# Evidence for Homologous Peptidergic Neurons in the Buccal Ganglia of Diverse Nudibranch Mollusks

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## SUMMARY

**The buccal ganglia of seven nudibranches (*Aeolidia papillosa, Armina californica, Dirona albolineata, D. picta, Hermissenda crassicornis, Melibe leonina,* and *Tritonia diomedea*) were examined to explore possible homologies between large cells that reacted with antibodies directed against small cardioactive peptide B ($SCP_B$). The buccal ganglion of each species possessed a pair of large, dorsal–lateral, whitish neurons that contained an $SCP_B$-like peptide. We refer to these neurons as the *SLB* (*$SCP_B$-immunoreactive Large Buccal*) cells. In all species examined, the SLB cells project out the gastroesophageal nerves and appear to innervate the esophagus.**

**In each species, an apparent rhythmic feeding motor program (FMP) was observed by intracellular recording from both SLB neurons and other neurons in isolated preparations of the buccal ganglia. SLB cells often fire at a high frequency, and usually burst in a specific phase relation to the FMP activity. Stimulation of SLB cells enhances expression of the feeding motor program, either by potentiating existing activity or eliciting the FMP in quiescent preparations. Finally, perfusion of isolated buccal ganglia with $SCP_B$ excites the SLB cells and activates FMPs. Thus, both the immunohistochemical and electrophysiological data suggest that the SLB cells within three suborders of the opisthobranchia (*Dendronotacea, Arminacea, and Aeolidacea*) are homologous. A comparison of our data with previously published studies indicates that SLB cell homologs may exist in other gastropods as well.**

***Keywords:*** **$SCP_B$, immunohistochemistry, nudibranchs, peptides, buccal ganglia, feeding motor program, central pattern generators, homologous.**

## INTRODUCTION

A primary feature of gastropod mollusks that has promoted their use in neurophysiology is the individual identifiability of their central nerve cells, based upon both morphological and physiological criteria. Studies on diverse species have led to identification of many specific neurons and determination of roles they play in behavioral responses. As such information becomes available from a sufficient number of different preparations, it is possible to look across species for evidence of conservation and for evolution of neural functions at the cellular level. For example, one distinctive pair of neurons on or near the anterior face of the cerebral ganglia can be identified in two subclasses based upon morphological, physiological, and pharmacological criteria (Senseman and Gelperin, 1974; Weiss and Kupfermann, 1976) suggesting that these serotonin-containing metacerebral giant cells are homologous (Weiss and Kupfermann, 1976; Granzow and Kater, 1977). Homologies have also been reported amongst neurons controlling gill movements in opisthobranch mollusks by Dickinson (1979, 1980).

The buccal ganglia of several gastropod mollusks contain a pair of large (100–300 $\mu$m in diameter), small cardioactive peptide B- ($SCP_B$) containing neurons. The opisthobranch *Aplysia californica* has two large, paired neurons in the buccal

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ganglion (B1 and B2) that innervate the gut and contain and synthesize SCPs ($SCP_A$ and $SCP_B$) in large amounts (Lloyd et al., 1985; Lloyd, Schacher, Kupfermann, and Weiss, 1986; Lloyd, Frankfurt, Stevens, Kupfermann, and Weiss, 1987). Both B1 and B2 are active during the swallowing cycle, but not during biting or food rejection, and appear to regulate gut motility (Lloyd, Kupferman, and Weiss, 1988a). The buccal ganglion of another opisthobranch, *Tritonia diomedea,* also has two distinctive pairs of neurons (B11 and B12) that contain SCP-like peptides (Lloyd, Masinovsky, and Willows, 1988b). Both B11 and B12 elicit cyclic motor output of the swallowing pattern generator and drive contractions of the gut (Lloyd and Willows, 1988; Willows, Lloyd, and Masinovsky, 1988). Identified SCP-containing neurons are also present in the buccal ganglion of *Tritonia festiva,* and they may be homologs to B11 and B12 (Masinovsky, Kempf, Calloway, and Willows, 1988). Two other nudibranches, *Hermissenda* and *Dentronotus,* also have very prominent $SCP_B$-immunoreactive neurons that project to the esophagus (Masinovsky et al., 1988). Pulmonate gastropods have also been shown to have large identifiable SCP-containing neurons in their buccal ganglia. *Limax maximus,* a terrestrial slug, contains a number of buccal ganglion cells with $SCP_B$ antigenicity. The largest of these are the lateral B1 cells. (Identification of neurons by particular numbers, e.g., B1 in *Aplysia, Limax, Helisoma,* and *Lymnaea,* does not imply that evidence for homology exists.) In *Limax,* the B1 cells modulate several aspects of feeding behavior (Prior and Watson, 1988). In *Helisoma trivolvis,* a freshwater pulmonate, seven to eight pairs of dorsal and four to five pairs of ventral $SCP_B$-containing neurons have been identified. The largest, located dorsolaterally, have been designated B1 (Murphy, Lukowiak, and Stell, 1985). The buccal ganglion of a close relative to *H. trivolvis, Lymnaea stagnalis,* contains two large and three medium-sized pairs of $SCP_B$-immunoreactive cells. The large lateral B1 cells and fibers appear to project to the anterior gut and salivary glands (Masinovsky et al., 1988). Thus, the available evidence indicates that many gastropod buccal ganglia have large identifiable SCP-containing neurons with similar biochemical, anatomical, and physiological properties. This has led to the hypothesis that they are homologous (Watson and Willows, 1986; Willows and Watson, 1986; Lloyd et al., 1988a,b; Masinovsky et al., 1988).

In order to explore this homology hypothesis further, we examined the buccal ganglia of *Aeolidia papillosa, Armina californica, Dirona albolineata, D. picta, Hermissenda crassicornis, Melibe leonina,* and *Tritonia diomedea.* We report here that the buccal ganglia of all seven species, representing three major nudibranch suborders (*Aeolidacea, Arminacea,* and *Dendronotacea*), have buccal ganglion neurons that are reidentifiable and probably homologous, based upon morphological characteristics, electrophysiological properties, and SCP-like immunoreactivity.

## METHODS

### Animals

*Tritonia* were obtained by trawling in Bellingham Bay, WA at depths of about 25 m. All other species were collected in near-shore locations near the Friday Harbor Laboratories, in the San Juan Islands of Washington, using SCUBA. Animals were maintained in flow-through seawater aquaria at approximately 10°C.

### Immunohistochemistry

Animals received an injection of 0.3 *M* $MgCl_2$ to anesthetize them 20 min prior to dissection. Buccal ganglia and associated gut tissue (esophagus, stomach, salivary glands, etc.) were removed, pinned out in a Sylgard-lined glass petri dish filled with cold seawater, and then fixed for 12 h in a solution of 4% paraformaldehyde in 0.1 *M* phosphate-buffered saline (PBS) (0.9% NaCl, pH 7.4). Fixations, and all the following procedures, were carried out on a shaker table at 8°C. After fixation, tissues were washed for 1 h in PBS, dehydrated through a graded series of alcohols, cleared in toluene or xylene, and then rehydrated (Costa, Buffa, Furness, and Soccia, 1980; Beltz and Kravitz, 1983; Prior and Watson, 1988). Following dehydration/rehydration, tissues were washed for 1 h in PBS containing 0.3% Triton X-100 and 0.1% sodium azide (PTA), and incubated for 12 h in PTA containing 6% nonimmune goat serum.

Spin media from hybridoma cell cultures producing IgG monoclonal antibodies against $SCP_B$ (Masinovsky et al., 1988) was diluted 1:20 in PTA containing 6% nonimmune goat serum, and tissues were incubated in this solution for 36–48 h. They were then washed for 24 h in PTA (four to six changes) and incubated for 24–36 h in goat anti-mouse secondary antibodies conjugated to fluorescein isothiocyanate (FITC) and diluted 1:100 in PTA containing 6% nonimmune goat serum. Finally, tissues were washed in PTA for 12 h and PBS for 1 h.

Preparations were mounted on slides using a medium that consisted of one part 50 m*M* Tris buffer (pH 9.5), and nine parts glycerol. Whole mounts were viewed on a

Nikon Optiphot microscope using epifluorescent illumination. A B2 excitation-barrier filter and reflector combination cube was used for FITC observations (460–490 nm interference excitation filter, 505 dichroic mirror, 511–545 barrier), and a G-cube (520–550 nm interference excitation filter, 575 nm dichroic mirror, 580 nm barrier) was used for visualizing antibodies conjugated to RITC (see below). Preparations were photographed with Tri-X film (ASA 400).

Staining with the monoclonal $SCP_B$ antibodies was partially inhibited by preincubation of the antibodies with $10^{-5}$ *M* $SCP_B$ for 24 h, and completely eliminated when we used $5 \times 10^{-5}$ *M* $SCP_B$. Preincubation with $10^{-5}$ *M* FMRFamide had no effect on subsequent immunohistochemical staining with $SCP_B$ antibodies. Further details about the $SCP_B$ monoclonal antibodies used in this study are available elsewhere (Masinovsky et al., 1988). Secondary antibodies and normal goat serum were obtained from Cappel Worthington Biochemicals (Malvern, PA).

### Lucifer Yellow Injections

The tips of microelectrodes (1 mm, thick walled) were filled with 4% Lucifer Yellow dissolved in 1% LiCl. Then, the electrode was backfilled with 3 *M* LiCl, and the tip was beveled to a resistance of 20–30 MΩ using a squirt bottle filled with a suspension of micropolish (0.05 μ gamma alumina, Buehler, Lake Bluff, IL). Dye was introduced into the cell by either pressure injection (Picospritzer, General Valve Co.), or application of hyperpolarizing current pulses (10 nA, 50% duty cycle). Buccal ganglia with injected cells were incubated in seawater overnight at 4°C and then fixed and processed as described above for immunohistochemistry, except with rhodamine-conjugated secondary antibodies. Lucifer and rhodamine were visualized and photographed in the same cells by switching filter cubes (G-cube for rhodamine and B2 cube for Lucifer Yellow).

### Electrophysiology

Buccal ganglia were removed from animals as stated above and pinned out in 1-ml Sylgard- or wax-lined recording chambers. Seawater at 12°C perfused the preparations at a rate of approximately 5 ml/min.

Neurons were impaled through the epineurium with 10–30 MΩ microelectrodes filled with 3 *M* KCl. Continuous intracellular records were obtained using Gould–Brush chart recorders. Neurons were stimulated intracellularly through the recording electrodes using a bridge–current circuit in the amplifiers.

$SCP_B$ stock solutions were made up in acidified distilled water each week. For pharmacological experiments, the stock solutions were diluted at least 1:1000 in seawater. $SCP_B$ was prepared by diluting a stock solution in 5–10 ml of filtered seawater and then perfusing the appropriate dilution through the recording chamber.

## RESULTS

### Immunohistochemistry

In every nudibranch we examined, there was a pair of large neurons in the buccal ganglion that contained $SCP_B$-like immunoreactive material (Figs. 1, 2). In live ganglia these neurons usually had a white or pale-white pigmentation when viewed with epiillumination. We call these neurons *$SCP_B$-immunoreactive Large Buccal* (SLB) cells. In some species they are also referred to by numbers. For instance, the B11 cells in *Tritonia diomedea* and the B1 cells in *Limax* are also SLB neurons, according to our terminology. In four species (*Tritonia, Melibe, Dirona albolineata,* and *Aeolidia*), we confirmed that the large, white cells were the same cells that contained an $SCP_B$-immunoreactive peptide by injecting them with Lucifer Yellow and then processing the buccal for SCP-like material using rhodamine-conjugated secondary antibodies.

In addition to the SLB cells, a number of other neurons in the buccal ganglion of each species reacted with the $SCP_B$ antibodies. There were three basic patterns of staining. The buccal ganglia of *Dirona picta, Dirona albolineata, Hermissenda, Tritonia,* and *Armina* each contained at least eight pairs of immunoreactive neurons in addition to the SLB cells (Fig. 1). Typically, there were two or three pairs of very intensely fluorescent cells, and the rest of the immunoreactive neurons were only weakly stained. In contrast, only three pairs of SCP-immunoreactive neurons were observed in the buccal ganglion of *Aeolidia;* one pair of SLB cells, and four much smaller cells that have not been identified (Fig. 1). Finally, in *Melibe,* which lacks an extensive buccal mass musculature and has a very small buccal ganglion, the paired SLB cells were the only buccal neurons that contained an $SCP_B$-immunoreactive peptide (Figs. 1, 2).

SLB cells had processes within the buccal ganglia (Fig. 1) and the peripheral nerves (Figs. 2, 3). Projections in the buccal ganglion produced a dense network within the ipsilateral hemiganglion, and sometimes passed to the neuropile of the contralateral side [note the contralateral projection of the SLB cell in *Melibe,* in Fig. 3(D)]. Large axons from the SLB cells project in the ipsilateral gastro-

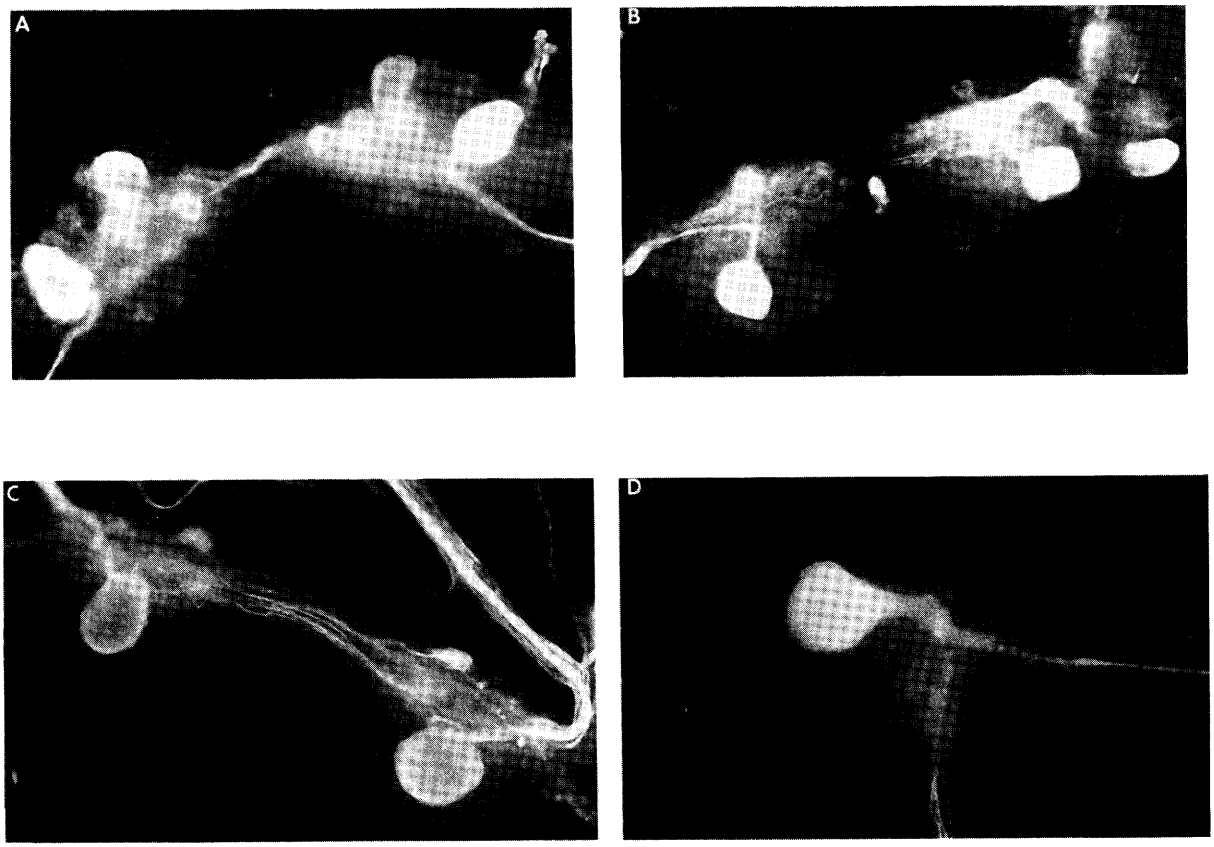


**Figure 1** $SCP_B$-immunoreactive neurons in the buccal ganglia of *Dirona picta* (A), *Hermissenda crassicornis* (B), *Aeolidia papillosa* (C), and *Melibe leonina* (D). All fixed whole mounts were treated with monoclonal antibodies directed against $SCP_B$. The SLB cells are the largest of the immunoreactive neurons in each ganglion. The large axons leaving the buccal ganglia in each figure arise from the SLB neuron somata. Other neurons containing an $SCP_B$-like peptide are also apparent in the ganglion of each species except *Melibe*. There are two additional pairs of small cells in *Aeolidia*, but one pair is not in focus, and six to eight smaller neurons in *Dirona* and *Hermissenda*. The pattern of staining in *Dirona picta*, shown here, is nearly identical to the pattern observed in *Dirona albolineata*, pictured in Figure 3. The large immunoreactive neuron just outside the right buccal ganglion of *Hermissenda* is located in one of the gastroesophageal ganglia. Anterior is up for all plates, but the large SLB cell axon in C was twisted during fixation so it appears to project anteriorly, although it actually projects posteriorly to the esophagus, as shown in Figures 2 and 3. Scale bar = 100 $\mu$m for A–C and 50 $\mu$m for D.

esophageal nerves to the esophagus and other regions of the gut (Figs. 2, 3). Some species (*Armina, Tritonia, Dirona, Aeolidia,* and *Hermissenda*) had a single large $SCP_B$-immunoreactive neuron in each gastroesophageal ganglion (GE-1) (Masinovsky et al., 1988) [Fig. 1(B) and 3(A)]. In these animals, the axons from the SLB cells and the $SCP_B$-positive neuron in the gastroesophageal ganglion project to the esophagus in parallel. Possible synaptic interactions between the gastroesophageal and SLB cells have only been examined in *Dirona,* and we found no observable morphological contacts between the cells, no electrical coupling, and no evidence of either excitatory or inhibitory synaptic interactions.

The peripheral SLB cell axons in all species gave rise to numerous branches that produced a dense network of processes and varicosities on the surface of the gut musculature (Fig. 4). In some species, such as *Armina* [Figs. 4(B)], a number of immunoreactive cell bodies were visible on the esophagus and they gave rise to a nerve plexus.

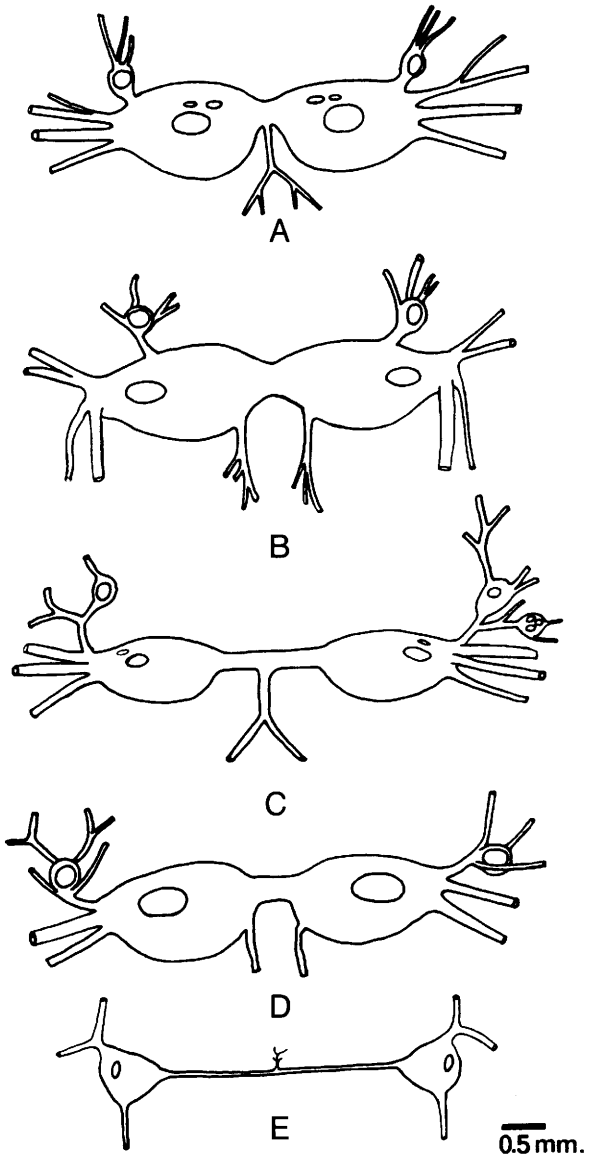


**Figure 2** Diagrammatic representation of the buccal ganglia of (A) *Tritonia,* (B) *Aeolidia,* (C) *Armina,* (D) *Dirona,* and (E) *Melibe* indicating the relative sizes and positions of the primary nerve trunks, and the major $SCP_B$ immunoreactive neurons. Scale bar = 1.0 mm for (A), 0.5 mm for (B–E).

Thus, while many of the varicosities on the esophagus represented SLB cell terminals, it is likely that some came from cells in the intrinsic nerve plexus.

## Electrophysiological Properties

***Relationship of SLB Cell Activity to the Feeding Motor Program.*** In all five genera, an apparent feeding motor program (FMP; identified by comparison with the FMP in *Tritonia diomedea,* Willows, 1980) could be recorded from any of several relatively large neurons in the buccal ganglion (examples from *Armina, Tritonia,* and *Melibe* shown in Fig. 5). These presumptive motor neurons were usually located dorsally, near the postero-medial margins of each ganglion. The burst pattern recorded across all species included spontaneous FMP bouts 2–10 min in duration which were composed of recurrent impulse bursts with 5- to 30-s interburst intervals, and each individual burst was often preceded by a hyperpolarizing wave.

When intracellular electrical activity in SLB cells was monitored during these spontaneous FMPs, a common pattern was observed (*Aeolidia, Dirona,* and *Tritonia* shown in Fig. 6). SLB cells which were silent, or firing irregularly, fired at a higher frequency during bouts of FMP. In some instances, SLB cells would also burst, and these bursts had a particular phase relationship with feeding motor neurons. Increases in the firing frequency of SLB cells often preceded increases in the intensity of motor neuron bursting (spikes per burst and/or burst frequency).

Changes in the rate of firing in both SLB cells occurred together, but spiking was not synchronous. In most species this was probably due to common synaptic input because we found no evidence for electrical coupling of left and right SLB cells, except in *Melibe.*

***Response of the FMP to Stimulation of SLB Neurons.*** In all the species, except for *Melibe,* intracellular stimulation of SLB neurons with sufficient current to cause firing at 5–10 Hz for more than 10 s usually elicited activity in previously inactive FMP neurons, or increased the rate and intensity of a spontaneously active preparation (Fig. 7). However, hyperpolarization of the SLB neurons, during spontaneously occurring FMP output, did not prevent expression of the FMP. Accordingly, SLB activity is sufficient, but not necessary for elicitation of FMP output.

***$SCP_B$ Exposure Excites SLB Neurons and Elicits FMP.*** Earlier work with the SLB neurons of *Tritonia diomedea* indicated that they contain and use $SCP_B$ (Lloyd et al., 1988b; Lloyd and Willows, 1988; Willows et al., 1988). Therefore, we perfused the buccal ganglion of each species with $SCP_B$ and examined the effect on the FMP and SLB cell activity. In each species, concentrations of $3 \times 10^{-5}$ to 3

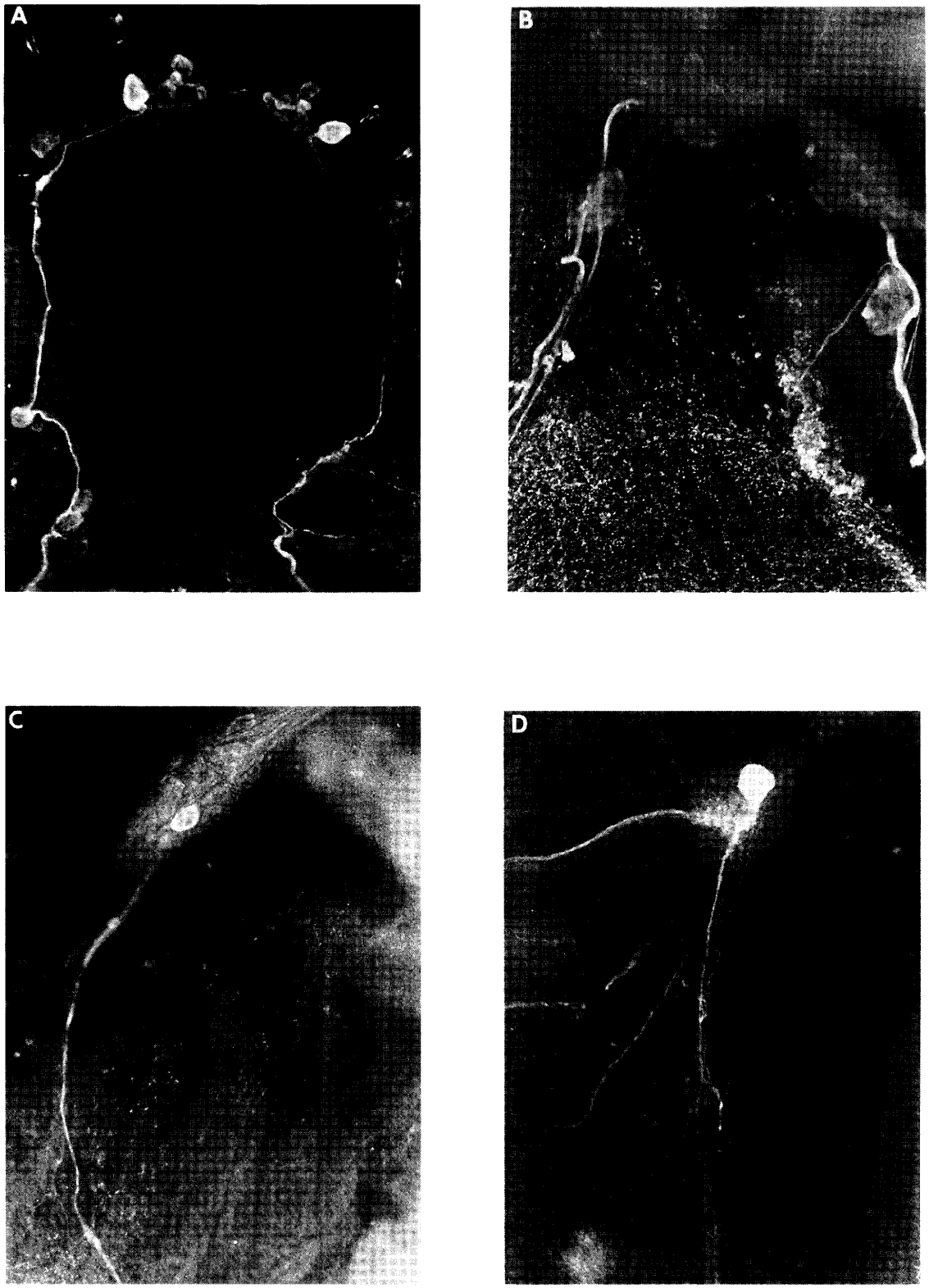


**Figure 3** Projections of SLB cells in *Dirona albolineata* (A), *Aeolidia* (B), *Armina* (C), and *Melibe* (D). These preparations were processed similarly to the ones shown in Figure 1, except the buccal ganglia remained attached to the esophagus during processing so the axonal projec-

$\times 10^{-7}$ *M*, elicited bouts of vigorous bursting and a large increase in the rate of SLB cell firing (Fig. 8). The threshold for this effect appeared to be related to the thickness (estimated by the opacity, and resistance to electrode penetration) of the sheath surrounding each ganglion. Species with a thin sheath, such as *Aeolidea, Dirona,* and *Hermissenda,* responded to concentrations as low as 3 $\times 10^{-7}$ *M*, whilst *Tritonia* and *Armina,* which have much thicker epineuria, required at least $3 \times 10^{-5}$ *M* $SCP_B$ to elicit the FMP.

## DISCUSSION

The results demonstrate that the buccal ganglia of seven nudibranch species from three suborders contain a pair of neurons, which we refer to as the *SLB* cells, that have similar morphological, biochemical, and physiological properties. In each species examined (1) the buccal ganglia are arranged as a bilaterally symmetric pair joined by a central commissure. (2) There is a relatively large pair of whitish pigmented neurons located symmetrically on the dorsal surface of each buccal ganglion. (3) These same neurons are intensely immunoreactive, suggesting they contain $SCP_B$-like peptides. (4) The axonal branches of these neurons project out the ipsilateral buccal nerves and innervate the esophagus and/or gut musculature. (5) The frequency of SLB cell firing increases during bouts of presumptive FMP. (6) Stimulation of the SLB cells (in most species) modulates the activity of the FMP. (7) Application of $SCP_B$ excites the SLB cells and activates the FMP. Based on these similarities, we conclude that the SLB cells in these nudibranches are possibly homologous.

Although the most striking feature about the SLB cells is their similarity in different nudibranch species, there are interspecies differences between SLB cells, and in the general pattern of $SCP_B$ immunoreactivity in the buccal ganglion. For example, the SLB cells in *Melibe* appear to be weakly electrically coupled, while in the other species we examined electrical coupling is not present. Stimulation of one *Melibe* SLB cell did not produce an obvious change in the FMP, as in most other species. Also, in *Melibe,* there is only one pair of $SCP_B$-immunoreactive neurons in the buccal ganglion, while *Aeolidia* has three pairs, and the other species examined have more than six pairs. These differences may be a function of the complexity of the different buccal ganglia (the *Melibe* buccal ganglion has less than 70 neurons, while all other ganglia have more than 200), the feeding strategies employed by the different animals, or the phylogenetic relationships between species. More detailed anatomical and biochemical studies presently underway may help to resolve these issues.

Large $SCP_B$-immunoreactive buccal ganglion neurons have also been reported in a number of other gastropods including *Aplysia, Lymnaea, Limax, Helix,* and *Tritonia hombergi.* A comparison of these findings with the present work indicates the strong possibility that homologies extend widely throughout the Gastopoda. Immunocytochemical and physiological studies of buccal ganglion neurons containing SCPs in the opisthobranch, anaspid *Aplysia californa* (Lloyd et al., 1988a), indicate that each buccal ganglion has a cluster of two pairs of large SCP-containing neurons and two to three additional pairs of smaller neurons located near the exit of the esophageal nerve. The largest immunoreactive cells, B1 and B2, are located, respectively, more laterally, and more proximal to the origin of the esophageal nerve. The axons from all these cells pass to the musculature of the gut in the esophageal nerves.

tions of the SLB cells would be apparent when observed at low magnification. In each species examined, the SLB cells innervated the esophagus. SLB cell axons branched extensively and gave rise to numerous varicosities on the surface of the esophagus, which are most apparent in *Aeolidia.* In *Dirona,* the SLB cell axon runs parallel to axons produced by several other large $SCP_B$-immunoreactive cells which are located outside the buccal ganglia. This is also true in all the other species with gastroesophageal ganglia. The buccal ganglia of *Armina* appears as if it only has a single cell containing $SCP_B$-immunoreactive material, but, in fact, it contains a number of putative $SCP_B$ cells. In *Melibe,* the left and right halves of the buccal ganglion are separated by a very long buccal–buccal connective and so only the right side is visible in D. The axon extending to the left of the ganglion is projecting through the connective to the left hemiganglion. The other axon projects posteriorly to the esophagus. The border of the esophagus is visible on the right side of the plate. Scale bar = 200 $\mu$m.

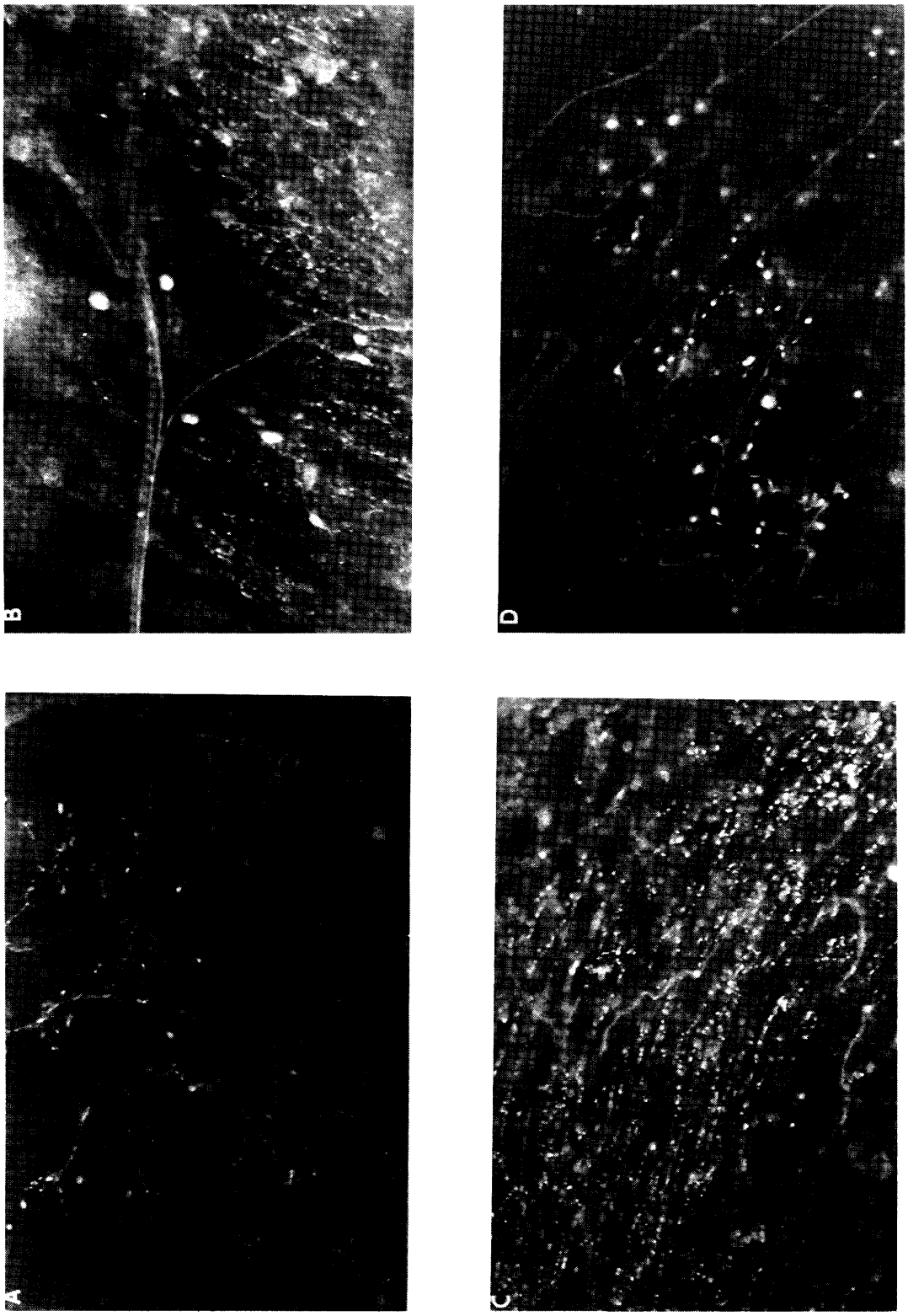


**Figure 4** $SCP_B$-immunoreactive varicosities on the esophagus of *Melibe* (A), *Armina* (B), *Aeolidia* (C), and *Dirona albolineata* (D). In each species examined, SLB cell axons innervate the esophagus and give rise to immunoreactive terminals on its surface. For example, in B it is possible to see SLB cell axons and the extent of the innervation. These plates also show three different innervation patterns. In *Armina* there is a very extensive $SCP_B$-immunoreactive plexus of small neurons on the surface of the esophagus. A few of these cells are visible in B. The functional relationship between the two $SCP_B$ systems (SLB input and intrinsic plexus) is not known. In *Aeolidia* there is very heavy staining of the esophagus with $SCP_B$ antibodies, while in *Melibe* (A) and *Dirona* (D), the $SCP_B$ immunoreactive fibers are more diffuse. Scale bar = A and D, 50 $\mu$m; B and C, 100 $\mu$m.

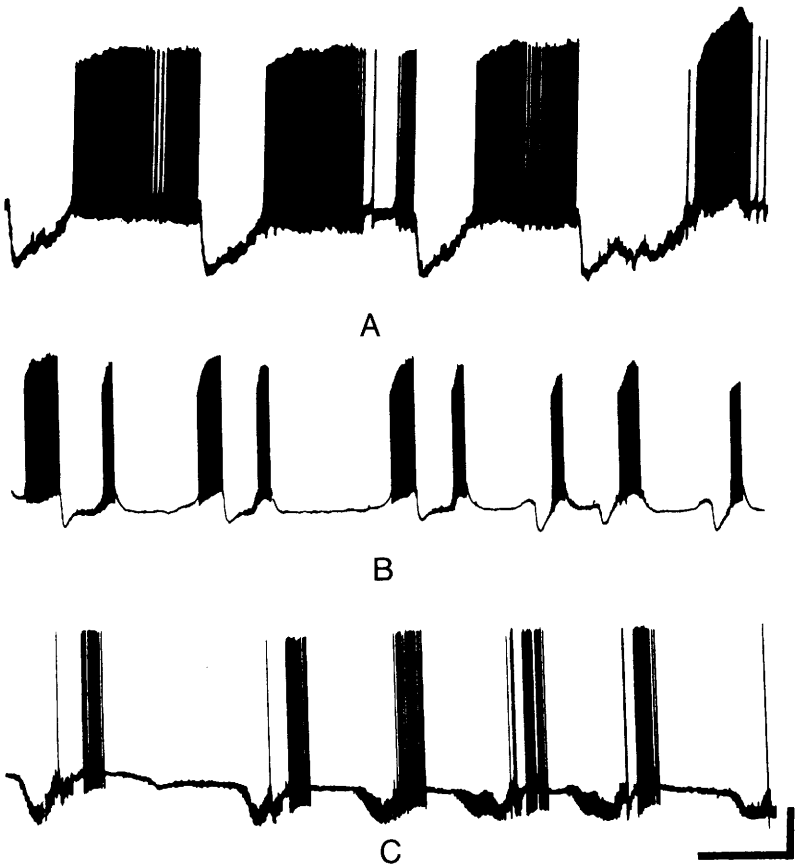


**Figure 5** The apparently homologous feeding motor pattern recorded in postero-medial buccal motor neurons in (A) *Melibe leonina*, (B) *Tritonia diomedea*, and (C) *Armina californica*. In each instance, as well as in the other species studied, the pattern includes spontaneous, cyclic impulse bursts that arise after a 5- to 20-mV psp volley which starts as a large hyperpolarizing wave, and ends with a growing excitatory postsynaptic potential (epsp) volley. Scale bar = 20 mV and 30 s.

Thus, the SCP-containing neurons in *Aplysia* appear to be closely homologous to those found in this study.

Since there are two pairs of large SCP-containing neurons in *Aplysia*, rather than the more commonly observed single pair in other opisthobranchs, and because the gastroesophageal ganglia seem to be incorporated into the buccal ganglion in this species, it may be that one of the two large neuron pairs is, in fact, homologous to the large pair in the gastroesophageal ganglia in other species [see Fig. 1(B) and 3(A) for examples of $SCP_B$-immunoreactive gastroesophageal cells]. This hypothesis is also supported by the observation that one occasionally observes the $SCP_B$-immunoreactive gastroesophageal neurons in *Tritonia* incorporated into the buccal ganglion (W. Watson, unpublished observation). Neuron B2 is located closer to the esophageal nerve and thus, based on location alone, might be considered homologous to the immunoreactive gastroesophageal neuron in other opisthobranches. However, neuron B2 (but not B1) has neurochemical characteristics (coexistence of acetylcholine and the SCPs) and physiological actions (Lloyd et al., 1985) which are quite similar to cell B11 of *Tritonia*. Thus, if one of the neurons in *Aplysia* is homologous to the SLB cells described in this paper, then the most likely candidate is B2.

Dorsett (1986) identified a pair of buccal neurons (SWC) in *Tritonia hombergi* that initiate and modulate the frequency of patterned spiking in buccal motor neurons that control aspects of feeding. Further studies (Dorsett, in preparation) indicate that the same $SCP_B$ antibodies used in the present work stain the SWC, as well as another pair of

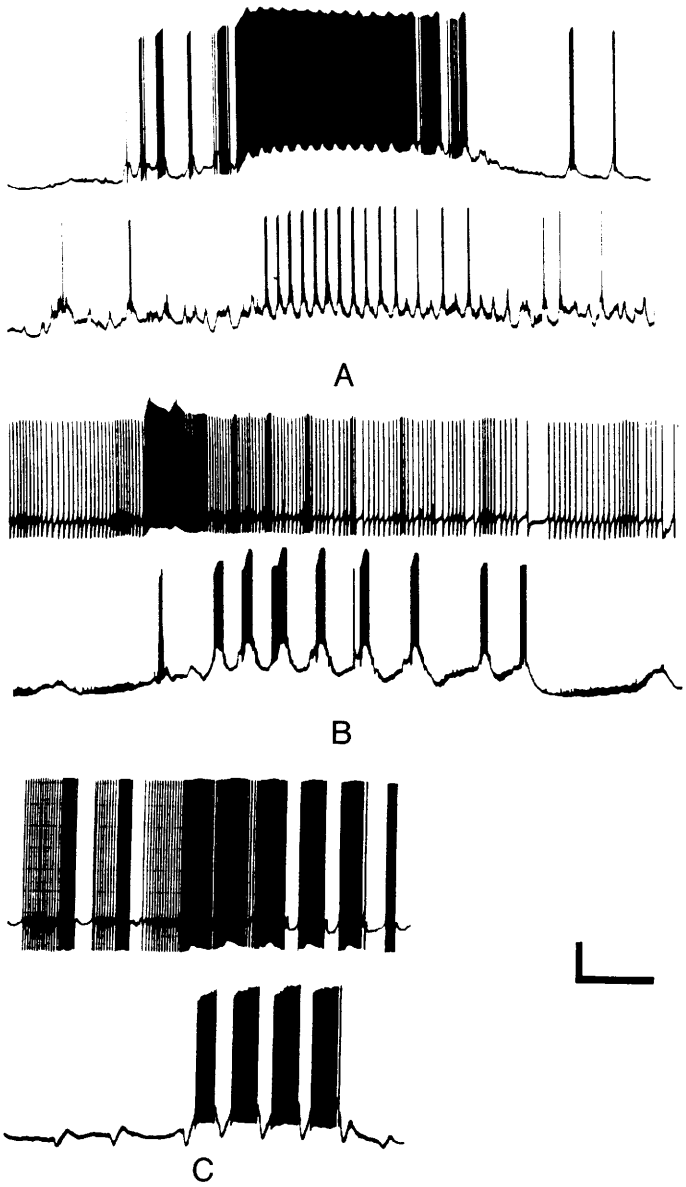


**Figure 6** Interactions between buccal motor neurons and SLB cells in (A) *Aeolidia,* (B) *Dirona,* (C) *Tritonia.* In each instance, and in the other species studied but not shown, the spontaneously occurring trains of FMP bursts in motor neurons (lower traces) are accompanied by an appropriately phased series of bursts in the SLB neurons (upper traces). An increase in the intensity of firing in the SLB neurons usually precedes an increase in the bursting rate or spike frequency in the motor neurons. Scale bar = 20 mV and 25 s.

buccal neurons and a large neuron pair in the gastroesophageal ganglia. Thus, the SWC resemble the B12 neurons of *Tritonia diomedea* (Masinovsky, Lloyd, and Willows, 1985) in terms of location, axonal distribution, and binding affinity for $SCP_B$ antibodies. The second pair of $SCP_B$-immunoreactive buccal ganglion neurons identified by Dorsett have axons in the central radular nerve and a branch that crosses the central commissure and ends in a dendritic field in the contralateral side of the ganglion. In its position, axonal distribution, and $SCP_B$ immunoreactivity, this neuron is identical to a ventrally occurring buccal neuron in *T. diomedea* identified by Masinovsky (1986) as vB1.

Several pulmonates also have presumptive SLB cells in their buccal ganglia. *Lymnaea stagnalis* has

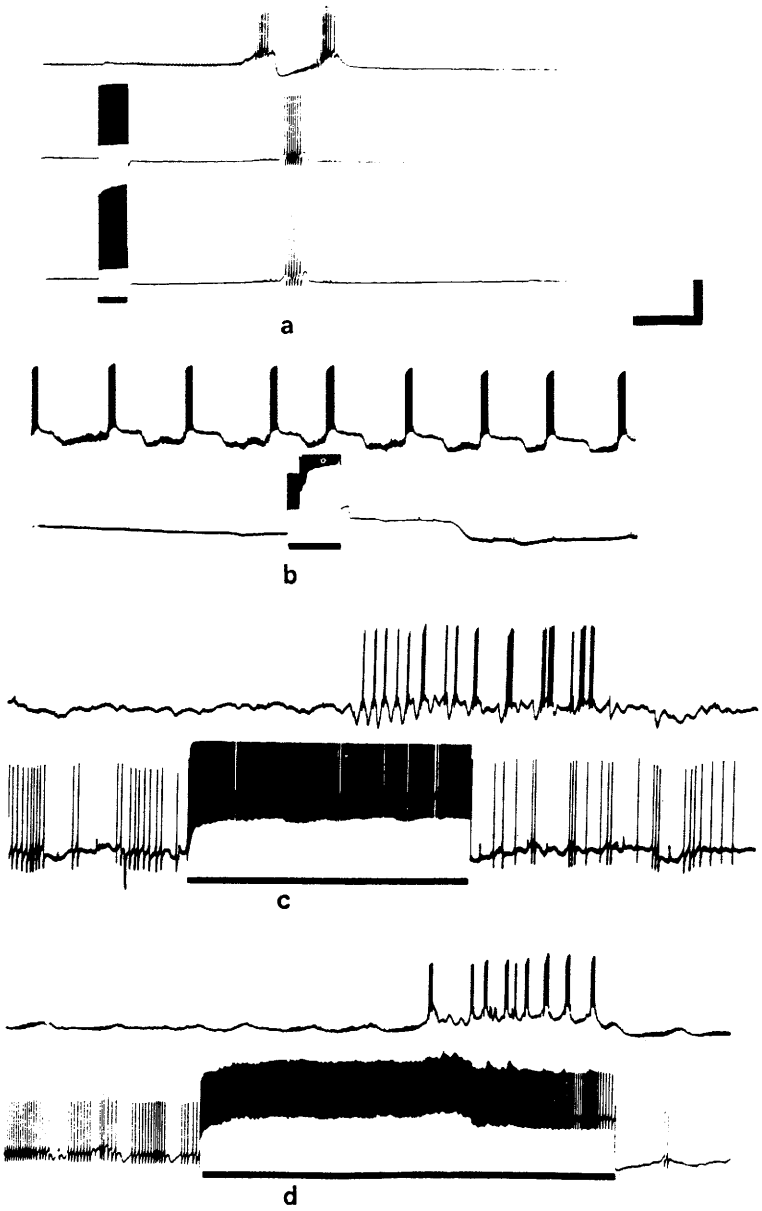


**Figure 7** Increased spiking driven in the SLB neurons by direct intracellular depolarization (bars) elicits the FMP in buccal neurons of (A,B) *Tritonia,* (C) *Dirona,* and (D) *Aeolidia.* In (A), both left and right SLB neurons are simultaneously driven at 3–5 Hz. for 2 s and a short FMP bout arises, including antiphase bursts in the SLB neurons. In (B), the FMP is spontaneously active, and SLB stimulation promotes a slight increase in burst rate and duration. Although not visible at this chart speed, the spiking rate also increases in response to SLB stimulation. In (C) and (D), prolonged SLB stimulation elicits a prolonged bout of FMP including the expected antiphase relations between spikes in the two neurons. Scale bar = 30 mV and 10 s (A), 50 s (B–D).

three pairs of reidentifiable neurons located on the dorsal surface of the buccal ganglia that react with $SCP_B$ antibodies (Masinovsky, 1986). The largest of the three pairs, located laterally, near the origin of the dorsobuccal nerve and cerebrobuccal connective, stains most densely and is called B1 by both Masinovsky (1986) and Benjamin and Rose (1979). This neuron pair fires synchronously dur-

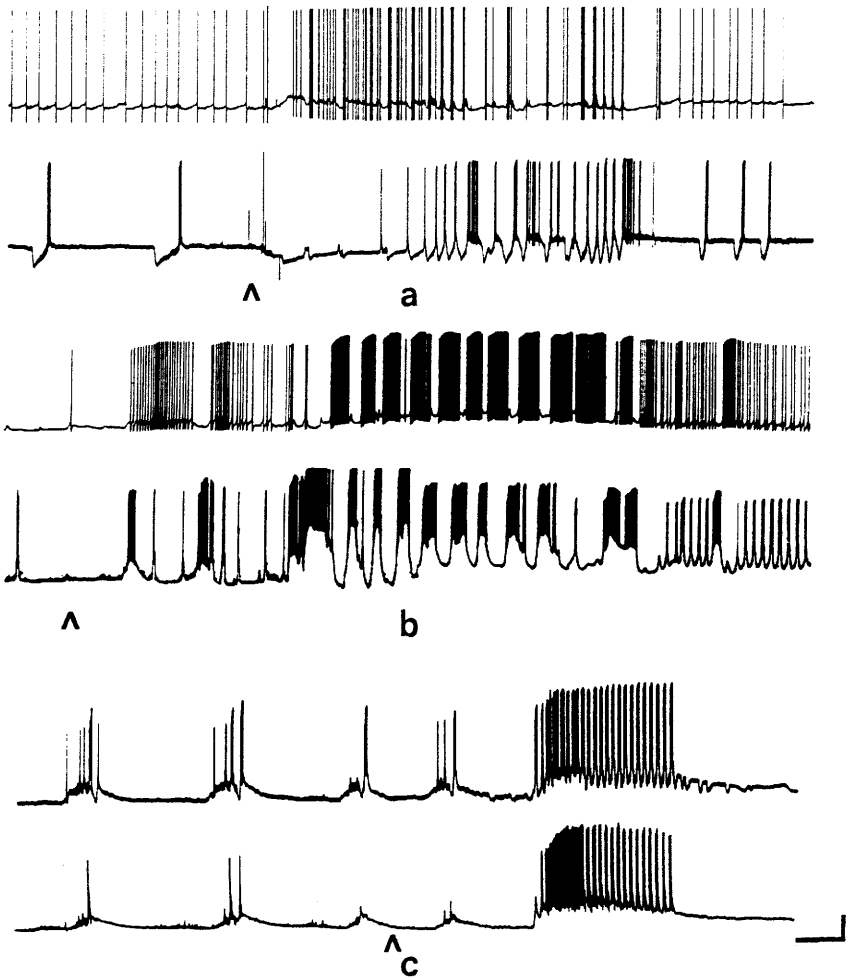


**Figure 8** Perfusion of the buccal ganglia of (A) *Armina,* (B) *Aeolidia,* and (C) *Hermissenda* with $10^{-5}$ *M* $SCP_B$ in seawater in each instance elicits a prominent increase in FMP output from presumed motor neurons (lower traces) and SLBs (upper traces). Preparations were perfused (5 ml/min) with $SCP_B$-free saline throughout the experiment. At the mark, 1 ml of $10^{-5}$ *M* $SCP_B$ was superfused over the preparation at the same flow rate, followed by a washout with drug-free saline. Scale bar = 20 mV and 50 s.

ing the protraction phase of the feeding cycle, is reciprocally coupled electrically, and innervates both the esophagus and salivary gland duct. *Helisoma trivolvis,* the pond snail, has two large and several smaller $SCP_B$-immunoreactive neurons. Superfusion of *Helisoma* buccal ganglia with $SCP_B$ enhances the likelihood of FMP expression and increases its rate and strength (Murphy et al., 1985). $SCP_B$ also modulates the FMP in the terrestrial slug *Limax maximus* (Prior, Watson, and Hess, 1985; Prior and Watson, 1988; Prior and Welsford, 1989). *Limax* has two large pairs of reidentifiable buccal neurons called *B1* and *B2,* and several smaller neurons, that react with $SCP_B$ antibodies (Prior and Watson, 1988). Direct stimulation of the B1 neurons produces FMP effects that mimic responses to exogenously applied $SCP_B$ (Prior and Delaney, 1986). Like *Lymnea,* the salivary glands of *Limax* are innervated by $SCP_B$-immunoreactive axons but the origin of these fibers is not known. Thus, while all putative SLB cells in gastropods appear to innervate the esophagus, projections of $SCP_B$-positive axons to salivary glands are most conspicuous in pulmonates.

In summary, it is apparent both within three suborders of the opisthobranchia (Dendronotacea, Arminacea, and Aeolidacea) and in other diverse gastropods that the buccal ganglion contains one

or two large, reidentifiable $SCP_B$-immunoreactive neurons that appear to be homologous. They all contain an $SCP_B$-like peptide (or peptides) and their pattern of innervation, electrical activity, influence on the FMP, and response to exogenously applied $SCP_B$ are similar. Recent studies indicate that many primitive gastropods also have SCP-immunoreactive neurons in their buccal ganglion (Pratt and Watson, in preparation). We conclude that the $SCP_B$-containing buccal neurons described in this paper and in previous contributions to the literature comprise a highly conserved neuronal system in the Gastropoda responsible for regulating central and peripheral aspects of gut motility. Further analysis of the similarities and differences between SLB cells in various gastropods may provide valuable information about both gastropod phylogeny and the evolution of identifiable neurons.

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