Functional Consequences of Compartmentalization of Synaptic Input

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Intra-axonal recordings of stomatogastric nerve axon 1 (SNAX1) indicate that there are synaptic inputs onto the SNAX1 terminals in the stomatogastric ganglion (STG) of the crab Cancer borealis (Nusbaum et al., 1992b). To determine whether this synaptic input only influenced SNAX1 activity within the STG, we identified the SNAX1 soma in the commissural ganglion (CoG). We found that this neuron has a neuropilar arborization in the CoG and also receives synaptic inputs in this ganglion. Based on its soma location, we have renamed this neuron modulatory commissural neuron 1 (MCN1). While intracellular stimulation of MCN1,., and MCN1,, has the same excitatory effects on the STG motor patterns, MCN1 receives distinct synaptic inputs in the STG and CoG. Moreover, the synaptic input that MCN1 receives within the STG compartmentalizes its activity. Specifically, the lateral gastric (LG) neuron synaptically inhibits MCN1,, initiated activity within the STG (Nusbaum et al., 1992b), and while LG did not inhibit MCN1,., initiated activity in the CoG, it did inhibit these MCN1 impulses when they arrived in the STG. As a result, during MCN1,.,-elicited gastric mill rhythms, MCN1,., is continually active in the CoG but its effects are rhythmically inhibited in the STG by LG neuron impulse bursts. One functional consequence of this local control of MCN1 within the STG is that the LG neuron thereby controls the timing of the impulse bursts in other gastric mill neurons. Thus, local synaptic input can functionally compartmentalize the activity of a neuron with arbors in distinct regions of the nervous system.

[Key words: crab, neuromodulation, presynaptic inhibition, projection neuron, rhythmic motor patterns, stomatogastric ganglion, synaptic compartmentalization]

Neurons often project long distances to influence their targets. Such is the case for many neurons that influence rhythmically active neural networks (Brodfuehrer and Freisen, 1986a; McCrohan, 1988; Grillner and Matsushima, 1991; Harris-Warrick et al., 1992b; Perreault et al., 1993; Rudomin et al., 1993). Intracellular recordings from the somata of these projection neurons have provided considerable information regarding their influence on neural network activity. However, the release sites at which they influence their network targets are electrotonically distant from the soma recording sites, providing little information regarding the activity occurring at these distant terminals.

Projection neurons may well be influenced at their distant terminals, since there is considerable evidence for presynaptic influences in the nervous system (Glantz et al., 1985; Brodfuehrer and Friesen, 1986b; Clarac et al., 1992; Nusbaum et al., 1992b; Watson, 1992). Such presynaptic influences provide for the possibility that these neurons could be functionally compartmentalized. Functional compartmentalization of neuronal activity has been addressed in several systems (Nelson et al., 1975; Nagy et al., 1981; Haydon and Winlow, 1982; Oland et al., 1987; Ross et al., 1990; Hounsfield and Kiehn, 1993; Skydsgaard and Hounsgaard, 1994). However, the consequences of this compartmentalization for the targets of these neurons remain to be determined in most systems.

One system in which the functional consequences of neuronal compartmentalization can be addressed is the stomatogastric nervous system (STNS) of decapod crustaceans (Selverston and Moulins, 1987, Harris-Warrick et al., 1992a). The STNS produces several rhythmically active motor patterns, including the pyloric and gastric mill rhythms. These two rhythms are produced by a neural network whose component neurons are located within the stomatogastric ganglion (STG), and which is strongly influenced by modulatory inputs from more anterior ganglia (Coleman et al., 1992, 1993; Harris-Warrick et al., 1992b; Marder and Weimann, 1992; Nusbaum et al., 1992a; Norris et al., 1993).

To examine the events occurring at the STG terminals of these projection neurons, Nusbaum et al. (1992b) recorded intra-axonally, at the entrance to the STG, from an identified modulatory neuron in the crab Cancer borealis. They reported that this neuron, called stomatogastric nerve axon 1 (SNAX1), arborizes throughout the STG neuropil, excites the STG network, and also receives synaptic inputs within the STG. Specifically, SNAX1 is electrically coupled to the lateral gastric (LG) neuron and it also receives inhibitory postsynaptic potentials (IPSPs) from LG that are strong enough to inhibit action potentials elicited in SNAX1.

We wanted to determine whether the synaptic inhibition of the SNAX1 terminals by LG only influenced SNAX1 activity within the STG, without affecting its activity in the CoG. We report here the identitication of the SNAX1 soma in the CoG, and that this neuron has an arborization within the CoG onto which it receives synaptic inputs. We have therefore renamed this neuron modulatory commissural neuron 1 (MCN1). To
simplify the nomenclature regarding the two MCN1 recording sites, we label intrasomatic recordings "MCN1 soma", and intraxonial recordings of SNAX1 "MCN1 SNAX". By performing simultaneous intracellular recordings from these two sites, we found that MCN1 receives distinct synaptic inputs in the STG and CoG. Moreover, we found that the inhibitory input from the LG neuron onto MCN1 SNAX only locally influences MCN1 activity, causing this neuron to have different activity patterns in the CoG and STG. A functional consequence of this local inhibition is that, by regulating the timing of the MCN1 excitatory effects onto its STG neuron targets, the LG neuron controls the timing of the impulse bursts in other STG neurons during MCN1-elicited gastric mill rhythms.

Some of these data appeared previously in abstract form (Coleman and Nusbaum, 1992).

Materials and Methods

Animals. Adult male Cancer borealis were obtained from Neptune Lobster and Seafood Company (Boston, MA), and the Marine Biological Laboratory (Woods Hole, MA) and were maintained in circulating, aerated artificial or natural seawater (10–13°C) until used. Crabs were cold anesthetized by packing in ice for 20–40 min prior to dissection. Data were obtained from a total of 88 animals.

Solutions. C. borealis physiological saline contained (mM) NaCl, 440; KCl, 11; MgCl2, 26; CaCl2, 13; Trizma base, 10; and maleic acid, 5 (pH 7.4–7.5). Proctolin was obtained from Sigma Chemical Co. (St. Louis, MO). Locustatychkinin 1 and II were obtained from Peninsula Labs (Belmont, CA). These peptides were stored as frozen aliquots of stock solution (10–2 M or 10–3 M) and were diluted to the final working concentration in saline immediately before they were used.

Electrophysiology. The stomatogastric nervous system was dissected from the crab stomach and pinned to a saline-filled, Sylgard (Dow Corning)-lined petri dish. The STNS consists of four ganglia plus their connecting and motor nerves. The four ganglia include the paired CoGs, the unpaired esophageal ganglion (OG), and the unpaired STG (Fig. 1). To facilitate intracellular recordings, the STG and CoGs were dehydrated and viewed with white light transmitted through a dark-field condensor (Nikon). Individual STG neurons were identified by their axonal pathways, activity patterns, and their interactions with other neurons (Weimann et al., 1991, 1993; Nusbaum et al., 1992b). Preparations were superfused continuously (7–12 ml/min) with physiological saline warmed to 10–13°C. Intracellular and extracellular recordings were made using routine methods for the STNS (Selverston and Moulins, 1987). Intracellular recordings were made with microelectrodes (15–30 MΩ) that were filled with 4 M potassium acetate plus 20 mM KCl. Extracellular recordings were made using stainless steel wire electrodes that were pressed into the Sylgard alongside individual nerves and isolated from the bath by Vaseline. Data were collected directly onto chart recorder paper (Astromed MT-95000) and stored on videotape (Vetter Instruments). Figures were prepared by scanning data into a graphics program (COREDLRAW, version 3.0) using Scanjet IIC (Hewlett Packard).

To block action potential propagation reversibly through the superior esophageal nerves (sone; Fig. 1), the sones were desheathed and a Vaseline well was placed around the desheathed area. Impulse propagation was blocked by replacing the saline in the well with an isotonic sucrose solution (750 mM). The impulse propagation block was effectively reversed by replacing the sucrose with saline. In order to superpose neuromodulatory peptides selectively onto the STG, this ganglion was isolated from the CoGs and OG by a Vaseline wall built across the middle of the stn. In these experiments, separate superfusion lines and switching manipulations were used for each half of the preparation.

Lucifer yellow dye fills and immunocytochemistry. Intracellular Lucifer yellow (LY-CH; Sigma) dye fills were accomplished using microelectrodes (40–80 MΩ) whose tips were filled with 5% LY in water. The electrode shaft was filled with 1 M LiCl, with an air bubble separating the two solutions. LY iontophoresis and subsequent processing for visualization, followed the techniques in Nusbaum et al. (1992b). In some preparations, the signal from the LY-fill was amplified using a polycrystalline anti-LY antiserum (gift of J. Y. Kuwada, Department of Biology, University of Michigan) Processing for LY immunocytochemical staining in whole-mounts followed the techniques of Beltz and Kravitz (1983).

Results

MCN1 soma identification

Previously, we used anatomical techniques to show that most of the inputs to the crab STG originate from somata in the paired CoGs, and that only two or three of these somata project through the inferior esophageal nerve (ion) to reach the STG (Coleman et al., 1992). Nusbaum et al. (1992b) showed, electrophysiologically, that MCN1 SNAX projected through the stn and ion into the CoG (Fig. 1). Using our anatomical localization of the CoG neurons as a guide, we identified the soma of MCN1 SNAX electrophysiologically. MCN1 soma was commonly found on the dorsal surface of the CoG, anterior to the previously identified L neuron (Robertson and Moulins, 1981), which has the largest soma in the CoG. The MCN1 soma is approximately 45 μm in diameter. There appears to be only one MCN1 in each CoG, since in recordings from approximately 65 CoGs we never found more than one MCN1 per ganglion.
tiated at both of these sites within 20 msec of one another, the MCN1, and MCN1, sites. When impulses were initiated at these sites, the latency from one intracellular recording site to the other was approximately 26 msec.

As a further confirmation that the MCN1, and MCN1, have the same projection within the stomatogastric nervous system, and cause time-locked action potentials in each other, the MCN1, impulse was recorded only in the ion and the MCN1, impulse was recorded only in the stn. The location as well as the occurrence of the collision could be changed by altering the delay between initiation of the two impulses. We found no indication from either dye fills or electrophysiological recordings that MCN1, projected to the contralateral CoG.

There was little difference between the excitatory effects of MCN1, and MCN1, on the pyloric and gastric mill motor patterns in the STG. For example, as shown in Figure 4, MCN1, activation caused an increase in the pyloric cycle frequency and also initiated a gastric mill rhythm. Similar results were shown for activation of MCN1, (Nusbaum et al., 1992b). In this recording, the pyloric rhythm was monitored with an extracellular recording of the pyloric dilator nerve (pdl), in which the activity of the two pyloric dilator (PD) neurons is selectively recorded. These neurons are members of the pyloric motor pattern pacemaker group (Selverston and Moulins, 1987; Weimann et al., 1991). The MCN1, elicited gastric motor patterns in Figure 4 is represented by the alternation in activity of the dorsal gastric (DG) and the coactive LG and gastric mill (GM) motor neurons. Although not shown, MCN1, stimulation also shared with MCN1, the ability to initiate the pyloric rhythm.

MCN1, and MCN1, receive distinct synaptic input

Since MCN1, has an arborization in both the CoG and the STG, it was possible that this neuron received distinct synaptic inputs in the two ganglia. Nusbaum et al. (1992b) showed that MCN1, receives input from both pyloric and gastric mill neurons in the STG, and that during MCN1, stimulation, the region of the neuron showed activity that was time locked to the STG motor patterns. This included a reduction or suppression of MCN1, activity by LG neuron impulse bursts during the gastric mill rhythm. Interestingly, MCN1, initiated activity in the CoG was not inhibited by the LG neuron impulse bursts (Fig. 4). LG does not project to the CoGs. Nevertheless, it is evident in Figure 4 that the MCN1, elicited activity in the CoG was time locked to both the pyloric and gastric mill rhythms. The pyloric timing was such that MCN1, was not active during PD neuron activity. This pyloric timing was replaced by nearly tonic activity in MCN1, during each

![Figure 2](image-url) Confocal image of an intracellular Lucifer yellow dye fill of MCN1 soma in whole-mount. A relatively thin neurite connects the MCN1 soma (arrow) with an expanded neurite within the neuropil. The CoG neuropil branches of MCN1 all originate from this expanded neurite. MCN1 projects from the CoG through the ion (upper right). The son is at the lower right. The small bleb alongside the MCN1 soma resulted from damage to the soma during removal of the intracellular electrode. The Lucifer yellow dye fill was intensified by processing the preparation with anti-Lucifer yellow antiserum, visualized with a fluorescein-conjugated secondary antiserum (see Materials and Methods). Scale bar, 100 μm.

![Figure 3](image-url) Superimposed oscilloscope sweeps showing that MCN1, and MCN1, have the same projection within the stomatogastric nervous system, and cause time-locked action potentials in each other. A, Each action potential elicited in MCN1, produced constant-latency action potentials in the stn, ion, and MCN1, . B, Each action potential elicited in MCN, is followed by fixed latency action potentials in the ion, stn, and MCN1, . A and B are from the same recordings.

Figure 3. Superimposed oscilloscope sweeps showing that MCN1, and MCN1, have the same projection within the stomatogastric nervous system, and cause time-locked action potentials in each other. A, Each action potential elicited in MCN1, produced constant-latency action potentials in the stn, ion, and MCN1, . B, Each action potential elicited in MCN1, is followed by fixed latency action potentials in the ion, stn, and MCN1, . A and B are from the same recordings.
the MCN_1 SOma recording in Figure 4 had suggested, we found COG, which is approximately 3 cm distant from the STG. As initiation zones near the COG and at the STG (Nusbaum et al., recorded in MCN_1, could be recorded in MCN_1 in the MCN_1x (Fig. 6) or MCN_1 (Fig. 7). MCN_1 activity resulted from the injection of constant-amplitude depolarizing current. Arrowhead indicates that MCN_1 stimulation began prior to the start of this recording segment, and persisted throughout this segment. DG neuron is the largest unit in the dgn recording, and the GM neurons are the smallest units in dgn. Membrane potential, LG, -54 mV to -69 mV (peak to trough of slow wave).

Figure 4. MCN_1 IOmS excites the pyloric rhythm and initiates a gastric mill motor pattern. Left. While MCN_1 was inactive, there was an ongoing pyloric rhythm (monitored with the pdn recording) and no gastric mill motor pattern. The active unit in the dgn is the anterior gastric receptor (AGR) neuron, a sensory neuron that is tonically active throughout both halves of the dgn recording. Membrane potentials: LG, -64 mV; MCN_1, -48 mV. Right, MCN_1 activity (firing frequency, 28 Hz) increased the pyloric rhythm frequency and elicited a gastric mill motor pattern. MCN_1 activity resulted from the injection of constant-amplitude depolarizing current. Arrowhead indicates that MCN_1 stimulation began prior to the start of this recording segment, and persisted throughout this segment. DG neuron is the largest unit in the dgn recording, and the GM neurons are the smallest units in dgn. Membrane potential, LG, -54 mV to -69 mV (peak to trough of slow wave).

Figure 5. Pyloric- and gastric mill-timed synaptic input onto MCN_1 occurs in the CoG. A. With the ipsilateral son intact, MCN_1 showed both pyloric- and gastric-mill-timed activity. MCN_1 activity occurred without intracellular current injection. B. When the son was transected, MCN_1 activity became tonic, despite the continued presence of the pyloric and gastric mill rhythms in the STG. The pyloric and gastric mill rhythms are represented by the short-duration, high-frequency bursting (PD neurons, small units) and the long-duration, low-frequency bursting (GM neurons, large units) in the dlvn recording, respectively. MCN_1 membrane potential: A, -45 mV to 50 mV (peak to trough of pylorically-timed inhibition); B, -43 mV.

There was no evidence of these IPSPs in the MCN_1 recording (Fig. 6A). Impulse activity was no longer recorded at MCN_1 during LG stimulation in Figure 6A because the impulses had been originating at the STG and propagating toward the CoG. The lack of LG-mediated IPSPs in MCN_1 is also evident in Figure 6B, which shows a series of superimposed oscilloscope traces triggered by impulses elicited in LG. In this experiment, the contralateral MCN_1 and MCN_1 were recorded during LG stimulation. Each LG impulse elicited two events in MCN_1 including an initial depolarization that results from electrical coupling, followed by an IPSP (Nusbaum et al., 1992b). Neither of these events were recorded in MCN_1.

Using MCN_1 recordings, we were able to study the effects of LG on MCN_1-initiated activity without impaling and depolarizing MCN_1. This was possible because, in some preparations, the STG spike initiation zone of MCN_1 became activated following MCN_1 stimulation. This occurred despite the fact that the depolarization produced by current injection into MCN_1 never reached the MCN_1 recording site (see Fig. 8). Meyrand et al. (1992) showed that the same phenomenon sometimes occurs in an STG neuron following intrasomatic stimulation. The switch in the site of MCN spike initiation is evident in the ion recording in Figure 7, where the change in the direction of propagation of the MCN spikes is reflected in a change in its shape (see also Fig. 3). This switch in the site of spike initiation was confirmed using oscilloscope sweeps to observe directly the time of arrival of the MCN action potentials at the different intracellular and extracellular recording sites. When the STG spike initiation zone of MCN_1 was active, stimulation of LG again effectively suppressed MCN activity at this location (Fig. 7). Once again, the LG-mediated inhibition produced no membrane potential change at the MCN_1 recording site. Also shown in Figure 7 is a recording of the contralateral MCN_1, showing the IPSPs produced by the LG action potentials in these neurons within the STG. This dual recording of both MCN_1 neurons also shows that MCN activity had no direct influence on the contralateral MCN_1 in the STG.

Local control of MCN_1 in the STG

In order to determine whether LG could locally control MCN_1 activity within the STG when MCN_1 action potentials were initiated in the CoG, we again used simultaneous recordings.
A. mcn'snax

B. mcn somalft

MCN1 SOMALFT

LG

MCN1 SOMALFT

MCN1 SOMALFT

LG

Membrane potentials: MCN1 SOMALFT, -56 mV; LG, -60 mV. A and B are from different preparations.

Figure 6. Synaptic inputs onto MCN1 SOMALFT are not recorded in MCN1 SOMALFT, which then propagated to the MCN1 SOMALFT recording site. During MCN1 SOMALFT depolarization, LG was stimulated (between arrowheads; firing frequency, 24 Hz). The IPSPs from LG onto MCN1 SOMALFT were not seen at the MCN1 SOMALFT recording site. MCN1 activity was time locked to the pyloric rhythm (dvn). MCN1 SOMALFT stimulation began prior to the start of this figure and persisted for the duration of the figure. LG neuron activity is also seen as the largest-amplitude spikes in the dvn recording. Membrane potentials: MCN1 SOMALFT, -56 mV; LG, -60 mV. B. Superimposed oscilloscope sweeps triggered by impulses elicited in LG show that each LG action potential produced an EPSP followed by an IPSP in MCN1 SOMALFT, but produced no response in MCN1 SOMALFT, -45 mV; MCN1 SOMALFT, -60 mV. A and B are from different preparations.

Figure 7. LG neuron activity suppresses MCN1 spikes initiated in the STG. Intracellular stimulation of MCN1 SOMALFT (between arrowheads; firing frequency, 10 Hz) increased the pyloric-timed oscillations in the LG neuron. Following termination of MCN1 SOMALFT stimulation, MCN1 activity persisted but the direction of impulse propagation was reversed (note change in shape of MCN1 spikes in the ionr recording). During this time, LG was stimulated (arrowheads; firing frequency, 10 Hz), causing an inhibition in MCN1 activity. Note also that during LG activity, IPSPs appeared in the contralateral MCN1 SOMALFT and MCN1 spikes no longer appeared in the ionr. After LG stimulation ended, activity resumed in MCN1 SOMALFT. Membrane potentials: MCN1 SOMALFT, -56 mV; LG, -45 mV to -50 mV (peak to trough of slow wave); MCN1 SOMALFT, -58 to -62 mV (peak to trough of slow wave).

The inability of LG activity to inhibit the MCN1 SOMALFT-elicited spikes at the MCN1 SOMALFT recording site, if not completely suppressed them. As is evident in Figure 8, the synaptic inhibition from LG instead appeared to be only passively spread from the STG neuropil to the MCN1 SOMALFT recording site, since the MCN1 action potentials were slightly larger in amplitude during the IPSP barrage. Although not yet documented, the synaptic inhibition from LG to MCN1 SOMALFT is likely to result from a conductance increase since (1) it inhibits MCN1 SOMALFT-initiated spikes, (2) its reversal potential is between -60 mV and -65 mV (P. Meyrand, M. J. Coleman, and M. P. Nusbaum, unpublished observations), and (3) it is reversibly suppressed by picrotoxin (10^-5 M; Meyrand et al., unpublished observations). LG is glutamatergic and in crab STG neurons glutamate inhibition produces picrotoxin-sensitive increases in either chloride or potassium conductance (Marder and Paupardin-Tritsch, 1978; Marder, 1987).

Despite the fact that LG activity did not inhibit MCN1 SOMALFT-elicited spikes at the MCN1 SOMALFT recording site, it appeared likely that the LG neuron was, indeed, interfering with these MCN1 spikes after they passed the MCN1 SOMALFT recording site. If so, this would have at least decreased the amplitude of the MCN1 SOMALFT action potentials at the MCN1 SOMALFT recording site, if not completely suppressed them. As is evident in Figure 8, the synaptic inhibition from LG instead appeared to be only passively spread from the STG neuropil to the MCN1 SOMALFT recording site, since the MCN1 action potentials were slightly larger in amplitude during the IPSP barrage. Although not yet documented, the synaptic inhibition from LG to MCN1 SOMALFT is likely to result from a conductance increase since (1) it inhibits MCN1 SOMALFT-initiated spikes, (2) its reversal potential is between -60 mV and -65 mV (P. Meyrand, M. J. Coleman, and M. P. Nusbaum, unpublished observations), and (3) it is reversibly suppressed by picrotoxin (10^-5 M; Meyrand et al., unpublished observations). LG is glutamatergic and in crab STG neurons glutamate inhibition produces picrotoxin-sensitive increases in either chloride or potassium conductance (Marder and Paupardin-Tritsch, 1978; Marder, 1987).

The inability of LG activity to inhibit the MCN1 SOMALFT-elicited spikes at the MCN1 SOMALFT recording site was not due to synaptic fatigue, because several seconds later the LG neuron was able to inhibit MCN1 spikes initiated at the STG (Fig. 8). As was the case in Figure 7, the STG spike initiation zone of MCN1 became active after MCN1 SOMALFT stimulation was terminated. This switch in the site of MCN1 spike initiation is evident in the ion recording (see also Fig. 7).
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Figure 8. LG does not inhibit MCN1stonel-elicited action potentials in either the COG or at the MCN1SNAX recording site. During depolarizing current injection into MCN1stonel (between arrowheads), LG stimulation (between arrowheads) did not inhibit MCN1stonel spikes at either MCN1 recording site. LG activity, however, did hyperpolarize MCN1SNAX. MCN1 activity persisted following MCN1stonel stimulation, but this latter activity originated at the STG (note change in shape of MCN1 impulses in the ion). The STG-originating spikes in MCN1 were inhibited by LG stimulation (arrowheads). Note the similar hyperpolarization of the MCN1stonel membrane potential during both LG stimulations. During intracellular stimulation, the MCN1 firing frequency was 14 Hz while the LG firing frequency was 24 Hz. Membrane potentials: MCN1stonel, -56 mV; MCN1SNAX, -44 mV to -50 mV (peak to trough of slow wave); LG, -60 mV.

If LG did inhibit the effects of MCN1stonel-elicited spikes within the STG, then this inhibition would reduce the MCN1 excitation of DG (Fig. 9). Not only should the MCN1stonel-elicited excitation of DG be reduced when LG is active, but it should be increased when LG is not active. As shown in Figures 10 and 11, this is indeed the case. In Figure 10A, MCN1stonel stimulation excited DG and increased the amplitude of the pyloric-timed oscillations in LG. LG was then depolarized during the MCN1stonel stimulation and, while MCN1stonel activity continued, the DG neuron stopped firing until LG stimulation was terminated. This ability of LG to control the MCN1stonel excitation of DG was routinely observed (n = 23). This effect appeared to result from the LG inhibition of the MCN1 terminals within the STG, because when MCN1stonel was not active, LG stimulation did not inhibit DG activity (Fig. 10B). In addition, when LG activity was suppressed by hyperpolarizing current injection, MCN1stonel stimulation caused a stronger excitation of DG than when LG was active (Fig. 11). Thus, LG neuron activity does inhibit the effects of MCN1stonel-elicited activity within the STG.

The IPSPs produced by LG in MCN1SNAX provided a mechanism for the LG inhibition of the effects of MCN1stonel-elicited activity within the STG. It remained possible, however, that the ability of LG to interfere with MCN1stonel effects in the STG was at least partly the result of a postsynaptic, instead of a presynaptic mechanism. Specifically, perhaps one or more of the MCN1 transmitters were enhancing the strength of the LG synapses. If this were the case, then while LG activity did not inhibit DG in normal saline, perhaps it would do so in the presence of the MCN1 transmitters. We tested this possibility by bath applying proctolin and locustatachykinin (LomTK) to the STG. MCN1 exhibits both proctolin and LomTK-like immunoreactivity (Christie et al., 1993), and bath application of either proctolin or LomTK to the crab STG excites the STG network (Marder et al., 1986; Nusbaum and Marder, 1989; Blitz A.)

Figure 9. Schematic illustration of synaptic interactions within the STG neuropil of MCN1 and gastric mill neurons LG and DG. MCN1 excites both LG and DG, and it is also electrically coupled to LG. LG inhibits MCN1. Based on data from Nusbaum et al. (1992b).

Figure 10. LG inhibits MCN1 excitation of DG within the STG. A, Intracellular stimulation of MCN1stonel (arrowheads) excited the DG neuron and increased the pyloric-timed oscillations in the LG neuron. During MCN1stonel stimulation, intracellular depolarization of LG (arrowheads) suppressed activity in the DG neuron. LG resumed firing after LG stimulation was terminated. Membrane potentials (A and B): MCN1stonel, -59 mV; LG, -68 mV (most hyperpolarized potential); DG, -64 mV. A and B are from the same recordings.
from a site electrotonically distant from the cell body. Based on neuron, MCNI, studied previously via intra-axonal recordings. We have identified the cell body of a modulatory projection DG activity was prematurely terminated. bursts that were occurring in MCNI-elicited gastric mill rhythms, DG activity. In contrast, when we stimulated LG during DG fired an impulse burst and again controlled the timing of the DG neuron, we considered the possibility that the LG-me-

Figure 11. LG hyperpolarization enhances the excitatory effects of MCNI on the DG neuron. A. Intracellular stimulation MCNI soma (arrowheads; firing frequency, 30 Hz) excited both LG and DG. B. Hyperpolarization of LG (downward arrowhead; -4 nA) prior to and during intracellular stimulation of MCNI soma (arrowheads; firing frequency, 33 Hz) increased MCNI excitation of DG. Membrane potentials: MCNI soma, -54 mV; DG, -72 mV; LG, -60 mV to -68 mV (peak to trough of slow wave in A). A and B are from the same recordings.

and Nusbaum, 1993). When proctolin (10^-6 M) and LomTK (10^-6 M) were applied either alone or in combination to the STG, however, there was still no inhibitory effect of LG onto DG (n = 6).

Functional significance of presynaptic inhibition of MCNI in the STG
Since LG activity interfered with the ability of MCNI to excite the DG neuron, we considered the possibility that the LG-mediated synaptic inhibition of MCNI soma controlled the relative timing of the impulse bursts in the LG and DG neurons during MCNI-elicited gastric mill rhythms. In the crab STG, there is no known synaptic pathway involving STG neurons that could otherwise account for the alternation in LG and DG activity that occurs during these gastric mill motor patterns (Fig. 10B; Weimann, 1992; Coleman and Nusbaum, unpublished observations). To test this possibility, we manipulated LG activity during MCNI soma-mediated gastric mill motor patterns (n = 5). We found that by either activating or suppressing activity in LG, the timing of the impulse bursts in DG was changed. For example, as shown in Figure 12, suppressing LG activity during an ongoing MCNI soma-elicited gastric mill rhythm enabled the DG neuron to increase the frequency of its rhythmic impulse bursts. As soon as LG was released from hyperpolarization, it fired an impulse burst and again controlled the timing of the DG activity. In contrast, when we stimulated LG during DG bursts that were occurring in MCNI-elicited gastric mill rhythms, DG activity was prematurely terminated.

Discussion
We have identified the cell body of a modulatory projection neuron, MCNI, studied previously via intra-axonal recordings from a site electrotonically distant from the cell body. Based on both anatomical and electrophysiological data, MCNI is likely to be the Cancer borealis equivalent to the P neuron, identified previously via intrasomatic recordings in both the spiny lobster, Panulirus interruptus (Selverston et al., 1976), and the European lobster, Homarus gammarus (Cardi and Nagy, 1994; Nagy and Cardi, 1994; Nagy et al., 1994). There is insufficient information available regarding the P neuron in the spiny lobster for a direct comparison. The P neuron in the European lobster does share with MCNI at least some of the same characteristics, including its axonal projection pattern, its pyloric-timed activity in the CoG, and its ability to excite the pyloric motor pattern. The pyloric-timed activity in the P neuron results at least partly from activity in the anterior burster (AB) neuron, which projects to the CoG from the STG and inhibits the P neuron (Selverston et al., 1976; Nagy et al., 1994). We have shown that the pyloric-timed inhibitory input to MCNI in the CoG also originates from the STG and occurs during the time of AB neuron activity, because the AB and PD neurons are coactive during pyloric rhythms and PD does not project to the CoG. Thus far, the P neuron influence on the gastric mill system has not been examined, and while the P neuron and MCNI both provide modulatory excitation to the pyloric rhythm, the MCNI influence on the pyloric rhythm has not yet been studied as extensively as has been done for the P neuron.

Flexibility in neural network output
Previous studies in many systems, including the S1NS, have shown that modulatory inputs impart considerable flexibility to neural network output (Harris-Warrick and Marder, 1991; Harris-Warrick et al., 1992a; Pearson and Ramirez, 1992; Steriade et al., 1993). This usually results from modulation of the membrane properties and/or synaptic strengths of network neurons. In the case of the crab gastric mill rhythm, another mechanism underlying the flexibility in motor pattern production results from the ability of some projection neurons also to provide timing cues that help determine the form of the motor pattern. Thus, one consequence of the lack of synaptic connections between LG and DG is that the timing of their activity patterns relative to each other depends on extrinsic input. For example,
in contrast to their pattern of alternating impulse bursts during MCN1-elicted gastric mill rhythms, these two neurons burst together during a distinct form of the gastric mill rhythm that occurs with activation of modulatory commissural neuron 7 (MCN7; Coleman et al., 1993). LG and DG can also exhibit alternating impulse bursts during a third form of the gastric mill rhythm that involves activation of modulatory commissural neuron 2 (MCN2: Norris et al., 1993). When MCN2 exhibits gastric mill–timed bursts, it excites LG and, independent of LG activity, inhibits DG. This constrains DG to fire in alternation with LG. This means of controlling the timing of the LG and DG bursts during gastric mill rhythms in Cancer is distinct from that occurring in the lobster P. interruptus, where there are reciprocal inhibitory connections between these two gastric mill neurons (Mulloney, 1987; Elson and Selverston, 1992).

Functional compartmentalization of neuronal activity

We have found there to be a functional compartmentalization of MCN1 during MCN1soma-elicted gastric mill motor patterns. This compartmentalization results from local synaptic inputs onto the MCN1 terminals in the STG. Thus, during times when the gastric mill rhythm is elicited by maintained MCN1soma activity, LG neuron impulse bursts rhythmically inhibit the MCN1 effects within the STG neuropil.

Nusbaum et al. (1992b) showed that LG suppresses MCN1 spike initiation within the STG. In this article, we found that LG also interferes with MCN1 spikes that are initiated in the CoG and propagate to the STG. However, because our MCN1soma recordings were outside of the STG neuropil, we were not able to determine whether the LG inhibition of the MCN1soma effects in the STG resulted from its elimination of the MCN1 action potentials or by reducing their amplitude to a level that was ineffective in mediating transmitter release. In either case, there are several lines of evidence that suggest that the MCN1soma spike only spreads passively into the STG neuropil. If this is indeed the case for MCN1, then it would increase the effectiveness of the LG inhibition, since passively spread spikes are more susceptible to inhibition than are actively propagated ones (Segev, 1990). Supporting this possibility is the fact that the spikes of all pyloric and gastric mill STG neurons spread passively into the STG (Mulloney and Selverston, 1979; Meyrand et al., 1992). Additionally, in another crustacean preparation, intraaxonal recordings from the central projection of primary afferents within the thoracic ganglion indicate that the spikes in these neurons also spread passively once they enter the ganglion (Cattaert et al., 1992). Cattaert et al. (1992) also showed that the passively spread spikes in crayfish sensory neurons are reduced in amplitude by presynaptic inhibition, resulting in a decrease in the amplitude of the PSPs that they evoke in their target neurons. Thus, even if the LG inhibition only reduces the amplitude of the MCN1 spikes, this could still be sufficient to reduce or eliminate the postsynaptic effects of MCN1. This is further supported by the fact that the ability of the passively spread spikes of STG neurons to evoke PSPs within the ganglion is determined by the level of the underlying membrane potential (Meyrand et al., 1992).

As suggested by Chiel et al. (1988), in neurons where the spike initiation zone is close to the release sites of that neuron, the distinction between pre- and postsynaptic inhibition becomes blurred. This is the case for LG inhibition of MCN1, where there is postsynaptic inhibition of MCN1 spikes initiated in the STG, and presynaptic inhibition of its STG release sites.

terms of MCN1soma-initiated spikes that propagate to the STG to evoke transmitter release, LG inhibition is postsynaptic only. It was not surprising that we did not observe an inhibition of the MCN1soma spikes at MCN1soma since this recording site preceded the site of synaptic inhibition. A previous ultrastructural study by King (1976a,b) showed that all synaptic interactions in the STG occur within the neuropil. In a modeling study of presynaptic inhibition, Segev (1990) showed that presynaptic inhibition onto neuropil terminals would not be effective at a recording site that was as close as a few tenths of a space constant away from the site of inhibition. Although we have not determined the MCN1 space constant, the MCN1soma recording site was sufficiently distant from the site of LG inhibition that the IPSPs appeared to be only passively spread to this recording site.

It remains unclear why MCN1 receives rhythmic input from the STG network onto its terminals in both the CoG and the STG. While the pyloric-timed input to MCN1 results in the same pattern in both ganglia, the gastric mill–timed input is quite different. During LG impulse bursts, MCN1soma is inhibited but MCN1soma actually shows slightly increased activity. Presumably, the purpose of the spatially separated inputs onto MCN1 pertains to its functions in each ganglion. For example, while LG is suppressing MCN1 actions in the STG, it may be appropriate for MCN1 to continue synchronically influencing targets in the CoG. The spatially separate inputs onto MCN1 may also relate to the ability of MCN1 to initiate action potentials at either the CoG or the STG. These separate inputs would ensure that MCN1 activity would be correctly timed, regardless of which end of the neuron was initiating activity.

In conclusion, our results indicate that the neuronal activity recorded intrasomatically does not necessarily reflect the activity pattern of the recorded neuron at electrotonically distant sites. Such a functional compartmentalization increases the complexity of neuronal signaling without necessitating an increase in the number of interacting neurons.

References


