The Molluscan Neuropeptide, SCP_B, Increases the Responsiveness of the Feeding Motor Program of *Limax maximus*

DAVID J. PRIOR[1]* and WINSOR H. WATSON III[2]

[1]Physiology Group, School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506 and [2]Department of Zoology, University of New Hampshire, and the Friday Harbor Laboratories, University of Washington, Seattle, Washington 98105

Received March 2, 1986; accepted July 6, 1987

SUMMARY

Small cardioactive peptide B (SCP_B) has an excitatory effect on both buccal neurons and musculature in numerous molluscan species. The present study reports the effects of SCP_B on the activity of specified buccal neurons and the expression of the feeding motor program of the terrestrial slug, *Limax maximus*. Superfusion of an isolated CNS preparation with 10^{-6} *M* SCP_B results in a 3–4-fold increase in the burst frequency of the fast salivary burster neuron (FSB), while having no effect on the activity of another endogenous burster, the bilateral salivary neuron (BSN). The response of the FSB to SCP_B is dose dependent, with a threshold concentration of $2 \times 10^{-8} M$. The response of the FSB to SCP_B showed no indication of desensitization, even after long-term exposure (20 min).

The feeding motor program (FMP) in *Limax* is a discrete pattern of cyclical motor activity that can be initiated by lip nerve stimulation. In the presence of SCP_B a previously subthreshold stimulus can initiate the full FMP. The pattern of the FMP, once initiated, appears unaffected by SCP_B. Thus it is the responsiveness of the initiation process that is enhanced by SCP_B. Histochemical studies revealed a number of buccal neuron somata and fibers that stain for SCP_B-like immunoreactive material (SLIM).

INTRODUCTION

Small cardioactive peptide B (SCP_B; Lloyd 1978, 1979) has been shown to have excitatory effects on both neurons and muscles in several gastropod species. For example, application of SCP_B to the isolated buccal ganglia of *Tritonia* (Willows et al., in press), *Helisoma* (Murphy et al., 1985), *Dirona, Aeolidia, Armina, Archidoris,* and *Hermissenda* (Willows and Watson, 1986) enhances patterned motor output associated with feeding activity. In addition, in *Aplysia* and *Limax* SCP_B can enhance the contractility of feeding musculature (Lloyd et al., 1984) and, in *Limax*, crop musculature (Krajniak et al., 1985). In the case of *Helisoma*, $10^{-6}M$ SCP_B increases the burst frequency of spontaneously active feeding motoneurons (B5 and B19) and

*Present address: Dept. of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011.

Journal of Neurobiology, Vol. 19, No. 1, pp. 87–105 (1988)
© 1988 John Wiley & Sons, Inc. CCC 0022-3034/88/010087-20$04.00

also is capable of initiating patterned motor activity (PMA; Murphy et al., 1985). In each of the aforementioned systems the presence of SCP_B-like peptides in the buccal ganglia has been demonstrated with immunohistochemistry. Furthermore, in *Aplysia*, SCP_B immunoreactivity has been localized to discrete sets of dense core vesicles that occur in identified buccal neurons (e.g., B2) and in processes and terminals in the esophagus, salivary gland, and radula closer muscle (Kreiner et al., 1986). Thus, both physiological and histological data suggest an involvement of SCP_B-like peptides in the control of molluscan feeding.

In many isolated buccal ganglion preparations considerable spontaneous patterned activity is often observed. As a result, it is often difficult to determine what aspect of buccal activity and pattern generation is being affected by a peptide modulator. This is not the case with the feeding motor program (FMP) of *Limax maximus*. In *Limax* the central pattern generator underlying feeding is quiescent until feeding is initiated by either application of a chemical stimulus to the lips (e.g., Cooke et al., 1985) or by electrical stimulation of the lip nerves (e.g., Phifer and Prior, 1985). Such stimulation results in initiation of a discrete cyclical pattern of buccal ganglion activity that controls feeding movements (see Fig. 5). This feeding motor program consists of bursts of efferent activity that correspond to the alternating protraction/retraction movements made by the radula during feeding (Gelperin et al., 1978; Reingold and Gelperin, 1980). Thus, in *Limax* there is a discrete pattern of feeding activity that usually continues for only 20–50 cycles, after which the preparation again becomes quiescent. We have taken advantage of this characteristic to investigate the possibility that SCP_B could exert a modulatory effect on the responsiveness of the FMP, in addition to the direct excitatory effects seen in other preparations. This experimental design has been used by Gelperin et al. (1985) to demonstrate an inhibitory effect of the peptide FMRFamide on the FMP of *Limax*. In the present study we have also used immunohistochemical techniques to identify buccal ganglion neurons that contain SCP_B-like peptides. A preliminary report of these findings has appeared in abstract form (Prior et al., 1985).

METHODS AND MATERIALS

Animals

Specimens of the slug, *Limax maximus*, were collected for us in the Seattle area by Drs. I. Deyrup-Olsen and A. Martin. Slugs were kept in vented plastic refrigerator boxes lined with moist paper towels and were fed ad lib with pellets of dog chow. The culture boxes were kept in the general laboratory (18–23°C) and were thus exposed to approximately the natural light/dark cycle (February–June, 1985).

Electrophysiology

The central nervous system (e.g., cerebral ganglia, circumesophageal ring, and the buccal ganglia) was dissected and pinned to a Sylgard-lined recording chamber having a volume of 0.5 mL. Preparations were continuously superfused with slug saline (see Prior and Grega, 1982) at a rate of 0.5 mL/min. Standard suction electrode techniques were used to record from, or stimulate, nerve branches. Intracellular recordings were made using glass micropipettes filled with either 2.0 *M* potassium acetate or 2.5 *M* potassium chloride. SCP_B was obtained from Pennisula Labs

(Belmont, CA) and was prepared in slug saline immediately before use or by dilution of a frozen stock (2×10^{-4} *M*, prepared each week). No difference in response was observed between the use of peptide prepared by the two methods. Preparations were dissected in the morning, and experiments were performed in the afternoon and evening of the same day.

Immunohistochemistry

Tissues were removed, pinned out in Sylgard-lined glass petri dish filled with cold saline, and then fixed for 12 h in 4% paraformaldehyde in 0.1 *M* phosphate-buffered (Sorenson's, pH 7.4) saline (PBS, 0.9% NaCl). Fixations, and all the following procedures, were carried out on shaker table in a cold room maintained at 8°C. After fixation, tissues were washed for 1 h in PBS. Some preparations were rinsed in 0.2 *M* glycine for 1 h prior to the PBS wash to aid in removal of residual fixature (A. Spencer, personal communication).

In order to facilitate penetration of whole mounts by the antibody, tissues were dehydrated through a graded series of ethanol (30, 50, 70, 80, 90, 95, and 100%, 10 min each), rinsed in toluene or xylene and then rehydrated by passing them through the same ethanol series in reverse order. Following dehydration/rehydration, tissues were washed for 1 h in PBS containing 0.3% Triton X-100 and 0.1% sodium azide (PTA). Tissues were then incubated for 12 h in PTA containing 6% nonimmune goat serum.

Primary antibody was diluted in PTA and 6% nonimmune goat serum (1:20). Tissues were incubated in diluted antisera for 36–48 h. They were then washed for 24 h in PTA (4–6 changes). Goat antimouse secondary antibody labeled with FITC was diluted 1:100 in PTA and 6% nonimmune goat serum. Secondary antibody incubations lasted for 24–36 h. Tissues were then washed in PTA for 12 h and PBS for 1 h.

Preparations stained with FITC were mounted on a slide using a medium that consisted of 1 part 50 m*M* Tris buffer (pH 9.5) and 9 parts glycerol. Whole mounts were viewed and photographed (Tri-X, 400 ASA) with a Nikon Optiphot microscope using epifluorescent illumination (B2 filter block, 460–485 interference excitation filter, 505 dichroic mirror, 511–545 barrier filter).

The SCP_B monoclonal antibody was a gift from B. Masinovsky and A.O.D. Willows of the University of Washington. Staining was partially (approximately 80%) inhibited by preincubation of the antibody with 10^{-5} *M* SCP_B for 24 h and completely eliminated when 5×10^{-5} *M* SCP_B was used. The secondary antibody and normal goat serum were obtained from Cappel (Cooper Biomedical, Malvern, PA).

RESULTS

Salivary Motoneurons

We initially examined the effects of SCP_B on the fast salivary burster neuron in order to compare this preparation with others in which SCP_B can modify ongoing activity (FSB; e.g., Prior and Gelperin, 1977). The FSB is an endogenously active salivary motoneuron that has a single axon in the ipsilateral salivary nerve (SN; Figs. 1 and 3). As seen in Figures 1–3 the baseline burst frequency of the FSB is highly variable, ranging from less than 1 burst/min up to 6–7 burst/min [see Fig. 2(A, B)]. Exposure of an isolated CNS preparation to 2×10^{-6} *M* SCP_B for 2 min resulted in a rapid increase in the burst frequency of the FSB which persisted for nearly 10 min (Figs. 1 and 2). Intracellular recordings have revealed no consistent effect of SCP_B on the "apparent" membrane potential of the FSB. Although in the example shown in Fig. 1(B) there is a slight depolarization, in other preparations there was no change in potential and in others even a slight hyperpolarization. It has now been shown that the increase in burst frequency is due to an increase in the rate of interburst depolarization of the FSB rather than a steady change in membrane potential (Hess and Prior, 1986).

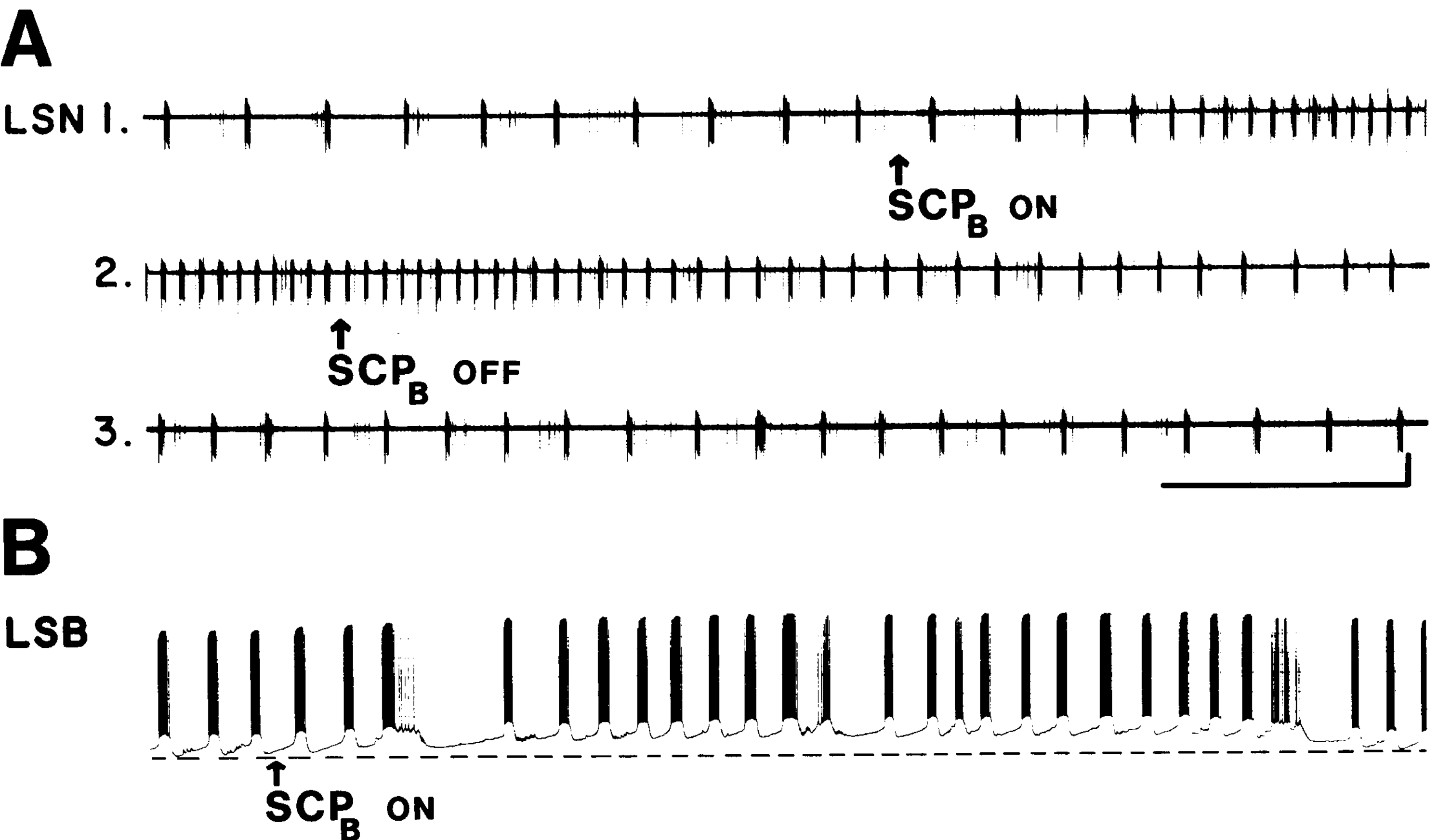


Fig. 1. (A) A continuous extracellular recording from the left salivary nerve (LSN) of an isolated buccal ganglia-brain preparation is shown in 1–3. The prominent bursting unit in this record is the fast salivary burster (FSB; each burst consists of 12–15 spikes). Within 20 s of the application of 2×10^{-6} *M* SCP$_B$ to the preparation (first arrow), the burst frequency of the FSB increases. Following removal of SCP$_B$ from the superfusion medium (second arrow), burst frequency of the FSB returns to the pretreatment level. (B) an intracellular recording from the fast salivary burster neuron (LSB) showing the increase in burst frequency and, in this case, progressive depolarization, in response to 2×10^{-6} *M* SCP$_B$ (the dashed line indicates the level of the interburst hyerpolarization before exposure to SCP$_B$). Bar = 30 s (A) and 20 mV (B).

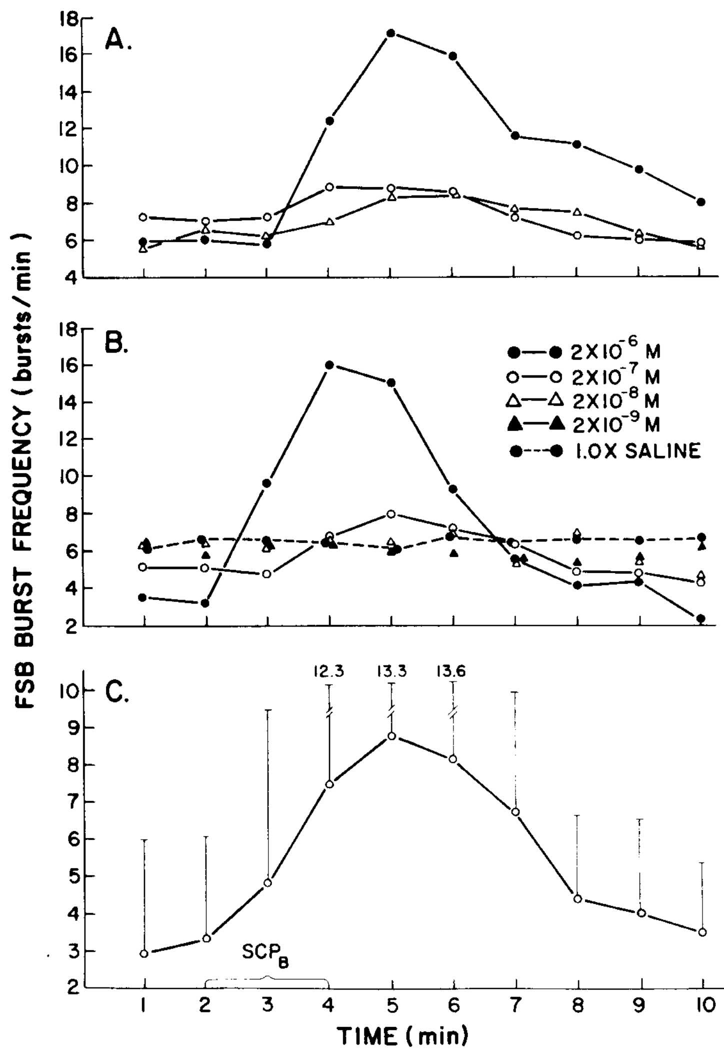


Fig. 2. The responses of the fast salivary burster neuron (FSB) to varying concentrations of SCP$_B$ are presented by plotting burst frequency as a function of time during the experiment. In each case SCP$_B$ was superfused over an isolated buccal ganglion-brain preparation between minutes 2 and 4. The preparation was superfused with saline for 20 min between each trial. (A) The responses obtained in three trials with the same preparation using various concentrations of SCP$_B$ are shown. Each point represents the burst frequency of the FSB in the preceeding 60 s. (B) The responses of a second preparation to SCP$_B$. In this case four different concentrations of SCP$_B$ were used as well as a control saline trial. (C) The extent of the variability between preparations is illustrated by plotting the mean (SD) burst frequency at each time point for 29 trials in 12 preparations during exposure to 2×10^{-6} *M* SCP$_B$.

In each of the two experiments illustrated in Fig. 2(A, B), 2×10^{-6} *M* SCP$_B$ caused a 3–4-fold increase in FSB burst frequency. Although there was considerable variation between preparations, in each case (12 preparations) the threshold concentration of SCP$_B$ was about 2×10^{-8} *M* [Fig. 2(A, B)]. The time course of the FSB response to SCP$_B$ is summarized in Fig. 2(C). Each point is the mean (SD) of 29 trials in 12 preparations. The maximum response

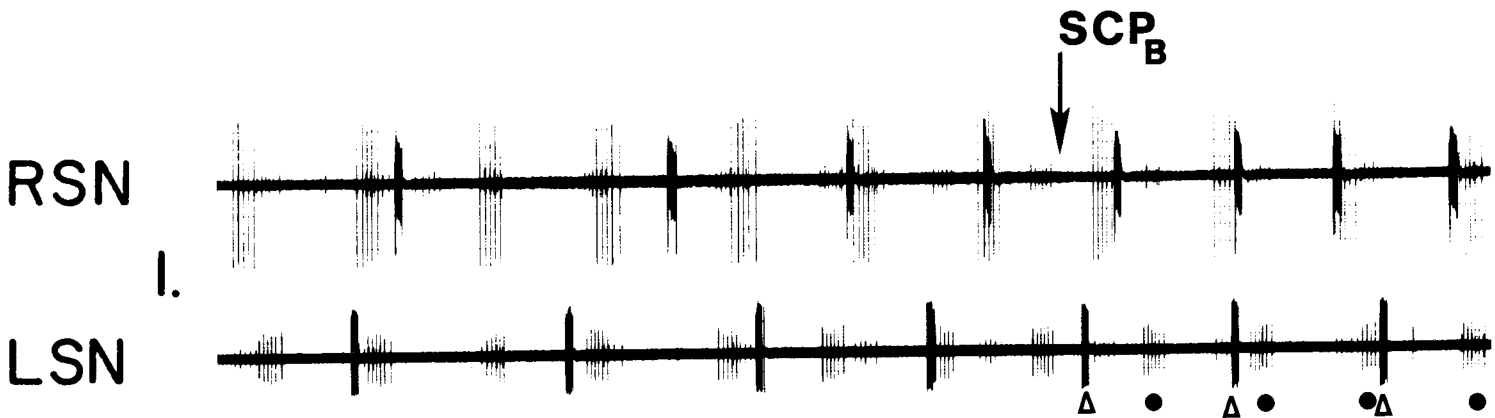


Fig. 3. Continuous extracellular recordings (1–3) from the right (RSN) and the left (LSN) salivary nerves of an isolated buccal ganglia-brain preparation. Superfusion of 2×10^{-6} *M* SCP_B was begun at the arrow. The burst frequency of the fast salivary burster (open triangles in LSN 1 and 2 is seen to increase while that of the bilaterial salivary neurons (BSN, solid circles, in LSN 1 and 2) remained unchanged.

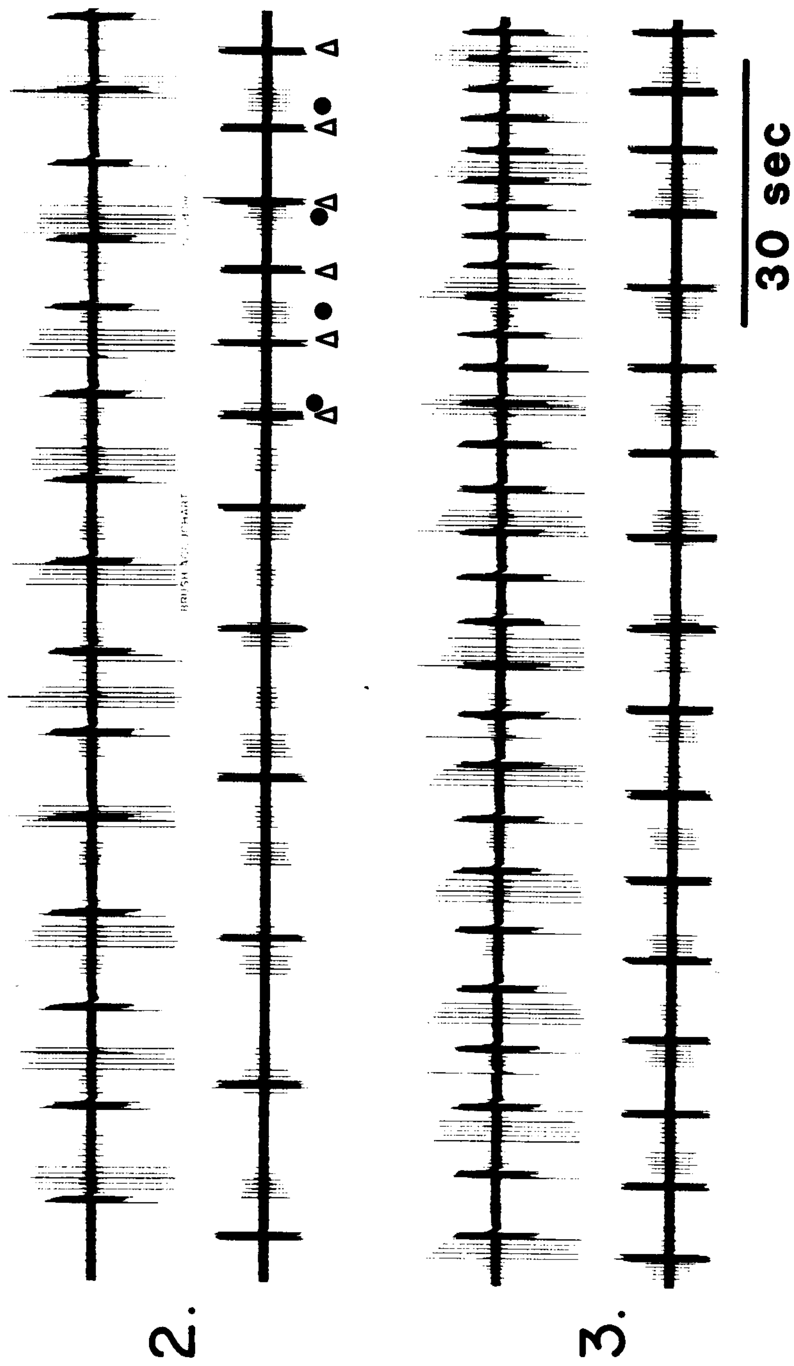


Fig. 3. *(Continued from previous page.)*

to a 2 min exposure to SCP_B (2×10^{-6} *M*) occurred at 3 min and returned to the pretreatment level within 10 min. Much of the variability was due to the large variation in the initial burst frequency of individual FSBs (e.g., in some cases the FSB was silent). Nevertheless, in 26 of 29 trials 2×10^{-6} *M* SCP_B caused an increase in FSB burst frequency, which was often several fold.

There appears to be a certain degree of specificity to the action of SCP_B in the *Limax* feeding system. Several other peptides that have been tested with the FSB have either no effect (Angiotensin II, Oxytocin; Prior, unpublished) or a strong inhibitory effect (FMRFamide; Cooke et al., 1985) on the activity of the FSB. In addition, although SCP_B has an excitatory effect on the FSB, it has no effect on the activity of another autoactive buccal neuron, the bilateral salivary neuron, (BSN; Copeland and Gelperin, 1983). As seen in Figs. 3 and 4, SCP_B caused a rapid increase in the burst frequency of both the right and left FSBs but resulted in no change in the burst frequency of either BSN (Figs. 3 and 4). Even prolonged superfusion of the CNS with 2×10^{-6} *M* SCP_B resulted in no alteration in the activity of the BSNs, even though the response of the FSBs was sustained (Fig. 4). Thus, in *Limax*, as in the other gastropods that have been examined, SCP_B has an excitatory effect on the ongoing activity of specific buccal neurons.

Effects of SCP_B on the Feeding Motor Program

Using the same type of isolated CNS preparation described above, the effect of SCP_B on the responsiveness of the feeding motor program was examined. As illustrated in Fig. 5, stimulation of the anterior lip nerve can

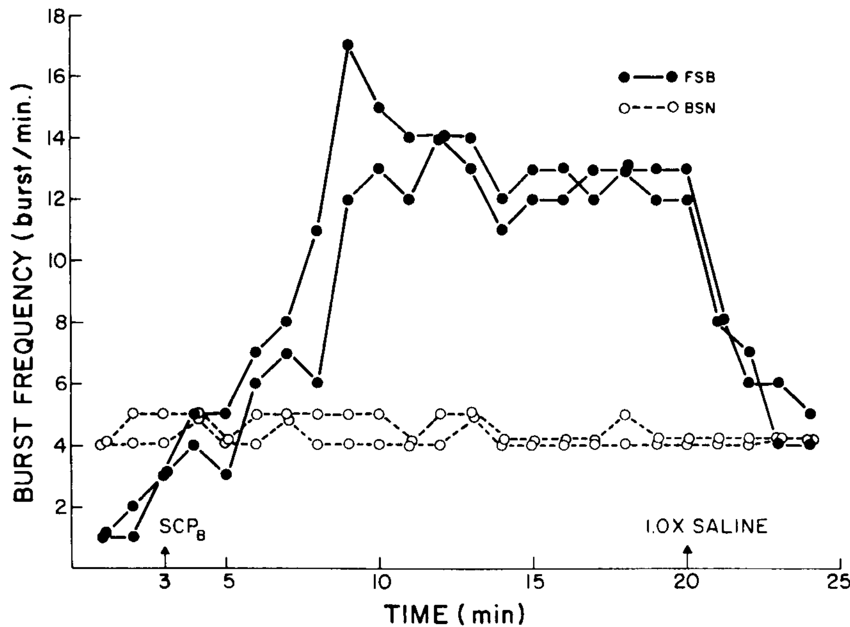


Fig. 4. Activity of the fast salivary bursters (FSB) and the bilateral salivary bursters (BSN) during long-term exposure to 2×10^{-6} *M* SCP_B is illustrated. From minutes 3 to 20 the preparation was superfused with 2×10^{-6} *M* SCP_B. The burst frequencies (the mean over 1-min periods) of the right and left FSBs and the right and left BSNs in one preparation are shown.

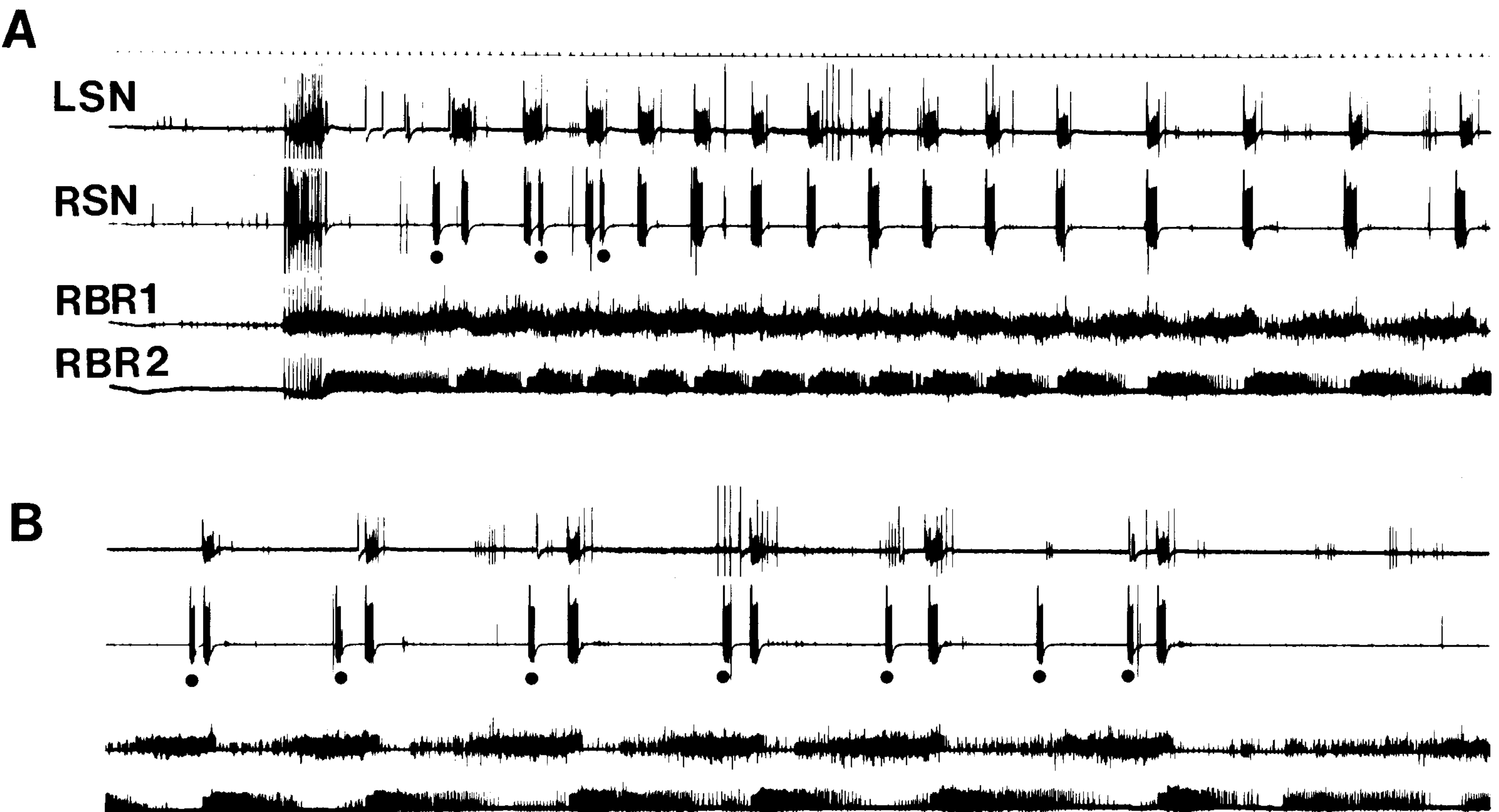


Fig. 5. Figure 5 illustrates activation of the feeding motor program (FMP) in an isolated buccal ganglia-brain preparation by electrical stimulation (artifacts at beginning at A) of an external lip nerve. The FMP is characterized by alternation of efferent bursts correlated with protraction (buccal nerve 1: RBR1; and the right and left salivary nerves: RSN, LSN) and retraction (buccal nerve 2:RBR2). The nonfeeding endogenous bursts of the right FSB are noted with dots. The upper calibration trace indicates one mark/second.

initiate the feeding motor program, which can be readily identified by extracellular recordings from buccal nerves. Efferent activity in the right and left salivary nerves (RSN, LSN) and buccal nerve 1 (RBR1) occurs during the protraction phase of feeding, whereas activity in buccal nerve 2 (RBR2) occurs during the retraction phase (Fig. 5 and Gelperin et al., 1978). Occasionally, at the beginning and at the termination of an FMP, endogenous bursts of a FSB occur amid the synchronized feeding bursts. These nonfeeding bursts (see the dots in Fig. 5, RSN) occur when the synaptic drive of the FMP is insufficient to completely override the endogenous activity of the FSB. Usually the FSB bursts are in synchrony with the late phase of protraction throughout an FMP (see Fig. 5, LSN).

To test the possible modulatory action of SCP_B on the responsiveness of the FMP, a threshold level of lip nerve stimulation was established for each preparation. The FMP was first initiated by suprathreshold stimulation of the anterior lip nerve [Fig. 6(A)]. After a period of 20 min the number of stimuli in the train was reduced to produce a subthreshold level of stimulation [Fig. 6(B)]. After 20 min the preparation was exposed to 2×10^{-6} M SCP_B, and the same subthreshold stimulation [as in Fig. 6(B)] was again applied. As seen in Fig. 6(C), in the presence of SCP_B the previously ineffective stimulation resulted in initiation of a complete FMP. When the preparation was returned to normal saline, the same subthreshold stimulation was once again insufficient to initiate an FMP. The entire experimental sequence was repeated with the same results in fine preparations. Thus, exogenous SCP_B is capable of increasing the responsiveness of the feeding motor program to lip nerve stimulation.

Immunohistochemistry

The overall distribution of SCP_B-like immunoreactive material (SLIM) in the paired buccal ganglia and salivary glands of *Limax* is illustrated in Fig. 7. the most notable feature is the presence of SLIM in the large, laterally positioned B1 cell bodies and their ipsilateral axons within the cerebrobuccal connectives (CBC). In addition, several other large somata, such as those in the B2 cluster and the protractor motoneuron, B7, react positively for SLIM. These are visible in the left buccal ganglion in Fig. 7 and in the right buccal ganglion in Fig. 8. In addition to the large, identifiable neurons containing SLIM, we observed several groups of small neurons that reacted with the SCP_B monoclonal antibodies. These are most obvious in the right buccal ganglion in Figs. 7 and 8.

SCP_B-like immunoreactive fibers were also observed in association with other tissues of the digestive system. The salivary gland was innervated by a number of axons which course over the duct and could be followed into the duct tissue [Figs. 7 and 9(A)]. In no preparations were SLIM-containing cell bodies observed on or within the duct musculature. The surface of the foregut was also heavily innervated by SLIM-containing fibers [Fig. 9(B)]. Although many fibers could be traced in these tissues, we have yet to identify the specific cell bodies in the buccal ganglia which give rise to them.

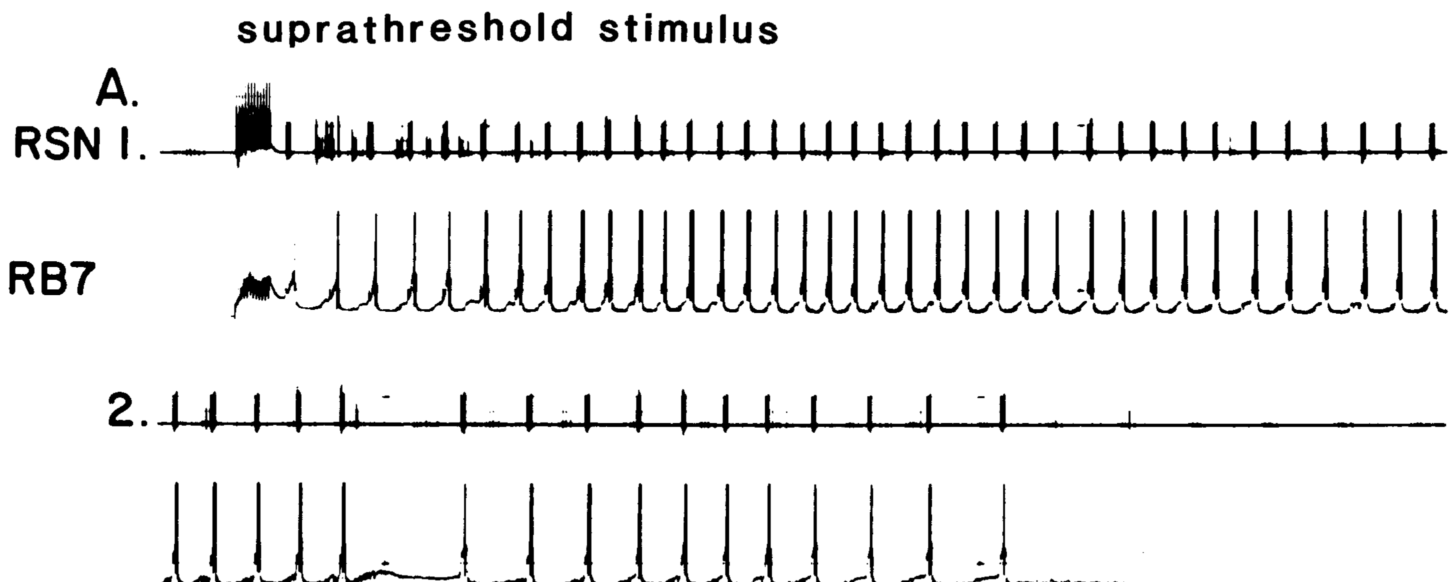


Fig. 6. Simultaneous extracellular recordings from the right salivary nerve (RSN) and intracellular recordings from a protractor motoneuron (RB7) during an FMP is shown. (A) 1–2: a train of electrical shocks applied to an external lip nerve initiated an FMP of 37 cycles. The RB7 bursts, each of which was composed of 4–7 impulses, were separated by a series of high frequency IPSPs. (B) A subthreshold (fewer stimuli) train of stimuli was applied to the external lip nerve. Although 3–4 bursts of salivary nerve activity and the corresponding compound EPSPs in RB7 were observed, an FMP was not initiated. After 20 min the preparation was bathed in 2×10^{-6} *M* SCP$_B$, and the same subthreshold stimulation used in B was repeated. In this case a full FMP was initiated. The calibration Bar = 30 (A, B, C) and 60 mV (C).

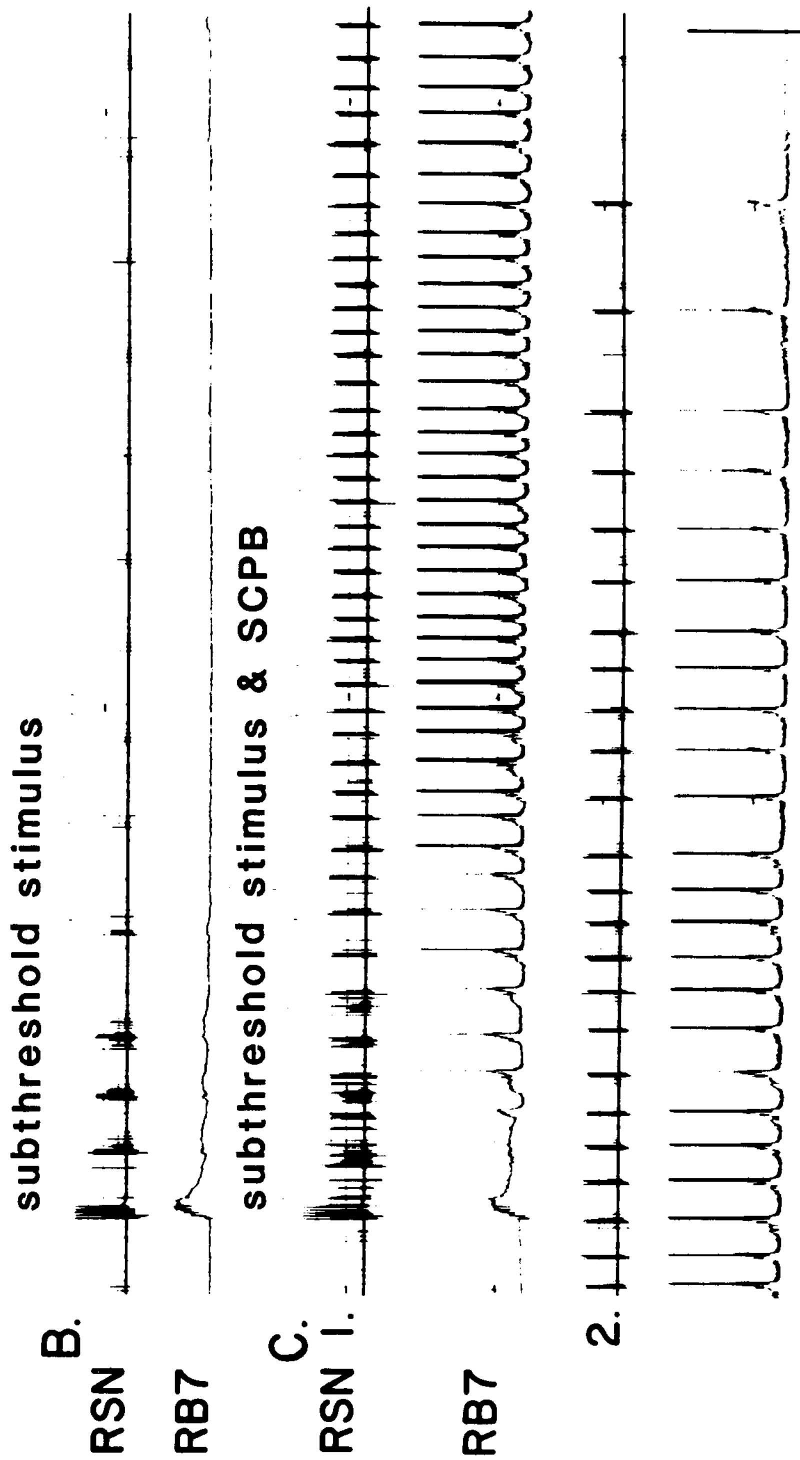


Fig. 6. (Continued from previous page.)

In *Lim*
burster a
stimulati
frequency
is unaffe
SCP_B sim
sive neur
10^{-9} *M* S
sure of a
an FMP.

It has r
fast saliv
input (He
Mg^{++}/Lo
in burst f
nous burs

Our ob
certain b
phy et al
and Wat
ongoing
patterned
preparati
SCP_B, ev
feeding a
the neura
shown in
can initia
siveness

It shou
more sen
SCP_B inc
effect on

In *Trit*
noreactiv
which co
marine g
son and
ganglia
made fo
promine
activity c
al. (198
located S
be that a
B11 in *T*
and Wil
occur in

DISCUSSION

In *Limax* SCP_B can increase the burst frequency of the fast salivary burster and can enhance the responsiveness of the feeding motor program to stimulation of a chemosensory pathway. Although SCP_B increases the burst frequency of the FSB several fold, the activity of the bilateral salivary neuron is unaffected (Figs. 3 and 4). This observation suggests that rather than SCP_B simply having a general excitatory effect, there exist subsets of responsive neurons. This is further supported by the observation that from 10^{-7} to 10^{-9} *M* SCP_B does not initiate the feeding motor program, although exposure of a preparation to 2×10^{-6} or 10^{-5} *M* SCP_B does occasionally result in an FMP.

It has recently been demonstrated that the excitatory effect of SCP_B on the fast salivary burster appears not to involve modification of chemical synaptic input (Hess and Prior, in preparation). FSBs in preparations bathed in high Mg^{++}/Low Ca^{++} saline responded to SCP_B with the characteristic increase in burst frequency. Thus SCP_B appears to be directly affecting the endogenous bursting of the FSB neuron.

Our observation that SCP_B causes an increase in bursting activity of certain buccal neurons is consistent with recent studies on *Helisoma* (Murphy et al., 1985), *Tritonia, Dirona, Aeolidia*, etc. (see Introduction, Willows and Watson, 1986). In all these cases SCP_B has an excitatory effect on ongoing patterned activity. In *Helisoma*, 10^{-6} *M* SCP_B can also initiate patterned motor activity in buccal neuron B19 that continues as long as the preparation is exposed to SCP_B (Murphy et al., 1985). However, in *Limax*, SCP_B, even at a concentration of 5×10^{-6} *M*, does not reliably "trigger" feeding activity. SCP_B does, however, reliably increase the responsiveness of the neural network underlying initiation of the feeding motor program. As shown in Fig. 6, in the presence of SCP_B an otherwise ineffective stimulus can initiate a complete FMP. Thus, in *Limax* SCP_B can modulate the responsiveness of the FMP.

It should be noted that even though SCP_B renders the initiation process more sensitive, the pattern of the FMP is unaffected. Thus, even though SCP_B increases the burst frequency of the fast salivary burster, it has little effect on the burst frequency or impulses/burst of the FSB during an FMP.

In *Tritonia*, stimulation of both B11 cells (which contain SCP_B-like immunoreactive material) elicits rhythmic bursting in buccal motoneuron B5, which corresponds to swallowing (Willows et al., submitted). In several other marine gastropods stimulation of homologous SLIM-containing cells (Watson and Willows, 1986) also elicits rhythmic output from isolated buccal ganglia (Willows and Watson, 1986). Recently a similar observation was made for buccal neuron B1 in *Limax*. Interacellular stimulation of this prominent SLIM-containing neuron results in an increase in the endogenous activity of the FSB (Prior and Delaney, 1986). In a recent report by Murphy et al. (1985) on *Helisoma*, their Fig. 6 clearly shows a prominent laterally located SLIM-containing neuron that could correspond to B1 in *Limax*. It may be that activation of this cell in *Helisoma*, like buccal neuron B1 in *Limax* and B11 in *Tritonia*, can modulate feeding activity. In fact, as proposed by Watson and Willows (1986), these large SCP_B-like immunoreactive neurons that occur in many gastropods may well be homologous.

(A)

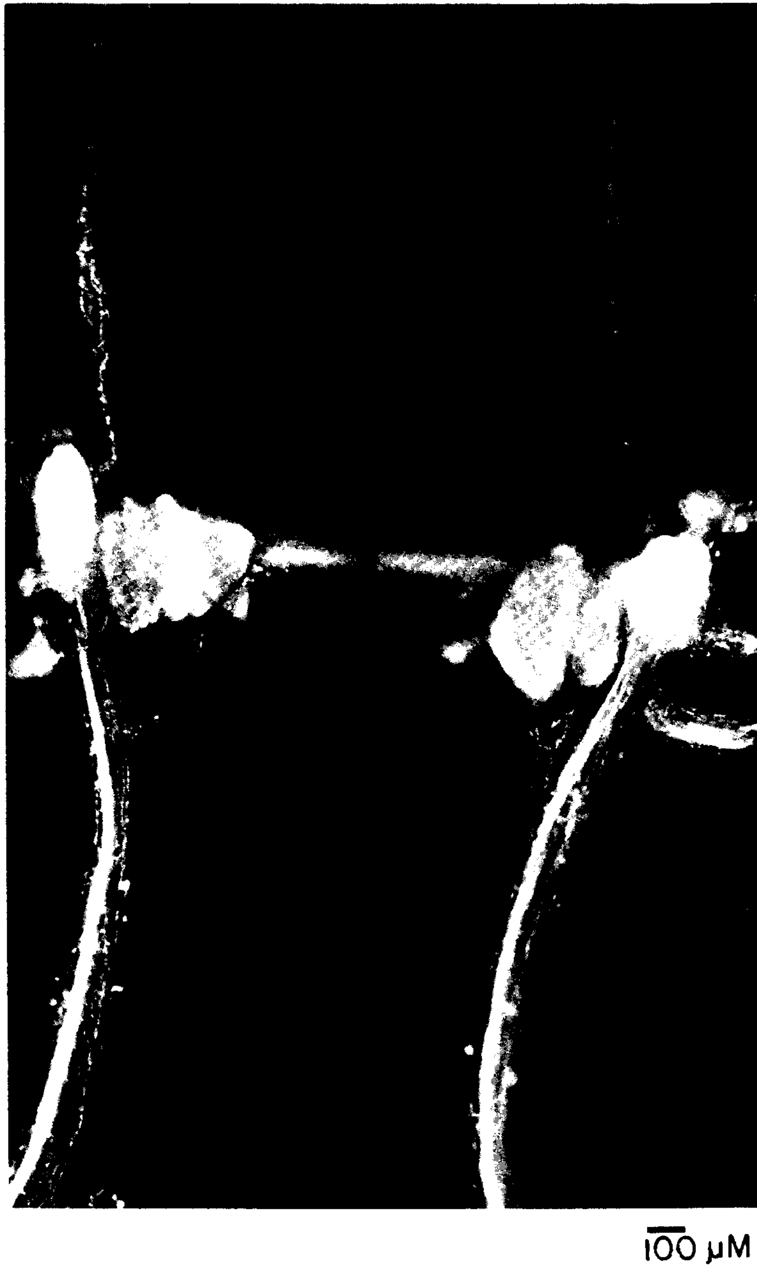


Fig. 7. Distribution of SCP_B-like immunoreactivity in the *Limax* buccal ganglion and salivary ducts. (A) A photomicrograph of a whole mounted pair of buccal ganglia stained for SCP_B-like peptides. (B) A schematic of A identifying prominent features, including buccal neuron B1; buccal-buccal connective (BBC); buccal nerves BR1, BR2, and BR3; gastric nerve (GN); salivary nerve (SN); cerebrobuccal connective (CBC; note the large axon of the ipsilateral buccal neuron B1 in each of the CBCs).

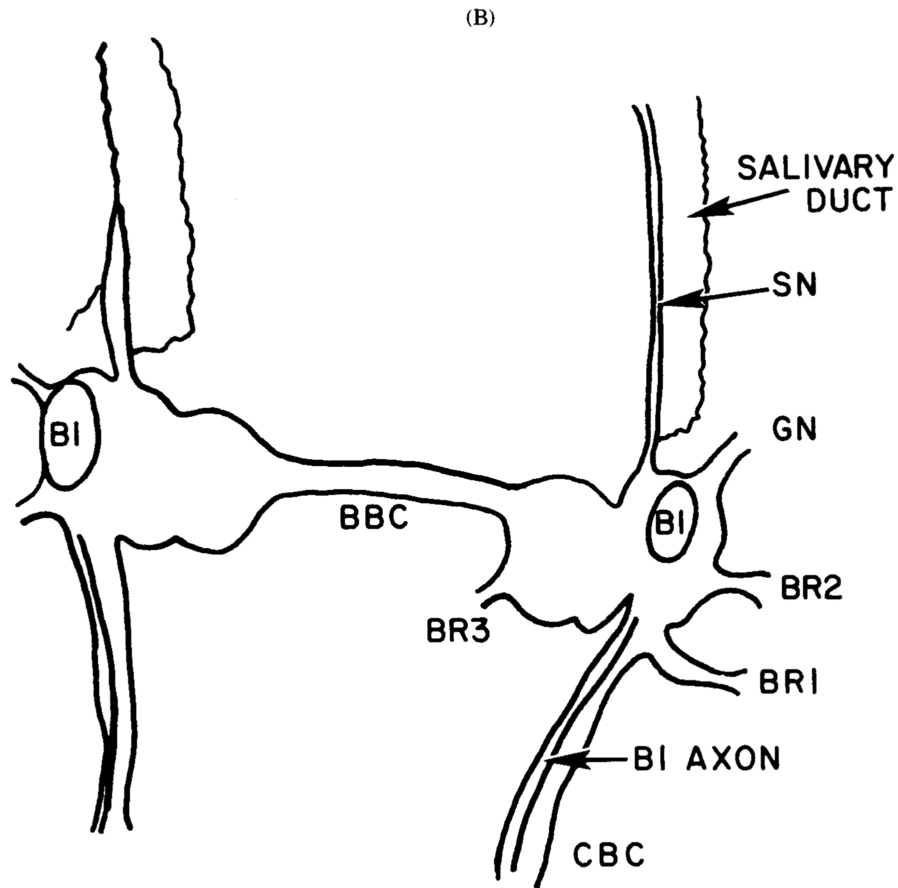


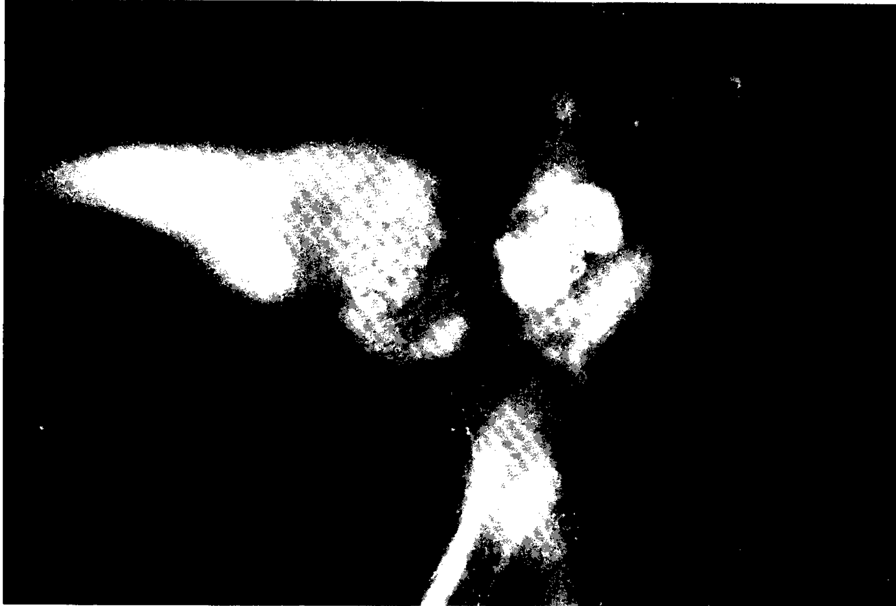
Fig. 7. *(Continued from previous page.)*

Immunohistochemistry

In all the gastropod species examined thus far, SLIM has been found the buccal ganglia and the gut (*Aplysia*, Lloyd et al., 1984; *Helisoma*, Murphy et al., 1985; *Tritonia, Lymnaea*, Masinovsky, submitted; 12 species of marine gastropods, Watson and Willows, 1986). In *Limax* there is also an extensive network of SCP$_B$-like immunoreactive varicosities on the surface of the foregut. In most of the other species studied, SCP$_B$-like immunoreactivity on the gut is localized in fibers that originate from large buccal ganglion neurons (B11 in *Tritonia*, Willows et al., submitted; B1 in *Lymnaea*, Masinovsky et al.; *Hermissenda, Dirona, Melibe*, and others, Watson and Willows, 1986). Since the B1 in *Limax* is known to have axons in several visceral ganglion nerve branches and appears to be homologous to the large SLIM cells in other gastropods, it may give rise to some of the SCP$_B$-like immunoreactive fibers observed on the gut (Prior and Gelperin, unpublished observations).

The present immunohistochemical studies were carried out with a monoclonal antibody directed against SCP$_B$. Our controls and RIA data (unpub-

A. SCP_B



B. Drawn from A. (SCP_B)

100 μM

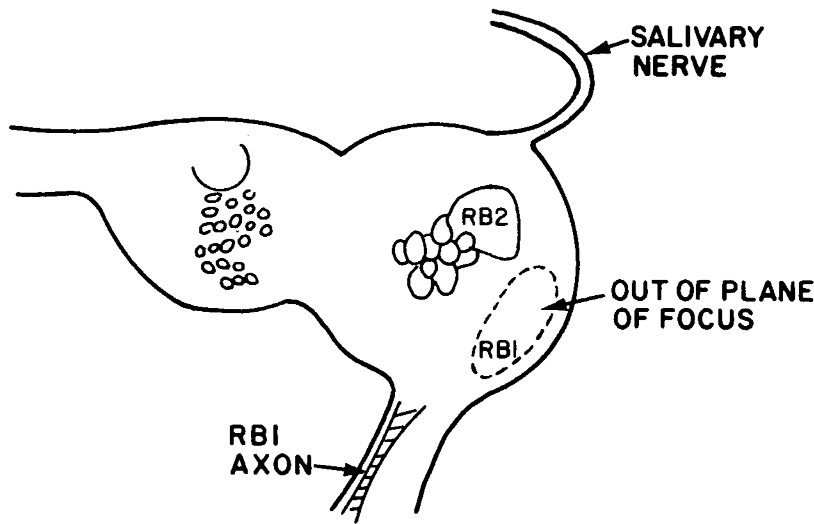
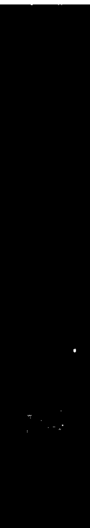
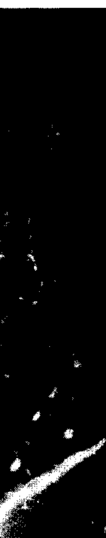
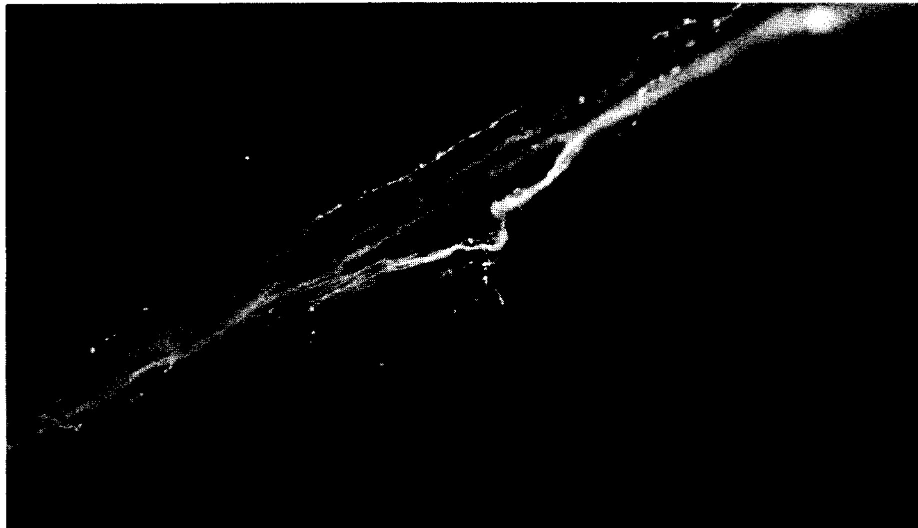


Fig. 8. Localization of SCP_B-like immunoreactivity in the *Limax* buccal ganglion is illustrated at a higher magnification. (A) A photomicrograph of a whole mounted right buccal ganglion, showing SCP_B-like immunoreactive cells and processes. The cell body of the large lateral neuron B1 was left slightly out of focus to allow visualization of neuron RB2 and its associated cluster of medium-sized neurons. Several structures seen in A are drawn and labelled in B.





A. Salivary Gland



B. Foregut

100 μM

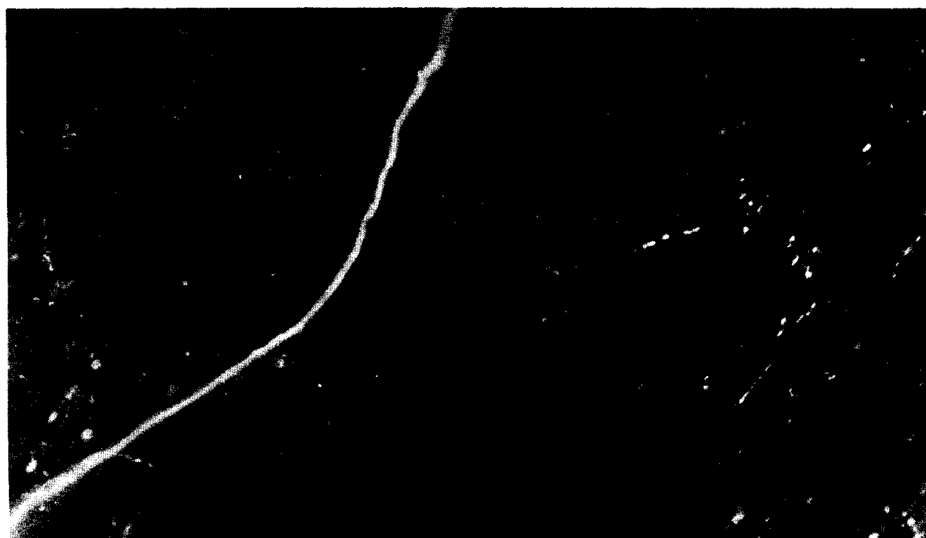


Fig. 9. Presence of SCP$_B$-like immunoreactive material in the salivary gland and foregut of *Limax*. The whole mount of a salivary gland shown in A reveals several fibers containing SCP$_B$-like immunoreactive material which runs along the length of the gland. The foregut of *Limax* (B) is covered with SCP$_B$-like immunoreactive fibers and varicosities.

lished) indicate that the antibody does not cross-react with the peptide FMRF-amide. However, it is likely that it does react with SCP_A (Lloyd et al., 1984). At least in *Aplysia*, SCP_A and SCP_B are found in the same cells (Lloyd et al., 1985) and appear to be derived from a common precursor (Mahon et al., 1985). Thus, cells that stain positively with our monoclonal antibody may well contain both SCPs.

The present results, and those from several other gastropod species, are consistent with the notion that SCP_B-like peptides have excitatory effects on buccal neurons and musculature and may be involved in modulation of the responsiveness of the feeding network. In order to begin a more detailed analysis of the physiological role of this peptidergic system in the regulation of feeding in *Limax*, we are now investigating whether or not SCP_B-like immunoreactive neurons such as B1 contain authentic SCP_B.

We express our gratitude to the Director, Professor A.O.D. Willows, and the staff of the Friday Harbor Laboratories for their kind cooperation during the course of this research. This project was supported in part by an N.I.H.-R.C.D.A. and a grant from the Whitehall Foundation to D.J.P. and N.I.H.-19053 to W.W. This is contribution No. 271 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association.

REFERENCES

COOKE, I., DELANEY, K., and GELPERIN, A. (1985). Complex computation in a small neural network. In *Memory Systems of the Brain*, N.M. Weinberger, J.L. McGaugh, and G. Lynch (Eds.), Guilford Press, New York, pp. 173–191.

COPELAND, J., and GELPERIN, A. (1983). Feeding and a serotonergic interneuron activate an identified autoactive salivary neuron in *Limax maximus*. *Comp. Biochem. Physiol.* **76A**: 21–30.

GELPERIN, A., CHANG, J.J., and REINGOLD, S. (1978). Feeding motor program in *Limax maximus* neuromuscular correlates and control by chemosensory input. *J. Neurobiol.* **9**:285–300.

HESS, S.D., and PRIOR, D.J. (1986). Small cardioactive peptide B modulates feeding motoneurons in the terrestrial slug, *Limax maximus*. *Neurosci. Abst.* **12**:1318.

KRAJNIAK, L.G., GREENBERG, M.J., DOBLE, K.E., and PRICE, D.A. (1985). Localization of FMRF-amide-related peptides in the slug, *Limax maximus*, and their effects on the isolated crop and penis. *Am. Zool.* **25**:15A.

KREINER, T., SOSSIN, W., and SCHELLER, R.H. (1986). Localization of *Aplysia* neurosecretary peptides to multiple populations of dense core vesicles. *J. Cell Biol.* **102**:769–782.

LLOYD, P.E. (1978). Distribution and molecular characteristics of cardioactive peptides in the snail, *Helix aspersa*. *J. Comp. Physiol.* **128**:269–276.

LLOYD, P.E. (1979). Central peptide containing neurons modulate gut activity in *Tritona*. *Soc. Neurosci. Abst.* **5**:252.

LLOYD, P.E., KUPFERMANN, I., and WEISS, K.R. (1984). Evidence for parallel actions of a molluscan neuropeptide (SCP_A) and serotonin in mediating arousal in *Aplysia*. *PNAS* **81**:2934–2937.

MAHON, A.C., LLOYD, P.E., WEISZ, K.R., KUPFERMANN, and SCHELLER, R.H. (1985). The small cardioactive peptides A and B of *Aplysia* are derived from a common precursor molecule. *PNAS* **82**:3925–3929.

MASINOVSKY, B., KEMPF, S., CALLAWAY, J., and WILLOWS, A.O.D. (in press). Monoclonal antibodies to the small cardioactive peptide SCP_B: Immunolabelling of neurons in diverse invertebrates.

MURPHY, A.D. LUKOWIAK, K., and STELL, W.K. (1985). Peptidergic modulation of patterned motor activity in identifiied neurons of *Helisoma*. *PNAS* **82**:7140–7144.

PHIFER, C.B., and PRIOR, D.J. (1985). Body hydration and haemolymph osmolality affect feeding and its neural correlate in the terrestrial gastropod, *Limax maximus*. *J. Exp. Biol.* **118**: 405–426.

PRIOR, D.J., and DELANEY, K. (1986). Activation of buccal neuron B1 in the edible slug, *Limax maximus*, mimics the action of exogeneous SCP_B. *Am. Zool.* **26**:126A.

PRIOR, D.J., and GELPERIN, A. (1977). Autoactive molluscan neuron: Reflex function and synaptic modulation during feeding in the terrestrial slug, *Limax maximus*. *J. Comp. Physiol.* **114**: 217–232.

PRIOR, D.J., and GREGA, D.S. (1982). Effects of temperature on the endogeneous activity and synaptic interactions of the salivary burster neuron in the terrestrial slug, *Limax maximus*. *J. Exp. Biol.* **98**:415–428.

PRIOR, D.J., WATSON, W.H., and HESS, S.D. (1985). The molluscan neuropeptide, SCP_B, increases the responsiveness of the feeding motor program of *Limax maximus*. *Am. Zoo.* **25**:16A.

REINGOLD, S.C., and GELPERIN, A. (1980). Feeding motor programme in *Limax II*. Modulation by sensory inputs in intact animals and isolated central nervous systems. *J. Exp. Biol.* **85**:1–19.

WATSON, W.H, III., and WILLOWS, A.O.D. (1986). Homologous peptidergic modulatory neurons in the buccal ganglia of marine molluscs: I. Immunohistochemistry. *Soc. Neurosci. Abst.* **12**:241.

WILLOWS, A.O.D., LLOYD, P.E., and MASINOVSKY, B.P. (in press). Multiple transmitter neurons in *Tritonia*: III. Modulation of central pattern generator controlling feeding.

WILLOWS, A.O.D., and WATSON, W.H. (1986). Homologous peptidergic modulatory neurons in the buccal ganglia of marine molluscs: II Physiology. *Soc. Neurosci. Abstracts* **12**:241.