

SEROTONERGIC MODULATION OF SWIMMING SPEED IN THE PTEROPOD MOLLUSC *CLIONE LIMACINA*

III. CEREBRAL NEURONS

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Summary

Swim acceleration in *Clione limacina* can occur via central inputs to pattern generator interneurons and motor neurons and through peripheral inputs to the swim musculature. In the previous paper, peripheral modulation of the swim muscles was shown to increase wing contractility. In the present paper, central inputs are described that trigger an increase in swim frequency and an increase in motor neuron activity. In dissected preparations, spontaneous acceleration from slow to fast swimming included an increase in the cycle frequency, a baseline depolarization in the swim interneurons and an increase in the intensity of motoneuron firing. Similar effects could be elicited by bath application of 10^{-5} mol l⁻¹ serotonin. Two clusters of cerebral serotonin-immunoreactive interneurons were found to produce acceleration of swimming accompanied by changes in neuronal activity. Posterior cluster neurons triggered an increase in swim frequency, depolarization of the swim

interneurons, an increase in general excitor motoneuron activity and activation of type 12 interneurons and pedal peripheral modulatory neurons. Cells from the anterior cerebral cluster also increased swim frequency, increased activity in the swim motoneurons and activated type 12 interneurons, pedal peripheral modulatory neurons and the heart excitor neuron. The time course of action of the anterior cluster neurons did not greatly outlast the duration of spike activity, while that of the posterior cluster neurons typically outlasted burst duration. It appears that the two discrete clusters of serotonin-immunoreactive neurons have similar, but not identical, effects on swim neurons, raising the possibility that the two serotonergic cell groups modulate the same target cells through different cellular mechanisms.

Key words: serotonin, swimming speed, modulation, cerebral neurons, mollusc, *Clione limacina*.

Introduction

There are four possible behavioral states in the swimming system of the pteropod mollusc *Clione limacina*; these include no swimming activity (passive sinking), slow swimming, fast swimming and escape swimming (Arshavsky *et al.* 1985a–d, 1989, 1992; Satterlie and Spencer, 1985; Satterlie, 1991a,b, 1993). The primary focus of this series of papers is on transitions between the different states: specifically, initiation of swimming, changes from slow swimming to fast swimming and variations of speed within slow and fast swimming speeds. Escape swimming involves different neural mechanisms and its examination is beyond the scope of the current study.

In the previous paper, a cluster of pedal serotonin-immunoreactive neurons (Pd-SW cells) was described which produced peripheral modulation of muscle contractility in the swimming system of *Clione limacina* (Satterlie, 1995). Since

these cells did not produce any detectable alteration of swim interneuron or motoneuron activities, it was suggested that the role of the immunoreactive neurons was in producing subtle changes of speed, such as acceleration within the limits of slow or fast swimming, but not a change from one to the other. The *Clione limacina* locomotory system is capable of more dramatic speed changes that involve an increase in wing-beat frequency achieved, in part, by a reconfiguration of the pattern generator through the activation of two previously inactive interneuron types (most notably the plateau-potential-producing type 12 interneurons: Arshavsky *et al.* 1985d, 1989). This change is accompanied by the recruitment of general excitor motoneurons (Satterlie, 1993) which, in turn, activate fast-twitch fatigable parapodial muscle cells and directly mediate enhanced contraction of the slow-twitch muscle cells

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that are active during slow swimming (Satterlie, 1993). The change from slow to fast swimming thus includes enhanced parapodial contractility in addition to an increase in wing-beat frequency. A search for neurons that might be involved in such 'changes of gear' should focus on cells that produce, in whole or in part, similar changes in pattern generator interneurons (including those added to the pattern generator during fast swimming) and swim motoneurons. These responses can be produced through bath application of serotonin to intact animals and reduced electrophysiological preparations (Arshavsky *et al.* 1985a; Kabotyansky and Sakharov, 1990; Satterlie, 1991a,b). We therefore decided to initiate our search for swim-modulating neurons by concentrating on serotonin-immunoreactive cells. There are relatively few serotonin-immunoreactive neurons in the central nervous system of *Clione limacina* (Satterlie *et al.* 1995). Likely candidates included the two medial clusters of neurons found in the cerebral ganglion since these form axon tracts that run in the cerebro-pedal connectives (Satterlie *et al.* 1995). These cells have been found to exert an excitatory influence on central neurons of the swimming system as well as having more global effects over systems that are altered in parallel with swimming speed changes, such as heart acceleration. The two cerebral clusters, anterior and posterior, produce distinct but synergistic excitatory inputs to the various cells of the swimming system, presenting an interesting complication to the organization of the serotonergic system responsible for the modification of swimming speed in *Clione limacina*.

Materials and methods

Animal collection, maintenance, dissection, immunohistochemical and electrophysiological techniques are as described previously (Satterlie *et al.* 1995; Satterlie, 1995) with the following exceptions: combined dye-injection/immunohistochemical experiments (double-labelling experiments) were performed in two ways. In some preparations, a 4% solution of the fixable dye Cascade Blue (Molecular Probes, Inc.) was pressure-injected *via* the recording electrode. The primary advantage of using Cascade Blue is that the excitation/emission spectra do not overlap with those of the Rhodamine label on the secondary antibody used in immunohistochemistry, thus providing a superior comparison of filled cells with antibody-labelled cells during filter switching. The disadvantage was that although our Cascade Blue fills adequately illuminated the cell bodies of injected cells, they were not bright enough to give a clear picture of the axon branches of these cells. For this reason, we also used a 5% solution of Carboxyfluorescein (Molecular Probes, Inc.) as the injected cell marker, which produced extremely bright fills that marked axons and axon branches. Although there was some 'bleed over' when viewing the Rhodamine-labelled cell(s) with the Fluorescein filter, the Carboxyfluorescein produced a green color that was clearly distinct from the yellowish fluorescence of the Rhodamine-labelled immunoreactive neurons. Carboxyfluorescein was not

visible with the Rhodamine filters. Controls in which known non-immunoreactive neurons were injected with dye and subjected to immunohistochemical analysis supported our contention that a qualitative color difference could be used to counteract the 'bleed over' problem.

Results

Spontaneous acceleration from slow to fast swimming, which appeared both as an increase in cycle frequency and as an increase in the force of muscle contraction, was accompanied by characteristic changes in the activity of both pattern generator interneurons and swim motor neurons. Increases in cycle frequency in interneurons included a tonic depolarization that typically lasted for the duration of fast swimming (Fig. 1A). At the motor neuron level, the general excitator motor neurons were typically recruited into the spiking mode during the change to fast swimming or, if spiking, they showed significant increases in the number of spikes per burst (Fig. 1B; Satterlie, 1993). Small motor neurons typically did not show a significant change in firing activity apart from the frequency change and a slight increase in the intensity of burst activity in each half-cycle.

Bath application of the serotonin antagonist mianserin to reduced preparations blocked episodes of fast swimming and produced long periods of invariant slow swimming in which the frequency of wing movements stayed between 0.75 and 1 Hz (Fig. 1C). Washing with sea water produced a gradual return of the variability of swim frequency and the appearance

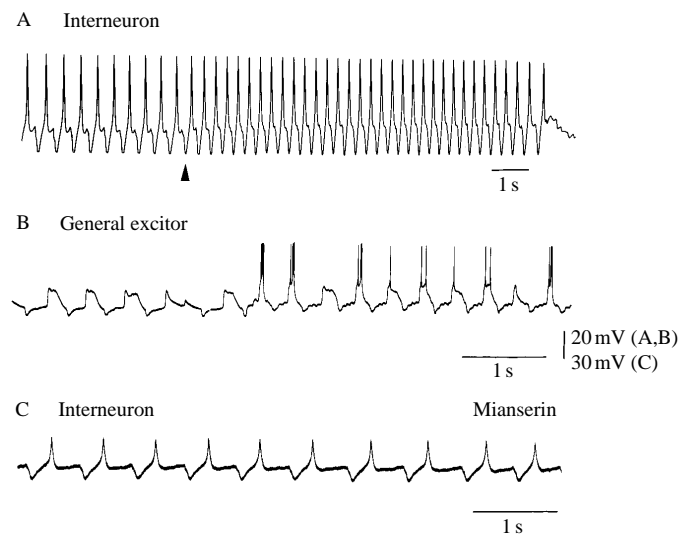


Fig. 1. (A) Spontaneous activity in a swim interneuron. An acceleration in swimming speed occurs at the arrow. Note the baseline depolarization that accompanies the increase in cycle frequency. (B) Spontaneous activity in a general excitator motoneuron. An acceleration in swimming speed changes the activity of the motoneuron from subthreshold synaptic activity to spiking activity. (C) Swim interneuron activity becomes slow and regular in the presence of 10^{-5} mol l^{-1} mianserin. No accelerations were noted until wash-out of the mianserin.

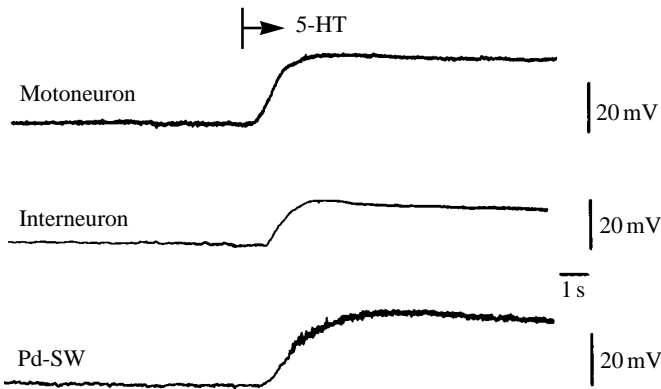


Fig. 2. Bath application of $10^{-5} \text{ mol l}^{-1}$ serotonin (5-HT) in high- Mg^{2+} saline results in depolarization of the swim motor neurons, swim interneurons and Pd-SW neurons.

of bouts of fast swimming. This suggested that serotonergic inputs to the swimming system played a role in central regulation of swimming speed.

To test whether the proposed target cells for serotonin modification were indeed responsive to serotonin, $10^{-5} \text{ mol l}^{-1}$ serotonin was bath-applied while recording from swim interneurons, swim motor neurons, pedal 5-HT cells, type 12 interneurons and heart excitor neurons. In all preparations, the cells were chemically isolated through the use of high- Mg^{2+} solutions. In all cases, the cells were depolarized following the addition of serotonin (Fig. 2) and returned to the normal resting membrane potential following wash-out (not shown). Furthermore, depolarizing responses were blocked by bath application of $10^{-5} \text{ mol l}^{-1}$ mianserin, suggesting that all major cell types in the swimming system were directly responsive to serotonin.

On the basis of the locations of the cerebral immunoreactive neurons reported in the first paper in this series (Satterlie *et al.* 1995), and a preliminary electrophysiological survey of cerebral immunoreactive neurons, we concentrated on two medial cell clusters. Both are located on the dorsal or dorso-lateral side of the cerebral ganglia and member neurons provide axon branches to each pedal ganglion. One cluster is located towards the anterior side of the cerebral commissure, while the other is to the posterior side. These cells will be referred to as posterior cluster cells (Cr-SP) and anterior cluster cells (Cr-SA) (see Fig. 3A).

Posterior cluster cells

Each posterior cluster contained three or four immunoreactive neurons slightly separated from one another. Depending on how the preparation was pinned, some of the cells were found on the margin of the ganglion and could be recorded from the ventral side. One of the Cr-SP cells, which was consistently located medial to the others, produced responses characteristic of swim acceleration and was capable of initiating swimming activity in non-swimming preparations. The majority of our recordings were conducted with this cell. Examinations of other cells in the posterior cluster indicated

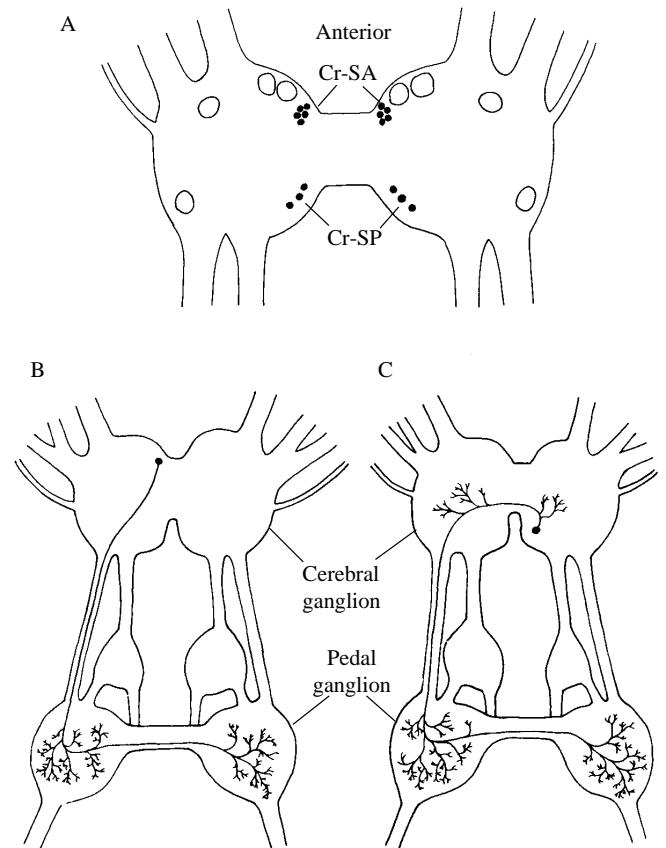


Fig. 3. Schematic diagram of the cerebral ganglia (A) and the central ring of cerebral, pedal and pleural ganglia (B,C) showing the location of cell bodies of the cerebral serotonergic anterior cells (Cr-SA) and the cerebral serotonergic posterior cells (Cr-SP) (filled circles in A) and the axonal morphology of individual Cr-SA (B) and Cr-SP (C) neurons. In all diagrams, anterior is to the top. The large open circles represent landmark somata used for orientation purposes.

that the cells located closer to the margin produced similar effects. Cr-SP cells were relatively small (soma diameter up to $25 \mu\text{m}$) and difficult to find. Their identification was based on their effect on swimming, which was a marked acceleration. Dye injections revealed that Cr-SP neurons sent a single axon to the contralateral cerebral ganglion through the cerebral commissure (Fig. 3C). The axon then descended to the contralateral pedal ganglion *via* the cerebro-pedal connective and across to the ipsilateral pedal ganglion through the pedal commissure. The axon produced extensive branching arborizations in both pedal ganglia and somewhat less extensive branching in the two cerebral ganglia.

To verify that the recorded/filled cells were indeed serotonin-immunoreactive neurons of the posterior cluster, the cells were injected with the dyes Cascade Blue or Carboxyfluorescein and subjected to serotonin immunohistochemistry (with Rhodamine-conjugated secondary antibody). In four out of four preparations, including both the medial and marginal cells, the filled cells also showed serotonin immunoreactivity (Fig. 4).

Cr-SP neurons were silent in about half of the preparations

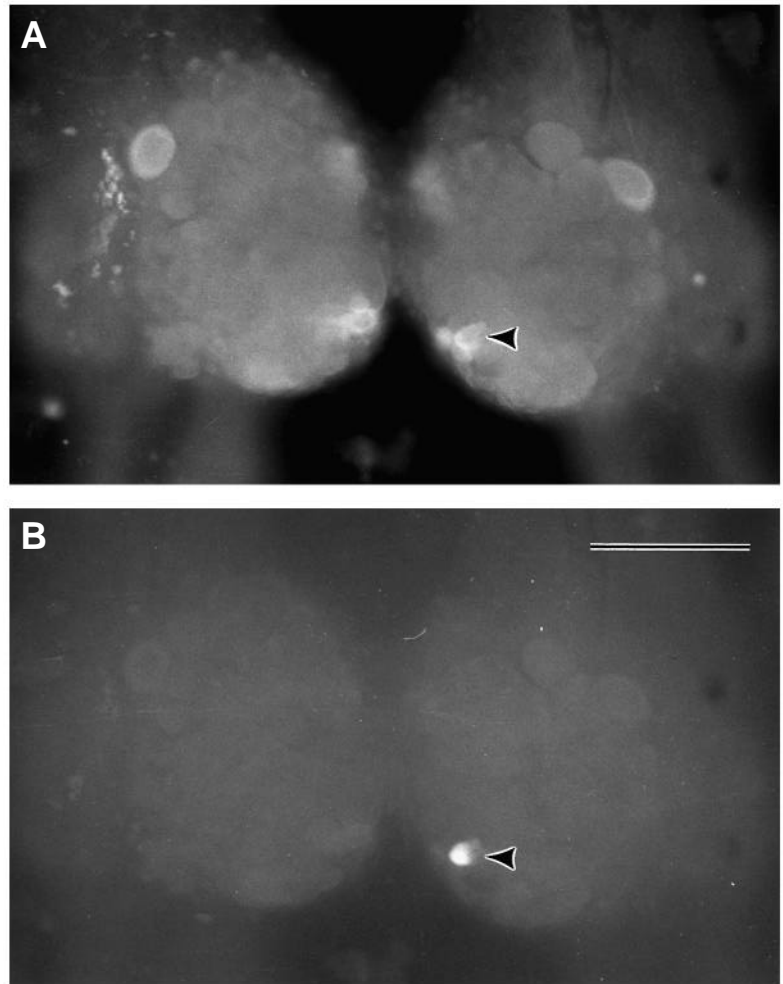


Fig. 4. Double labelling of a Cr-SP neuron. In A, the cerebral ganglia were subjected to serotonin immunohistochemistry using a Rhodamine-conjugated secondary antibody. The large cells at the top are the metacerebral cells. The arrowhead indicates the dye-filled cell, which is also shown in B, where the neuron has been filled with Cascade Blue. Note that the axon is not visible with this dye. Scale bar, 200 μm .

and showed regular or irregular firing activity during swimming activity in the others. When active, their firing activity could be roughly correlated with the activity of the swim neurons: increased frequency of swimming movements was accompanied by increases in firing frequency in Cr-SP neurons. During periods of active inhibition of swimming, the Cr-SP neurons displayed fast bursts of IPSPs (Fig. 5A). When these appeared while simultaneously recording from cells of the swimming system, including swim interneurons, swim motor neurons or pedal peripheral modulatory neurons, the active inhibition was always common to both recorded neurons, although the individual IPSPs did not always appear to be synchronous.

Anterior cluster cells

Each cerebral ganglion contained a tight cluster of seven or eight serotonin-immunoreactive neurons on the antero-medial margin near the cerebral commissure (Fig. 3A). All neurons from the cluster had soma diameters in the range 15–20 μm . These neurons were designated Cr-SA neurons. The main physiological effect of Cr-SA cell activity was the initiation of swimming or a significant acceleration of swimming. To verify that recorded cells were indeed serotonin-immunoreactive,

double-labelling experiments were conducted in which neurons were injected with Carboxyfluorescein and subjected to serotonin immunohistochemistry (Fig. 6).

Two sets of experiments strongly suggested that all the neurons from the anterior cluster had the same physiological effect as well as the same morphology. First, in each of five different preparations, three separate cluster neurons were penetrated, recorded and filled. Second, in each of four double-labelling preparations, two dye-filled neurons that exhibited serotonin immunoreactivity appeared in different areas of the cluster.

Each Cr-SA neuron had the same morphology, with one large axon running through the ipsilateral cerebral ganglion to the ipsilateral cerebro-pedal connective (see Fig. 3B). The axon extended to the ipsilateral pedal ganglion then across the pedal commissure to the contralateral pedal ganglion. In each pedal ganglion, the axon branched extensively, particularly in the region close to the origin of the wing nerve. Unlike Cr-SP neurons, Cr-SA neurons did not have noticeable branches in the cerebral ganglia (compare Fig. 3B and Fig. 3C). All Cr-SA neurons showed irregular spontaneous spike activity which correlated with the activity of the swim neurons and Pd-SW neurons.

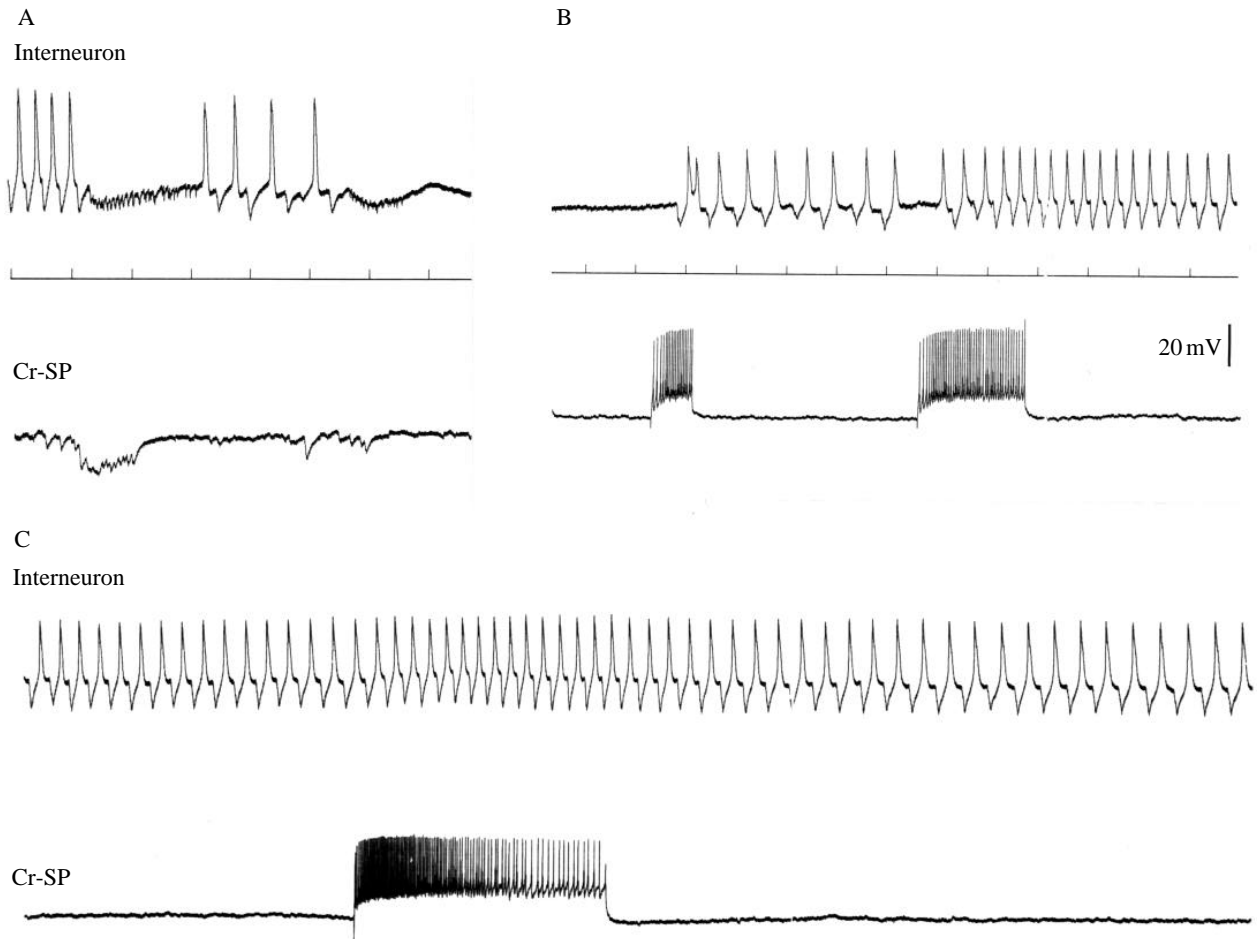


Fig. 5. Dual recordings from a Cr-SP neuron and a swim interneuron. (A) When swimming is inhibited, there is also an inhibition in the Cr-SP neuron. (B) Stimulated spike activity in a Cr-SP neuron was capable of initiating swimming activity. (C) During existing swimming activity, a burst of spikes in a Cr-SP neuron triggered an increase in swimming speed and a baseline depolarization in the swim interneurons (compare the depolarization here with that in Fig. 1A). The tick interval on the time trace is 1 s. The chart speed in C is the same as in B.

Influences on swim interneurons

In non-swimming preparations, induced bursting in Cr-SP neurons was able to initiate swimming provided that the spike burst included more than four or five action potentials (based on experimental firing rates of approximately 20 Hz; Fig. 5B). In swimming preparations, spike bursts in Cr-SP neurons triggered an immediate swim acceleration that appeared in the first cycle following the initiation of the burst (Fig. 5C). The acceleration, which was accompanied by a baseline depolarization in the swim interneurons, outlasted the stimulus by up to 10 s (for a burst of 1 s duration at about 40 Hz). The baseline depolarization was similar to that seen in interneurons during spontaneous speed changes (compare Fig. 5C with Fig. 1A).

When bathed in high-Mg²⁺, high-Ca²⁺ sea water, inputs from Cr-SP neurons to swim interneurons were reduced to depolarizing slow waves that were up to 8 mV in amplitude and which outlasted the stimulus burst by at least 1–2 s (data not shown). No responses were seen following a single spike in a Cr-SP neuron, nor following low-frequency bursts. Only

with bursts of approximately 10 spikes at or above 15 Hz did slow waves appear. Discrete synaptic potentials from Cr-SP neurons were never seen in swim interneurons.

Swim acceleration was blocked in preparations that were bathed in the serotonin antagonist mianserin (10^{-5} mol l⁻¹). Induced bursting in Cr-SP neurons that normally produced swim acceleration had no effect in mianserin-treated preparations. Furthermore, in preparations bathed in high-Mg²⁺, high-Ca²⁺ saline with added mianserin, the swim interneuron slow waves described above were not observed.

In non-swimming preparations, activation of an individual Cr-SA neuron produced initiation of activity in the swim central pattern generator and swim motor neurons. In preparations that showed swimming activity, activity in a Cr-SA neuron caused a marked acceleration in swim interneuron activity (Fig. 7A). In contrast with Cr-SP neurons, the period of swim acceleration typically did not greatly outlast the period of Cr-SA firing.

Following the application of high-Mg²⁺, high-Ca²⁺ sea water, Cr-SA cell activity produced a compound monosynaptic

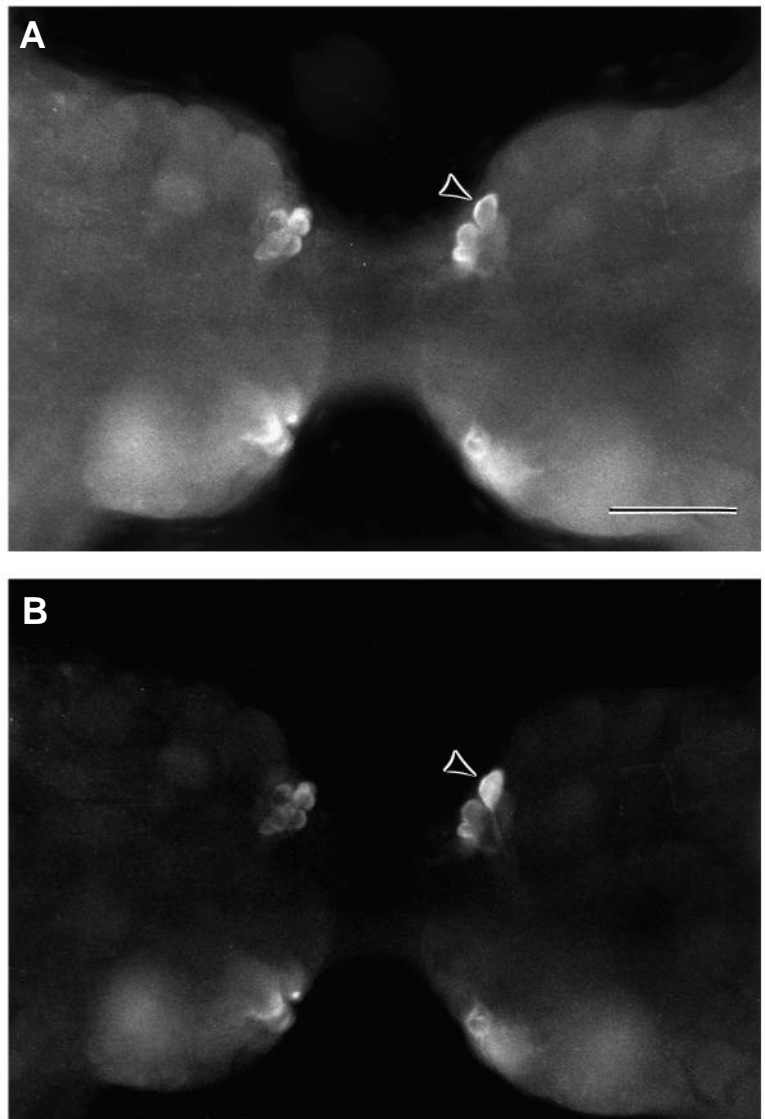


Fig. 6. Double-labelling experiments indicate that recorded Cr-SA neurons are also serotonin-immunoreactive. (A) Serotonin immunoreactivity demonstrated using a Rhodamine-conjugated secondary antibody. (B) Carboxyfluorescein fill of a recorded Cr-SA neuron. The Rhodamine label is visible in the Carboxyfluorescein filters, but Carboxyfluorescein is not visible in the Rhodamine filters. Scale bar, 50 μm . The arrowheads mark the same cell body in A and B.

PSP in the swim interneurons following each spike burst in Cr-SA cells (Fig. 7B). This PSP, as well as the acceleration of swimming activity in normal preparations, was blocked by $10^{-5} \text{ mol l}^{-1}$ mianserin.

Type 12 interneurons have cell bodies in the pleural ganglia and are recruited into the pattern generator during fast swimming (Arshavsky *et al.* 1985*d*, 1989). These cells normally receive only IPSPs from the pattern generator during slow swimming, but produce distinct cycle-locked plateau potentials when activated during the change to fast swimming. Cr-SP activity was able to initiate plateau activity in type 12 interneurons in non-swimming preparations (Fig. 8A) and to initiate plateau production in slow swimming preparations (Fig. 8B). In preparations in which type 12 interneurons regularly produced plateaus, Cr-SP stimulation produced a slow baseline depolarization similar to that found in swim interneurons.

High- Mg^{2+} , high- Ca^{2+} sea water reduced the Cr-SP-induced response to small (1–3 mV) slow wave depolarizations that

outlasted Cr-SP bursts by approximately 1 s (data not shown). As with other preparations, mianserin reversibly blocked Cr-SP cell inputs to type 12 interneurons.

As with Cr-SP neurons, induced firing in Cr-SA cells triggered plateau potentials in type 12 interneurons, typically for the duration of the induced burst. The excitatory effect of Cr-SA cells on type 12 cells was also blocked by $10^{-5} \text{ mol l}^{-1}$ mianserin.

Influences on motor neurons

Cr-SP neuron activity had a distinct affect on general excitor motor neurons (GEMNs). In slow swimming preparations in which the GEMNs were not spiking, but merely receiving subthreshold synaptic activity from the pattern generator, induced firing in Cr-SP neurons initiated cycle-locked general excitor spiking (Fig. 9A). In preparations in which GEMNs were producing a single spike or a few spikes per cycle, Cr-SP activity converted the GEMN output to robust bursts of spikes per cycle (Fig. 9B). In both cases, the period of

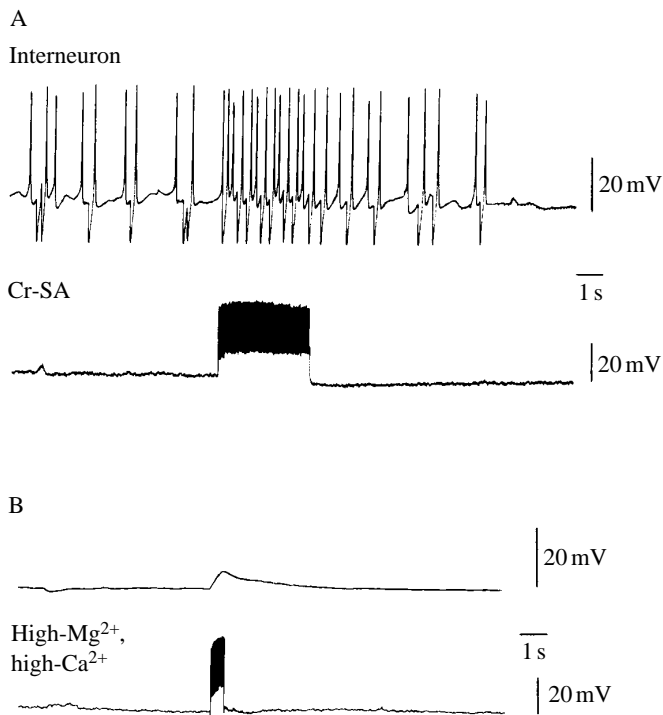


Fig. 7. (A) Induced activity in a Cr-SA neuron triggers an acceleration of activity in swim interneurons without a significant baseline depolarization. (B) This connection is maintained when the preparation is bathed in high-Mg²⁺, high-Ca²⁺ sea water.

increased firing in GEMNs did not greatly outlast the period of Cr-SP firing. In records in which GEMNs showed a spontaneous change from the non-spiking mode to the spiking mode, the Cr-SP cell exhibited spontaneous firing activity.

With preparations bathed in high-Mg²⁺, high-Ca²⁺ sea water, Cr-SP spikes produced small synaptic potentials (1–4 mV) in GEMNs (data not shown). Cr-SP bursts produced facilitating synaptic potentials in GEMNs; however, the synaptic potentials did not faithfully follow individual spikes one-to-one. Mianserin reversibly blocked the excitatory inputs to GEMNs.

Induced firing in individual Cr-SA neurons also produced significant activation of general excitor motor neurons (Fig. 10A). During existing swimming activity, Cr-SA bursts triggered an acceleration of general excitor activity as well as a baseline depolarization of the motor neurons (Fig. 10B). Spontaneous swim accelerations showed similar increases in both general excitor and Cr-SA activities (Fig. 10B). When the motor neurons were spiking, Cr-SA activity greatly increased the intensity of the spike bursts. The connection was deemed monosynaptic by the same test used for swim interneurons; however, in this case, each Cr-SA spike produced a single large PSP in the general excitor (Fig. 11A). The EPSPs had a short constant latency, typically under 5 ms. EPSPs in these tests, and increases in general excitor activity in normal preparations, were blocked by bathing the preparation in 10⁻⁵ mol l⁻¹ mianserin (Fig. 11B).

Small motor neurons were relatively unresponsive to Cr-SP

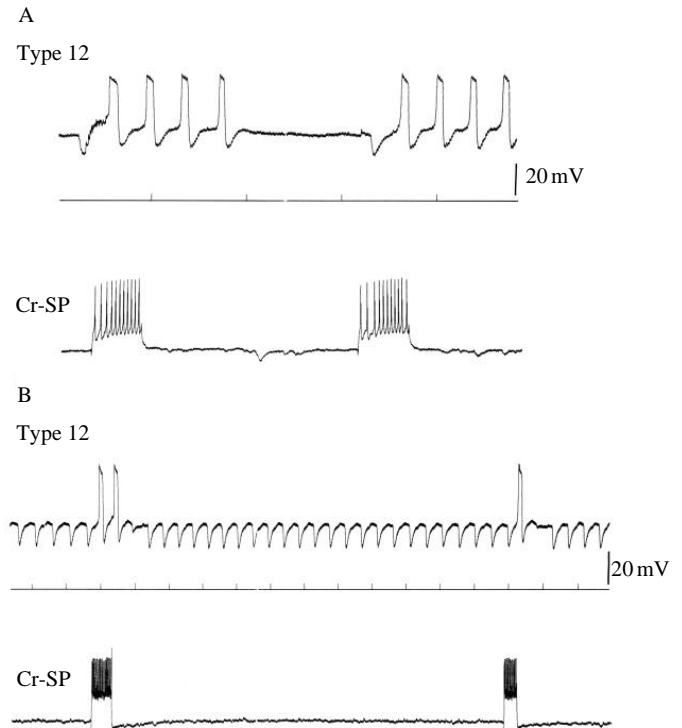


Fig. 8. (A) Type 12 interneurons produce phase-locked plateau potentials that occur in the depressor phase (ventral movement) of the swim cycle. Cr-SP neuron bursts were capable of initiating swimming activity with the type 12 interneurons in the plateau mode. (B) During normal slow swimming, type 12 interneurons received an IPSP during the elevator phase (dorsal movement) of the wing cycle, with no significant input during the depressor phase. Cr-SP bursts produced a short-term shift of type 12 activity to plateau activity. The tick interval on the time traces is 1 s.

neuron activity. Typical responses included a slight baseline depolarization similar to that seen in swim interneurons and a frequency increase presumably imposed by the pattern generator. The burst intensity of small motor neurons was occasionally increased following Cr-SP activity. However, small motor neurons were more strongly activated by Cr-SA activity *via* monosynaptic connections (Fig. 12A,B). The small motor neuron responses did not significantly outlast the Cr-SA burst. Bathing the preparations in high-Mg²⁺, high-Ca²⁺ sea water revealed large-amplitude (up to 10 mV) compound EPSPs with each Cr-SA spike burst (Fig. 12B). The connections from Cr-SA cells to small motor neurons were blocked by mianserin.

Influences on pedal 5-HT neurons

In the previous paper (Satterlie, 1995), the medial serotonergic neurons of the pedal ganglia (Pd-SW cells) were shown to have a strictly peripheral modulatory influence over the swimming system and their spontaneous firing activity was shown to be loosely correlated with swimming speed. Spontaneous spike activity in Cr-SP cells was likewise loosely tied to spike activity in Pd-SW cells (Fig. 13A). Active inhibition of swimming resulted in inhibition of both cells

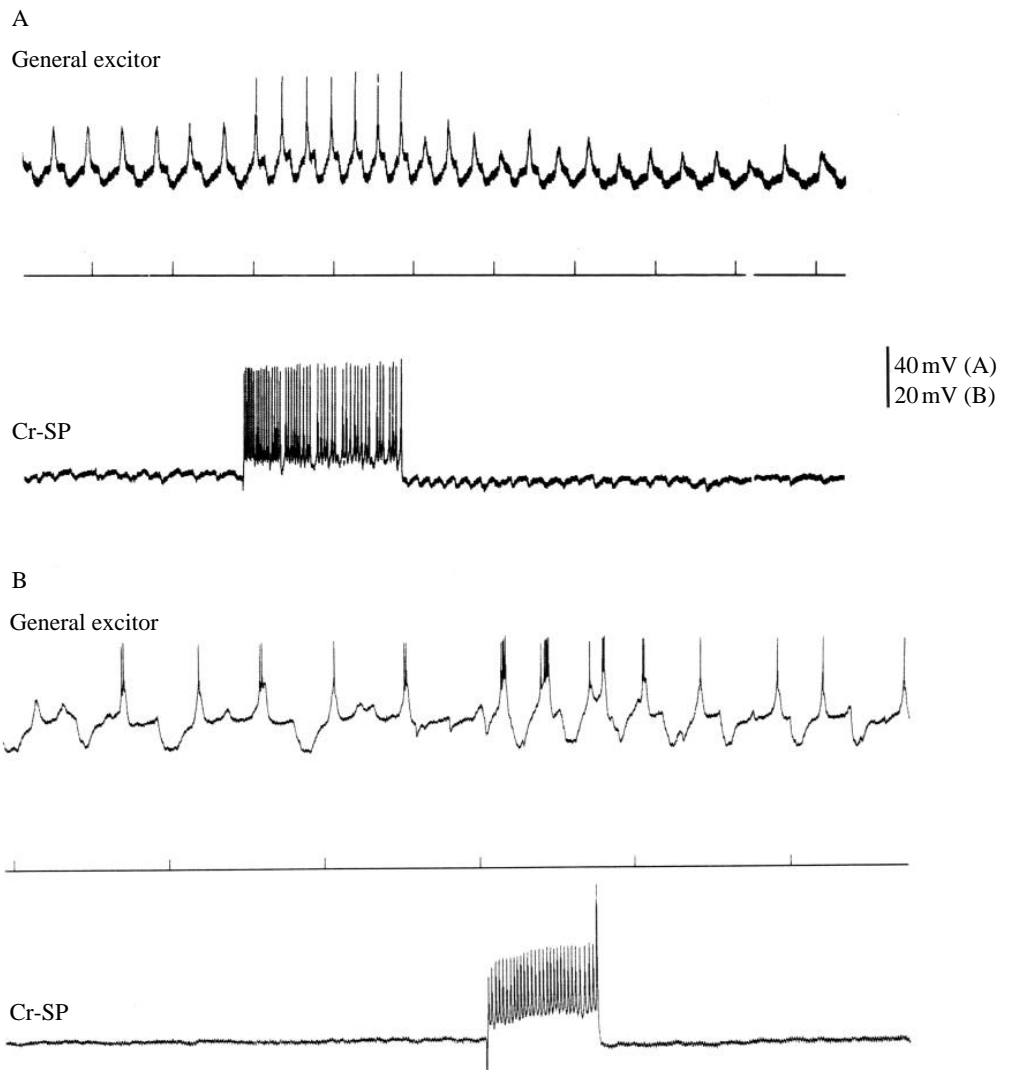


Fig. 9. Cr-SP neurons activate general excitor motor neurons. (A) Induced Cr-SP bursts change the general excitor activity from phasic subthreshold synaptic activity to phasic spiking activity. (B) When general exciters are spiking, Cr-SP activity triggers an increase in firing intensity in each half-cycle of swimming activity. The tick interval on the time traces is 1 s.

simultaneously (Fig. 13A). Induced firing in Cr-SP cells was capable of initiating spike activity in Pd-SW cells (Fig. 13B). Bathing the preparation in high-Mg²⁺, high-Ca²⁺ sea water clearly revealed that two types of synaptic responses were produced in Pd-SW cells by Cr-SP inputs: fast synaptic potentials which were followed by slow depolarizing waves (lasting 2–5 s and up to 15 mV in amplitude; Fig. 13C). Pd-SW cells did not influence Cr-SP activity in these or untreated preparations. As with other cells, Cr-SP inputs to Pd-SW cells were blocked by 10⁻⁵ mol l⁻¹ mianserin.

Cr-SA neurons also produced weak excitatory responses in Pd-SW neurons. In spontaneous recordings, there was a positive correlation between spike activity in the two neuron types (Fig. 14A). When stimulated to burst, Cr-SA activity either initiated Pd-SW cell spiking or increased its frequency (Fig. 14B). The effect persisted in high-Mg²⁺, high-Ca²⁺ sea water and was blocked by mianserin.

Arshavsky *et al.* (1990) have identified an asymmetrical neuron in the left pedal ganglion that provides excitatory inputs to the heart. An asymmetric immunoreactive soma was found in the same position as the heart excitor in the left pedal

ganglion. Through a combination of physiological recording and double labelling, the heart excitor was shown to be immunoreactive for serotonin (Fig. 15C,D). Heart excitor neurons were excited by Cr-SA activity (Fig. 15B) with a connection that was weak and blocked by mianserin. There was a loose correlation between the firing activity in Cr-SA neurons and heart exciters (Fig. 15A).

Discussion

Cerebral serotonin-immunoreactive neurons from two different clusters were found to produce acceleration of swimming speed in *Clione limacina* through global excitatory effects on swim interneurons, swim motor neurons, type 12 interneurons, pedal serotonin-immunoreactive neurons and heart excitor neurons (Fig. 16). One of the cerebral cell types (Cr-SP) was undoubtedly found by Arshavsky *et al.* (1992) and was referred to as a 'cerebral locomotion excitor'. The cerebral cells are believed to utilize serotonin as a neurotransmitter/neuromodulator on the basis of several lines of evidence. First, double-labelling experiments show that the recorded and filled

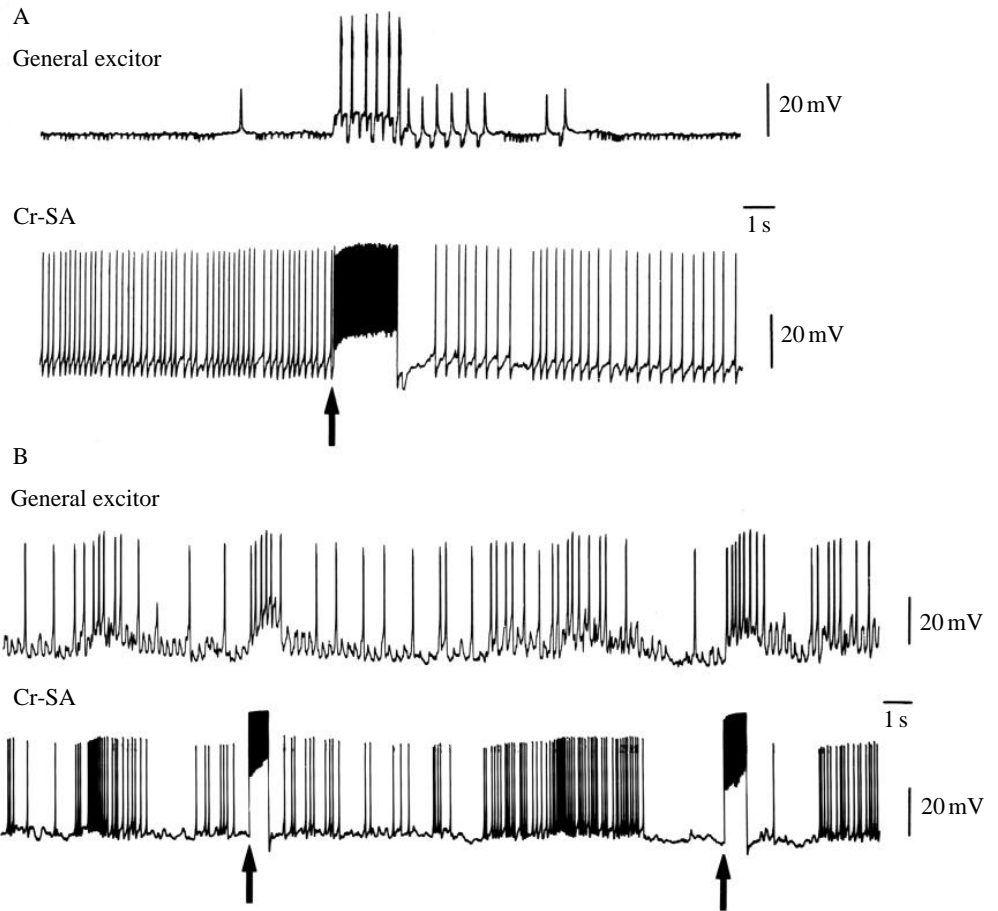


Fig. 10. Induced (A,B) and spontaneous (B) increases in Cr-SA neuron activity both produce an acceleration of swimming and spiking activity in general excitor motor neurons. The arrows indicate bursts triggered by intracellular current injection.

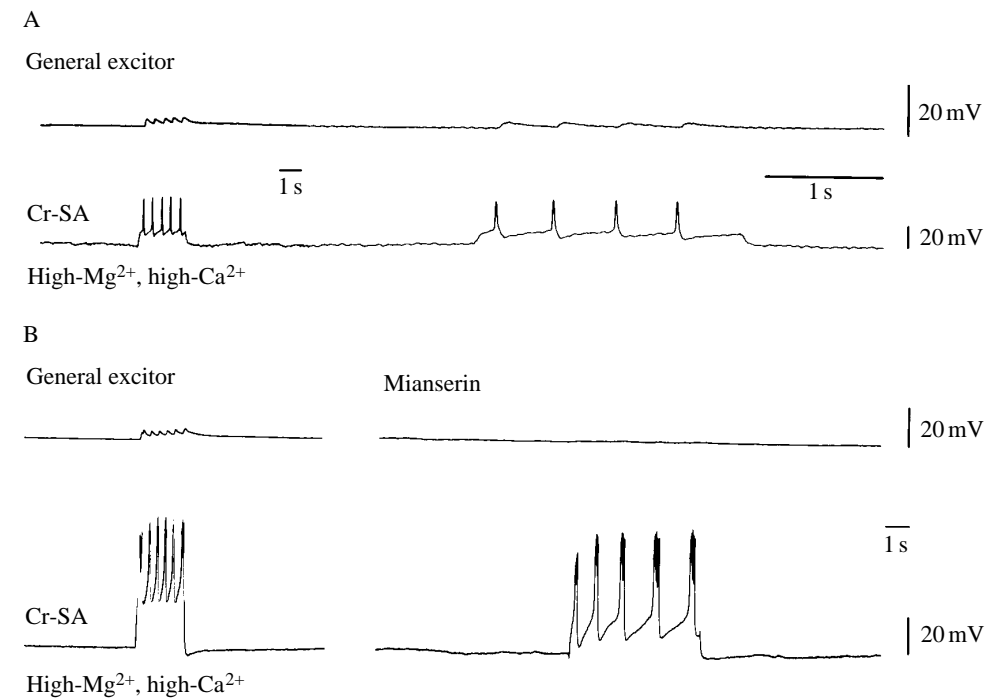


Fig. 11. (A) Cr-SA neuron to general excitor inputs persisted when the preparation was bathed in high-Mg²⁺, high-Ca²⁺ sea water. EPSPs occurred 1:1 with presynaptic spikes (shown at two chart speeds). (B) The putative monosynaptic connections were blocked when 10⁻⁵ mol l⁻¹ mianserin was added to the recording dish.

cells are immunoreactive for serotonin. Second, activity in the cerebral neurons produces acceleratory responses that mimic those induced by bath-applied serotonin as well as those

observed during spontaneous swim accelerations. Finally, all responses, including responses to bath-applied serotonin and interactions between cerebral neurons and the various cell

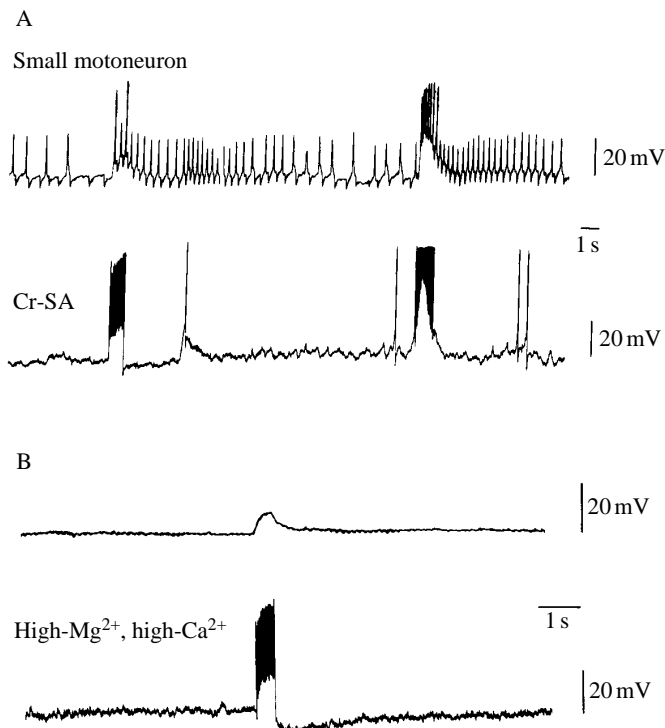


Fig. 12. (A) Cr-SA neuron activity produced strong activation of small motor neurons. (B) The connection persisted when the preparation was bathed in high-Mg²⁺, high-Ca²⁺ sea water.

types associated with the swimming system, are reversibly blocked by the serotonin antagonist mianserin. The cerebral neurons could release cotransmitters in addition to serotonin; however, no evidence for multiple transmitter action from these cells exists to date.

Individual neurons from both cerebral clusters are capable of *initiating* activity in the swimming system. In addition, they have a powerful effect in accelerating existing swimming activity, primarily through their effects on the pattern generator interneurons of the pedal and pleural ganglia. The net effect of this interaction is an increase in wing-beat frequency. Inputs from the cerebral neurons to the swim motoneurons also directly result in an increase in the force of muscle contractions since cerebral neuron activity either induces spike activity in non-spiking general excitor motor neurons or increases the intensity of bursting in active general excitors. These motor neurons are either silent (no spikes) or produce one or two spikes per swim half-cycle during slow swimming and are recruited into spiking activity during the change to fast swimming, when they typically produce robust bursts in each half-cycle (Satterlie, 1993). Cerebral cell effects on small motor neurons, which are active during both slow and fast swimming, are also excitatory.

Cerebral serotonin-immunoreactive neurons (Cr-SP and Cr-SA) do not have any direct inputs to the swim musculature. In contrast to the pedal serotonin-immunoreactive neurons (Pd-SW), which produce only peripheral modulation of muscle activity (Satterlie, 1995), the cerebral neurons produce

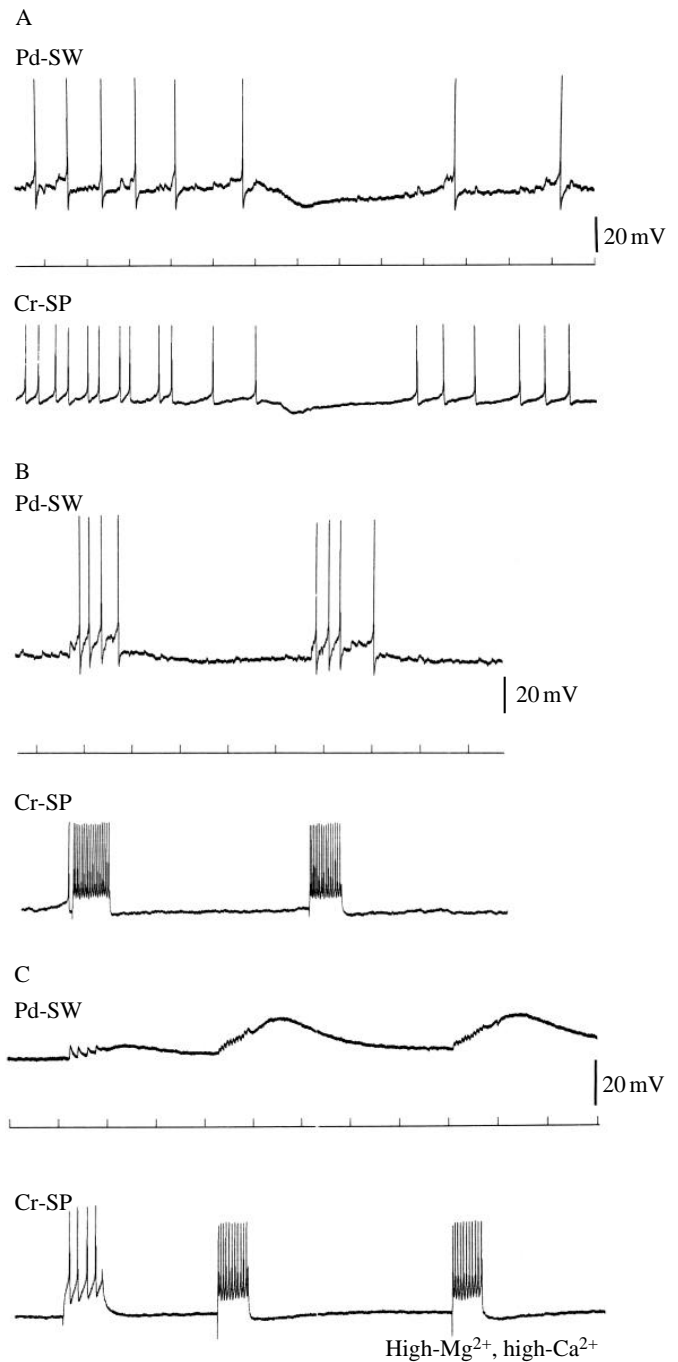


Fig. 13. (A) The spontaneous activities of Pd-SW neurons and Cr-SP neurons were very similar, including periods of swim inhibition. (B) Activation of a Cr-SP neuron induced spiking in Pd-SW neurons. (C) The Cr-SP to Pd-SW cell connections persisted when the preparation was bathed in high-Mg²⁺, high-Ca²⁺ sea water. The responses included phasic EPSPs and slow wave depolarizations. The tick interval on the time traces is 1 s.

widespread central effects (Fig. 16). In addition to excitatory inputs to the swim interneurons and motor neurons, they also excite the Pd-SW neurons as well as the serotonergic heart excitor neuron. The heart excitor has been shown to produce

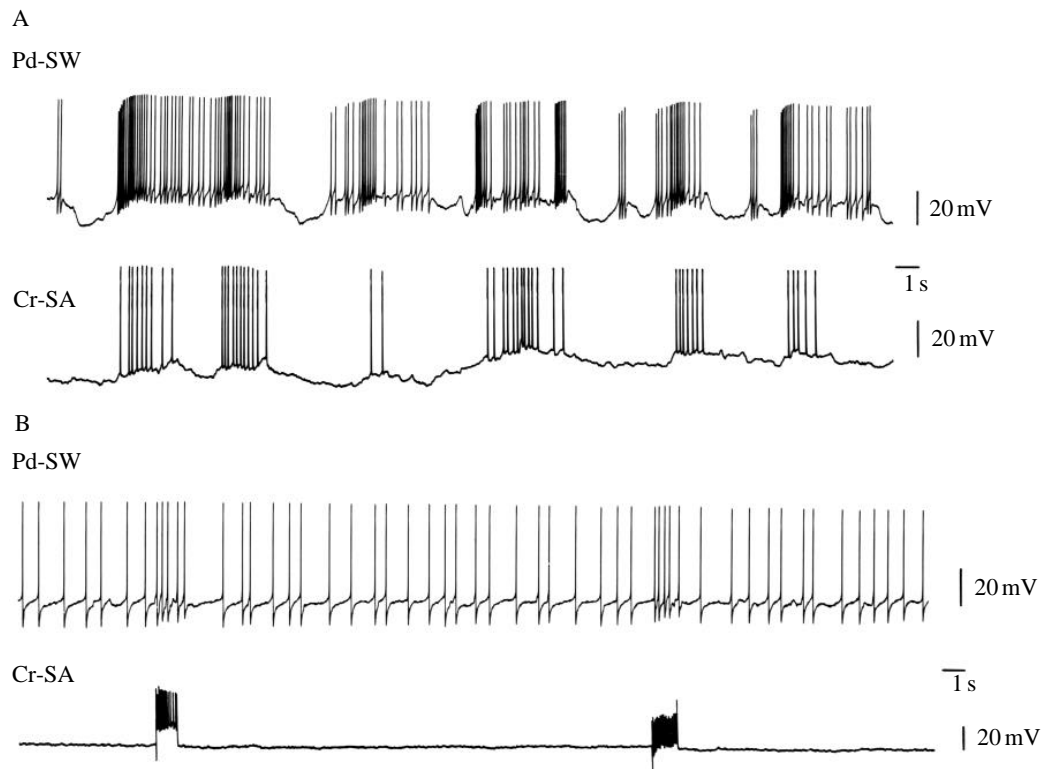


Fig. 14. (A) The spontaneous activities of Cr-SA neurons and of Pd-SW neurons were usually similar in that periods of firing tended to coincide in the two cell types. (B) Induced spiking in Cr-SA neurons triggered increases in firing frequency in Pd-SW neurons.

acceleration of heart activity coincident with increases in swimming activity in *Clione limacina* (Arshavsky *et al.* 1990).

The identification of cerebral serotonergic neurons that initiate and accelerate swimming is interesting in a comparative sense. Serotonin is used as a modulator in a variety of locomotory systems, including *Aplysia californica* pedal locomotion (Mackey and Carew, 1983), *Aplysia brasiliana* swimming (McPherson and Blankenship, 1991), leech swimming (Nusbaum and Kristan, 1986; Nusbaum, 1986), insect flight (Claassen and Kammer, 1986), lamprey swimming (Harris-Warrick and Cohen, 1985; Grillner and Matsushima, 1991), swimming in embryonic amphibians (Woolston *et al.* 1994; Sillar and Simmers, 1994) and cat locomotion (Barbeau and Rossignol, 1990). In *Aplysia brasiliana*, both bath-applied serotonin and stimulation of cerebro-pedal connectives were found to be effective stimuli for the initiation of rhythmic parapodial flapping (Parsons and Pinsker, 1989; McPherson and Blankenship, 1991). Furthermore, a pair of cerebral neurons has been found in the C cluster region; they are capable of initiating weak swim motor programs (Blankenship *et al.* 1993). These cells not only trigger swimming movements but also produce direct EPSPs in POP neurons, which are similar in function to the Pd-SW neurons of *Clione limacina*. It is possible, therefore, that the cerebral neuron pair of *A. brasiliana* may be at least functionally similar to one type of cerebral serotonin-immunoreactive neuron of *Clione limacina*. If so, it is interesting that putative swim 'command' neurons of animals that swim infrequently not only serve that function in animals that swim nearly continuously but also have a function in the acceleration of swimming, most notably to change the pattern generator and motor neuron activities to produce a change from

slow to fast swimming. This change involves a reconfiguration of the pattern generator through the activation of two types of previously 'inactive' interneurons, including type 12 interneurons, whose cell bodies lie in the pleural ganglia (Arshavsky *et al.* 1985*d*, 1989). Type 12 interneurons normally receive only rhythmic IPSPs during slow swimming, but produce plateau potentials during each half-cycle of fast swimming activity. Inputs from the cerebral neurons of *Clione limacina* are capable of shifting the activity of type 12 interneurons to the plateau mode, thus helping to contribute to a shortening of the cycle period and allowing an increase in the swim frequency.

The existence of two separate populations of serotonin-immunoreactive neurons that both influence the same target cells of the swimming system raises interesting questions, particularly since the modes of action of the two groups are slightly different. In general, the posterior cluster neurons (Cr-SP cells) produce excitatory effects that tend to outlast the period of Cr-SP cell activity. In monosynaptic tests, these cells tend to produce slow depolarizing wave-type voltage changes in their targets, including swim interneurons, Pd-SW cells and type 12 interneurons. Exceptions included both the general excitor and the small motor neurons, which received fast EPSP-type inputs. The most notable interaction was between Cr-SP cells and Pd-SW cells, since they included fast EPSPs followed by a large slow wave. In contrast, anterior cluster cells (Cr-SA cells) tend to produce fast EPSP-type responses that do not outlast the period of Cr-SA cell firing. Monosynaptic EPSPs were recorded from the swim interneurons, general excitor motoneurons, small motoneurons and Pd-SW cells. Cr-SA cells produce particularly strong excitation of both the general

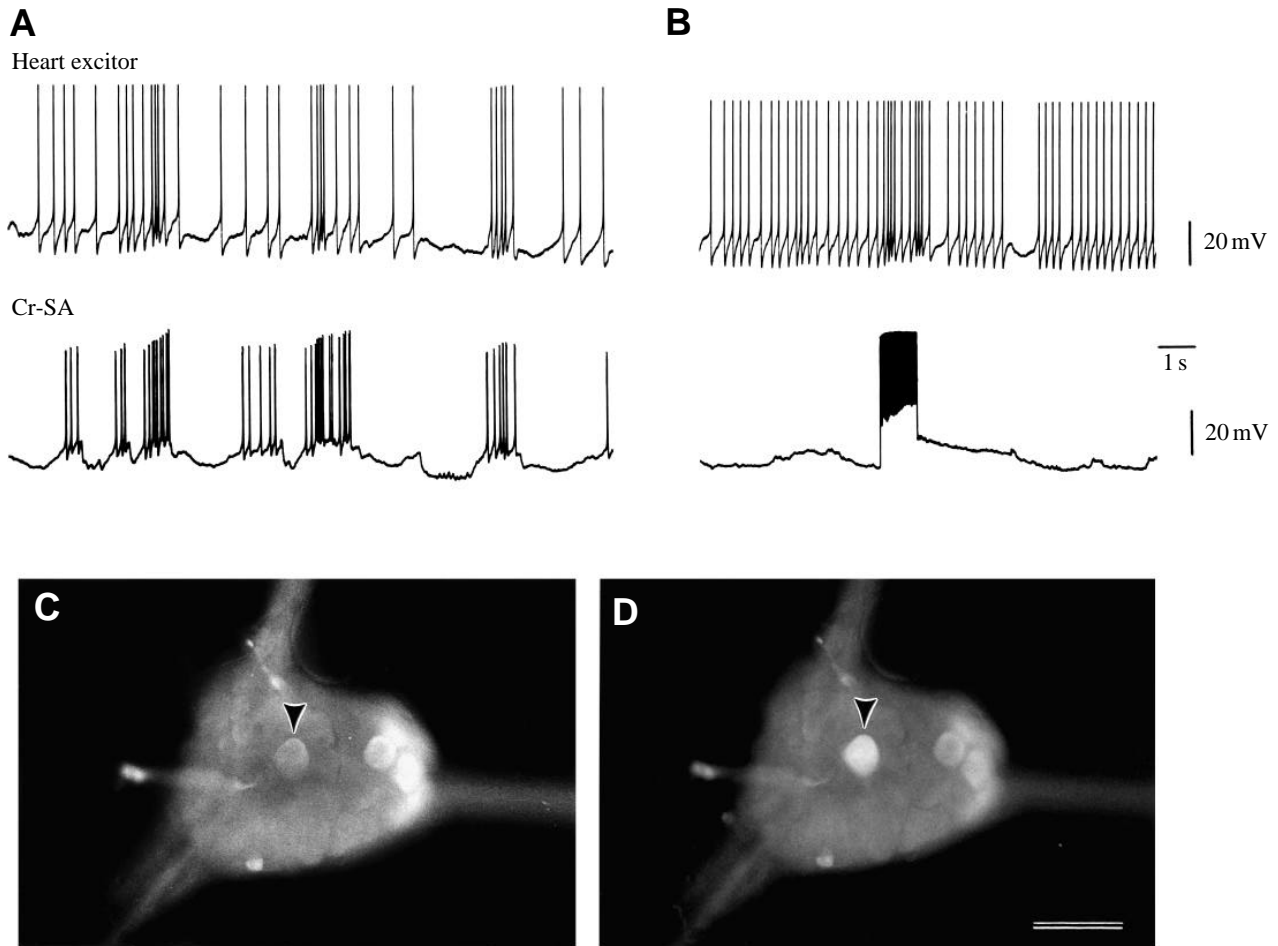


Fig. 15. (A) As with Cr-SA and Pd-SW cells, Cr-SA cells and heart excitor neuron activities were similar. (B) Induced firing in a Cr-SA neuron triggered an acceleration in heart excitor spiking. Double-labelling experiments (C,D) show that the heart excitor (arrowhead) is serotonin-immunoreactive. (C) Serotonin immunohistochemistry with a Rhodamine-conjugated secondary antibody. (D) Carboxyfluorescein injection of the heart excitor neuron. Scale bar, 100 μm (C,D).

excitor and small motoneurons. A similar situation exists in the leech swimming system, in which several serotonergic neurons provide excitatory drive to the swim central pattern generator (CPG), including neurons that provide direct synaptic input to CPG neurons, and Retzius cells which produce a longer-term

excitation *via* a humoral mechanism (Willard, 1981; Nusbaum and Kristan, 1986; Nusbaum, 1986). The exact function of the dichotomy in activation parameters in the *Clione limacina* cerebral serotonin system is not clear at present, but it appears that one could serve more of a gain-setting function (posterior) and the other an immediate activation function (anterior). An intriguing aspect of this arrangement, both behaviorally and mechanistically, is the notion that two populations of activator/modulator cells releasing the same neuroactive agent

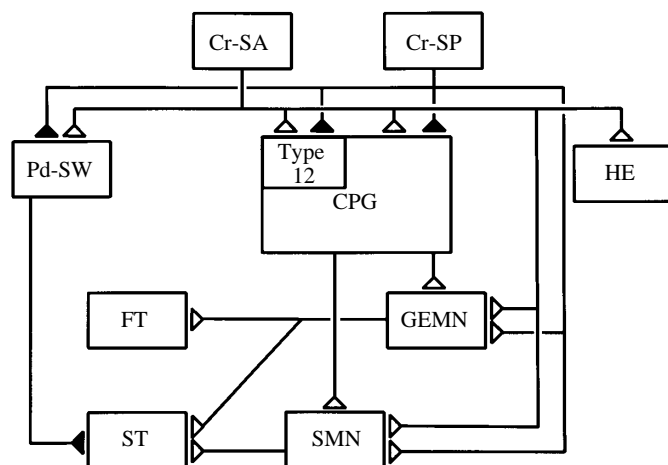


Fig. 16. Schematic diagram showing the influence of Cr-SA and Cr-SP neurons on the various cells of the swimming system. Only half of the swimming system is shown. All connections are excitatory; open triangles represent fast EPSP-type inputs, filled triangles represent connections that utilize slow depolarizing waves and are modulatory. The swimming system is simplified so that it does not show the mixture of excitatory and inhibitory connections that produce the alternation of dorsal and ventral wing movements. CPG, central pattern generator; GEMN, general excitor motor neuron; SMN, small motor neurons; FT, fast-twitch musculature; ST, slow-twitch musculature; HE, heart excitor neuron. Other abbreviations as given in the text.

on identical target cells are able to produce different responses in those target cells. The conditions of activation of the two cerebral groups during normal behavior patterns are not known. In particular, it is not known whether the two groups are sometimes activated together and sometimes activated separately. At least three behavior patterns involve a dramatic increase in swimming speed: 'normal' fast swimming, escape swimming and the acquisition phase of feeding. The roles of the two cerebral groups in these and other behavior patterns are currently being evaluated.

Both groups of cerebral neurons provide excitatory input to the Pd-SW neurons, which have been shown to modulate swim muscle contractility (Satterlie, 1995). Cerebral serotonin neurons can thus increase muscle contractility in two ways: by activation of Pd-SW neurons and by activation or enhancement of the activity of the swim motoneurons, particularly the general excitor motoneurons.

Thus far, we have discussed four different groups of serotonin-immunoreactive neurons, including the two cerebral clusters (Cr-SP and Cr-SA), a cluster of pedal neurons (Pd-SW) and the heart excitor (also a pedal neuron). All of these cells have similar firing characteristics. In particular, the frequency of spike production increases as swimming speed increases, and all are similarly inhibited when swimming is inhibited. Within this group, the cerebral 5-HT clusters exert an excitatory influence over the pedal cells. The pedal cells can presumably also operate independently. The widespread inhibition suggests that there is a distributed inhibitory system that is designed to suppress swimming activity. Cerebral neurons have been found in the cerebral ganglion near both the anterior and posterior clusters that send axons to the pedal ganglion and that can inhibit swimming activity. Similar cells have been described by Arshavsky *et al.* (1992) and referred to as 'cerebral locomotion inhibitors'. Cerebral inhibitory cells have not been systematically studied to determine whether they produce widespread or more localized effects.

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