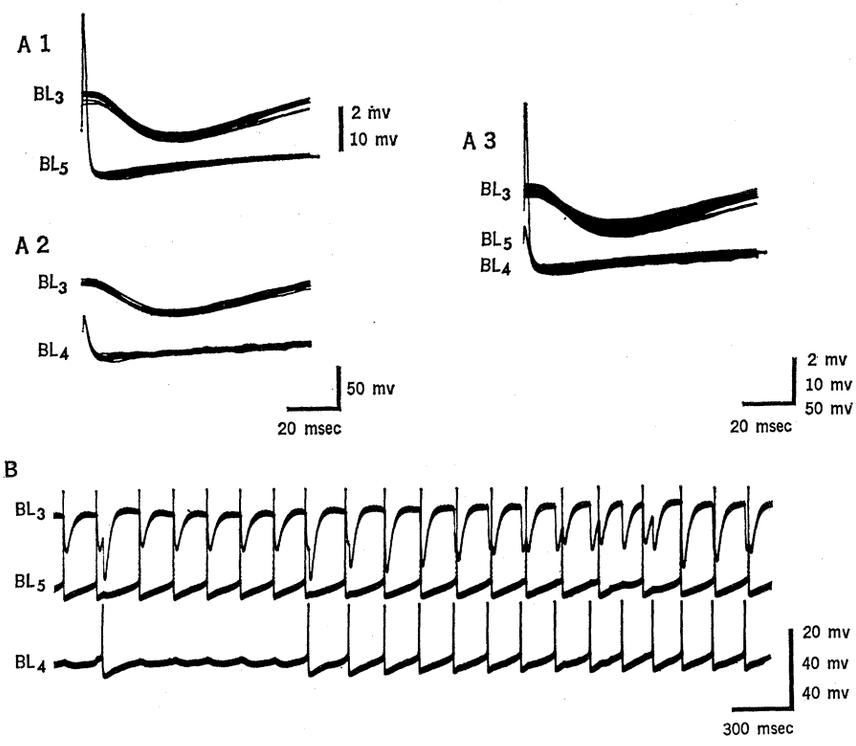

Fig. 4. (A) Feed-forward summation in the buccal ganglia of *Aplysia*. Intracellular multiple-sweep recordings from two ipsilateral interneurons and an inhibitory follower cell. (A1) With interneuron BL_4 hyperpolarized to prevent firing, action potentials in interneuron BL_5 produce IPSP's in BL_3. (A2) With interneuron BL_5 hyperpolarized, action potentials in interneuron BL_4 produce IPSP's in BL_3. (A3) Traces from parts A1 and A2 are photographically superimposed to show the similarity of PSP's produced in a follower cell from each of the two ipsilateral interneurons. (B) Recordings from two interneurons and a member of their common follower population. An action potential in either interneuron alone is capable of producing an IPSP in BL_3 and a small electrical coupling potential in the other interneuron. Closely spaced action potentials in the two interneurons produce summated PSP's. Synchronous action potentials in the two interneurons produce PSP's which summate so as to give the appearance of a large, unitary IPSP. Ganglia were bathed in seawater containing 60 m*M* Ca^{2+}. Upper trace (BL_3) was capacitively coupled; time constant, 0.1 second.

rocal excitatory electrical connections between, the two ipsilateral interneurons. This scheme provides for feed-forward summation of interneuronal actions upon the common follower cell population. Shared inputs to ipsilateral pairs of interneurons are of the same sign, permitting a cascading of activity leading to amplified synaptic output from the interneuron pair. Because each interneuron mediates the same synaptic action upon a given follower cell as does its ipsilateral partner, all follower cells participate in receiving summated output. As a consequence of this arrangement, an apparent redundancy of interconnections is revealed to be capable of summation, permitting more effective synaptic action than could be provided by either a single pathway, or two totally independent parallel channels.

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4. The interneurons mediate two types of depolarizing synaptic potentials to different follower cells. The depolarizing synaptic potentials to some as yet poorly identified cells (BL_{1a} and BR_{1a}) are purely excitatory chemical PSP's. In these cells, ACh also elicits a purely excitatory response. By contrast, the depolarizing synaptic potential in other cells (BL_2 and BR_2) consists of two components: an early excitatory component and a late inhibitory component. At the resting level of membrane potential, the PSP and the ACh response are primarily depolarizing. However, at depolarized membrane potentials, the interneuron or ACh pulses produce biphasic depolarizing-hyperpolarizing responses. The properties of this dual PSP will be described in a later paper (D. Gardner and E. R. Kandel, in preparation).
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C1 Inhibitor: Evidence for Decreased Hepatic Synthesis in Hereditary Angioneurotic Edema

Abstract. *Although the $C\bar{1}$ inhibitor was detected in 5 to 10 percent of normal hepatic parenchymal cells by means of the immunofluorescent technique, none was seen in liver biopsies from two individuals with hereditary angioneurotic edema having low concentrations of $C\bar{1}$ inhibitor in the serum. In contrast, the percentages of cells which reacted with fluorescent antiserums to C4 and transferrin were normal. These data suggest that in most subjects with hereditary angioneurotic edema, there is decreased synthesis of the $C\bar{1}$ inhibitor but normal synthesis of C4, and that the disease results from this biosynthetic error.*

Hereditary angioneurotic edema (HANE) is inherited as an autosomal dominant trait. Afflicted individuals tend to sustain recurrent episodes of circumscribed noninflammatory edema of the skin and the gastrointestinal and respiratory tracts (*1*). All patients with the disease are deficient in serum inhibition of the activated first component of complement, $C\bar{1}$ (*2*). The $C\bar{1}$ inhibitor of normal plasma has been isolated (*3*); it inhibits plasmin, PF/dil, and kallikrein as well as $C\bar{1}$ (*4*). In approximately 85 percent of affected kindred, the concentration of $C\bar{1}$ inhibitor protein is low, ranging from 5 to 30 percent of the normal amount as judged by immunochemical methods. In the remaining families there is a dysfunctional protein, the concentration of which is normal or elevated (*5*). The electrophoretic mobility of the dysfunctional protein varies from family to family; therefore there are probably several different aberrant structural genes producing this form of the disease (*6*).

In affected individuals, serum concentrations of the fourth and second components of complement (C4 and C2) may be low between attacks and decline during attacks (*7*). The decreased concentration of C4 is primarily related to increased catabolism, although synthetic rates may be low (see *8*).

It is not known whether the low concentrations of $C\bar{1}$ inhibitor in serums of patients with the common form of HANE are the result of diminished synthesis or accelerated destruction of the molecule. We have examined this question through the use of fluorescein-labeled antibody to localize $C\bar{1}$ inhibitor in normal liver and in liver of two patients with HANE and low serum concentrations of $C\bar{1}$ inhibitor. Biopsies of liver, duodenum, stomach, and lymph node were obtained from a 54-year-old male patient with HANE (W.K.) during hemigastrectomy for an obstructing duodenal ulcer. Liver biopsy from another patient, a 39-year-old female (G.G.), was obtained dur-

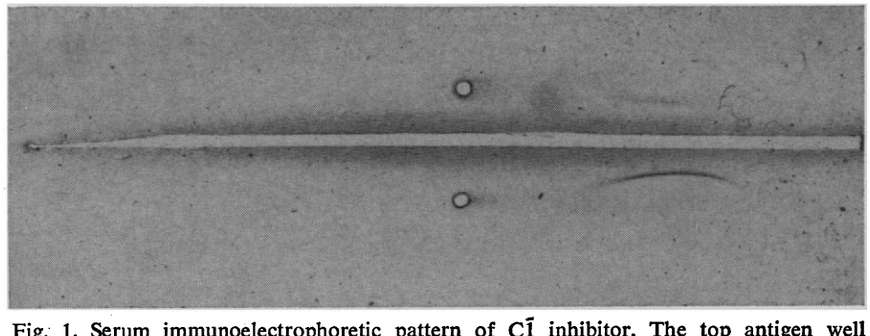

Fig. 1. Serum immunoelectrophoretic pattern of $C\bar{1}$ inhibitor. The top antigen well contained serum from a patient with hereditary angioneurotic edema (G.G.); the bottom well contained pooled serum from normal adults. The pattern was developed with goat antiserum to human α_2-neuraminoglycoprotein ($C\bar{1}$ inhibitor). A single immunoprecipitin arc is present for each sample.